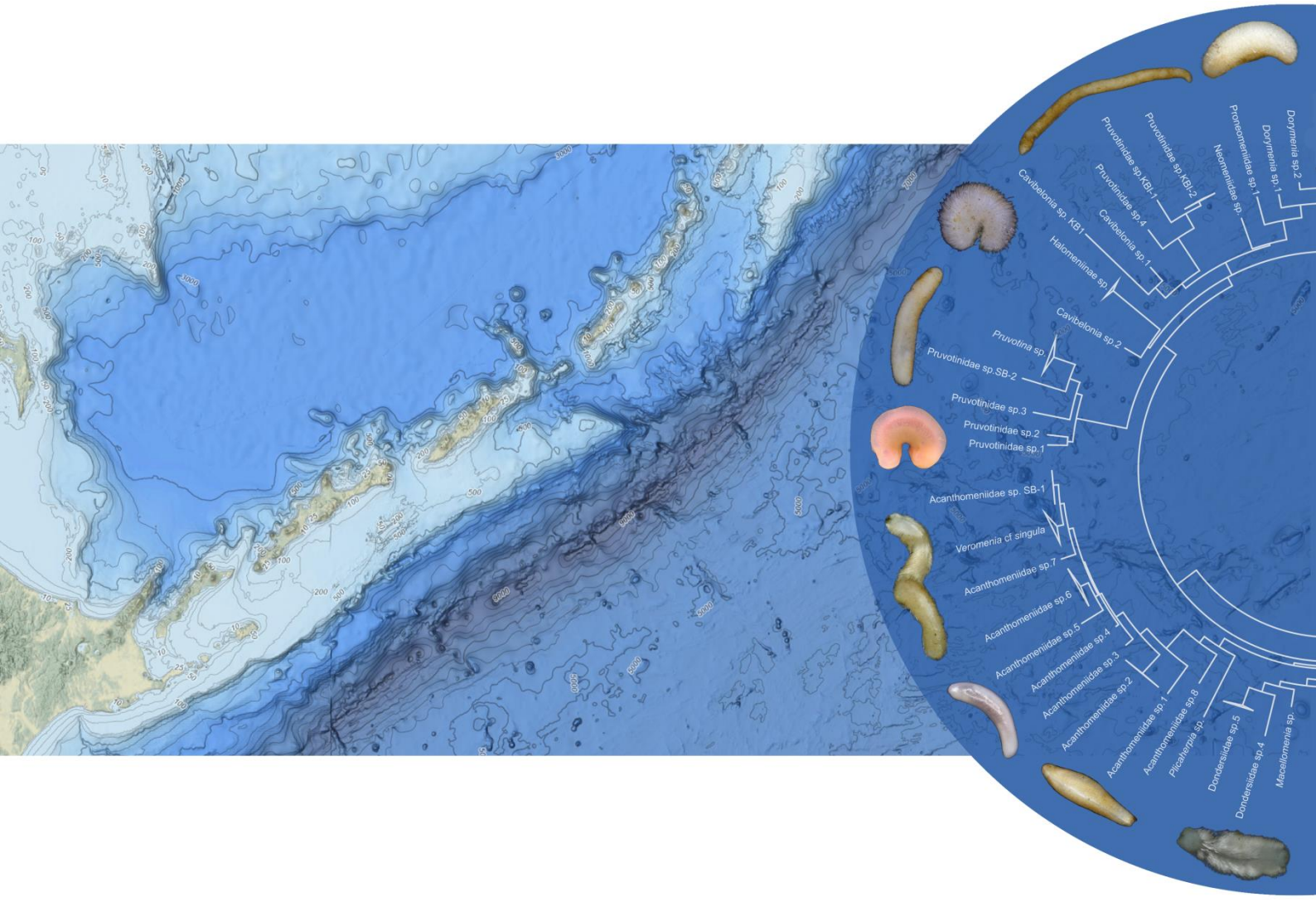
From shallow sands to deep-sea trenches: Towards integrative systematics of Solenogastres (Aplacophora, Mollusca)



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Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

Ich habe mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen.

München, den 31.05.2021

(Franziska S. Bergmeier)

Diese Dissertation wurde angefertigt unter der Leitung von
Prof. Gerhard Haszprunar
und Dr. Katharina M Jörger
an der Fakultät für Biologie der Ludwig-Maximilians-Universität München.

Titelbild: Bathymetrische Karte (Gebco_2020 NOAA NCEI Visualization) des Ochotskischen Meeres und des Kurilen-Kamtschatka-Grabens im Nordwestpazifik, mit Lebendbildern einiger dort gefundener Solenogastres.

To my family: Helmut and Luise, Ferdinand and Jakob.

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Summary

The marine realm encompasses a plethora of habitats: from light-flooded tropical coral reefs down to chemosynthetic vents and seeps to oceanic trenches several kilometers below the ocean's surface. Habitat destruction, pollution, and effects of climate change accelerate rates of species extinction and pose a massive threat to marine ecosystems and biodiversity. Lack of baseline knowledge on species diversity is the key shortfall of current biodiversity research and especially prevalent among small-size invertebrates which constitute the larger part of global, metazoan biodiversity.

Solenogastres (or Neomeniomorpha), an enigmatic class of molluscs are one of those understudied and neglected marine taxa. Instead of bearing a shell, these worm-shaped molluscs are densely covered in aragonitic spicules (the scleritome). They have been found from the tropics to the poles and occur from shallow waters down to the deep sea, with a peak in diversity along the continental shelfs. Despite their circumglobal occurrence, less than 300 species of Solenogastres have been described during the last 150 years since their first discovery. However, natural history collections alone have been estimated to contain at least ten-times more undescribed species than are currently known. Taxonomy of Solenogastres is bulky, requiring a mosaic of morphological and anatomical characters even for higher classification and is thus considered notoriously complex among zoologists. Novel approaches to characterize solenogaster diversity are urgently needed in order to catch up with discovery rates and modernize the taxonomic process.

During my dissertation, I aimed to explore the diversity and evolution of Solenogastres in two understudied marine environments: the shallow-water interstitial habitat (i.e. the pore spaces between sand grains) and the deep oceans beyond the bathyal zone. For this purpose, I developed a novel integrative taxonomic workflow combining morphological characters of traditional taxonomy with DNA barcoding for molecular approaches to species delineation, supplemented with state-of-the-art anatomical 3D reconstructions of selected key lineages. My dissertation research is based on Solenogastres collected by colleagues and myself during sampling trips targeting marine interstitial malacofauna in Bermuda, Hawaii, Azores, Honshu and Okinawa (Japan). I joined two out of a series of four international deep-sea expeditions collecting benthic fauna in the Northwest Pacific, sampling across a depth range from 1,600 m down to almost 10,000 m in the Kuril-Kamchatka Trench. Overall, these expeditions covered different areas in the Northwest Pacific of varying geological age and stages of isolation. Additional material was made available through the natural history collection of the Section Mollusca, Bavarian State Collection of Zoology (SNSB-ZSM München), resulting in a total of 347 Solenogastres investigated during the course of my dissertation.

Based on my work we are now able to identify main clades of meiofaunal Solenogastres, in a first step towards elucidating their global diversity of the clade in the interstitial habitat. The discovery of a putative widely distributed mesopsammic lineage of Dondersiidae (order Pholidoskepia) at sampling sites in the Atlantic and Pacific is challenged by the presence of co-occurring morphologically cryptic species revealed through anatomical 3D reconstructions. This highlights 1.) the risk of chimeric species descriptions if several individuals are used to extract all sets of taxonomically relevant characters and 2.) the importance of molecular data to reliably test hypothesis on conspecificity and distribution patterns in this taxonomically challenging group.

Northwest Pacific Solenogastres were delineated based on unique morphological characters (i.e. scleritome data) and, if possible, cross-validated via molecular-based phylogenetic analyses. This integrative approach resulted in 60 candidate species across regions and depth zones in the

Northwest Pacific (additional 13 candidate species lack molecular data), with the majority constituting species new to science. Their diversity covers all four orders, at least nine families, and 15 genera – therein presenting an immense boost in regional diversity.

On a global scale, the number of abyssal Solenogastres has been more than doubled by these studies, and the animals collected from the bottom of the Kuril-Kamchatka Trench provide the first evidence of this molluscan class from the hadal zone and hold its depth record at almost 10,000 meters. The established baseline dataset of alpha-diversity from adjacent areas and depths zones enabled a first glimpse into distribution patterns. While there was overall little faunal overlap between the investigated regions and depths, several unique links were revealed: 1.) across depth by an eurybathic species occurring in the Kuril Basin (3,350 m) and at the bottom of the trench (9,580 m); 2) across the Kuril-Kamchatka Trench: *Kruppomenia genslerae* Ostermair, Brandt, Haszprunar, Jörger & Bergmeier, 2018 was found in the Sea of Okhotsk and on the open abyssal plain, thereby indicating that a hadal trench does not pose an insurmountable dispersal barrier for benthic invertebrates; and 3) potentially across oceans: anatomical investigations suggest that an abyssal species from the Atlantic is also present on the Northwest Pacific Plain, albeit molecular data from the putative Atlantic conspecifics to support pan-oceanic distribution is lacking. In order to gain insights into the feeding ecology of deep-sea Solenogastres, we sequenced their gut contents from genomic DNA extracts. This molecular-based approach showed that they are highly specialized micropredators with taxon-specific prey preferences. While anthozoan and hydrozoan cnidarians have been generally assumed as the main food source of Solenogastres, Siphonophora, Nemertea, Annelida and Bivalvia have now been added to their menu.

The molecular phylogeny used as a backbone for our integrative approach to characterize their diversity has also several implications for solenogaster systematics. As two fast evolving mitochondrial markers were used in its analyses, without counterbalancing conservative markers the phylogeny cannot reliably resolve deep relationships within a group that has been hypothesized to date back to the early Paleozoic. Nevertheless, as our dataset contains multiple species and genera across several families, we were able to test the validity of existing taxonomic units: several classificatory entities (i.e. the largest order Cavibelonia, families Acanthomeniidae and Pruvotinidae) were retrieved as polyphyletic which will thus necessitate major systematic revisions in the future.

The integrative approach developed during my dissertation allows for fast and efficient species delineation. Scleritome characters were chosen as the main morphological trait, as they are comparatively easy to access and provide the necessary link to the existing classificatory system to prevent a parallel system of DNA-based taxonomy. At the same time, reducing the amount of required characters presents an efficient solution when confronted with small-sized animals and high proportions of singletons that hamper the use of single individuals for multiple lines of investigation (e.g. morphology, anatomy, DNA). The set-up of our community-curated online database AplacBase currently serves as an openly accessible repository and initial identification tool, providing supporting information and guiding researchers through the essence of aplacophoran taxonomy. However, in order to overcome the taxonomic deficits prevalent in Solenogastres, novel approaches need to aim beyond the characterization of their diversity and consequently provide efficient solutions to the currently complicated process of species descriptions and diagnosis. Based on a backbone phylogeny stabilized by mitochondrial genomes, a streamlined approach combining “deep taxonomy” with rapid, DNA-based taxonomy is proposed to tackle the emerging wealth of novel Solenogastres species.

1. Introduction

1.1 The Biodiversity Crisis and Taxonomic Impediment

Global biodiversity is lost at ever increasing rates. Conservative estimates suggest that at current extinction rates, almost 1% of existing species go extinct every ten years (Stork, 2010). Pollution, habitat fragmentation and loss through industrial development and the over exploitation of limited natural resources all have led to accelerated extinction in plant and animals species, when compared to natural extinction rates (Pimm & Joppa, 2015; Valiente-Banuet et al., 2015). Climate change is projected to act furthermore as a global synergistic factor, speeding up extinction rates in the future beyond their current linear progression (Brook et al., 2008; Stork et al., 2009; Coll et al., 2020). The 'Biodiversity Crisis' threatens diversity not just on a numerical scale, but affects entire ecosystems by cascading effects through the loss of ecological interactions (Valiente-Banuet et al., 2015). The deforestation of tropical rainforests and the threats posed by ocean acidification to coral reefs are the most commonly used examples to illustrate the dangers and effects of the biodiversity crisis on hotspots of species richness (see e.g. Barlow et al., 2016; Hoegh-Guldberg et al., 2017).

However, global change and anthropogenic disturbances do not stop at beaches or ocean surfaces and economic focus is shifting towards the deep oceans (e.g. Fischer et al., 2015; Thompson et al., 2018). The discovery of polymetallic nodules has sparked considerable economic interests to mine the abyssal plains and hydrothermal vents of the deep seabed for rare-earth minerals (Van Dover et al., 2017; Simon-Lledó et al., 2019). Long-lasting effects on midwater and benthic communities are inevitable through mining activities and especially the diversity of bottom-dwelling fauna is predicted to suffer massive losses (Niner et al., 2018; Drazen et al., 2020; Leray & Machida, 2020; Vonnahme et al., 2020).

The foundation for effective conservation of biodiversity is knowledge on species richness. Estimates suggest that marine environments and especially the deep-sea sea floor still harbor an unprecedented magnitude of yet undiscovered and undescribed diversity (Grassle & Maciolek, 1992; Appeltans et al., 2012). Huge efforts towards faunal inventories and species descriptions are required to reveal this 'dark diversity' and provide reliable biodiversity surveys and subsequent assessments. Taxonomy is the fundamental discipline of biology, dedicated to "postulating hypotheses of identity and relationships" of organisms (Wägele et al., 2011). However, funding and positions in taxonomic fundamental research are lacking and taxonomic training is almost absent from academia, all contributing to the extinction of expertise with far reaching consequences - a predicament termed 'Taxonomic Impediment' (de Carvalho et al., 2007; Wägele et al., 2011). Even large-scale projects like the Census of Marine Life (CoML), a decade long effort dedicated to understanding marine life by more than 2,700 scientists, has resulted in the description of merely 1,200 + species (CoML, 2010; Alexander et al., 2011). This emphasizes the fact, that while rates of discovery are fast especially in unexplored ecosystems like the deep sea, rates of species descriptions are not: a newly discovered species remains "sitting on a scientist's shelf" for an average of 20.7 years before it is formally described (Fontaine et al., 2012). Overall the predicament persists that while rates of species descriptions have steadily inclined over the last years (Costello et al., 2013; Bouchet et al., 2016), the rate of describing diversity is still slower than its projected extinction (Flowers, 2007).

In many taxa, traditional taxonomy tends to be bulky and time-consuming, contributing to slow rates of species descriptions. The advent of molecular barcoding and high through-put sequencing (Hebert et al., 2003; Hebert & Gregory, 2005) has led to initial calls to replace 'traditional' taxonomy with a

DNA-centered system to speed up the taxonomic progress (Tautz et al., 2003). By now, an integrative approach to taxonomy combining several lines of evidence is regarded as the golden standard (e.g. Dayrat, 2005; but see Padial et al., 2010; Riedel et al., 2013 for a different interpretation). While species delineation based on DNA sequences may be streamlined, molecular methods have not been able to circumvent the temporal bottleneck of the formal, peer-reviewed process of describing species (see e.g. Monaghan et al., 2005; Tänzler et al., 2012). Reducing species descriptions to a minimum of characters combined with DNA sequences might work well for some taxa (Riedel et al., 2013), but it remains difficult to integrate these novel datasets into classificatory systems of diversity that may be centuries old.

'Museomics' approaches tackle this challenge by extracting DNA from historical (type) material. Initial specimen fixation and a storage history unsuitable for molecular work lead to degradation and fragmentation of 'old' DNA and have thus long hampered successful barcoding of (type) material in museum collections through PCR amplifications (Zimmermann et al., 2008). However, adapted and specialized protocols and sequencing techniques have led to a breakthrough in 'museomics' for a diverse range of taxa (see e.g. Guschanski et al., 2013; Jaksch et al., 2016; Der Sarkissian et al., 2017; Derkarabetian et al., 2019; Ferreira et al., 2020; Scherz et al., 2020). Nevertheless, 'museomics' approaches are developed with taxon-specific protocols and not yet applicable across a wide range of groups.

Huge taxonomic bias still exists at the expense of invertebrates (Cardoso et al., 2011; Donaldson et al., 2016). This knowledge deficit is especially prevalent in the marine realm, where somewhere between conservative 300,000 to (much more probable) more than 10,000,000 estimated species remain undiscovered (Costello et al., 2010; Ramirez-Llodra et al., 2010; Appeltans et al., 2012). The state-of-knowledge index, taking into account estimations on described species, availability of identification guides and current taxonomic expertise (see Costello et al., 2010), is rated "poor" for the majority of invertebrates in the marine environment. The phylum Mollusca fares comparably well with an index above the mean value, but the accumulation curve of described valid mollusc species is still far from levelling out (Costello et al., 2010; Bouchet et al., 2016). Molluscs account for about 20% of overall marine species diversity and often form a dominant faunal component (Bouchet, 2006; Rosenberg, 2014). This is mostly owed to the hyper-diverse class of Gastropoda and also to Bivalvia, both with long taxonomic history and in many cases relatively straight forward identification based on externally visible characters (e.g. shells). Both groups are used to uncover and study gradients and patterns of diversity and distribution (see e.g. Rex et al., 2005a; Schwabe et al., 2007; Yahagi et al., 2017). Yet, scientific progress in the 'minor' molluscan classes has been slow and with the persisting lack of baseline knowledge in their alpha-diversity their biology and ecological roles also remain in the dark.

1.2 Who are Solenogastres?

Solenogastres is a small, exclusively marine class benthic molluscs. To date, only 293 species have been formally described (MolluscaBase, 2021). The majority of species are very small animals of only a couple of millimeters body size and in general look like inconspicuous 'worms'. Only a handful of Solenogastres, like reef-dwelling *Epimenia* or coral-mimicking *Anamenia amabilis* Saito & Salvini-Plawen, 2010, are of striking size and color. Solenogastres occur circumglobally from the poles to the tropics and have successfully colonized unique habitats like the interstitial of marine sediments or sulfide-rich hydrothermal vents (Salvini-Plawen, 1985b; Scheltema & Kuzirian, 1991; García-Álvarez et al., 2000; Scheltema, 2008; Todt, 2013).

Solenogastres are one of the two primary shell-less groups of Mollusca ('Aplacophora'). The first described solenogaster *Neomenia carinata* Tullberg, 1875 was initially placed among 'worms' by its discoverer, while the caudofoveate *Cheatoderm nitidulum* Lovén, 1844 was classified as an echinoderm. Since their discovery 150 years ago, evolutionary relationships among Aplacophora and their molluscan relatives have been subject to competing hypothesis. Aplacophorans have been traditionally placed at the base of the molluscan tree by morphocladistic analyses, either as a monophylum (Testaria-Conchifera concept, see Waller, 1998) or a paraphyletic grade, with either of them as the earliest offshoot and the respective other class as sister group to all other molluscs (Hepagastralia-Testaria concept see e.g., Salvini-Plawen, 1972b; Salvini-Plawen, 1980; Haszprunar, 2000; Haszprunar & Wanninger, 2012; Salvini-Plawen & Steiner, 2014). Following the antithetical Aculifera concept, Mollusca are split into a dichotomy of monophyletic Aplacophora and Polyplacophora (as Aculifera) and all other shell-bearing molluscs (as Conchifera) (Scheltema, 1988; Scheltema, 1993). In this case, it has been suggested that Aplacophorans originated from their last common ancestor through progenesis (Scheltema, 1993). In the last decade, approaches based on large-scale phylogenomic datasets (Kocot et al., 2011; Smith et al., 2011; Kocot et al., 2020) and housekeeping genes (Vinther et al., 2012b) converged towards the Aculifera hypothesis (but see e.g. Stöger et al. (2013) for a large-scale multi-marker study resulting in contrary 'Variopoda'). Following these two antithetical hypothesis, the two aplacophoran classes have been assigned a key role in understanding the evolution of Mollusca: they may be interpreted to represent either the ancestral or highly derived condition of Mollusca. Comparative developmental studies of polyplacophorans and aplacophorans (Scherholz et al., 2013; Scherholz et al., 2015), as well as gene expression patterns (Redl et al., 2016), and data from the fossil record (see e.g. Sutton et al., 2004; Sutton et al., 2012; Vinther et al., 2012a) further support the evolutionary scenario of a chiton-like ancestry of Aculifera.

Aplacophorans are weird molluscs and their strange morphology and anatomy are one of the main reasons why their evolutionary history is so contentious among malacologists. Solenogastres have a distinctively worm-shaped body and only few species show slight modifications of this bauplan with external dorsal ridges and keels (see for example *Entonomenia tricarinata* (Salvini-Plawen, 1978), *Sandalomenia papilligera* Thiele, 1913*, Lyratoherpia californica* (Heath, 1911)), or with an elongated and narrowly tapering tail (e.g., some species of the genus *Dorymenia*, see García-Álvarez et al. (1998); García-Álvarez et al. (2009)). The foot emerges from the anterior glandular pedal pit and runs as a thin, ciliated band along the midventral side of the animal. Only a recently found deep water solenogaster from Iceland has completely reduced its foot (hence the name *Apodomenia enigmatica* Kocot, Todt, Mikkelsen & Halanych, 2019), blurring the morphological line to foot-less Caudofoveata, albeit molecularly and anatomically firmly placed within Solenogastres (Kocot et al. 2019). The pallial cavity is small, lacks true ctenidia, and is located in the posterior part of the animal (or completely lost in the case of *A. enigmatica* (Kocot et al., 2019)). In contrast to conchiferan molluscs, Solenogastres do not bear a shell but are densely covered by calcareous sclerites which are embedded in a chitinous cuticle (Scheltema & Ivanov, 2004). Elements of the scleritome occur in diverse shapes and arrangements: scales are solid and usually arranged flatly against the body surface, while spicules (referring to all needle-like elements) can be either solid or (partially) hollow with hooks or intricate microstructures like serrations on their distal portions (e.g. see some representatives of Pruvotinidae (Zamarro et al., 2013)). Spicules often protrude in steep angles from the cuticle in multiple layers, giving the respective animal a shaggy to fuzzy appearance, in contrast

to the smooth and often almost translucent look of Solenogastres covered in imbricated scales (García-Álvarez & Salvini-Plawen, 2007).

As with their external vermiform habitus, solenogaster anatomy is relatively uniform across the currently known taxa. They have a tetraneural nervous system, with paired ventral and lateral medullary cords (Heath, 1904; Salvini-Plawen, 1967; Todt et al., 2008). True ganglia (i.e. with distinct neuropil-perikarya striation) are present as a fused cerebral ganglion and paired lateral, buccal, and pedal ganglia (Faller et al., 2012). Albeit lacking eyes, Solenogastres have other unique sensory organs. In the head region, the vestibular sense organ and peri-atriobuccal cirri are potential chemo- and mechanosensors for food detection (Schwabl, 1955; Salvini-Plawen, 1968b; Salvini-Plawen, 1985a; Haszprunar, 1986). Some taxa have a pedal commissural sac enclosed between the pedal ganglia, which potentially serves as a statocyst-like movement receptor (Scheltema, 1981; Haszprunar, 1986). The dorsoterminal sense organ is located above the pallial cavity in the dorsal integument and can occur as a single, fused or paired, or multiplied structure (*Lyratoherpia californica* (Heath 1911) reportedly bears eleven such organs (Heath, 1911; Scheltema et al., 2012)). While the chemosensory dorsoterminal sense organ has often been homologized with the osphradium of other molluscs (Salvini-Plawen, 1972b; Salvini-Plawen, 1981b; Haszprunar, 1987), it has also been suggested that it presents a separate structure homologous to the 'posterior organ' (sensu Lindberg & Sigwart, 2015) of Caudofoveata and Polyplacophora (Lindberg & Sigwart, 2015).

Solenogastres are simultaneous hermaphrodites with internal fertilization, as derived from sperm morphology (Buckland-Nicks, 1995; Buckland-Nicks & Scheltema, 1995) and anatomy. The paired gonads are directly connected to the pericard via short gonopericardioducts. Only a single species (*Phyllomenia austrina* Thiele, 1913) is known to have true gonoducts which circumvent the pericard and lead directly into the glandular spawning duct (Thiele, 1913; Salvini-Plawen, 1970). As observed in few species, animals intertwine their trunks and press the openings of the pallial cavities together during mating (Scheltema & Jebb, 1994). Reproductive output correlates with size, thus small taxa only lay few eggs (Morse, 1994; Todt & Wanninger, 2010) while large animals spawn close to a hundred eggs at a time (Hadfield, 1979; Okusu, 2002). Fertilized eggs are spawned into the surrounding water and after an initial, short period of floating they will sink to the ground and adhere to the substratum with their sticky egg hull (Todt & Wanninger, 2010). Few species brood their young in their pallial cavities until the juvenile animals are released at a crawling stage (e.g., Thiele, 1913; Heath, 1918; Baba, 1938; Salvini-Plawen, 1978a; Salvini-Plawen, 1978b; Scheltema & Jebb, 1994; Todt & Kocot, 2014). In non-brooding species, lecithotrophic larvae hatch from their eggs and 'swim' upwards in the water column through ciliary movement. Settlement begins with the onset of metamorphosis up to ten days after hatching, but some larvae retain their swimming abilities for several more days (Okusu, 2002; Todt & Wanninger, 2010).

Larger-sized Solenogastres are occasionally found in close association with anthozoans or hydrozoans, tightly coiled around the stalks of the cnidarians and feeding on their soft tissue (Salvini-Plawen & Benayahu, 1991; Scheltema & Jebb, 1994; Sasaki & Saito, 2005; Mifsud et al., 2008; Saito & Salvini-Plawen, 2010). Based on the presence of undigested cnidocysts in the midgut of Solenogastres, it has been suggested that the majority of species feed on living cnidarians or scavenge on jelly plankton (Salvini-Plawen, 1972a; Todt, 2013). Only Simrothiellidae have presumably specialized on different food sources like polychaetes (Handl & Todt, 2005; Todt & Salvini-Plawen, 2005). In contrast to all other molluscs, the midgut of Solenogastres is functionally and histologically undifferentiated. As a straight tube, the midgut fills up most of the body cavity in the middle part of

the animal and simultaneously functions as a stomach, intestine, and storage organ (Todt & Salvini-Plawen, 2004). The majority of Solenogastres have a radula, most commonly consisting of paired teeth (distichous type), which functions as a tweezer to pinch and tear off prey tissue. The radula has been lost in 20% of species (Scheltema et al., 1994), and in these cases the muscular pharynx serves as a suction pump (Sasaki & Saito, 2005). By far the highest degree of anatomical diversification is found in glandular organs associated with the foregut, which are highly variable in their position, number, and histology. To accommodate the complexity and diversity of these glands, eight types can be differentiated based on e.g. their position relative to the foregut, the cytology of the gland cells, the position of the gland cells in relation the foregut epithelium, associated muscle layers, and the morphology of the gland ducts (Handl & Todt, 2005). The various gland produce unique combinations of secretions, probably related to feeding adaptations (Todt, 2006): they potentially prevent the discharge of cnidocysts and/ or produce enzymes for pre-digestion of tissue (Salvini-Plawen, 1988; Sasaki & Saito, 2005; Todt, 2006). The importance of these glands for the classification of Solenogastres has been pointed out early on (Nierstrasz, 1905; Salvini-Plawen, 1972; Salvini-Plawen, 1988). Especially their lateroventral foregut glands play a major role in the taxonomy of the group and are one of the main characters for the identification of most solenogaster families (Salvini-Plawen, 1978a; Salvini-Plawen, 1978b; García-Álvarez & Salvini-Plawen, 2007).

Based on his monographic work on Antarctic Solenogastres, Salvini-Plawen (1978a; b) classified Solenogastres into four orders: Cavibelonia Salvini-Plawen, 1978 (13 families, 59 genera, 188 spp.), Pholidoskepia Salvini-Plawen, 1978 (6 families, 20 genera, 61 spp.), Neomeniamorpha Salvini-Plawen, 1978 (2 families, 3 genera, 29 spp.), and Sterrofustia Salvini-Plawen, 1978 (3 families, 8 genera, 13 spp.). Cladistic approaches based on morphological and anatomical characters have not been able to resolve internal relationships among Solenogastres, but have already hinted at doubts about the validity of parts of this classification (Scheltema & Schander, 2000; Salvini-Plawen, 2003b). The first phylogenomic study by Kocot et al. (2019) based on a dataset of more than 500 genes of representatives of all four solenogaster orders, has shown major conflicts with this classification system and has rendered the most speciose order Cavibelonia polyphyletic.

Solenogaster taxonomy

Solenogaster taxonomy relies on the morphology of hard-parts (i.e. the scleritome and the radula) to differentiate between the four orders, but for identification on family level and beyond anatomical characters (especially of the reproductive and digestive system) are indispensable (García-Álvarez & Salvini-Plawen, 2007). Out of approximately 43 characters used in Solenogastres taxonomy, 31 are based on anatomy (Scheltema, 1992). However, preparing specimens for histological sectioning requires time, appropriate equipment and expert knowledge on their anatomy and cytology and is impossible for the untrained. Even among accomplished invertebrate taxonomists, they are thus often still regarded as “obscure and difficult’” (Todt, 2013).

The advent and rise of molecular barcoding (Hebert et al., 2003; Hebert & Gregory, 2005) has considerably accelerated species delineation and identification of marine invertebrates, especially in groups with challenging taxonomy, e.g. due to high rates of morphological crypsis (see e.g. Grant et al., 2011; Brasier et al., 2016). For many newly discovered taxa, DNA sequences of commonly used barcodes (e.g. nuclear ribosomal 18S and 28 S rRNA, histone H3, mitochondrial cytochrome *c* oxidase subunit 1 COI, ribosomal 16S rRNA) accompany new species descriptions and are routinely deposited in online repositories like GenBank or BOLD. However, in Solenogastres only the most recent species descriptions contain molecular markers: of 27 species described during the last decade, only three

include DNA sequence data (Kocot & Todt, 2014; Kocot et al., 2019). Prior to this thesis, only 21 solenogaster sequences have been deposited in GenBank – including six not identified to species level, and two contaminated sequences at least partially amplified from food organisms. Solenogaster nuclear genes like the popular barcoding markers 18S and 28S rRNA cannot be amplified using standard PCR procedures (Okusu & Giribet, 2003; Meyer et al., 2010). The respective sequences contain high guanine-cytosine contents, which result in secondary structures that hamper amplification and lead to the formation of potentially contaminated chimeric DNA sequences (Meyer et al., 2010). While mitochondrial COI and 16S rRNA are easier to amplify, universal primer pairs like Folmer's HCO-LCO (Folmer et al., 1994) still lead to contaminations and overall low success rates in Solenogastres (own observation). A reliable reference library with broad coverage of different taxa is a prerequisite for molecular barcoding as a tool for species identification (Collins & Cruickshank, 2013) – however, the much needed reference barcodes of Solenogastres are still lacking.

As a direct consequence of their difficult and impractical taxonomy, Solenogastres often remain unidentified when they are routinely collected as part of marine benthic samples (Gutt et al., 2015; Girard et al., 2016) and are underrepresented in biodiversity surveys, even though they can occur in high abundance and might constitute a major part of benthic malacofauna (Scheltema, 1990).Estimates propose that natural history collections alone harbor a number of species at least one magnitude higher than their currently known global diversity (Glaubrecht & Maitas, 2005; Todt, 2013). The impediment to solenogaster taxonomy is further amplified by many species descriptions based on poorly preserved material and insufficient scleritome or anatomical data, which hamper follow-up research and re-identification of freshly collected material. Out of all 90 solenogaster genera, more than a third (35 genera) are monotypic (MolluscaBase, 2021). Many of these taxa have been described based on single animals ('singletons') and intraspecific variability of traditionally used taxonomic characters (e.g. like the scleritome) remains unexplored (see e.g. Salvini-Plawen, 1978a; Salvini-Plawen, 1978b).

1.3 Habitats and Distribution

Solenogastres occur circumglobally, from tropical to polar waters and from the shallow intertidal down to the deep sea. In terms of their bathymetric distribution, their currently known diversity peaks along the continental slope (Scheltema, 1990; García-Álvarez & Salvini-Plawen, 2007; Todt, 2013), and two-thirds of solenogaster species (189 spp.) have been described from 200 to 1,000 meters. The number of described species rapidly declines when moving upwards towards the shallow intertidal and of 73 species from 200 m or less, only two have been described from less than 10 m (Salvini-Plawen, 1985b; Salvini-Plawen & Benayahu, 1991; García-Álvarez & Salvini-Plawen, 2007). Among the few shallow-water forms collected at 100 m or above are ten interstitial lineages of tiny Solenogastres, inhabiting the minute spaces between sand grains (García-Álvarez et al., 2000; Kocot & Todt, 2014). Marine sediments and their interstices form a continuous habitat extending across depth zones and geographical distance. They provide key ecosystem functions and are home to a diverse and highly specialized fauna which has been proposed to be among the most speciose within the marine realm (Snelgrove, 1999; Rundell & Leander, 2010). In gastropods, global patterns of diversity and evolutionary pathways into the marine mesopsammon are beginning to emerge (see e.g. work by Swedmark, 1968; Swedmark, 1971; Neusser et al., 2009; Brenzinger et al., 2013; Brenzinger et al., 2014; Jörger et al., 2014a; Jörger et al., 2014b; Jörger et al., 2020), while knowledge on interstitial Solenogastres is restricted to the descriptions of those few species. They have been mainly described from marine research stations around the world which share historical focus and

expertise on meiofauna research (for example on Bermuda, around Banyuls-sur-Mer, Roscoff (France), Plymouth (UK), and Friday Harbor Lab (USA) (Marion & Kowalevsky, 1886; Salvini-Plawen, 1968a; Salvini-Plawen, 1985b; Kocot & Todt, 2014)). This indicates that Solenogastres are potentially a worldwide but nearly entirely neglected part of the marine mesopsammon. Consequently, the currently low number of shallow-water species especially from the interstitial, rather reflects the lack of exploration than a true absence of Solenogastres from this highly diverse habitat.

Solenogastres are often termed a "deep-sea clade", due to their peak in diversity and abundance along the lower bathyal (Ramirez-Llodra et al., 2010; Todt, 2013). Remarkably few records exist from the lower abyssal zone and beyond. These depth zones are extreme environments: lacking light and nutrients, the abyssal and hadal regions were long thought void of any life. Even though less than 0.01% of the deep ocean floor below 4,000 meters have been explored, this tiny fraction has been shown to be teeming with life (Blankenship-Williams & Levin, 2009; Ramirez-Llodra et al., 2010; Danovaro et al., 2017). Prior to this thesis, only 18 species of Solenogastres have been known from the lower abyssal below 4,000 m, mostly recorded from the Atlantic. The deepest recorded solenogaster is the simrothiellid *Plawenia schizoradulata* (Salvini-Plawen, 1978) from 5,931 m in the South Shetland Trench (Scotia Sea, Southern Ocean) (Salvini-Plawen, 1978b; Scheltema & Schander, 2000; Gil-Mansilla et al., 2009). No Solenogastres have ever been reported from the hadal zone, which begins at depths of 6,000 meters and accounts for the deepest 45% of the oceans (Jamieson & Stewart, 2021). Such depths are often (but not exclusively) found along the slopes and bottoms of deep oceanic trenches. V-shaped with steep slopes and terraces, trenches are extremely structured geological features and act as traps for accumulating organic matter (Blankenship-Williams & Levin, 2009; Ramirez-Llodra et al., 2010). Trench fauna is characterized by high levels of endemism due to their bathymetric isolation (Wolff, 1959; Wolff, 1970; Belyaev, 1989).

Large parts of oceanic trenches lie below the calcium carbonate compensation depth, which strongly limits the vertical distribution of organisms with calcareous elements, like all shell- or sclerite-bearing molluscs. Despite their dependence on calcium for biomineralization of their shells, bivalves and gastropods are prominent components of hadal fauna and have adapted to these conditions by maintaining very thin and soft shells and through protection with a thick layer of organic periostracum (Jamieson, 2015; Fukumori et al., 2019). The origin of this deep fauna is still contentious. Source-sink dynamics explain the sustenance of abyssal populations through equilibrium of influx from bathyal "source" populations into abyssal, non-reproductive "sink" populations (Rex et al., 2005b). However, this model does not seem to apply to several well-studied and successful deep sea taxa or to trenches found in considerable distance from continental margins (Young, 2003; Smith et al., 2008; Hardy et al., 2015). To elucidate potential colonization processes and distribution patterns in the deep sea, it is paramount to gather comparable data on the faunal compositions of adjoining depth zones and pair it with knowledge on dispersal biology and bathymetric ranges of its taxa – all data which is still lacking for the majority of Solenogastres. Bathymetric ranges of a few hundred to round 2,000 m along the continental shelf and down to the upper bathyal are not rare in Solenogastres (García-Álvarez & Salvini-Plawen, 2007; Scheltema et al., 2012). Only a single species has been documented with a remarkable vertical distribution of more than 3,500 m (*Pruvotina longispinosa* Salvini-Plawen, 1978 in Salvini-Plawen (1978b)). It remains to be confirmed with molecular data if species like *P. longispina* are actually able to sustain depth ranges of almost 4,000 m, or whether there are separate but morphologically cryptic species present in different depth zones.

Distribution records of Solenogastres are mostly highly restricted both in terms of their vertical and geographical distribution. Only few species have been recorded outside their type localities: *Proneomenia sluiteri* Hubrecht, 1880, which was originally described from the Barents Sea and has recently been rediscovered at several spots in Icelandic waters (Hubrecht, 1880; Todt & Kocot, 2014), extending its potential distribution range from the Arctic to the Temperate Northern Atlantic (following the marine ecoregions of Spalding et al. (2007)). According to datasets published in the Ocean Biodiversity Information System OBIS, *Neomenia* Tullberg, 1875 is an almost circumglobally distributed genus, although missing from Arctic waters (Ivanov & Scheltema, 2014; Cooper & Barry, 2017; NMNH, 2018). Based on these datasets, its type species *Neomenia carinata* Tullberg, 1875 can be found on both sides of the North Atlantic, but the taxonomic backbone of many of these published datasets is unclear. *Micromenia amphiatlantica*, Cobo & Kocot, 2020, a deep-sea species recently described from the Guinea (East Atlantic) and Brazil Basins (West Atlantic), is currently the only solenogaster whose transoceanic distribution is backed by morphological and anatomical work from aplacophoran taxonomists – but molecular data is still lacking to confirm conspecificity (Cobo & Kocot, 2020). Geographical and bathymetric distribution records of Solenogastres thus currently remain scattered and comparisons between different regions and depth zones are still missing. However, such baseline studies are much needed to uncover first population dynamics and gradients of species richness. Ultimately, this also contributes to a better understanding of their biology (e.g. dispersal abilities) ecology and potential vulnerability towards disturbances in their natural habitat.

In terms of their global distribution, half of all solenogaster species are found in the Southern Hemisphere (145 out of 291 spp.), and 25% (77 spp.) from the seas surrounding Antarctica. The strong bias towards the Southern Ocean results from the monographic work “Antarktische und subantarktische Solenogastres” by Luitfried von Salvini-Plawen, who almost exclusively described all Antarctic species, mostly based on historical material collected during numerous expeditions from the late 19th to the middle of the 20th century (Salvini-Plawen, 1978a; Salvini-Plawen, 1978b). Likewise, almost a third of all species (88 spp.) have been described from the Mediterranean (29 spp.) and the North Atlantic (59 spp.) mostly along the coasts of Europe. This reflects historical and recent strongholds of aplacophoran taxonomy, for example by Kowalevsky, Nierstrasz, Pruvot, and Wirén from the late 19th century (Kowalevsky, 1880; Kowalevsky & Marion, 1887; Pruvot, 1890; Wirén, 1892; Pruvot, 1899; Nierstrasz, 1905), also recent work by Salvini-Plawen (Salvini-Plawen, 2003a; Salvini-Plawen, 2006), Amelie Scheltema (see e.g. Scheltema, 1999; Scheltema & Schander, 2000), and Spanish taxonomists (see e.g. García-Álvarez & Salvini-Plawen, 2001; García-Álvarez et al., 2001; García-Álvarez & Urgorri, 2001; Zamarro et al., 2019).

In contrast to the Antarctic, Atlantic, and Mediterranean, there is a prominent gap in knowledge on Solenogastres in the Northwest Pacific – a region that has been intensely studied for decades by a series of Russian expeditions (Belyaev, 1989; Ebbe et al., 2010; Brandt & Malyutina, 2015). While these expeditions have led to numerous publications on taxonomy and topography of the region (see e.g. Zenkevitch, 1963; Belyaev, 1989), no published records on any Solenogastres collected during these scientific endeavors are available. Until the start of my thesis, only elven species have been described from the Northwest Pacific during more than a century, mostly scattered along the coast and continental shelf of Japan between 40 and 1,500 meters, while the regions beyond remained unexplored (Heath, 1911; Baba, 1940; Baba, 1975; Salvini-Plawen, 1997; Saito & Salvini-Plawen, 2010; Sirenko, 2013; Saito & Salvini-Plawen, 2014).

1.4 Main study region: the deep Northwest Pacific

The Northwest Pacific (NWP) is one of the most productive and biodiverse marine regions in the world (Grebmeier et al., 2006; Saeedi et al., 2019). It is characterized by a homogeneous abyssal plain with a mean depth of 5,000 m (Zenkevitch, 1963) and a system of oceanic trenches, formed since the Cretaceous by the subduction of the Pacific Plate beneath the Okhotsk Plate (Gnibidenko et al., 1983; Chen et al., 2008). Due to the high level of primary production in the surface layers, the abyssal plain carries higher benthic biomass and diversity than other regions of the NWP (Ebbe et al., 2010; Brandt et al., 2015; Brandt et al., 2018a).

The Kuril-Kamchatka Trench (KKT) extends from the western part of the Aleutian Trench along the Kamchatka Peninsula and the Kuril Island Arc until it connects to the Japan Trench off the coast of Hokkaido (Fig. 1). Along its length of 2,200 km, the trench has a mean width of 120 km (Angel, 1982) and with its deepest point lying 9,604 meters below sea level it is one of the five deepest trenches on our planet (Blankenship-Williams & Levin, 2009; Dreutter et al., 2020). Due to its proximity to the continental margin, its huge dimensions and steep slopes, the KKT traps large quantities of surface-related organic matter at its bottom (Thistle, 2003; Blankenship-Williams & Levin, 2009). The trench can thus sustain potentially higher benthic diversity and species richness than more oligotrophic trenches in other parts of the world (e.g. the Atlantic Puerto Rico Trench). In general, hadal trenches like the KKT are proposed to act as distribution barriers to benthic species that cannot overcome the immense depth differences posed by the trench. Comparing faunal compositions of shallower regions adjoining the KKT consequently allows inference of dispersal abilities of selected taxa.

Several marginal seas of varying geological age and isolation stages are connected to the open NWP (Fig. 1). The Sea of Okhotsk is a semi-isolated marginal sea of the cold temperate Northwest Pacific, between the far eastern parts of continental Russia, the Kamchatka Peninsula, Hokkaido, and Sakhalin Island. Most of the northern parts of the Sea of Okhotsk are spread across shallow shelf area and its mean depth is only 812 meters (Tyler, 2002). The Kuril Basin, a back-arc basin of the Kuril Island Ridge formed during the Early Oligocene to Late Miocene (32-7 million years ago) (Terekhov et al., 2008), is the oldest and deepest part of the Sea of Okhotsk, averaging at around 3,300 meters depth (Gnibidenko et al., 1983). Anoxic bottom waters have formed repeatedly during several interglacial periods for the last 500,000 years (Liu et al., 2006), potentially wiping out bottom-dwelling organisms. Distribution, diversity and biomass of today's benthic fauna in the Sea of Okhotsk are shaped by several factors. While primary production is high in the northern shelf region, the central part and the Kuril Basin are characterized by less primary production and bottom waters with low oxygen saturation (Tyler, 2002; Kamenev, 2018).

The region is influenced by two main currents. The warm, northbound Kuroshio Current originates in the Equatorial Pacific, while the southbound East Kamchatka/ Oyashio Current flows southwards from the Bering Sea (Fig. 1) (Qiu, 2001; Fujikura et al., 2010; Kawabe & Fujio, 2010). Two deep-water passages, the Krusenstern Strait at 1,920 m and the Bussol Strait at 2,318 m, connect the Kuril Basin of the Sea of Okhotsk to the open Northwest Pacific. Arctic deep waters transported by the East Kamchatka/ Oyashio Current enter through the Krusenstern Strait and sink to the bottom of the Kuril Basin. A large, counter clockwise gyre circulates the water masses through the Sea of Okhotsk, before they move out through the Bussol Strait. As a result of this water exchange, the benthic fauna of the Basin is linked to the open Pacific through supposedly widely distributed species (Zenkevitch, 1963; Tyler, 2002; Kamenev, 2018). However, these distribution patterns have been challenged by recent faunal comparisons between the Sea of Okhotsk and the KKT region, suggesting instead that

these putative wide distributions result from taxonomic lumping, faulty identifications, or cryptic species complexes (e.g. Fukumori et al., 2018; Kobayashi et al., 2018).

The Northwest Pacific has been extensively studied in the middle of the 20th century by a series of Russian expeditions, collecting data on hydrology, physical chemistry, and faunal composition (Brandt & Malyutina, 2015). Since the time of the *Vityaz* expeditions, new sampling gear and techniques to explore the deep-sea benthos have emerged. In the framework of a German-Russian (and vice versa) series of cooperative expeditions, the area has been revisited between 2011 and 2016 applying standardized sampling techniques (KuramBio I expedition 2012, SokhoBio expedition 2015, KuramBio II expedition 2016) (Brandt & Malyutina, 2015; Malyutina & Brandt, 2015; Brandt & Malyutina, 2016; Brandt et al., 2018a; Malyutina et al., 2018). The investigated area of the NWP offers diverse geological features with unique properties across a large bathymetric range. Through comparisons of species diversity and distribution between these regions (i.e. open NWP abyssal plain, semi-isolated Sea of Okhotsk, slopes and bottom of the KKT) we can uncover patterns of biogeography and connectivity for different bottom-dwelling taxa, as well as possible directions of colonization.

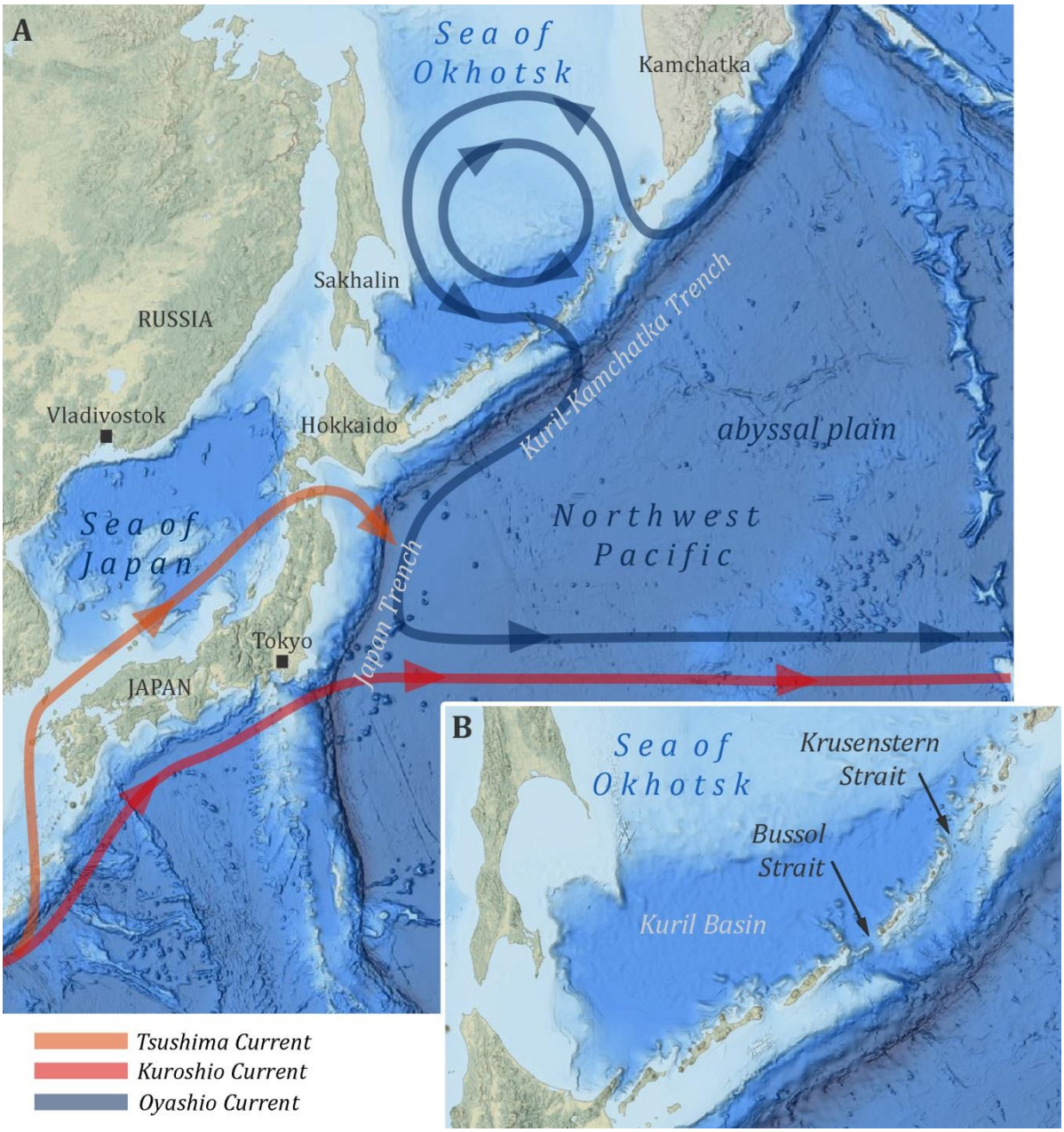


Figure 1. Study area in the Northwest Pacific with **A.** main bathymetric features and surface water currents. **B.** Southern part of the Sea of Okhotsk with bathyal passageways to the open Northwest Pacific. For details on sampling stations of the different expeditions see chapter 6 – 8. Base map: Gebco_2020 (NOAA NCEI Visualization), accessed through the NOAA Bathymetric Data Viewer at https://maps.ngdc.noaa.gov/viewers/bathymetry/.

1.5 Aims of the thesis

The shallow-water mesopsammon is a circumglobal ecosystem, harboring a high diversity of specialized fauna. Only few and scattered records exist of a handful of solenogaster species inhabiting the marine interstitial, with few to no anatomical data on these lineages. **Part I** of my thesis explores the diversity of Solenogastres in this understudied habitat (Chapter 1 – 4). For this purpose, I investigated the only solenogaster family exclusively known from the mesopsammon. State-of-the-art 3D microanatomical studies from a type locality revealed the presence of additional, externally cryptic species (Chapter 1). This pointed to the risk of creating chimeric species in small-sized Solenogastres, if several individuals are used to extract all taxonomically relevant characters (i.e. one animal for scleritome analysis, another one for histology) and highlighted the urgency to incorporate molecular data into novel integrative approaches. In the framework of a survey of shallow-water mesopsammic malacofauna, Chapter 2 and 3 of my dissertation assess the regional alpha-diversity of Solenogastres from São Miquel and Santa Maria in the Azores archipelago. Isolated, oceanic islands like the mid-Atlantic Azores can offer unique insights into putative colonization routes and the origin of island diversity (Chapter 3). Chapter 4 of my thesis is a guide to the diversity of shallow-water, meiofaunal aplacophoran molluscs (Solenogastres + Caudofoveata). This guide compiles all global aplacophorans from the meiofaunal size class, provides guidance on how to collect them and a taxonomic key to their identification. To tackle the taxonomic difficulties and pitfalls outlined in the previous chapters, an integrative taxonomic approach is proposed in Chapter 5. Specifically aimed at minute animals, it combines high-magnification scanning-electron-microscopy with subsequent DNA extraction. Molecular species delineation methods are thus backed up by detailed scleritome characters, which in most cases allow integrating these animals into the existing classificatory system and also enable identification on higher taxonomic level. This approach ultimately paves the way for a streamlined characterization of solenogaster diversity, with the option of future re-identification through molecular barcoding and formal species descriptions of selected key lineages.

The diversity of Solenogastres in the deep Northwest Pacific (NWP) has remained hitherto unknown, with only few scattered records from the shelf region. **Part II** of my thesis (Chapters 6 to 9) has the objective to establish baseline knowledge on the solenogaster fauna of the deep NWP. For this purpose, I investigated specimens deposited at the SNSB-ZSM München as well as freshly collected material through the streamlined taxonomic approach of combining morphological and molecular data with selected anatomical investigations. The compiled large-scale data set enabled me to comparatively study regional alpha-diversity, and for the first time explore bathymetric and geographical distribution ranges backed up by molecular data (Chapters 6 – 9). All animals were sampled during several expeditions to different regions of the NWP between 30°- 60° N and 135° - 165° E, spanning depth ranges from the lower bathyal down to the bottom of an oceanic trench. Applying the combined approach of scleritome characters and molecular data, I investigated the abyssal solenogaster fauna of the NWP plain in Chapter 6. Among the wealth of novel species, I identified key lineages for detailed anatomical investigations from the regionally most species-rich families. Among them, a putative link is revealed between the solenogaster fauna of the abyssal plain of the NWP and the Southeast Atlantic abyssal Angola Basin (Chapter 6). Chapter 7 characterizes the solenogaster diversity of the Sea of Okhotsk, with the aim to compare the faunal composition of its semi-isolated basin with the open abyssal plain of the NWP. The hadal zone of oceanic trenches has never before been explored for Solenogastres. The Kuril-Kamchatka Trench is one of the world's five deepest trenches, and its solenogaster fauna is for the first time investigated in Chapter 8. The slopes

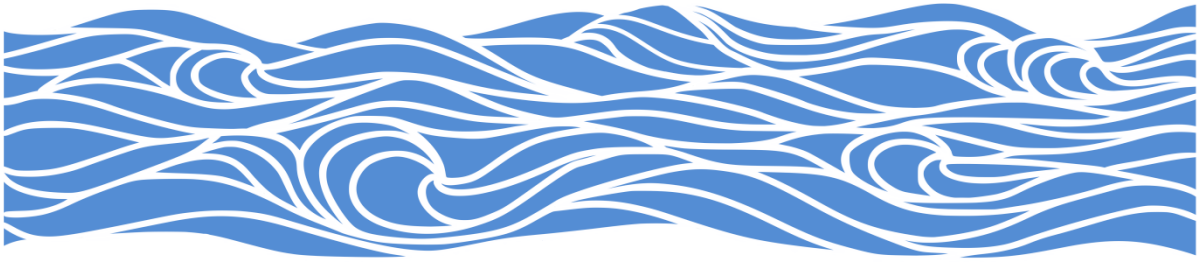
and bottom of the trench harbor a unique diversity of Solenogastres mostly new to science. Analyses of the combined data set from the previous chapters hint at an endemic abyssal and hadal solenogaster fauna and elucidate first patterns of connectivity between the different investigated regions of the NWP. The resulting two-marker phylogeny revealed the need to partially revise traditional solenogaster systematics (Chapter 8). As part of the collaborative Beneficial project ("Biogeography of the NW Pacific fauna − A benchmark study for estimations of alien invasions into the Arctic Ocean in times of rapid climate change") the compiled data set is made publicly available within the Ocean Biodiversity System (OBIS). Published within the "Biogeographic Atlas of the deep NW Pacific", Chapter 9 contains a synthesis of the diversity and distribution patterns of Solenogastres in the deep sea of the NWP.

The aim of **Part III** (Chapter 10) of my thesis is to contribute to a better understanding of the interactions within deep-sea communities. Indirect sequencing of gut contents of the Solenogastres investigated in Chapter II revealed highly specialized feeding strategies, thereby shedding light on their feeding ecology and the role of these molluscs within deep-sea food webs.

Part IV (Chapter 11 and 12) focuses on communicating highly specialized marine biodiversity research to a wider audience, also outside of the scientific community. To counteract the challenges of solenogaster taxonomy and the resulting negligence among the scientific community, the newly established online database AplacBase (Chapter 11) provides resources on Aplacophorans. AplacBase offers among others a searchable database (filters include systematics, type localities, marine provinces according to Spalding et al. (2007), depth distribution), interactive map of species records, a compilation of 150 years' worth of aplacophoran literature, quick ID guides to family level (using external characters which are straight-forward to access) and a photo gallery of formally described and also unknown species. The platform is aimed at experts and non-experts alike, with the objective to boost research on aplacophorans, and make these two classes of molluscs more accessible beyond the currently small group of trained specialists. Communicating science to the general public comes with its own challenges, especially if the audience consists of primary school children and the research area of almost invisible, tiny animals. Chapter 12 gives an example of different activities that aim to engage young children in the discovery of sand-dwelling meiofauna and promote ocean literacy.

2. Results

Part I. Solenogastres from shallow sands: navigating cryptic species and the diversity of interstitial Solenogastres



Chapter 1. Bergmeier FS, Haszprunar G, Todt C & Jörger KM (2016) **Lost in a taxonomic Bermuda Triangle: comparative 3D-microanatomy of cryptic mesopsammic Solenogastres (Mollusca)**. *Organisms Diversity & Evolution*, 16: 613-639.

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ORIGINAL ARTICLE

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Lost in a taxonomic Bermuda Triangle: comparative 3D-microanatomy of cryptic mesopsammic Solenogastres (Mollusca)

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Abstract Solenogastres (Mollusca) have a quite uniform bodyplan and an evolutionary history with few shifts out of their deep-water habitat and beyond their epibenthic lifestyle. Consequently, few clades inhabit the shallow subtidal mesopsammon; only Meiomeniidae (order Pholidoskepia) is entirely restricted to this habitat. What was initially designed as a comparative microanatomical redescription of Meiomeniidae to explore the diversity of this clade with its unique evolution, developed into a taxonomic nightmare of cryptic, co-occurring lineages: three out of four valid species of Meiomeniidae co-occur in coarse sands in the Bermuda archipelago and were re-collected at the respective type localities. We analyzed the material combining three-dimensional (3D) reconstructions from histological serial sections and ultrastructural data, providing novel insights into meiomeniid anatomy and discussing potential phylogenetic implications. However, not all collected material could be unambiguously assigned to known lineages of mesopsammic Solenogastres. In addition to meiomeniids, we discovered another co-occurring, externally highly cryptic but anatomically distinguishable lineage. It is provisionally placed within Dondersiidae, but its taxonomic assignment remains problematic due to an exclusive character mosaic and a unique foregut gland complex. Our study reveals the risk of creating chimeric taxa in small-bodied Solenogastres, as morphological characters needed for species delineation cannot be extracted from single individuals, while conspecifity based on external features is risky to assume with cryptic species co-occurring. Molecular markers will be needed to reliably retrieve Meiomeniidae from their current Bermuda Triangle of taxonomy and to proceed in solenogaster taxonomy confronted with a wealth of poorly known lineages especially in meiofaunal forms.

Keywords Neomeniomorpha · Taxonomy · Species delineation · Worm-molluscs · Aplacophora

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Introduction

Solenogastres (also known as Neomeniomorpha) is a clade of marine, vermiform aplacophoran molluscs, which currently comprises close to 300 valid species (Todt 2013). They were traditionally considered as archaic molluscs that branched off close to the base of the molluscan tree, based on their vermiform body covered by a cuticle with aragonitic scales and spicules, a reduced foot that can be retracted into the ventral (=pedal) groove and a simple organization of the gut (Salvini-Plawen 1980; Salvini-Plawen and Steiner 1996; Haszprunar 2000; Salvini-Plawen 2003a). Alternatively, these putative "primitive" character states were interpreted as derived traits with both clades of worm-molluscs (Solenogastres and Caudofoveata) originating from a common ancestor via progenesis (e.g., Scheltema 1993). So far, molecular datasets have not yet finally settled the dispute on the systematic placement of Solenogastres, although most recent approaches based on broad phylogenomic datasets (Kocot et al. 2011; Smith et al. 2011; Smith et al. 2013) or housekeeping genes (Vinther et al. 2012) seem to converge on the Aculifera hypothesis, i.e., in monophyletic Aplacophora (Solenogastres

and Caudofoveata) as sister to Polyplacophora. This is only contradicted by the largest taxon sampling for phylogenetic analyses of Mollusca based on four molecular standard markers (Stöger et al. 2013). Excluding fast evolving mitochondrial markers, Stöger et al. (2013) also retrieve monophyletic Aplacophora, but they cluster with cephalopods and scaphopods in a clade termed Variopoda. Even though sister-group relationships are not finally settled, hypotheses based on molecular data currently favor Solenogastres in a derived position in the molluscan tree of life (for recent reviews, see, e.g., Kocot 2013; Giribet 2014; Schrödl and Stöger 2014; Vinther 2015).

Traditional systematics relying primarily on three key characters (scleritome, foregut glands, and radula) has classified Solenogastres in four main orders: aplotegmentarian Pholidoskepia and Neomeniamorpha and pachytegmentarian Sterrofustia and Cavibelonia (Salvini-Plawen 1978; García-Álvarez and Salvini-Plawen 2007). So far, internal relationships among Solenogastres could not be well resolved based on cladistic analyses of morphological characters, but doubts about the existing classification system have been expressed, e.g., on the monophyly of Cavibelonia (Scheltema and Schander 2000; Salvini-Plawen 2003a). Preliminary molecular analyses support traditional groupings but corroborate a polyphyly of Cavibelonia (Kocot et al. 2013). The poor resolution in morphology-based phylogenetic analyses might be influenced by the generally homogeneous anatomical bauplan of these vermiform molluscs, which remained in striking morphological stasis despite their world-wide distribution and estimated early Paleozoic origin (Vinther et al. 2012; Stöger et al. 2013). This comparatively small variation in the general bauplan is combined with a lack of detailed knowledge on the microanatomy of several lineages. Such knowledge is important for better understanding the general biology of these animals and might also provide additional characters for phylogenetic analyses. While molecular approaches in the future will hopefully contribute substantially to our understanding of the origin and evolutionary relationships of the different solenogaster lineages, reconstructing the evolutionary history of Solenogastres is currently further complicated by the lack of a general concept on character evolution, i.e., on plesiomorphic versus derived character states within solenogaster lineages (Todt et al. 2008a).

Most known solenogaster species inhabit deeper zones of the continental shelf and bathyal depths (i.e., 100–2000 m), and only few lineages are found in shallow subtidal depths ranging from 0 to 20 m, but only poor data exist on bathymetric ranges of the clades (Scheltema 1990; Todt 2013). Some solenogaster species have an epizoic lifestyle, living directly on their cnidarian prey such as Octocorallia (e.g., Pruvot 1890; Salvini-Plawen 1967a; Scheltema and Jebb 1994) or Hexacorallia (Sasaki and Saito 2005). The majority of Solenogastres live epibenthic and glide on the surface of soft (coarse, muddy, or silty) sediments (see lab experiments of Salvini-Plawen 1968), and only few direct observations also report on species burrowing in the sand (Kocot and Todt 2014). Currently, ten taxa (see García-Álvarez et al. 2000; Kocot and Todt 2014; Klink et al. 2015) are considered to inhabit the marine mesopsammon, i.e., the interstitial spaces between sand grains (Swedmark 1964, 1968). Since this resembles one of the few habitat shifts in the evolutionary history of Solenogastres, it is of special interest for exploring the morphological and anatomical diversity of Solenogastres. Mesopsammic Solenogastres include the smallest representatives of the clade and among molluscs in general, challenging the minimum limits of a successful bauplan, and potentially bear morphological adaptations to the mesopsammic environment (such as a scaly scleritome or posterior adhesive organ (Salvini-Plawen 1985a; García-Álvarez et al. 2000). The ten putative mesopsammic species belonging to six families (see García-Álvarez and Salvini-Plawen 2007) have likely—at least in parts—shifted independently to an infaunal lifestyle based on a preliminary molecular phylogenomic hypothesis based on EST transcriptome data (Kocot et al. 2013).

Meiomeniidae present the only entirely mesopsammic clade, which thus displays a diversification within the interstitial habitat (García-Álvarez et al. 2000). However, out of the four valid species, only the description of the type species *Meiomenia swedmarki* Morse, 1979 contains details on internal anatomy (based on whole mount preparations) (Morse 1979). The other species (re)descriptions are limited to external characters, such as the scleritome, dorsoterminal sense organ, or abdominal stylets and to the radula morphology (Salvini-Plawen 1985a). Furthermore, the single serially sectioned specimen supplementing some anatomical data was not re-collected at the type locality (Morse and Norenburg 1992). *M. swedmarki* is known from the US Pacific Northwest Coast (Morse 1979) and *Meiomenia arenicola* Salvini-Plawen and Sterrer, 1985 was described from the US East Atlantic Coast (Salvini-Plawen 1985a) and later also reported from the shallow subtidal waters off Bermuda (García-Álvarez and Salvini-Plawen 2007). A second genus *Meioherpia* Salvini-Plawen, 1985 was erected based on slight differences in the scleritome (Salvini-Plawen 1985a) and a common atriobuccal opening vs. two separate openings in *Meiomenia* (García-Álvarez and Salvini-Plawen 2007). Both valid species—*Meioherpia stygalis* Salvini-Plawen and Sterrer, 1985 and *Meioherpia atlantica* Salvini-Plawen, Rieger and Sterrer, 1985—co-occur with *M. arenicola* in Bermuda, and the diagnostic features used to delineate meiomeniid genera and species have been under dispute between several authors (Salvini-Plawen 1985a; Morse and Norenburg 1992) (see "Discussion" for details).

Modern morphological methods, such as transmission electron microscopy, computer-aided 3D-reconstructions based on histological semithin sectioning, immunolabeling of neurotransmitters, and phalloidin labeling have addressed

the evolution of aplacophoran diversity and demonstrated new possibilities for species (re)descriptions (see, e.g., Scherholz et al. 2013; Señarís et al. 2014). We re-collected mesopsammic Solenogastres from Bermuda at the meiomeniid type locality and surroundings. Preliminary examination in the field via light microscopy identified all specimens as Meiomeniidae based on external characters of the scleritomes. Taxonomic identification in Meiomeniidae is highly problematic however, due to a very low interspecific variation and because of characters like copulatory spicules, whose presence depends on the ontogenetic stage of the specimen (Morse and Norenburg 1992). Our study presents a comparative microanatomical approach, based on serial semithin sections visualized in 3D reconstructions, to contribute to the taxonomic clarification of three different co-occurring lineages currently lost in a "taxonomic Bermuda Triangle." We highlight morphological differences to other solenogaster species and therein contribute to the search for additional character sets to reconstruct the evolutionary history of this exceptional molluscan clade. Modifications to the general solenogaster bauplan are discussed in regard to the habitat shift from an epibenthic to a mesopsammic environment.

Material and methods

Material

Material was collected during fieldtrips to the Bermuda archipelago in 1999, 2002, and 2011 from two different collecting sites, one of which corresponds to the type locality of *M. stygalis* (see Table 1 for summary of material, including museum numbers from the Bavarian State Collection of Zoology (ZSM)). All specimens were preliminary investigated in the field for external characters of the scleritome via light microscopy and identified as belonging to the order Pholidoskepia. Based on microanatomical data, we were later able to assign five specimens to Meiomeniidae (M1 to M5) and two

Table 1 Specimens investigated in this study with information on collectors, sampling localities (all on Bermuda), and processing

I.D.	Field I.D. via light microscope	ZSM no.	Location	Coll.	Fixation	Embedded	Used for
M1	*Meiomenia arenicola*	20150445	Flatt's Inlet 32°19′22.6″ N 64°44′14.8″ W	ChT	Glutaraldehyde	Spurr's	3D (1 μm sections)
M2	*Meioherpia stygalis*	20150446	Flatt's Inlet 32°19′22.6″ N 64°44′14.8″ W	ChT	Glutaraldehyde	Spurr's	3D (1 μm sections)
M3	*M. stygalis*	Lost during preparation	Flatt's Inlet 32°19′22.6″ N 64°44′14.8″ W	ChT	EtOH	–	Radula
M4	*Meioherpia atlantica*	20150448	Castle Roads[a] 32°20′10.7″ N 64°40′05.3″ W	GH	Glutaraldehyde	Spurr's	Histology (1.5 μm sections)
M5	*Meioherpia atlantica*	20150449	Castle Roads[a] 32°20′10.7″ N 64°40′05.3″ W	GH	Glutaraldehyde	Spurr's	Histology (1.5 μm sections)
D1	*Meioherpia* sp.	20150452	Castle Roads[a] 32°20′10.7″ N 64°40′05.3″ W	AF, MS, GH	Glutaraldehyde	Spurr's	3D (1 μm sections)
D2	*Meioherpia atlantica*	20150450	Castle Roads[a] 32°20′10.7″ N 64°40′05.3″ W	GH	Glutaraldehyde	Spurr's	TEM (70–80 nm sections)
Ph1	*M. stygalis*	20150447	Flatt's Inlet 32°19′22.6″ N 64°44′14.8″ W	ChT	EtOH	–	SEM
Ph2	*Meioherpia atlantica*	20150451	Flatt's Inlet 32°19′22.6″ N 64°44′14.8″ W	ChT	Glutaraldehyde	–	Scleritome, light microscopy

ChT Christiane Todt, GH Gerhard Haszprunar, MS Michael Schrödl, AF Alexander Fahrner, M1-M5 meiomeniid specimens, D1 and D2 dondersiid specimens, Ph1 and Ph2 pholidoskepian specimens not identified to family level

[a] Type locality of M. stygalis

specimens to Dondersiidae (D1 and D2) (see Table 1). However, our microanatomcial study (see details below) shows that external morphology alone is insufficient to reliably assign specimens to a family. For two specimens (Ph1 and Ph2), no additional characters were available, and thus they could not be identified to family level. Seven specimens were fixed in 2.5 % glutaraldehyde buffered with sodium cacodylate buffer and two in 70 % ethanol (see Table 1). Additional specimens were fixed in ethanol for future molecular analyses.

Scleritome and radula analyses

We used light microscopy with birefringence filters as well as scanning electron microscopy (SEM) to visualize and re-examine the external characters of the scleritome. We documented the whole mounted Ph2 with a differential interference contrast filter on a BX 51 compound microscope (Olympus). Ph1 was first stepwise rehydrated in 80 and 70 % ethanol and then dehydrated in a graded acetone series (70, 80, 90, 100 %). We critical point dried the specimen in 100 % acetone in a Baltec CPD 030 (Leica Microsystems) in carbon dioxide atmosphere and mounted it on a SEM stub with a self-adhesive carbon sticker. It was sputter-coated with gold in a Polaron Sputter Coater (GaLa Gabler Labor Instrumente Handels GmbH) in Argon atmosphere for 240 s. We took SEM pictures with a LEO 1430 VP (Zeiss) SEM at 1-10kV and edited the pictures in Photoshop CS6 (Adobe Systems Software Ireland Ltd.). For investigation of the radula, we dissolved the soft body of one specimen (M3) in potassium hydroxide (we started with 10 % KOH and steadily increased the concentration, until the tissue started to slowly dissolve), dissected the radula and took pictures with a SP25 camera (Olympus) mounted on a CX41 compound microscope (Olympus).

Histology and 3D-microanatomy

After initial fixation (see Table 1), the animals used for histological (M1, M2, M4, M5, and D1) and ultrastructural investigations via TEM (D2) were decalcified in 3.5 % ascorbic acid overnight, post-fixed using osmium tetroxide, and embedded in Spurr's low-viscosity resin (Spurr 1969) (see Table 1). We serially sectioned M1, M2, and D1 with 1 μm thickness, and M4 and M5 with 1.5 μm section thickness using a diamond knife on a RMC MT 7000 microtome (Leica AG). We applied contact cement to the lower edge of the resin blocks to obtain ribbons of sections for the 3D reconstruction (Ruthensteiner 2008). The ribbons were collected on microscopic slides, stained using azure II/methylene blue (Richardson et al. 1960), and sealed with cover glasses.

Digitalization and 3D-reconstruction

We took digital photographs with ×60 magnification of every section of M2 at the University Museum of Bergen using a DFC 420 Camera (Leica Camera AG) mounted on a 6000B compound microscope (Leica Microsystems), and of M1, M4, M5, and D1 at the LMU Biocenter using a SP25 camera (Olympus) on a CX41 compound microscope (Oympus). The photographs were processed in Bergen with the software Leica Application Suite V3.10 (Leica Camera AG) and the Cell^D software (Olympus) at the Biocenter. All digital photos of the series of M1, M2, and D1 were converted to 8-bit greyscale format, contrast enhanced, and unsharp masked with Photoshop CS 2 (Adobe Systems Software Ireland Ltd.). For comparison of the microanatomy, the three series were imported into the 3D-visualization software AMIRA 5.3.3 (Visage Imaging, Berlin, Germany). Computer based 3D reconstructions of all major organ systems were then conducted of M1, M2, and D1 mainly following the workflow described by Ruthensteiner (2008).

For ultrastructural analysis, D2 was sectioned alternating into semi- (1 μm) and ultrathin (60–80 nm) sections. Ultrathin sections were stained with uranyl acetate and lead citrate after Reynolds (1963) and studied using an EM 900F (Zeiss) transmission electron microscope.

Results

General morphology of mesopsammic Pholidoskepia from Bermuda

All nine investigated pholidoskepian specimens are of vermiform shape with body lengths varying between 0.4 and 1 mm. In all studied specimens, the body cross-section is more or less circular and varies between 60 μm (in M1) and 120 μm (in D1) in diameter. A ciliated foot is located in the pedal groove running along the midventral line of the body. The scleritomes of meiomeniids as well as the co-occurring dondersiids *incertae sedis* are highly similar: all specimens are covered in pholidoskepian-type scales with some interspersed laterally projecting spines (Fig. 1a). Examination via SEM shows that these scales are mostly leaf shaped in individual Ph1 with their distal tips varying in roundness (Fig. 1b). Few interspersed lanceolate scales (length up to 20 μm) with a medial keel project from the mantle (Fig. 1b–d) in posteriolateral position. The ventral pedal groove is laterally surrounded by paddle-like scales (Fig. 1e).

Despite their external uniformity (as identified via light microscopy in the field and reported above), histological investigations uncovered two principally distinct lineages comparatively described in the following (for taxonomic assignment, see "Discussion").

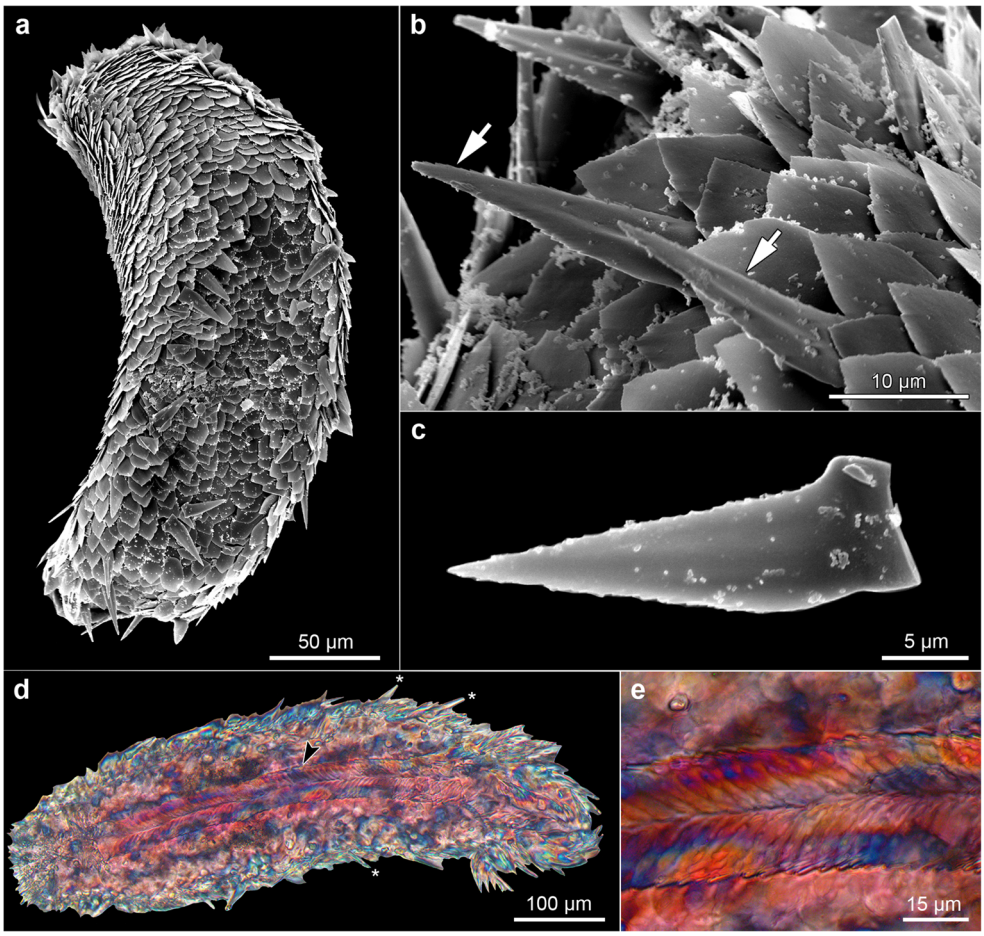

Fig. 1 Scleritome of mesopsammic Pholidoskepia from Bermuda. **a–c** SEM micrographs of specimen Ph1. **d–e** Light microscopic images of Ph2 using bipolarized light. **a** Dorsal view of entire specimen. Anterior side facing to the top. **b** Keeled, laterally projecting lanceolate scales (arrows) and leaf-shaped scales from the posterior part of the body; crystallized structures are preparation artifacts. **c** Isolated, lanceolate scale. **d** Ventral view of entire specimen, with projecting lateral mantle scales (asterisks) and the pedal groove (arrowhead). Anterior side to the left. **e** Paddle-shaped scales flanking the pedal groove

Microanatomy of an undescribed Dondersiidae *incertae sedis* (D1 and D2)

The histologically studied specimen D1 is of approximately 550 µm length and exhibits a slightly more dorsoventrally keeled body cross-section than the meiomeniids described below. The indention of the pedal pit marks the beginning of the ciliated foot approximately 55 µm from the rostral end, and the narrow pedal groove extends until the posterior end along the midventral line of the body (Fig. 2a). The atrium (also called vestibular chamber or vestibulum) is located 15 µm from the anterior end and extends for 20 µm into the body. It is lined by a continuous border of microvilli and clearly separated from the more posterior positioned mouth opening (Fig. 2b). It is surrounded by 11 periatrial stereo-cirri which measure up to 20 µm from their distal tip to their point of insertion in the integument (Fig. 2b, d, d'). In light microscopic investigations, the cuticle appears as a homogeneous structure (see, e.g., Fig. 2g); however, in transmission electron microscopy, it is revealed to contain numerous inclusion bodies (possible bacteriocytes, see Fig. 3a, b). Voids in the cuticle clearly show the former location of sclerites, which were dissolved during the embedding process (Fig. 3b, asterisks). The epidermis consists of isoprismatic epidermal cells and numerous glandular cells distributed over the entire body. Some of these glandular cells contain either electron light or dense secretions, whereas other cells contain a combination of droplets of varying electron density (Fig. 3a, arrowhead, b). Subepidermal, unicellular sole glands with dark-blue staining, drop-like secretions are positioned on both sides of the foot groove and open to the outside laterally of the ciliated foot (Fig. 3c'). In the posterior third of the body an additional type of gland cells with lighter staining secretions is found ventrally. These subepidermal gland cells increase in number towards the posterior end and form a dense accumulation (approx. 45 × 35 µm) below the rectum (Fig. 2a). In the immature specimen D2, neither pallial cavity nor gonads were yet developed.

Nervous system and sensory structures

The nervous system consists of fused cerebral ganglia and paired lateral, buccal, and pedal ganglia (Fig. 2e). The lateral and pedal ganglia give rise to the paired lateral and ventral nerve cords, therein forming the tetraneural nervous system

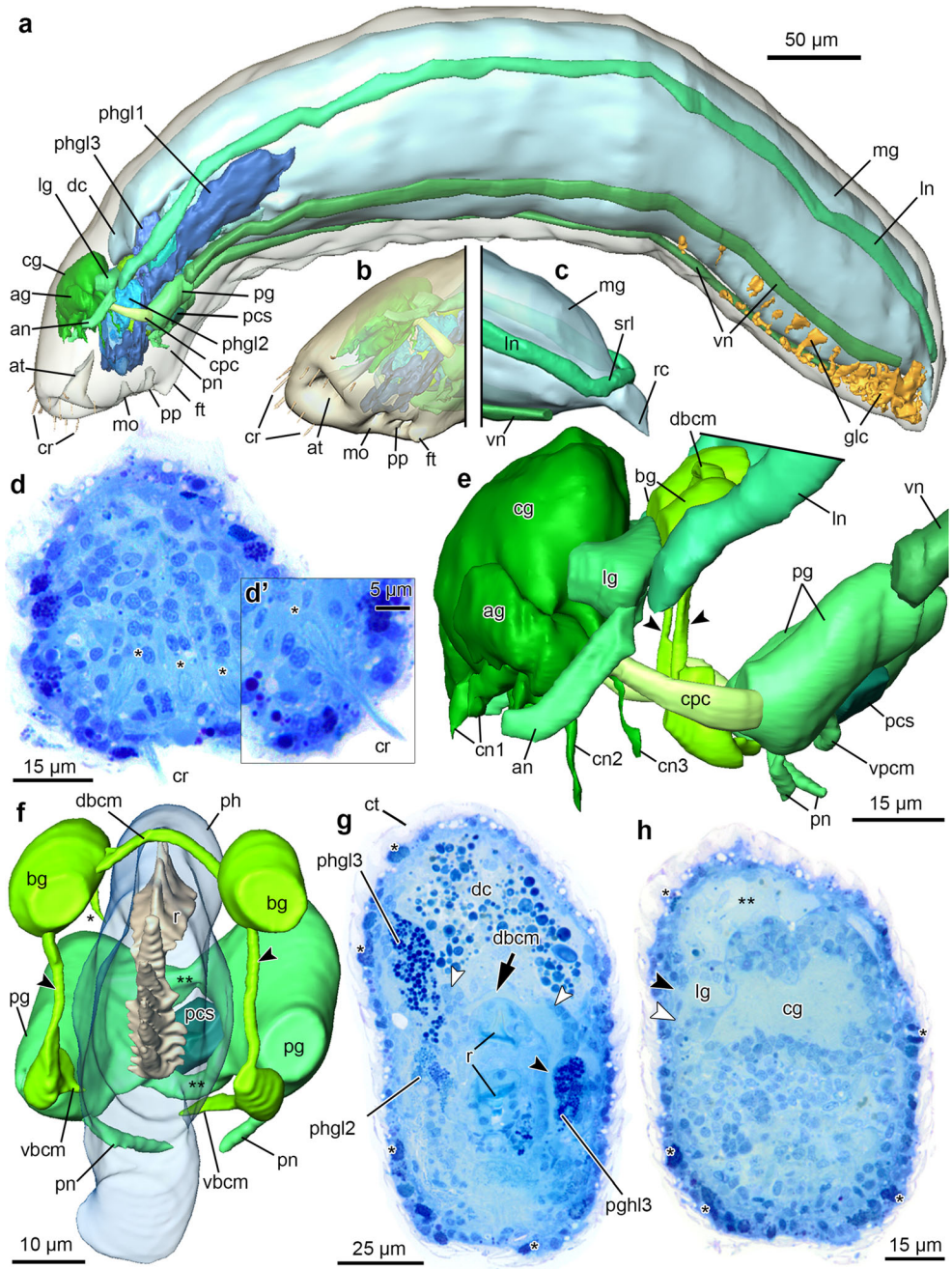

Fig. 2 General morphology, microanatomy, and histology of the nervous system of dondersiid *incertae sedis* D1. **a–c, e, f** 3D-reconstructions. **d, d', g, h** Histological cross-sections of the nervous system. **a** Overview of all reconstructed organ systems. Body surface transparent. Green nervous system, blue digestive system. Lateral view. **b** Head region, showing atrium surrounded by atrial cirri, mouth opening, and pedal pit. Lateroventral view. **c** Posterior end with midgut (transparent), rectum, and suprarectal loop. Body surface not shown. **d** Anterior edge of the atrium showing cirri. Base of cirri marked with asterisks. **d'** Close-up of longitudinal section through a periatrial cirrus. Base of cirrus marked with asterisk. **e** Nervous system, lateral view. Arrowheads mark ventral buccal commissure. **f** Frontal view of pedal ganglia, and buccal ring surrounding the pharynx (transparent blue) with radula. Single asterisk marks putative radula nerve, and double asterisks mark dorsal and ventral pedal commissure. Black arrowheads point to putative ventral buccal commissure. **g** Cross-section at the level of radula, buccal ganglia (white arrowheads), dorsopharyngeal-buccal commissure (arrow) and putative ventral buccal commissure (black arrowhead). Asterisks mark epidermal glandular cells. **h** Cross-section at the level of the cerebral ganglia with lateral nerve cord (black arrowhead) and anterior nerve (white arrowhead) emerging from the right lateral ganglion. Single asterisks mark epidermal glandular cells, and double asterisks mark tissue formed by largely vacuolated cells. Abbreviations: at atrium, ag accessory ganglion, an anterior nerve, bg buccal ganglion, cg cerebral ganglion, cn1-3 cerebral nerves 1–3, cpc cerebropedal connective, cr cirri, dbcm dorsopharyngeal-buccal commissure, ct cuticle, dc dorsal caecum, ft foot, glc glandular cells, lg lateral ganglion, ln lateral nerve cord, mg midgut, mo mouth opening, pcs pedal commissural sac, pg pedal ganglion, ph pharynx, phgl 1–3 pharyngeal gland types 1–3, pn pedal nerve, pp pedal pit, r radula, rc rectum, srl suprarectal loop, vbcm putative ventral buccal commissure, vn ventral nerve cord, vpcm ventral pedal commissure

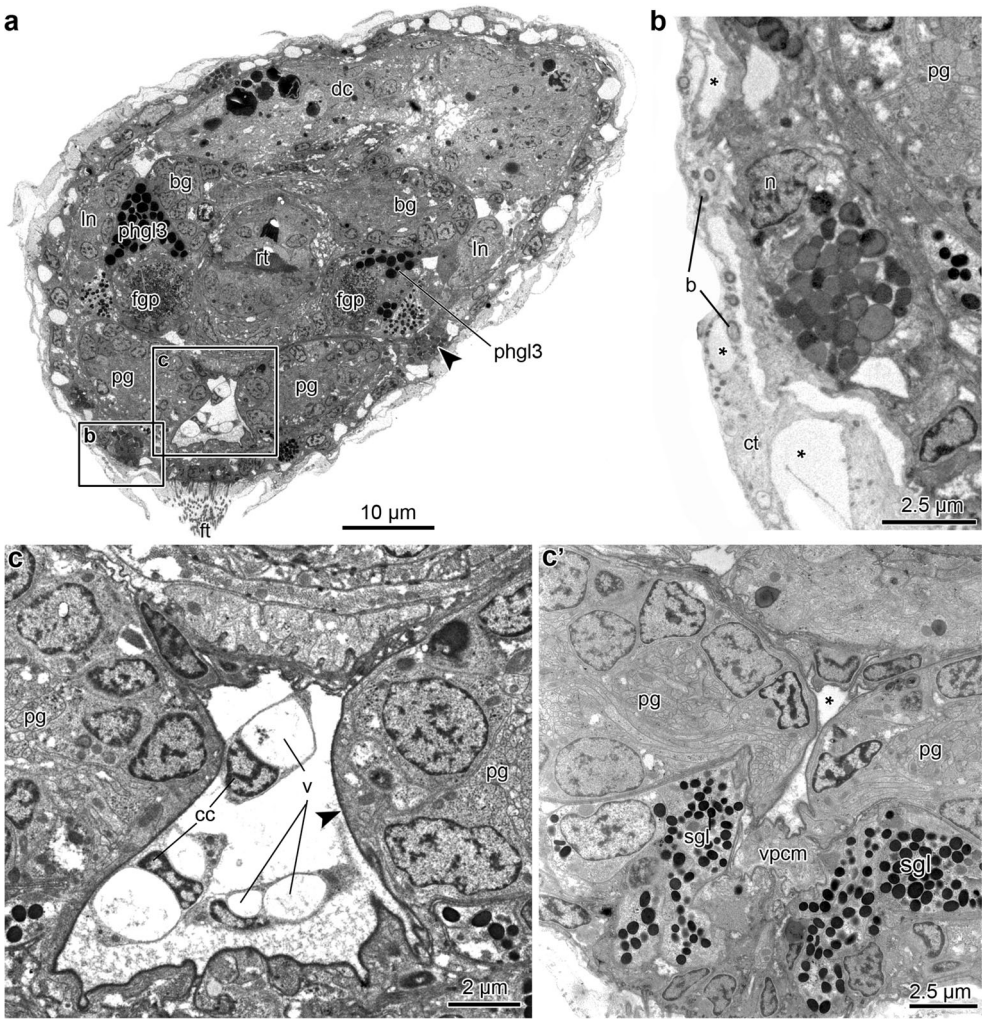

Fig. 3 TEM-micrographs of dondersiid *incertae sedis* D2. **a** Cross-section through pharynx, buccal, and pedal ganglia. Boxes indicate respective area of close-up micrographs. Arrowhead points to epidermal glandular cell. **b** Detail of the integument showing the heterogeneous cuticle containing putative endocuticular bacteriocytes, voids of dissolved sclerites (asterisks), and an epidermal glandular cell. **c** Pedal ganglia and enclosed pedal commissural sac with three central cells in the lumen. Central membrane of pedal commissural sac is indicated by arrowhead. **c'** Section posterior to **c**; pedal ganglia with ventral pedal commissure and posterior end of the lumen of the pedal commissural sac (asterisk). Abbreviations: b putative endocuticular bacteria, bg buccal ganglion, cc central cell, ct cuticle, dc dorsal caecum of midgut, fgp foregut gland pouch, ft cilia of foot, ln lateral nerve cord, n nucleus of epidermal glandular cell, phgl3 pharyngeal gland 3, pg pedal ganglion, rt radula tooth, sgl sole gland, v vacuole, vpcm ventral pedal commissure

typical for Solenogastres. The fused cerebral ganglia (40 µm width and 30 µm length) are positioned dorsoanterior to the pharynx, and a dorsal cleft indicates their fused nature (see Fig. 2h). A clear separation into central neuropil and multiple surrounding layers of perikarya in the cerebral ganglia is discernible histologically (Fig. 2h). Three cerebral nerves emerge ventrally from the cerebral ganglia (Fig. 2e) and run into an additional formation of presumably nervous tissue. But perikarya and neuropil are randomly distributed within this tissue, and no clear structural boundaries could be ascertained. Anterior to the cerebral ganglia towards the atrium, similar supposedly nervous tissue is present, densely filling the majority of the body cavity (Fig. 2d). Here, the tissue forms more or less spherical accessory ganglia-like structures. But again, no clear boundaries could be detected and we thus refrained from including it in the reconstruction. Anteriolateral to each side of the cerebral ganglia, another pair of accessory ganglia is present (ag in Fig. 2e), clearly delimited from the surrounding tissue but again with homogeneously distributed neuropil and perikarya. The lateral ganglia (approx. 14 × 8 µm) are positioned posteriolateral to each side of the cerebral ganglia and are not fully separated from them (see Fig. 2h). From each lateral ganglion, a thick lateral nerve (up to 3 µm diameter) emerges (Fig. 2h, white arrowhead) and extends anterior for 25 µm, but unfortunately we were unable to follow its further course. Dorsal to each lateral ganglion, the lateral nerve cords (characterized by neuropil with sparsely distributed nuclei) emerge (Fig. 2h, black arrowhead) and proceed between epidermis and midgut epithelium towards the posterior end, where they form the suprarectal loop (Fig. 2c). The paired buccal ganglia are located posterior to the lateral ganglia, positioned towards the center of the body, lateral to the pharynx (Fig. 2f, g). These ganglia are spherical (12 × 15 µm) and connected by a dorsopharyngeal-buccal commissure (dbcm; Fig. 2f, g). Additionally, from each buccal ganglion a nerve emanates in ventral direction lateral to the pharynx (black arrowheads in Fig. 2f, g). Ventrally both nerves thicken into ganglion-like swellings (about 6 µm diameter, no clear separation of neuropil and perikarya) and then proceed towards the midline of the body, possibly forming a ventropharyngeal-buccal commissure (vbcm, Fig. 2f). However, due to folds in the histological sections in this region, the point of

connection could not be traced. A single buccal nerve emerges from the buccal ganglia (only visible on the right side) and leads towards the radula (single asterisk, Fig. 2f). From each posterior part of the cerebral ganglia, the cerebropedal connectives proceed posterior towards the paired pedal ganglia. The pedal ganglia are elongated (approx. 40 μm length) and in their posterior part give rise to the ventral nerve cords, which proceed towards the posterior end in the ventral half of the body and seem to terminate slightly anterior to the suprarectal loop (Fig. 2a, c). From each pedal ganglion a pedal nerve emerges in direction of the foot (Fig. 2e, f). The pedal ganglia are connected by one dorsal and one ventral pedal commissure (Fig. 2f, marked with two asterisks). No posterior ganglia are present in the studied dondersiid specimen. Two structures presumably serve as sensory organs: the stereo-cirri surrounding the atrium (Fig. 2a, b, d, d') and the pedal commissural sac (pcs) enclosed between the pedal ganglia. The latter is positioned between the two pedal commissures (see Figs. 2f and 3a, c, c' for ultrastructure). This organ (approx. 11 μm in length and 14 μm at widest point in D1) is delimited by a well-defined central membrane and a subsequent, compressed layer of connective tissue on the ventral side (Fig. 3c). The commissural sac contains large, vacuolated free-floating central cells (Fig. 3a, c). In D1, we counted nine of these central cells. An innervation of the pedal commissural sac from any of the described ganglia could not be detected, neither via light microscopy nor via ultrastructural investigation. No dorsoterminal sense organ could be detected in the studied specimens.

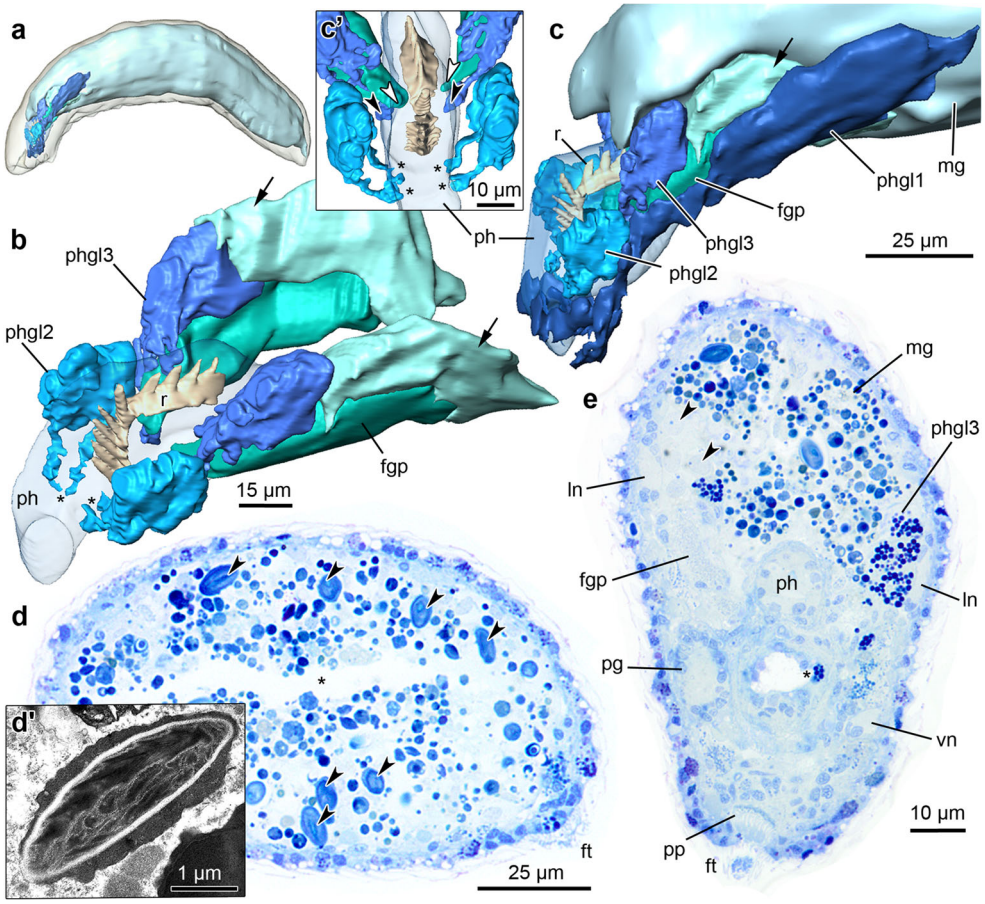

Fig. 4 3D-reconstruction (**a–c'**) and histology (**d, e**) of the digestive system of dondersiid incertae sedis D1, including TEM-close-up of an undischarged cnidocyst (**d'**) in the midgut of D2. **a** Overview of the digestive system. Body transparent. Lateral view. **b** pharynx (transparent) with radula, pharyngeal glands 2 and 3, and foregut gland complex (pharyngeal gland 1 omitted). Asterisks mark openings of ducts of pharyngeal gland 2, and black arrows point to tissue composed of cells with enlarged nuclei, associated with foregut gland pouches. Dorsolateral view. **c** 3D reconstruction of the anterior part of the digestive system. Pharynx transparent. Black arrows point to tissue composed of cells with enlarged nuclei, associated with foregut gland pouches. Lateral view. **c'** Anterior view of pharynx (transparent) and radula, showing openings of the ducts of pharyngeal glands 2 (asterisks) and 3 (black arrowhead), as well as foregut gland pouches (white arrowheads). Pharyngeal gland 1 omitted. **d** Cross-section through the midregion of the body, showing digestive epithelium of the midgut. Arrowheads point to cnidocysts contained in the midgut epithelium. **d'** Unidentified (stenotele?) cnidocyst in the midgut epithelium of D2. **e** Cross-section through the pharynx posterior of the radula. Asterisk marks opening of pharyngeal gland 2 into pharynx. Black arrowheads point to enlarged nuclei of cells associated with foregut gland pouches. Abbreviations: fgp foregut gland pouch, ft foot, ln lateral nerve cord, mg midgut, ph pharynx, phg1-3 pharyngeal gland 1–3, pg pedal ganglion, pp pedal pit, r radula, vn ventral nerve cord

Digestive system

The digestive system of the 3D-reconstructed specimen D1 comprises three types of pharyngeal glands (phgl 1–3), the foregut gland complex and a midgut which fills the majority of the body cavity (Fig. 4a). The oral tube and pharynx are both ciliated and the latter contains the monoserial radula (Fig. 4b-c'). The radula is approximately 45 μm in length and consists of ten triangular teeth (each approx. 12 μm in length) resting on odontophores (Fig. 5a, a'). No putative denticles on the teeth could be reliably reconstructed based on the histological sections. Even TEM could not detect a radula membrane (Fig. 5a, a')—i.e., a structure clearly discernible from the radula teeth. All three pairs of pharyngeal glands are loose multicellular aggregations of glandular cells, which unite in common ducts leading into the pharynx. They can be differentiated by their position relative to the pharynx, as well as by the staining properties of their secretions. The largest pair of pharyngeal glands (phgl 1) extends from a ventral position from the beginning of the pharynx (i.e., in front of the radula, where the gland opens via two small ducts laterally into the pharynx) in posterior and midlateral direction for

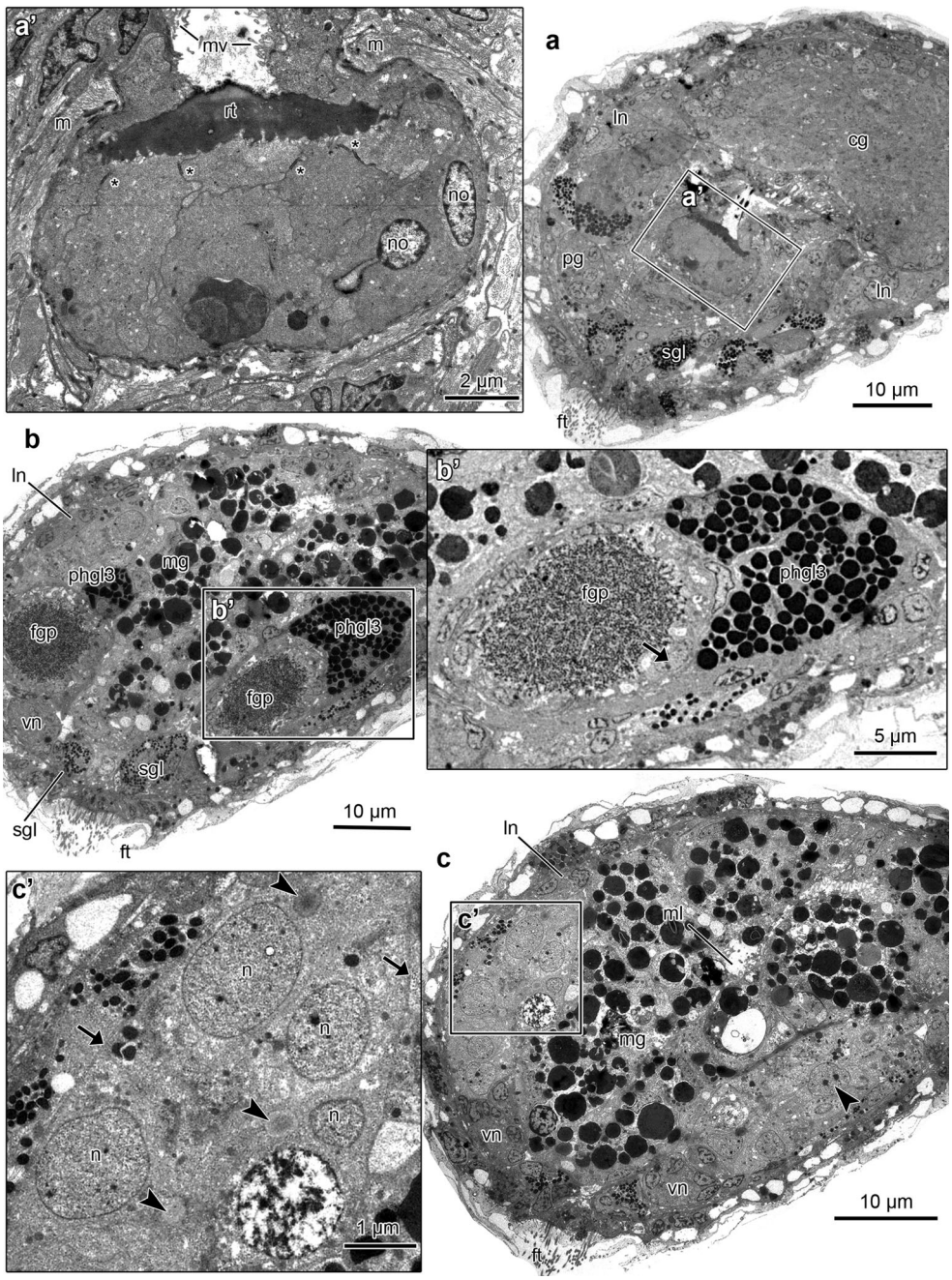

Fig. 5 TEM-micrographs of dondersiid incertae sedis D2, showing details of the digestive system and associated structures. Boxes indicate respective area of close-up micrographs. **a** Cross-section through cerebral ganglia, pharynx, and radula with odontophore. **a'** Detail of radula and odontophore. Asterisks indicate desmosomal junctions between odontophore cells. **b** Cross-section at line of foregut gland pouches and midgut. **b'** Detail of foregut gland pouches and pharyngeal gland 3. Arrow points to nucleus of one epithelial cell, forming the foregut gland pouch. **c** Cross-section posterior to the foregut gland, through tissue with enlarged nuclei (arrowhead). **c'** Detail of tissue, delimited by membrane (arrows). Arrowheads point to mitochondria. Abbreviations: cg cerebral ganglia, fgp foregut gland pouch, ft cilia of foot, ln lateral nerve cord, m muscle, mg midgut, ml midgut lumen, mv microvilli of pharynx, n enlarged nucleus, no nucleus of odontophores, phgl3 pharyngeal gland 3, pg pedal ganglia, rt radula tooth, sgl sole gland, vn ventral nerve cord

about 140 μm, therein extending beyond the foregut gland complex described below (see Fig. 4c). The secretions of phgl 1 stain light blue in azure II/methylene blue and are the smallest secretions in comparison to the secretions of the other pharyngeal glands of this dondersiid. The second type of pharyngeal glands (phgl 2, Fig. 4b, c) measures around 30 μm and opens into the pharynx posterior to phgl 1 but still anterior to the radula via two ducts on each side (asterisks in Fig. 4c'). The droplet shaped secretions of phgl 2 are larger and stain in darker blue than those of phgl 1.The third type of pharyngeal glands (phgl 3, Fig. 4b, c) is positioned posterior to phgl 2 and dorsal to the pouches of the foregut gland complex and extends posterior for 20 μm. These glands open via a single duct on each side into the posterior part of the pharynx lateral to the radula (black arrowhead in Fig. 4c'). Their drop-like secretions form the largest of the three pharyngeal glands' secretions and stain dark blue on our histological sections. The multicellular foregut gland complex (see Fig. 4e for histology of D1 and Fig. 5b, b' for ultrastructure of D2) consists of conspicuous pouches (Figs. 4b and 5b, b'), which open on both sides into the pharynx at the level of the posterior half of the radula, roughly at the same position as phgl 3. Attached to these pouches are cells characterized by comparatively enlarged nuclei (up to 2 μm in diameter) with loose heterochromatin (Fig. 5c, c'). They are located posterior to the phgl 3, halfway over the pouches and extend posterior for 45 μm (Fig. 4b). The pouches are formed by a thin, non-muscular and non-glandular cuboidal epithelium, and are 60 μm in length (D1). In histological sections, their content stains light blue (Fig. 4e). Ultrastructural investigations show that the substance contained within these pouches is homogeneously electron dense (Fig. 5b, b') and consists of numerous rod-shaped elements. The nature and origin of the content of the pouches remains unclear (see "Discussion").

The midgut fills the entire body cavity and spans until the posterior end of the animal. Anterior, small paired caeca extend for 5 μm dorsally of the pharynx (Fig. 4c). Towards the posterior end, the midgut narrows and in absence of a pallial cavity, the rectum opens directly to the exterior on the ventral side (Fig. 2a, c). The midgut is continuously comprised of large columnar digestive cells containing digestive vesicles of varying sizes (see Fig. 4d), which restrict the midgut lumen to a small slit. These digestive cells contain numerous undigested cnidocysts (Fig. 4(d, black arrowheads, d') for ultrastructure of an undischarged cnidocyst). The body cavity between midgut caeca and posterior to the cerebral ganglia is filled with a congregation of comparably large cells of undetermined origin (see Fig. 2h, double asterisks).

Reproductive system and excretory structures

The studied specimens D1 and D2 were immature and no structures of the reproductive system were developed. We were also unable to detect a heart or pericardium, which together with the reproductive system usually form the gonopericardial system. On a histological level, we were also unable to detect larval excretory structures in specimen D1. In individual D2, however, we ultrastructurally detected a subepidermal cell filled with numerous vesicles of different sizes (Fig. 6a) ventral to the lateral nerve cord and next to the pouch of the foregut gland complex. This cell is surrounded by longitudinal and diagonal muscle fibers and the proximal cell wall forms a system of basal infoldings (see Fig. 6a), which might present a putative ultrafilter (see "Discussion"). Additionally, we detected amoebocytes with possible excretory function (see "Discussion") between the epidermis and the

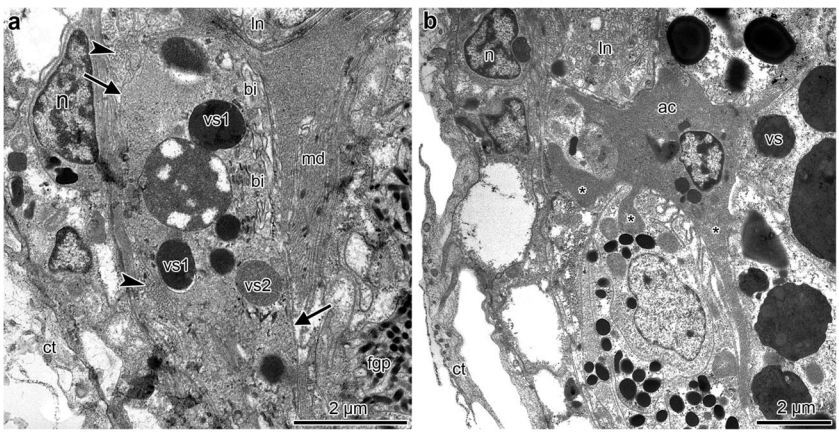

Fig. 6 TEM-micrographs of putative excretory structures of dondersiid incertae sedis D2. **a** Cross-section at the level of foregut gland pouches, with putative ultrafiltration cell. Arrows point to membrane delimiting cell. Black arrowheads point to longitudinal muscle fibers. **b** Amoebocyte positioned between integument and midgut epithelium with extending parapodia (asterisks). Abbreviations: ac amoebocyte, bi basal infoldings, ct cuticle, fgp foregut gland pouch, ln lateral nerve cord, md diagonal muscle fibers, n nucleus of epidermal cell, vs vesicle in midgut epithelium, vs1-2 vesicles within putative ultrafiltration cell

midgut epithelium, extending pseudopodia into the intercellular spaces (Fig. 6b).

General morphology and microanatomy of Meiomeniidae (M1–M5)

The general morphology of the five studied meiomeniids (M1–M5) is highly similar to the described dondersiid specimens. In all histologically investigated specimens (M1, M2, M4, M5), the atrium is clearly separated from the mouth opening (Fig. 7a, b). The size of the blind ending atrium differs between the specimens (lengths between 20 and 40 μm, diameter ranging from 25 to 40 μm). In contrast to the immature dondersiid (D1) and immature meiomeniids (M4, M5), a pallial cavity is present in the two mature meiomeniid specimens (M1, M2). In both individuals, it opens in a subterminal position at the end of the pedal groove. In meiomeniid M1 the pallial cavity forms a single pouch (Fig. 7d, f), whereas in meiomeniid M2, the pallial cavity divides into two equally large lateral pouches (Fig. 7e, g). The integument is formed by a chitinous cuticle of varying thickness and an underlying epidermis of isoprismatic cells and interspersed glandular epidermal cells containing blue staining droplets (Fig. 7c (asterisks)). We observed largely vacuolated cells in the midsection of the body cavity in between the various organs (double asterisks, Fig. 8c). This cell type is only visible in meiomeniid M1; it could not be detected in the other material, which might be due to contraction or fixation artifacts. The single posterior adhesive gland is distinctively present in two examined specimens (M1, M2, see Fig. 7g), and could not be observed in the other serially sectioned specimens. The gland is located posterior to the pallial cavity and opens via a single duct midventrally through the body wall. Its glandular secretion stains heterogeneously dark blue in azure II/methylene blue stained histological sections (Fig. 7g).

Nervous system and sensory structures

The central nervous system is described based on the 3D-reconstruction of individual M1 (Fig. 8) and compared with a partially reconstructed nervous system of M2.

In general, the basic pattern of the reconstructed meiomeniid nervous system (Fig. 8a) corresponds to the one found in the investigated dondersiid. The fused cerebral ganglia (in meiomeniid M1, they measure approx. 50 μm at widest point and 35 μm in length, Fig. 8d) are located dorsal to the oral tube and pharynx. The nervous system also comprises paired buccal and pedal ganglia, as well as paired lateral ganglia. In M1 and M2, we detected precerebral accessory ganglia, as found in the dondersiids, with homogenously distributed nuclei and neuropil (Fig. 8b). In contrast to the dondersiid nervous system, an additional set of clearly differentiated paired ganglia–the basal ganglia–is present in the meiomeniids. The basal ganglia (approx. 8 μm diameter; 19 μm length) are located ventroposterior to the accessory ganglia in proximity to the cerebral ganglia (Fig. 8d). From the posterior part of each cerebral ganglion, the cerebropedal connective arises. The pedal ganglia (in meiomeniid M1 approx. 16 μm in diameter and 27 μm length) are positioned ventrolateral to the pharynx (Fig. 8c) and extend into the ventral nerve cords to both sides of the pedal groove (Fig. 8d). Pedal commissures could not be detected. The paired buccal ganglia (in meiomeniid M1 approx. 14 × 16 μm) are positioned at the posterior end of the cerebral ganglia, dorsolateral to the pharynx (Fig. 8c, d). The lateral nerve cords exit the cerebral ganglia midlaterally through the lateral ganglia, which are not fully separated from the cerebral ganglia (Fig. 8d), and extend to the posterior end of the animal along the dorsolateral parts of the body cavity. A suprarectal loop connecting the two lateral nerve cords was clearly visible in meiomeniid M1 (Fig. 7a, f). At the same level they merge with the ventral nerve cords (Fig. 7a) and posterior to the suprarectal loop the lateral nerve cords bend ventrally (Fig. 7a). As in the reconstructed individual D1, no posterior ganglia are present in the meiomeniid nervous system. A pedal commissural sac is enclosed between pharynx and pedal ganglia, and contains approximately five central cells (Fig. 8c (asterisks)). Some small periatrial cirri are present at the border of the atrium, but these are less prominent than in the dondersiid. A dorsoterminal sense organ could not be detected in the studied Meiomeniidae.

Digestive system

In contrast to the Dondersiidae described above, the digestive systems of the two meiomeniids (M1 and M2) studied in histological detail, lack pharyngeal glands and contain only comparably simple ventrolateral foregut glands (Fig. 9a). The mouth opening is located posterior to the atrium, and followed by a short and ciliated oral tube. The muscular pharynx is lined by a ciliated epithelium and contains the radula (Fig. 9b, c). In contrast to the monoserial radula of the dondersiids, the meiomeniid radula is distichous with at least nine rows of paired teeth. Each radula tooth is slightly curved, with a single large lateral denticle and three smaller median denticles (Fig. 9c').

In meiomeniids, the ventrolateral foregut glands only consist of one histologically differentiated multicellular structure. These foregut glands of M2 are positioned on both sides of the pharynx and extend in dorsoposterior position for 50 μm (Fig. 9a) before opening at the level of the radula. In M1 and M2, the glandular cells of these foregut glands are filled with globular, purple to dark-blue staining vesicles, yet no distinctive cell borders are recognizable within the glands and they are not surrounded by a muscular layer (Fig. 9c). The straight midgut forms at its dorsoanterior end either a

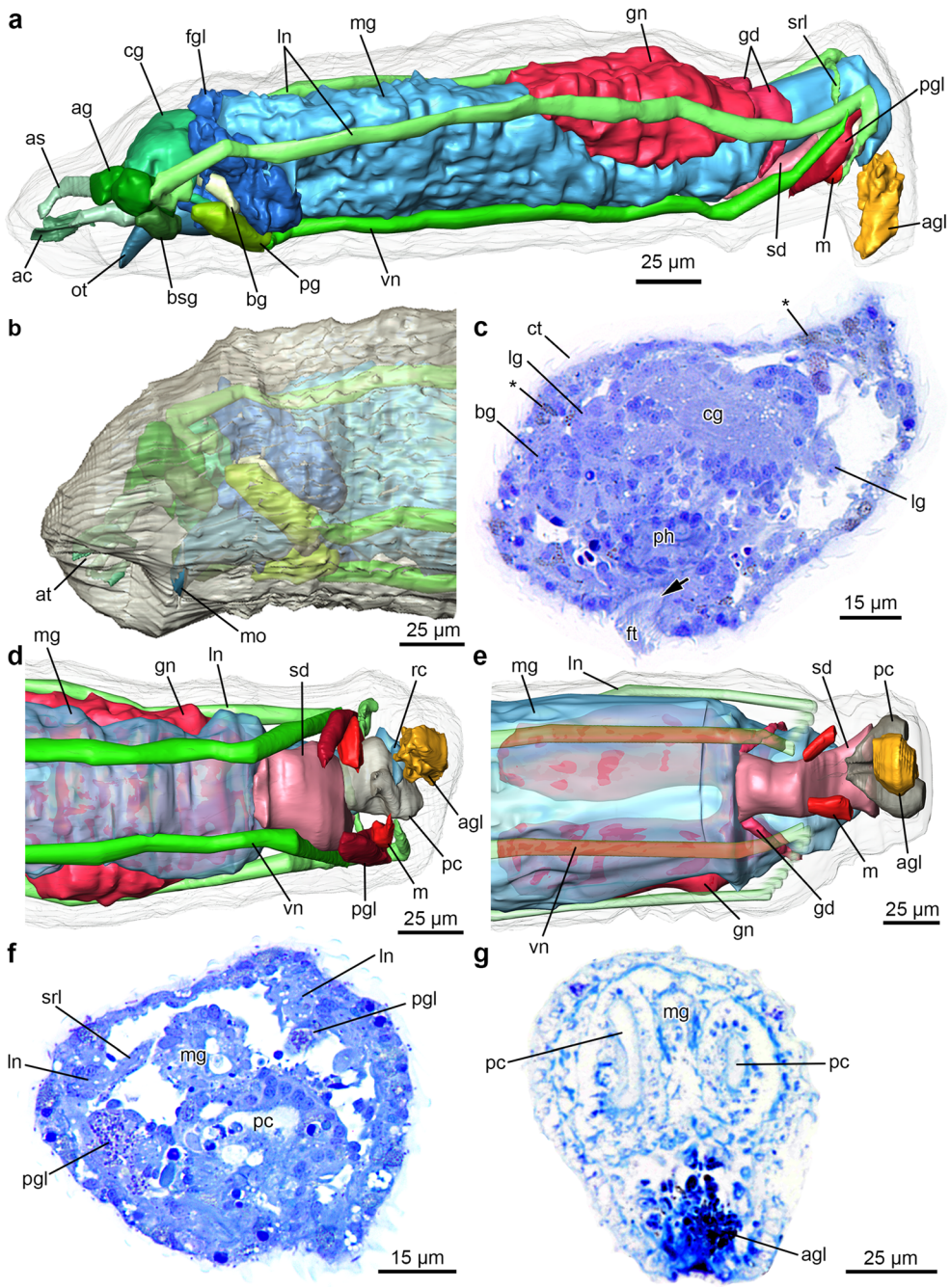

Fig. 7 Microanatomy and histology of two mesopsammic Meiomeniidae (M1, M2). **a**, **b**, **d**, **e** 3D-reconstructions. **c**, **f**, **g** Histological cross-sections. **a** Overview of M1, showing all reconstructed organ systems, body surface transparent. Green nervous system, red reproductive system, blue digestive system. Lateral view. **b** Head region of M1 with separated atrium and mouth opening. **c** Cross-section at the level of the fused cerebral ganglia and the pedal pit (black arrow) of M1. Asterisks indicate epidermal glandular cells. **d** Posterior part of M1 in ventral view showing single pallial cavity. **e** Posterior part of M2, ventral view, showing bilobed pallial cavity (the connection between ventral and lateral nerve cords could not be fully reconstructed due to missing sections, and is therefore only dotted). **f** Cross-section through the posterior part of M1 showing the suprarectal loop and single pallial cavity. **g** Cross-section through M2, at the level of the posterior adhesive gland showing the bilobed pallial cavity. Abbreviations: ac atrial cirri, ag accessory ganglion, agl adhesive gland, as atrial sensory nerve, at atrium, bg buccal ganglion, bsg basal ganglion, cg cerebral ganglia, ct cuticle, fgl foregut gland, ft foot, gd gonoduct, gn gonad, lg lateral ganglion, ln lateral nerve cord, m muscle strands probably associated with the copulatory spicules, mg midgut, mo mouth opening, ot oral tube, pc pallial cavity, pg pedal ganglion, pgl prostatic gland, ph pharynx, rc rectum, sd spawning duct, srl suprarectal loop, vn ventral nerve cord

paired (meiomeniid M2, Fig. 9b) or a single (meiomeniid M1, Fig. 9c) caecum, which extends anteriorly. The midgut tube fills the majority of the body cavity, and as in the dondersiid, its tissue is comprised of large columnar digestive cells. These digestive cells are filled with numerous vesicles (Fig. 10f) and constrict the lumen of the gut to a small, slit-like space. They

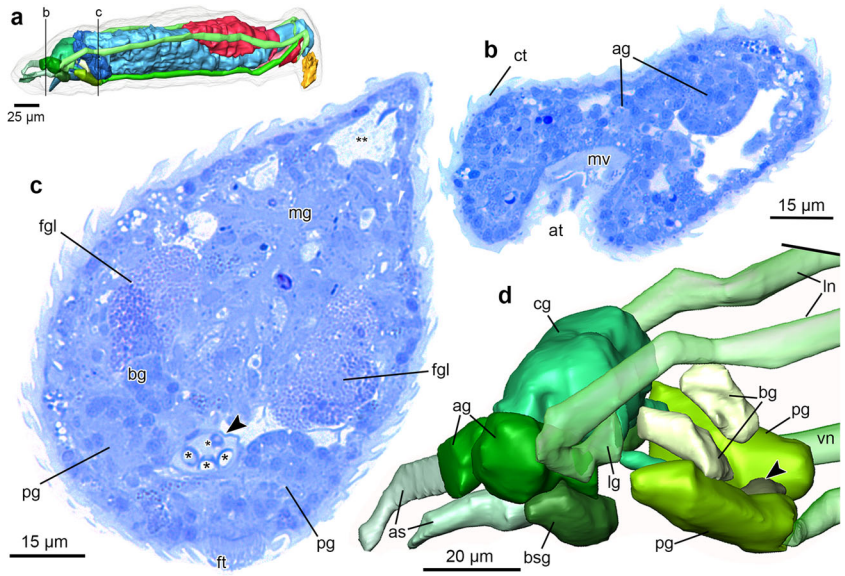

Fig. 8 3D-reconstruction and histology of the nervous system of Meiomeniidae based on M1. **a, d** 3D-reconstructions. **b, c** Histological cross-sections. **a** Overview of M1, lateral view. Lines indicating levels of the respective cross-sections. **b** Atrium and accessory ganglia. **c** Pedal ganglia and pedal commissural sac (black arrowhead) containing central cells (asterisks). Double asterisks mark largely vacuolated cells in the dorsal body cavity. **d** Anterior central nervous system of M1, lateral view. Black arrowhead points to pedal commissural sac. Abbreviations: ag accessory ganglion, as atrial sensory nerve, at atrium, bg buccal ganglion, bsg basal ganglion, cg cerebral ganglia, ct cuticle, fgl foregut gland, ft foot, lg lateral ganglion, ln lateral nerve cord, mg midgut, mv microvillous border, pg pedal ganglion, vn ventral nerve cord

contain engulfed but still undigested cnidocysts (Fig. 10f (asterisks)), indicating that the compared meoimeniid and dondersiid lineages are both cnidarivores. The posterior part of the midgut of meiomeniid M1, lined by isoprismatic cells, loops laterally around the single pallial cavity and the rectum opens into the pallial cavity on the ventral side (Fig. 7d). In meiomeniid M2, the posterior part of the midgut proceeds medially between the paired pouches of the pallial cavity (Fig. 7e) and significantly reduces its diameter, yet the rectum could not be differentiated histologically.

Reproductive system and excretory structures

In meiomeniid M1 and M2, the gonads are paired tubular organs, located dorsal of the midgut in the posterior third of the body (Fig. 10a, b, e). Blindly emerging as two separate structures, they fuse towards the posterior end of the animal and lead via paired, ciliated gonoducts into an unpaired spawning duct. The spawning duct leads into the anterior part of the pallial cavity (Fig. 10b). This duct is comprised of ciliated, glandular epithelial cells. The gonads of meiomeniid M1 contain four large oocytes in different stages of vitellogenesis with sizes varying between 16 and 25 µm (Fig. 10c). In meiomeniid M2 the gonads contain packages of sperms at the dorsal and lateral gonadal walls, and no oocytes are present (Fig. 10f). Next to the pallial cavity, muscle strands and large putative prostatic glands, presumably associated with copulatory spicules, are present in lateroventral position (Figs. 7d–f and 10d). Neither a pericardium, nor a heart—in Solenogastres typically connected with the gonads in a gonopericardial system—could be detected histologically in any of the studied specimens. The cavity enclosed by the paired gonads in M1 and M2 is interpreted as part of the body cavity rather than a pericardium, due to its continuous dimensions extending over large parts of the body and the absence of a delimiting pericardial epithelium (see "Discussion").

Discussion

Taxonomic challenge

Dondersiidae

All material that we extracted from sand samples externally showed the scleritome typical for Meiomeniidae, i.e., with at least three different types of scales including lanceolate ones projecting laterally (see Fig. 1a). Meiomeniidae is also characterized, however, by a distichous radula and a comparably simple arrangement of foregut glands ("*Meioherpia*-type" in Handl and Todt 2005), which is clearly opposed by the monostichous (=monoserial) radula and the complex foregut glands (three types of pharyngeal glands and an unusual ventrolateral foregut gland complex with conspicuous pouches) detected in two of the individuals. Monoserial radulae are characteristic for several families of Solenogastres (e.g., Dondersiidae, Macellomeniidae, and Acanthomeniidae) and the majority of the ten amphimeniid genera (García-Álvarez

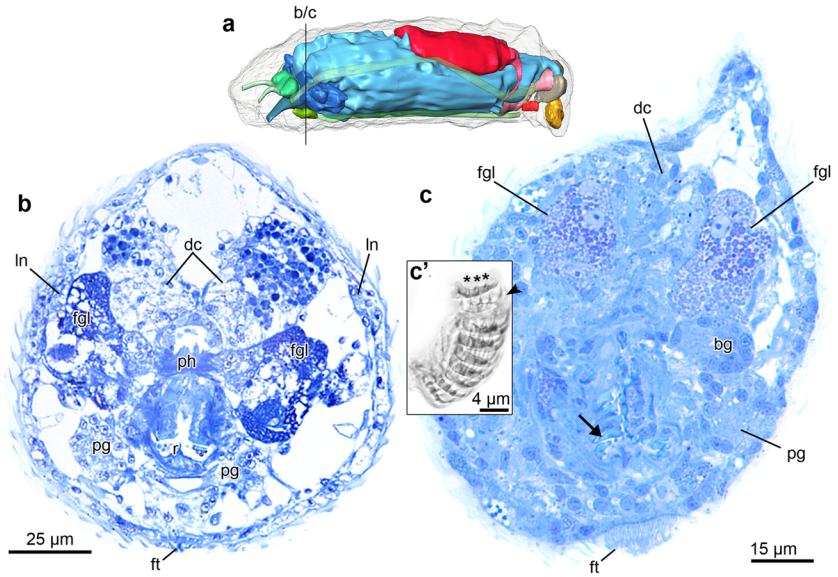

Fig. 9 Histology of the digestive system of meiomeniids, based on M1 and M2. **a** 3D-reconstruction of M2, lines indicating the relative levels of histological sections (for orientation only; c taken from M1). **b** Cross-section of M2 through pharynx with openings of the foregut glands and of the paired dorsal caeca of midgut. **c** Pharynx and single dorsal caecum of M1. Arrow points to single radula tooth of the distichous radula. **c'** Light microscopic image of one column of radula teeth from meiomeniid M3. Note large lateral denticle (black arrowhead) and three smaller median denticles (asterisks). Abbreviations: bg buccal ganglion, dc dorsal caecum of midgut, fgl ventrolateral foregut glands, ft foot, ln lateral nerve cord, pg pedal ganglia, ph pharynx, r radula

and Salvini-Plawen 2007). But in combination with the scaly scleritome lacking hollow needle-like sclerites, the cryptic lineage encountered in Bermuda sands currently can be best assigned to Dondersiidae. Monophyly of Dondersiidae is poorly supported, and the taxon currently serves as a "catch basin" for several lineages with similar radula and scleritome morphology (Scheltema et al. 2012). The generic classification of our dondersiid-like specimens from Bermuda remains unclear as putative small denticles of the radula cannot be reliably reconstructed based on histological section series and no material remains for SEM studies of the radula. The complex system of different types of pharyngeal glands and unique ventrolateral foregut glands with conspicuous pouches are unique for any of the described genera of Dondersiidae, and a similar setting is so far only known from a recently described species from the Azores provisionally classified within *Dondersia* (Klink et al. 2015). Neither the family nor the generic classification of the species from the Azores and its putative close relative encountered in the course of the present study in Bermuda are without conflict to established character sets characterizing these taxonomic entities (e.g., presence of a pedal comissural sac, unique ventrolateral forgut glands with pouches). We refrain from establishing novel categories, until gaps in the data matrix of the mysterious novel lineages can be filled and until Dondersiidae are comparatively reinvestigated also with the support of molecular markers. Therefore, we further abstain from formally describing the discovered novel lineage herein, as (1) detailed ultrastructural data on the scleritome cannot be reliably assigned between the co-occurring lineages, (2) the two single individuals are juveniles lacking a description of the gonopericardial system and above all, and (3) no material remains for molecular analyses. A formal taxonomic description is postponed until more material is available to fill these gaps.

Meiomeniidae

The two meiomeniid genera *Meioherpia* and *Meiomenia* were erected by Salvini-Plawen (1985a) based on the presence/absence of a dorsoterminal sense organ (DTSO) and absence/ presence of copulatory spicules. Both of these morphological characters are taxonomically highly problematic (Morse and Norenburg 1992): a DTSO is not mentioned in the original description of *M. swedmarki* which does not mean, however, that it is truly absent. Moreover, reinvestigation of *M. arenicola* from Florida revealed the presence of a DTSO (Morse and Norenburg 1992). Reinvestigation of the type material of *M. swedmarki* and/or additional material re-collected at the type locality ("topotypes") is required; *Meiomenia* might possess a DTSO, which can easily be overlooked with light microscopy or even SEM when mounted in a non-suitable angle. Therefore the use of this character to distinguish both genera is dubious at present. The presence vs. absence of copulatory spicules is also problematic. Morse (1994) reports that these spicules are not always present in adult specimens and might only be formed shortly before or lost during copulation in the male phase of the hermaphrodites, and thus cannot serve as a reliable

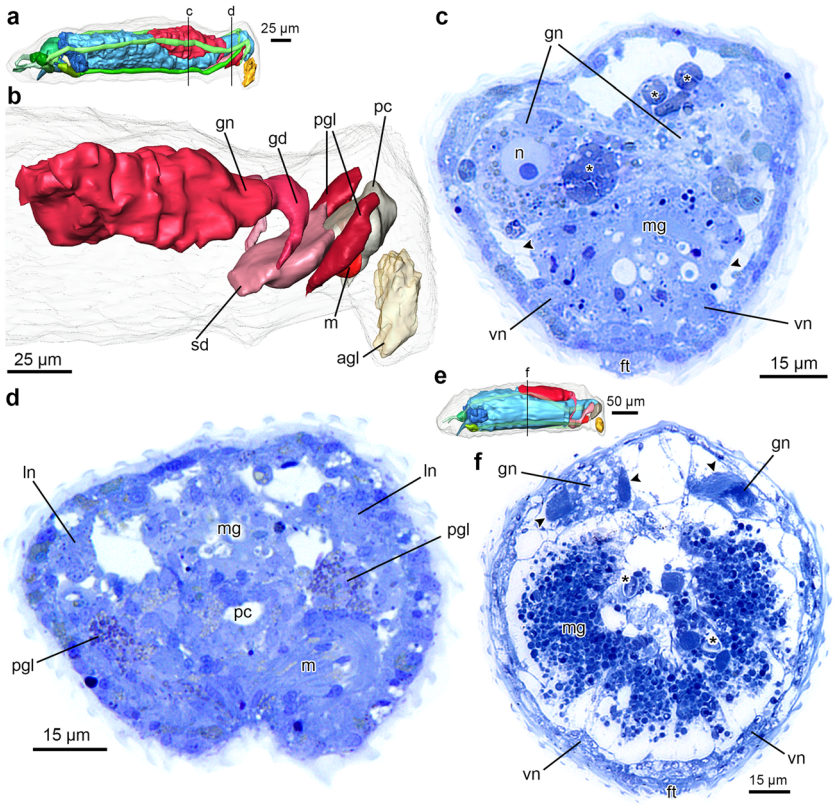

Fig. 10 3D-reconstruction (**a, b, e**) and histology (**c, d, f**) of the meiomeniid reproductive system, based on M1 and M2. **a** Overview of M1, laterodorsal view. Lines indicate levels of respective histological cross-sections (**b, c**). **b** Reconstructed reproductive system of M1, body surface transparent. **c** Cross-section through the gonads of M1. Asterisks mark yolky substance. **d** Cross-section through posterior end of M1 showing prostatic glands and muscles probably associated with the copulatory spicules, which are truly absent or have been dissolved during preparation. **e** Overview of 3D-reconstructed M2, laterodorsal view, indicating plane of section **f** Section through the midgut and male gonads of M2. Arrowheads point to sperm packages; asterisks indicate intracellular cnidocysts in the midgut. Abbreviations: agl adhesive gland, ft foot, gd gonoduct, gn gonad, ln lateral nerve cord, m muscle strands likely associated with the copulatory spicules, mg midgut, n nucleus with dark nucleolus of yolky oocyte, pc pallial cavity, pgl prostatic gland, sd fused spawning duct, vn ventral nerve cord

diagnostic character. Initially, both genera were described with a common atriobuccal opening by Salvini-Plawen (1985a), but later the separation of atrium and oral opening in *Meioherpia* was added as a diagnostic feature by García-Álvarez and Salvini-Plawen (2007), based on reinvestigations of *M. atlantica* (Todt 2006). Our own reinvestigation of the meiomeniid type species *M. swedmarki*, based on a series of semithin histological sections of material re-collected at the type locality by Dr. K. M. Kocot, confirmed a single anterior opening from which both atrium and pharynx diverge. Therefore, at present this is the only reliable character for discrimination of the two genera within Meiomeniidae. Our study points at further characters which might be useful in delineation of genera or species (i.e., presence/absence of a pericardium, simultaneous/sequential development of sperm and oocytes), but further comparative data is needed.

At species level, only *M. swedmarki* from the Pacific Northwest Coast of the USA is clearly delineated by geographic distribution and morphological characters (Morse 1979). All subsequently described meiomeniid species, however, which are at least partially co-occurring in the temperate Western Atlantic, are taxonomically problematic: *M. arenicola*, *M. stygalis*, and *M. atlantica* were all originally described based on scleritome and radula characteristics investigated via light microscopy based on only one or two specimens, lacking information on potential intraspecific variation (Salvini-Plawen 1985a). *M. arenicola* was originally described from North Carolina, USA (Salvini-Plawen 1985a) and later redescribed based on material collected from Florida, USA, adding data on live observations, and details of the scleritome via SEM and histological investigations of one serially sectioned specimen (Morse and Norenburg 1992). But since identification based on radula and scleritome characters is problematic (see present study and "Discussion"), the conspecificity of the meiomeniid from Florida and the original *M. arenicola* needs to be tested via molecular markers (DNA barocding) and morphological investigations from specimens collected at the type locality.

Based on the original description, there is only slight variation in the density of laterally projecting mantle sclerites and

in the shape of some body scales between the two *Meioherpia* species and the distinguishing occurrence of abdominal spicules in *M. atlantica*, which "could not be ascertained" in *M. stygalis* (Salvini-Plawen 1985a). Meiomeniid radulae show little variation useful for taxonomic purposes: both genera of Meiomeniidae are characterized by distichous radulae and only differ in the number of denticles (four to six in *Meiomenia*, three median denticles plus one lateral one in *Meioherpia*) and the number of rows (García-Álvarez et al. 2000). But the number of rows can vary during ontogeny with a possible intra-individual variation in the number of denticles (Scheltema et al. 2003), and therefore, this character is considered unreliable for species delineation.

The Meiomeniidae investigated herein all belong to the genus *Meioherpia*, based on the distinct separation of atrium and buccal opening. Unfortunately at this point, based on the absence of clearly distinguishing features and uncertainty on intraspecific variability of scleritome characters, the specimens of *Meioherpia* investigated in our study cannot be allocated to one of the existing species. A varying degree in the amount and visibility of abdominal spicules within a population might indicate that only one species of *Meioherpia* is valid. This is contradicted, however, by microanatomical variation in the shape of the pallial cavity and anterodorsal midgut caecum as well as the number of central cells within the pedal commissural sac investigated herein (albeit the intraspecific variation of these characters also still needs to be tested). Molecular barcodes (own unpublished data) of Bermudan Meiomeniidae also support the presence of at least two different co-occurring meiomeniid lineages. A reinvestigation of the type material is urgently needed to assign these anatomical differences to described species, but unfortunately the type material could not be found in the museum's collection and might be lost (late Prof. Salvini-Plawen, personal communication). Hopefully, the currently unavailable holotypes can be retrieved to reinvestigate the material for further characters. But even in case the types reappear in the future, it remains unclear whether we will be able to retrieve any microanatomical information or molecular data from these whole-mounts. A designation of neotypes (or lectotypes, if paratypes exist), which present unambiguous, distinguishing character states might be needed in the future to reliable solve the taxonomic dilemma and are necessary to retrieve the Meiomeniidae from this Bermudan triangle of taxonomy.

Cryptic lineages co-occurring

Our study provides another example that Solenogastres present a taxonomically especially challenging group, with a prejudice towards solenogaster taxonomy as "obscure" and "difficult" (see Todt 2013). Traditionally, taxonomic classification of Solenogastres heavily relies on microanatomical data, which requires histological sectioning (see, e.g., Nierstrasz 1902; Heath 1911, 1918; Salvini-Plawen 1978) and impedes identification in the field (Scheltema and Schander 2000). The presence of co-occurring, externally cryptic lineages—as detected in the present study—demonstrates the limitations of approaches to discriminate solenogaster species only through external morphology and scleritome characters (Scheltema and Schander 2000). Prior to histological investigations, large enough specimens can be carefully squashed between microscopic slides and cover glass to try and discern the radula type. In larger specimens one single individual can be divided for histological sectioning of the anterior and posterior end and scleritome analyses from the midbody, which in general bears few microanatomical characters. This combination nevertheless also presents a trade-off between the complete coverage of scleritome characters (e.g., surrounding atrium and mouth opening and the dorsoterminal sense organ) and quality of the obtained information (i.e., radula morphology only derived from histological section series). In meiofaunal lineages such a trisection of a single individual to obtain various character sets is often unfeasible. The aim of the present study is to raise awareness within the taxonomic community that externally highly cryptic species are co-occurring and that conspecifity of specimens collected in the same lot cannot be assured via external morphology—risking the creation of chimera via combining characters derived from multiple—non-conspecific—individuals. The frequency of cryptic lineages is currently still unknown, but with large parts of the ocean floor still unexplored, current species diversity of Solenogastres is likely underestimated by magnitudes (Scheltema and Schander 2000; Glaubrecht et al. 2005; Todt 2013) with a special deficit in meiofaunal forms. Therefore, efficient approaches that are able to reliably uncover cryptic lineages are needed (i.e., via molecular barcoding) and workflows have to be developed, which allow for a combination of molecular and high-end morphological approaches on single, minute individuals (see Bergmeier et al. 2015)

Comparative microanatomy of mesopsammic Pholidoskepia from Bermuda

Nervous system

The nervous system of Solenogastres shows a conserved general bauplan across the different major clades and is mainly characterized by large, fused cerebral ganglia, paired lateral ganglia that give rise to the lateral nerve cords, paired ventral (=pedal ganglia), from which the ventral nerve cords originate, and a buccal ring with paired buccal ganglia. At the posterior end, the lateral nerve cords of the tetraneural nervous system fuse in the suprarectal loop (see, e.g., Heath 1904; Salvini-Plawen 1967a, 1978; Scheltema et al. 1994).

The nervous systems of the two pholidoskepian lineages described herein confirm the typical bauplan of the tetraneural

nervous system of Solenogastres but differ in the number of ganglia with a pair of basal ganglia anterioventral to of the cerebral ganglia only present in the investigated Meiomeniidae. Basal ganglia are also reported, e.g., for the related pholidoskepian *Wirenia* (Todt et al. 2008b) where they are involved in the innervation of the "frontal ganglia." The presence vs. absence of basal ganglia among Pholidoskepia might be a useful character for phylogenetic reconstruction, but the lack of basal ganglia in the undetermined Dondersiidae herein and potentially related *Dondersia* (?) *todtae* Klink et al. 2015 still requires reinvestigation once adult specimens have been discovered. A mass of yet not fully differentiated putative nervous tissue is found in both of these dondersiid species anterior to the cerebral ganglia (Klink et al. 2015; present study), and it cannot be excluded that the basal ganglia form late during development. Rather unreliable for species delineation or phylogenetic purposes is also the varying degree of separation of lateral ganglia from the cerebral ganglia, observed between Meiomeniidae and Dondersiidae *incertae sedis*. In the later species, it was found to differ even within individuals between the left and right side of the body, suggesting high intraspecific variability and variation across different stages of development. Posterior ganglia, found in terminal position at the lateral nerve cords of *Wirenia argentea* Odhner, 1921, are lacking in the meiomeniid and the dondersiid nervous systems investigated herein. In *W. argentea*, these paired ganglia innervate the dorsoterminal sense organ, a sensory structure which could not be detected in the studied material (see "Discussion"). The absence of the posterior ganglia might be due to a true lack of a dorsoterminal sense organ, reduced because of different sensory requirements in the case of Meiomeniidae. Alternatively, it might develop later in ontogeny in our juvenile Dondersiidae *incertae sedis* if, for example, related to reproduction. Thus, further comparative microanatomical data on adult pholidoskepian nervous systems is needed to fully evaluate the potential of characters of the nervous system for phylogenetic reconstruction in Solenogastres.

Unfortunately, due to the minute sizes and contracted stages of many specimens, even our histology-based 3D reconstructions were limited in resolution and we were unable to reliably compare the innervation pattern and the number of cerebral nerves between the different pholidoskepian lineages. Recent studies have added valuable data on the development of the solenogaster nervous system via immunocytochemical staining against different neurotransmitters (Redl et al. 2015) and revealed previously unrecognized bundles of neurites, such as an unpaired one associated with the buccal ganglia—potentially corresponding to the buccal nerve detected in D1—running dorsally along the midgut (Faller et al. 2012). Both studies therein document the value of this technique for the detection of nerves in highly miniaturized nervous systems, since compressed stages of tissue hamper histological detection. Especially in minute mesopsammic Solenogastres, histological approaches should ideally be supported by immunocytochemical and ultrastructural analyses to allow for reliable assessment of delicate structures of the nervous system.

In *W. argentea*, large parts of the body cavity anterior to the cerebral ganglia are filled with supposedly nervous tissue, termed frontal ganglia (Todt et al. 2008b), like in all of our pholidoskepian material. These nervous structures have been mentioned in various solenogaster species as "precerebral ganglia" (see, e.g., Salvini-Plawen 1978; Scheltema et al. 1994) and are commonly associated with the cerebral nerves and the atrial sense organ. In our meiomeniid and dondersiid specimens, these more or less spherical structures clearly differ histologically from the main ganglia discussed above by lacking a distinct separation into neuropil and a surrounding layer of perikarya; the cell nuclei are homogeneously distributed (see Fig. 8b for histology). In *W. argentea,* these frontal ganglia do not react to any of the immunohistochemical stainings applied by Todt et al. (2008b) but were found to be innervated by the cerebral ganglia and to be formed mainly of the cell bodies of ciliary receptor cells located within the atrium. In the investigated Meiomeniidae cerebral nerves lead into and partially through these structures and they were thus, in concordance with previous studies on Solenogastres (Salvini-Plawen 1978; Todt et al. 2008b) identified as part of the nervous system. Nevertheless, the term frontal or precerebral ganglia should be treated with caution due to the evident discrepancies with the definition of ganglia. In position, histology and innervations these spherical structures closely resemble so-called accessory ganglia described from different groups of mesopsammic gastropods (Neusser et al. 2006; Jörger et al. 2008; Brenzinger et al. 2013a; Brenzinger et al. 2013b). Among gastropods, accessory ganglia have been hypothesized as a solution to a lack of space for neuronal processes in the cerebral ganglia due to the miniaturization of the slugs (Haszprunar and Huber 1990). However, this hypothesis is contradicted by their unique occurrence among mesopsammic lineages and absence in equally minute benthic gastropods (Haszprunar and Huber 1990; Jörger et al. 2008). Accessory ganglia probably represent an adaptation to process the stimuli of the three-dimensional interstitial habitat (Brenzinger et al. 2013a). In Solenogastres, accessory ganglia associated to the cerebral nerves seem to be common in many minute lineages regardless of the habitat (i.e., epibenthic vs. mesopsammic—see Todt et al. 2008a and present study) but have not been reported from the few "giant" Solenogastres (e.g., Scheltema and Jebb 1994; Salvini-Plawen and Paar-Gausch 2004). Thus, at present stage of data, they might rather be related to size constraints than presenting an adaptation to a specific habitat, i.e., accessory ganglia might present nervous tissue (putatively with neurosecretory function) "outsourced" from the cerebral ganglia due to the functional restraint on minimum brain size (Niven and Farris 2012). A brief

comparison of the ratio of cerebral ganglia to body length of the reconstructed meiomeniid M1 to the large-sized cavibelonian *Epimenia babai* Salvini-Plawen, 1997 (without using any volumetric calculations) shows that the length of the cerebral ganglia of M1 takes up almost a tenth of the entire body length, whereas this ratio is decreased in *E. babai* by more than the power of ten, when using measurements provided for the holotype (see Salvini-Plawen 1997), therein demonstrating the applicability of Haller's Rule to Solenogastres. This rule states that the ratio of the cerebral ganglia of small animals is larger in relation to their body size than in bigger animals (Rensch 1948). This indicates that there is a restrictive lower size limit (respectively a minimum number of neurons) to keep the cerebral ganglia functional.

Sensory structures

Sensory structures in Solenogastres are in general poorly understood. Even though the atrial sense organ, pedal commissural sac, and dorsoterminal sense organ have been studied on ultrastructural level (Haszprunar 1986, 1987b; Scheltema et al. 1994), their function often remains speculative. In the investigated pholidoskepian specimens, we found two structures presumably serving sensory function: the atrium (see Figs. 2b, d, 7b, and 8b) and the pedal commissural sac (Figs. 3(c) and 8c). But we were unable to detect a dorsoterminal sense organ—a prominent sensory feature described in many other solenogaster species and used for taxonomic purposes (Haszprunar 1986, 1987b; García-Álvarez and Salvini-Plawen 2007). This conspicuous "knob" (when protruded (Haszprunar 1987b)), otherwise more like a pit (Scheltema et al. 1994) occurs as a single (fused), paired or multiplied structure dorsal to the pallial cavity, but is obviously truly absent in certain taxa (Salvini-Plawen 1978). If present, it is innervated by the posterior ganglia of the lateral nerve cord (Todt et al. 2008b), and accordingly, Haszprunar (1987b) has interpreted it as an osphradial sense organ being homologous to organs at the same position in Caudofoveata, Polyplacophora and conchiferan Mollusca, although this view has been questioned recently (Lindberg and Sigwart 2015). It is usually visible externally by surrounding scales differing in size and orientation from the scleritome covering the remaining body region (see e.g., Morse and Norenburg 1992). Dondersiidae present different character states with a dorsoterminal sense organ present as a single organ in some genera (e.g., *Micromenia* or *Dondersia*), present in multiplied form (e.g., 11 DTSO in *Lyratoherpia*) and absence in others (e.g., *Heathia* or *Ichthyomenia*) (see García-Álvarez and Salvini-Plawen 2007; Scheltema et al. 2012). Based on developmental data on Solenogastres, the dorsoterminal sense organ forms comparably late in larval development (Heath 1914). Depending on its function, a speculative sensory role during reproduction such as in chitons or bivalves (Haszprunar 1987a), its development could be further delayed and might therefore not yet be developed in our juvenile dondersiid-like specimens, i.e., comparative examination of juveniles and adults is needed for clarification. In previous studies on Meiomeniidae, a dorsoterminal sense organ has been reported in members of both genera, *Meioherpia* and *Meiomenia* (see Morse and Norenburg 1992; García-Álvarez and Salvini-Plawen 2007), but it is not mentioned in the original description of *M. swedmarki* (see Morse 1979). Morse and Norenburg (1992) critically discuss the difficulties of a present or respectively absent dorsoterminal sense organ as a diagnostic feature, as its visibility might, e.g., depend on the stage of contraction of the animal and it can therefore be easily overlooked. The inadequate position of our material investigated via SEM (see Fig. 1a) does not allow for any interpretation of absence of this sensory organ in this specimen. Thin nerves originating from the suprarectal loop can be easily overlooked on histological sections, given the tiny size of the animals and partially missing sections in our investigated material, but it is surprising that no ganglion-like swellings (i.e., posterior ganglia) could be detected on any of the histological section series. This might support a true absence of the dorsoterminal sense organ in our material.

In the present dondersiid-like specimen D1, the atrium (also termed vestibule (e.g., Scheltema et al. 1994), vestibulum (e.g., Todt et al. 2008b), or atrial sense organ (e.g., Haszprunar 1986) is anteriolaterally surrounded by numerous stiff stereocirri (Fig. 2b, d), which consist of bundles of modified cilia (see Haszprunar 1986 for ultrastructure on *W. argentea*, as *Aesthoherpia glandulosa* (Gymnomeniidae)). We were unable to detect the innervations of the cirri because of the high density of accessory ganglia beneath the cirri (Fig. 2d). Based on the similarity in structure and position to the periatriobuccal cirri in *W. argentea*, we interpret these structures to have a sensory function, presumably of chemical or mechanical nature as suggested by Haszprunar (1986). The atrium itself is lined with microvilli and occasionally bears cilia, but does not form papillae as found in *W. argentea* (Haszprunar 1986). Observations on living meiomeniids showed that the atrium can be protruded when crawling (Morse 1979; personal observation) with possible chemosensorical probing of the environment (Morse 1979).

In both meiomeniid and dondersiid-like specimens investigated, we found a conspicuous pedal commissural sac (pcs) being enclosed between the paired pedal ganglia, ventral pedal commissure and the pharynx (Figs. 3a, c and 8c). This organ has been already mentioned for *M. atlantica* by Todt (2006, see Fig. 4a) and is herein studied in detail for the first time for Meiomeniidae (and a first ultrastructural account for Dondersiidae is provided). Haszprunar (1986) investigated the ultrastructure of the pcs in the pholidoskepian *W. argentea* (the same photos have been reprinted by Scheltema et al.

(1994) in better quality). Whereas the relative position and basic bauplan of the pcs in *W. argentea* corresponds to the one in our studied pholidoskepian specimens, there is some variation on ultrastructural level (see Fig 3c on D2). In *W. argentea*, the sac lumen contains largely vacuolated cells with a (decalcified) crystallineous body inside the vacuole. The sac lumen is delimited by an inner central membrane, surrounded by "loose surrounding tissue" and an outer membrane (Haszprunar 1986, Fig. 19; Scheltema et al. 1994, Fig. 16e). This loose surrounding tissue is also present in the description of the pedal commissural sac of *Genitoconia rosea* Salvini-Plawen, 1967 (Gymnomeniidae) (labeled as "Ventral Sinus"see Salvini-Plawen 1967a, Fig. 12a, b). Haszprunar (1986) detected nerves as well as other "nervous material," distinguishable by its accumulation into dense areas with small vesicles, forming seemingly empty vacuoles (see Haszprunar 1986, Fig. 19) within the loose surrounding tissue. The pcs of the dondersiid-like specimen D2 again shows a lumen with vacuolated cells inside, however, here we lack a layer of loose surrounding tissue and we were only able to detect a single membrane delimiting the pcs (Fig. 3c). The absence of this conspicuous tissue might also be a further result of the spatial limitations due to the minute body size of the juvenile dondersiid. Within our material, the number of central cells contained within the pedal commissural sacs varied between two and nine cells, but more comparable data is needed to evaluate the ontogenetic and interspecific variability of this character.

A conspicuous pedal commissural sac has so far been reported for representatives of the pholidoskepian Gymnomeniidae Odhner, 1921, that is for *W. argentea* (Haszprunar 1986), *G. rosea*, and *G. atriolonga* Salvini-Plawen, 1967 (Salvini-Plawen 1967a) and an undescribed *Gymnomenia* sp. in Scheltema (1981). Recently, it was also encountered for the first time in a mesopsammic representative of Dondersiidae with similar characteristics to our dondersiid-like specimens (Klink et al. 2015). Uniquely outside of Pholidoskepia, Scheltema et al. (1994) and Scheltema and Schander (2000) also described a pedal commissural sac for *Scheltemaia mimus* (Scheltema and Schander, 2000)—a pruvotiniid with uncertain phylogenetic affinities among the Cavibelonia. However, the monophyly of this traditional order of Solenogastres was already doubted by Scheltema and Schander (2000), a view being supported by first cladistic analyses (Salvini-Plawen 2003a) and by a first phylogenomic study (Kocot et al. 2013). The systematic position of *Scheltemaia* remains uncertain, but *S. mimus* has a very spiny scleritome with hollow, barbed upright spicules (Scheltema and Schander 2000) and foregut glands with intraepithelial glandular cells (*Epimenia*-type according to Handl and Todt (2005) and Type C following Salvini-Plawen's (2003b) original classification)—hence differing considerably from the other pcs-bearing pholidoskepian Solenogastres by external morphology and anatomical characters, therein objecting a possible sister-group relationship between *Scheltemaia* and Pholidoskepia. Based on the present data, the pedal commissural sac may be interpreted in various ways: (1) as a plesiomorphic character which already evolved in the last common ancestor of Pholidoskepia and *Scheltemaia* or potentially already in the solenogaster stem line. The pcs was subsequently reduced in many lineages or simply overlooked in many original descriptions, which frequently lack detailed histological investigations. (2) The pcs in both lineages is not homologous and presents a character which evolved in parallel. (3) *Scheltemaia* has been misplaced and rather represents a largely modified pholidoskepian Gymnomeniidae, although this is considered highly unlikely. A comparative reinvestigation across representatives of all major solenogaster lineages is needed to confirm the absence of a pedal commissural sac from other lineages and allow for further interpretation whether its absence/presence might be linked to a certain lifestyle. Moreover, microanatomical reinvestigation of the enigmatic *Scheltemaia incertae sedis* is needed in combination with ultrastructural details on its pedal commissural sac to evaluate a putative structural homology.

Due to its structural similarities to statocyst organs of other molluscs and its position between the pedal ganglia and their commissure, Haszprunar (1986) suggests that the pcs could serve as a movement receptor. The statocyst organ of other molluscs is—in its most basic form—a fluid-filled sac lined by sensory epithelial cells (Williamson 1993). So far such innervated sensory cells with cilia or microvilli have not been detected histologically or ultrastructurally in the pedal commissural sac of Solenogastres (Haszprunar 1986; Todt et al. 2008b) and could also not be detected herein, raising the question of how movement and acceleration are transmitted from the central cells over the pcs membrane.

Similar putative gravity receptor systems which lack a sensory epithelium with ciliary structures have been described for few other invertebrates, namely in the Acoelomorpha (Ferrero 1973, Ferrero and Bedini 1991; Achatz et al. 2013) and in the enigmatic *Xenoturbella* (Ehlers 1991, Israelsson 2007), where it has been argued that stimuli might be directly transmitted to an underlying "nerve cushion" (Ferrero 1973). Haszprunar (1986) found nerves in the investigated Solenogastres running from the pedal ganglia into the layer of loose tissue surrounding the pcs, but no such nerves were found in the present study. Since it is in close proximity and contact to the pedal ganglia—lacking loose surrounding tissue—in the pholidoskepian Solenogastres investigated herein, the best explanation at present stage of knowledge seems a direct excitation of the pedal ganglia by contact with the largely vacuolated central cells via the pcs surrounding membrane. Due to structural differences to other molluscan statocysts, the pedal commissural sac of Solenogastres might present a convergent development to sense gravity and movement. The complexity of

this putative equilibrium receptor system might be driven by their infaunal habitat where gravity is of major importance for orientation (Ferrero and Bedini (1991) on acoels), but more reliable data on the lifestyle of the different solenogaster lineages combined with detailed investigation on their sensory organs is needed to test for a putative correlation.

Reproductive system and excretory structures

All collected specimens of the undescribed dondersiid in the present study were immature and therefore data on their reproductive system is not available. The only known representative among Dondersiidae with large similarities to the ones analyzed herein–*Dondersia* (?) *todtae*–is unfortunately again only known from mesopsammic juveniles (Klink et al. 2015). In the studied mature meiomeniids M1 and M2, the paired and tubular gonads lead via paired gonoducts to the unpaired spawning duct, which opens into the pallial cavity (see Fig. 7d, e). All studied Solenogastres—immature and mature—entirely lacked a pericardium and therefore differ considerably from the general solenogaster bauplan, where a combined gonopericardial system is present. The only exceptions known so far are representatives of the sterrofustian Phyllomeniidae with true gonoducts, i.e., lacking a connection between gonads and pericardium (Salvini-Plawen 1970; García-Álvarez et al. 2010). Typically, the gonads are connected to the pericardial cavity via paired gonopericardioducts and paired pericardioducts then lead into the more or less fused spawning duct(s) (Scheltema et al. 1994). In this structural arrangement, the pericardium containing the heart is not only the site of ultrafiltration but can also serve as storage for ripe eggs (Todt and Wanninger (2010) on *W. argentea*). Reynolds et al. (1993) detected a small heart within a spacious pericardium, which bears podocytes in the epicardial wall, of an undescribed *Meiomenia* sp. from Fort Pierce (Florida, USA). However, the gonoducts of our studied meiomeniid specimens led directly from the gonads into the spawning duct, leaving no room for an interposed pericardium. The only comparable structure in our material refers to a cavity above the gonads (Fig. 10c), but it extends continuously over almost the entire body length, lacks a histologically detectable epithelium and is thus interpreted as part of the body cavity. However, ultrathin section series are necessary to unambiguously exclude the presence of a pericardium, as thin-walled structures can be easily overlooked, especially when they collapse during fixation. If truly absent, this character might contain taxonomic relevance as distinguishing character between the genera *Meioherpia* and *Meiomenia*, which requires specific re-examination on the other valid species. The putative genuine absence of a pericardium in our material calls for alternative sites of ultrafiltration. In Polyplacophora, the larval protonephridia are retained as a functional ultrafilter far after settlement until an early juvenile phase and the complex of metanephridial excretory system and heart/pericardium is completely formed late in development (Baeumler et al. 2011). However, *W. argentea* loses its larval protonephridia a few weeks after hatching, before the gonopericardial system develops (Todt and Wanninger 2010). Unsurprisingly, we were unable to histologically detect protonephridial structures in the immature dondersiid-like D1, but ultrastructural investigations of D2 revealed a single cell with a system of basal infoldings of the inwardly positioned parts of the basal membrane which might serve as a putative ultrafilter (Fig. 6a). Based on the location of this cell—subepidermal and in the anterior third of the body—it could be part of a retained larval protonephridial system. Since the ultrathin sections are not available as a complete series, we are unable to tell whether this cell is part of a fully functional protonephridium, a remnant of a reduced larval protonephridium, or actually an alternative ultrafiltrating cell, i.e., a rhogocyte. These cells are usually situated in the haemocoel and connective tissue, and present another cell type suspected to contribute to excretion in other molluscan taxa (Haszprunar 1996). Rhogocytes are also confirmed to exist in Caudofoveata (*Scutopus ventrolineatus* Salvini-Plawen, 1968) and Solenogastres (*Neomenia carinata* Tullberg, 1875, *Nematomenia banyulensis* (Pruvot, 1890), *Dondersia* sp. (GH personal observation)), but apart from the aforementioned single cell, we were unable to detect them via TEM in the investigated specimen. Epidermal papillae function as additional excretory organs in other groups of Solenogastres (Baba 1940), but papillae do not occur among pholidoskepian representatives (García-Álvarez and Salvini-Plawen 2007). Amoebocytes, as found between the midgut epithelium and integument during ultrastructural investigations of D2 see (Fig. 6b), have been observed in the cavibelonian *E. babai* (as *E. verrucosa* (Nierstrasz) in Baba (1940)). In *Epimenia*, the amoebocytes are reported to phagocytose particles and pass them into the midgut lumen from which they are excreted through the rectum (Baba 1940).

Based on histological sections of *M. arenicola* and *M. swedmarki*, Morse (1994) identified the male part of the gonad as positioned in front of the much larger female part. However, the gonads of our examined specimens contained either eggs in various stages of vitellogenesis (M1, see Fig. 10c) or packages of sperms formed in the dorsal and lateral gonadal walls (M2, see Fig. 10f). Male and female gametes did not co-occur, indicating sequential hermaphroditism—as has been generally proposed for Solenogastres by Salvini-Plawen (1978) in the form of protandry. Live observations on large-sized Soleongastres (in *Epimenia australis*) report pseudo-copulation during which specimens press the openings of the pallial cavities together, fastened by hook-like spicules at the margins of the pallial cavities (Scheltema and Jebb 1994). Both *Meiomenia* species bear a pair of protruding sclerites at their posterior end which are either "spoon

shaped" (in *M. swedmarki*) or "evenly taper to a point" (*M. arenicola*) (Morse 1994). Due to their association with "prostatic glands" and muscle tissue, they are referred to as "copulatory spicules" (Morse and Norenburg 1992; Morse 1994) and might serve as a fastening structure during mating and for transfer of the prostatic glands' secretions or but may play a role in sperm competition as the love dart in certain stylommatophoran gastropods (e.g., Davison et al. 2005; Chase 2007; Kimura et al. 2014). Both mature meiomeniid specimens M1 and M2 in our material possessed similar posterior muscle bundles (Figs. 7d–f and 10b, d).

Associated posterior glands were herein termed prostatic glands, according to the identical location and association with muscle bundles described by Morse (1979; 1994). However, prostatic glands were lacking in the male specimen M2 (Fig. 7e) but present in the female specimen M1 (Fig. 7d). Prostatic glands (together with copulatory spicules) might therefore be formed very late during the male stage and are not yet present in the male M2 and not yet reduced in the female M1. Alternatively, the prostatic glands are misinterpreted as prostatic, and rather belong to the female reproductive system. Eggs deposited by *W. argentea* are covered in a slightly adhering egg hull, which sticks to the substrate (Todt and Wanninger 2010). Due to the proximity of the glandular tissue to the pallial cavity of M1, the secretions of these glands might play a role in the formation of this sticky egg hull.

Calcareous spicules are usually dissolved during the process of embedding, but we were also unable to detect cavities, which would indicate a type of spicules differing from the remaining body scleritome. The presence (or absence in *Meioherpia*) of copulatory spicules is suggested as a taxonomic character to differentiate the two meiomeniid genera in the original description by Salvini-Plawen (1985a) and in the synopsis of García-Álvarez and Salvini-Plawen (2007). Their diagnostic value is doubtful, however (see "Discussion" above).

No data exists on the reproduction in meiofaunal Solenogastres, and it is likewise unknown whether self-fertilization occurs. Due to the protandry observed in the studied meiomeniids, however, the latter is rather unlikely. In contrast to the reproductive system of many other Solenogastres (see e.g., Todt and Wanninger 2010 for the pholidoskepian *W. argentea* and García-Álvarez et al. 1998, 2009 for cavibelonian representatives), there were no seminal vesicles or seminal receptacles present in the herein investigated lineages, leaving no structures for the storage of allo- or autosperm. As a result of the minute size of the meiomeniid pallial cavity (Fig. 10d) in relation to the large oocytes (Fig. 10c), brooding (as observed, e.g., in *Halomenia gravida* Heath, 1911 by Heath (1914) and *Proneomenia custodiens* Todt and Kocot, 2014 by Todt and Kocot (2014)) is considered unlikely and we rather assume that eggs are deposited shortly after fertilization as in, e.g., *W. argentea* (Todt and Wanninger 2010) or *M. swedmarki* (Kocot, personal communication). The large yolk coating surrounding the eggs as observed in M1 (see Fig. 10c) hints at a lecithotrophic development, and the small number of relatively large oocytes (found in M1) and absence of sperm storing organs suggests a continuous reproductive period with low reproductive output (Swedmark 1968; Morse 1994).

Digestive system

The solenogaster digestive system shows a series of important characters traditionally used in solenogaster systematics and taxonomy such as radula, formation and histology of the glands associated with the foregut and, in larger species, the formation of lateral pouches or constrictions of the midgut (Salvini-Plawen 1978; García-Álvarez and Salvini-Plawen 2007). Among our externally highly cryptic specimens from Bermuda, we encountered considerable differences to the general meiomeniid characters of the digestive system (i.e., distichous radula, no pharyngeal glands, foregut glands of *Meioherpia*-type), which had major responsibility in reclassifying some of the material as Dondersiidae (for taxonomy, see "Discussion" above).

The radula of the dondersiid-like individuals is clearly monostichous (=monoserial, see Figs. 4b and 5a'), which is the typical radula type of 21 % of the described genera of Solenogastres according to Scheltema et al. (2003). Four general types of monostichous radulae in Solenogastres can be distinguished based on fusion and size of central and lateral denticles (Scheltema et al. 2003). Our reconstruction of the monostichous radula from histological section series clearly shows one large elongated triangular tooth, but is insufficient to reliably exclude the presence of, e.g., small lateral denticles. In concordance with the radula of *Dondersia* (?) *todtae* (see Klink et al. 2015), the slight median depression of each tooth is interpreted as an incomplete fusion of central denticles, which then best reflects the radula known for the genus *Dondersia*, but ultrastructural data via SEM is needed for confirmation.

In Meiomeniidae, radulae are of striking similarity and both genera are characterized by a distichous type, which only varies in number of rows and denticles (see "Discussion" above). Distichous radulae are found among several families of Solenogastres (García-Álvarez and Salvini-Plawen 2007) and 47 % of the known genera (Scheltema et al. 2003) and are interpreted as plesiomorphic (Salvini-Plawen 1985b) or convergent developments (Scheltema 2014). The absence of a radula membrane was discussed as a feature distinguishing Solenogastres from other mollusc classes (Salvini-Plawen 1988; Wolter 1992); however, its presence is confirmed at least in certain genera (see e.g., Todt and Salvini-Plawen 2005). In the ultrastructurally studied dondersiid-like D2, we were not able to identify a radula membrane, possibly due to structural similarity between tooth and membrane.

Alternatively, the radula membrane might be truly absent in Dondersiidae, potentially providing a suitable character for phylogenetic reconstruction. The radula in Solenogastres is usually not used as a rasping organ as in other molluscs, but is more likely for hooking into prey tissue and ripping off pieces before they are ingested via a suction pump pharynx (Todt and Salvini-Plawen 2004). Based on the distichous radula type (see Fig. 9b, c) and morphology of the pharynx, we assume that at least the investigated meiomeniids also use this method of carnivore feeding. A monostichous radula like in our dondersiid-like specimens is likely used to pierce prey and then suck up the food, similar to herbivorous Saccoglossa (Gastropoda) feeding on algae (Salvini-Plawen 1967b). The presence of numerous undischarged cnidocysts in the gut (Figs. 4d and 10f) of all histologically and ultrastructurally (D2, see Fig. 4d') investigated meiomeniid and dondersiid specimens is in concordance with previous observations (Salvini-Plawen 1985a) on Meiomeniidae and indicates that all our studied lineages feed on cnidarians, in general considered the main prey of the majority of Solenogastres (Salvini-Plawen 1972; Scheltema and Jebb 1994; Okusu and Giribet 2003). In some preliminary molecular analyses (FSB, own unpublished data) on nuclear 28S rRNA sequences retrieved from entirely extracted meiomeniids (collected together with the material investigated herein), BLAST search against all available sequences deposited to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) revealed similarities to anthozoan and some hydrozoan cnidarians. Both of these lineages are represented in the interstitial (Thiel 1988), but their occurrence is scarce (Swedmark 1964) and we did not record any co-occurring cnidarians at our collecting sites. In Solenogastres the foregut glands (i.e., pharyngeal glands, esophageal glands, ventrolateral foregut glands and dorsal foregut glands) are highly variable and serve as an important taxonomic character for the higher classification of Solenogastres. Their initial categorization by Salvini-Plawen (1978) has been revised by Handl and Todt (2005) based on thorough, comparative ultrastructural analyses. All of the foregut glands produce secretions aiding in the feeding process (Todt 2006) and can be distinguished based on their cytology and position where they secret into the foregut in regard to the position of the radula.

In comparison with the encountered dondersiid-like specimens, our analysed meiomeniid material shows a relativly simple arrangement of foregut glands entirely lacking pharyngeal (as well as esophageal and dorsal foregut gland) and only possessing a *Meioherpia*-type ventrolateral foregut gland (i.e., a multicellular, endoepithelial foregut gland with extraepithelial glandular cells and an inner layer of musculature, following the revised terminology of Handl and Todt 2005). The necessary details to determine the correct type of foregut gland are difficult to detect histologically via semithin sectioning (at least for the majority of the specimens investigated herein) due to the preservation and the compact stage of all tissues and organ systems. It could only be reliably investigated via ultrastructural images such as conducted for Dondersiidae *incertae sedis* (Fig. 5b, b'), rendering this important taxonomic character difficult to access, at least for meiofaunal Solenogastres.

The foregut glands of the investigated dondersiid-like specimens are highly complex, consisting of three pairs of pharyngeal glands and an unusual ventrolateral foregut gland complex dominated by conspicuous lateral pouches (see Figs. 4b and 5b, b'). To our knowledge, a similar setting has been reported so far only for *Dondersia* (?) *todtae* from the Azores (Klink et al. 2015). The latter differs, however, from the foregut glands described herein as only two pairs of pharyngeal glands are present. Additionally, large glandular cells dorsally of the pouches were identified as "dorsal foregut glands," secreting via ducts into the pouches. Based on their position on top of the pouches, the dorsal foregut glands of *Dondersia* (?) *todtae* might be identical to the third pair of pharyngeal glands (phgl 3, Fig. 4a–c) of the undescribed dondersiid from Bermuda. However, the secretions produced in the dorsal foregut glands and pharyngeal glands 3 differ histologically: in *Dondersia* (?) *todtae,* they stain light pink (see Klink et al. 2015, Fig. 4e), whereas in the Bermudan dondersiid (Fig. 4e), the glands contain blue staining droplets (both stained using azure II/methylene blue). Identical in both lineages is a mitochondria rich tissue with comparably enlarged nuclei dorsoposterior to the pouches (Fig. 5c'). Whereas no connection between this tissue and the pouches was detected in the Azorean specimen (Klink et al. 2015), the cells forming this tissue in the Bermudan dondersiid seem to be opening into the pouches; however, the histology of this tissue negates glandular function (Fig. 5c').

Remarkable are the conspicuous central structures of the ventrolateral foregut glands (see Figs. 4b and 5b, b'), which we defined as pouches rather than ducts since we were unable to detect glandular cells in the vicinity with identical content, which might be secreting into the "duct": all surrounding glandular cells bear histologically distinguishable contents and the epithelium surrounding the pouches itself is non-glandular and non-muscular (see Fig. 5b'). Even our TEM images cannot reliably clarify the nature of the content of the pouches. We suspect a bacterial origin, but additional TEM images on higher resolution are needed to confirm this suspicion. So far, the only known bacterial symbiosis in Solenogastres is restricted to epi- and endocuticular bacteria of *Neomenia carinata* (Haszprunar in Scheltema et al. 1994, Fig. 11e) and the hydrothermal vent Solenogastres *Helicoradomenia* (see Katz et al. 2006). Microanatomical and ultrastructural investigation of the digestive system of *Helicoradomenia* detected no signs for microvory or symbiotic bacteria associated with the digestive system (Todt and Salvini-Plawen 2005). If the bacterial nature of the substance within the pouches can be confirmed, this is the first finding of

bacteria associated with the digestive tract in Solenogastres and due to their separation into lateral pouches of the foregut system a symbiosis is more likely than originating from the feeding process, e.g., by grazing along microbial mats or by suspension feeding.

The midgut of our material shows little distinguishing features between the investigated lineages and bears no lateral constrictions. In our material, we always detected single or paired dorsal caeca of the midgut extending anteriodorsal to the pharynx (see Fig. 4b, c). The histology of the caeca did not differ from that of the remaining midgut. Such caeca are common formations of the solenogaster digestive system (see, e.g., Salvini-Plawen 1981; Scheltema and Schander 2000), and their number can vary within a genus—e.g., *Pruvotina artabra* Zamarro, et al. 2013 with paired caeca (Zamarro et al. 2013) vs. *Pruvotina praegnans* Salvini-Plawen, 1978 with a single caecum (Salvini-Plawen 1978). To evaluate whether this character is subject to intraspecific variability or can serve for species delineation in Meiomeniidae, additional specimens need to be investigated histologically.

Adaptations to the mesopsammon in meiofaunal Solenogastres

Among the usually epibenthic Solenogastres, few representatives are known to inhabit the interstitial world of marine sands (Morse 1979; Salvini-Plawen 1985a; Morse and Norenburg 1992; García-Álvarez et al. 2000; Kocot and Todt 2014; Klink et al. 2015). Only scarce descriptions exist on the microanatomy of these mesopsammic species, which would allow evaluating whether the shift from an epibenthic to an infaunal lifestyle is connected to any anatomical modifications. Two of the histologically investigated Meiomeniidae from Bermuda (M1, M2, M5) possess an unpaired gland posterior to the pallial cavity (Fig. 7d, e), likely corresponding to the posterior adhesive organ in *M. swedmarki* (Morse 1979). Accumulation of glandular cells are also present in the dondersiid *incertae sedis* D1 (see Fig. 2a) and a similar species (*Dondersia* (?) *todtae*) described from the Azores, but in shape and histology, those glands slightly differ from the ones described for meiomeniids, i.e., in the Dondersiidae *incertae sedis* a series of unicellular glandular cells filled with single droplets increase in density towards the posterior end, where they form a dense aggregation (see Fig. 2a). In Meiomeniidae the posterior adhesive organ forms a multicellular glandular structure with heterogeneously staining secretion (Fig. 7d, e, g). Other supposedly interstitial Solenogastres lack similar adhesive organs (García-Álvarez et al. 2000; Kocot and Todt 2014). Given the uncertain phylogenetic affinities of the potential dondersiids and the unknown phylogenetic relationships among the different mesopsammic lineages of Pholidoskepia, it remains speculative at present stage of knowledge whether the posterior adhesive organ forms an apomorphy for the discovered lineages or rather presents independent, convergent developments as adaptation to the mesopsammic environment in Meiomeniidae and Dondersiidae *incertae sedis*.

García-Álvarez et al. (2000) discussed the oval scales of meiomeniids as a putative adaptation to move through the mesopsammic environment by pushing off from sand grains. Flatly arranged scales might also be preferable within this habitat, when compared with the partly long and needle-shaped sclerites of other solenogaster taxa, to not get stuck in the tight spaces between the sand grains. This might favor scaly scleritomes as investigated in the present lineages resulting in externally cryptic species. Nevertheless, interstitial taxa do not exclusively exhibit scaly scleritomes, e.g., *Biserramenia psammobionta* Salvini-Plawen, 1967 has acicular sclerites of up to 100 μm in length (García-Álvarez et al. 2000) and Kocot and Todt (2014) directly observed specimens of two species with acicular sclerites burrowing into the substrate, however, one of them (*Hypomenia sanjuanensis* Kocot and Todt, 2014) has distally curved sclerites, possibly facilitating burrowing head-first into the substrate. Moreover, scaly scleritomes are a common feature among pholidoskepian Solenogastres and are not restricted to infaunal forms. However, lack of data hinders a comparative evaluation of a potential correlation between sediment grain size, respectively size of interstitial spaces, and type of scleritome. Nevertheless, among the supposedly interstitial Solenogastres, Meiomeniidae, and the discovered—albeit juvenile—Dondersiidae *incertae sedis* with unclear phylogenetic affinities seem to be best adapted to the interstitial habitat by exhibiting next to the minute body sizes, scaly scleritomes and adhesive glandular organs at their posterior ends.

Conclusions

Detailed morphological (re)descriptions as presented herein are still indispensable to fill the gaps in knowledge on the evolution of characters states and the morphological diversity of Solenogastres. Our histological and ultrastructural study on mesopsammic Pholidoskepia shows that the microanatomy of Solenogastres bears additional characters with potential systematic and taxonomic value beyond the traditional ones of scleritome, radulae, and foregut glands. At present, variation between specimens concerning, e.g., organization of the pallial cavity or presence/absence of heart and pericardium are difficult to evaluate, however, lacking thorough comparative data on intraspecific and ontogenetic variation. Time-consuming 3D-reconstructions based on histology always present a trade-off to the analyses of numerous specimens.

Future developments in generating detailed datasets with micro-CTs might present more efficient means to address the morphological diversity of Solenogastres, when combined with targeted histological and ultrastructural sectioning.

The present study highlights taxonomic problems which might be symptomatic for Solenogastres (and potentially also for other meiofaunal organisms): minute and highly cryptic species co-occur—this is also corroborated by molecular barcodes for the meiofaunal Solenogastres at Flatt's Inlet (own unpublished data)—and the phenotypic characters needed for species delineation cannot be investigated in one individual, hindering reliable assignment to described species. Under ideal circumstances, one specimen can be studied externally via SEM and later on serve for radula preparation, but then it is destroyed for histology and ultrastructural analyses of the internal anatomy and vice versa. Light microscopic investigation allows for a combination of both character sets but immolates details and risks to damage structures prior to histological analyses. Thus, additional character sets which allow for unambiguous species identification such as molecular barcodes are urgently needed to stabilize solenogaster taxonomy and make Solenogastres more accessible to ecological and biogeographic studies. This will at first involve a joint effort of the community to establish molecular datasets of the valid type material to prevent that a parallel molecular-based taxonomy will be established without linkage to the existing morphology-based one.

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STRANDED ON A LONELY ISLAND: DESCRIPTION OF *DONDERSIA* (?) *TODTAE* SP. NOV., THE FIRST SHELF SOLENOGASTER (MOLLUSCA, APLACOPHORA) FROM THE AZORES

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ABSTRACT

Among molluscs, Solenogastres forms a comparably neglected clade with still many unexplored 'white spots' on their putative global distribution map. Only three species of Solenogastres have been recorded so far from Azorean waters, all in bathyal depths. Here we present the first finding of a pholidoskepian solenogaster extracted from sand samples of the shallow subtidal off São Miguel. The present study describes this new species *Dondersia* (?) *todtae* sp. nov. (Dondersiidae, Pholidoskepia) based on several specimens combining data from light- and scanning electron microscopy. We performed a computer-supported 3D-reconstruction of all major organ systems from serial semithin sections (0.75 and 1 μm). This new species can be clearly distinguished from its congeners and related pholidoskepids by its complex scleritome consisting of several different types of ovoid scales and lanceolate spines and a unique arrangement of complex foregut glands bearing conspicuous paired pouches lateral of the pharynx. Unfortunately, all investigated specimens are juveniles, thus no characters of mantle cavity or gonopericardial system could be studied. The systematic placement of the solenogastran lineage remains problematic as it unites characters from Meiomeniidae and Dondersiidae and at present cannot be placed unambiguously in any of the valid genera. Molecular data is needed in the future to validate our systematic hypothesis and to allow assignment to new collected adult specimens.

RESUMO

Entre os moluscos, os Solenogastres formam um clado comparativamente negligenciado, com muitos "pontos brancos" ainda inexplorados no mapa da sua putativa distribuição global. Até agora só três espécies de Solenogastres foram registadas para as águas Açorianas, todas em profundidades batiais. Aqui apresentamos a primeira descoberta de um solenogaster folidosquépio extraído de amostras de areia do subtidal baixo ao largo de São Miguel. O presente estudo descreve esta espécie nova *Dondersia* (?) *todtae* sp. nov. (Dondersiidae, Pholidoskepia) com base em vários exemplares, combinando dados de microscopia de luz e electrónica de varrimento. Executámos uma reconstrução 3D, com apoio de computador, de todos os sistemas orgânicos principais a partir de secções seriadas semi-finas (0,75 e 1 μm). Esta nova espécie pode ser claramente distinguida dos seus congéneres e aparentados folidosquépios pelo seu complexo escleritoma, constituído por vários tipos diferentes de escamas ovoides e espinhos lanceolados, e um arranjo único de complexas glândulas do tubo digestivo anterior possuindo conspícuas bolsas pares aos lados da faringe. Infelizmente, todos os exemplares investigados eram juvenis, pelo que os caracteres da cavidade palial ou do sistema gonopericárdico não puderam ser estudados. A colocação sistemática da linhagem solenogástria permanece problemática pois une caracteres dos Meiomeniidae e Dondersiidae e, por agora, não pode ser colocada sem ambiguidade em qualquer dos géneros válidos. É necessária informação molecular para, no futuro, validar a nossa hipótese sistemática e permitir atribuição apropriada a novos exemplares adultos que venham a ser colectados.

INTRODUCTION

The Azores are an isolated group of islands in the Atlantic midway between Europe and North America with interesting biodiversity as all native fauna and flora must have reached the islands via long-distance dispersal (Morton & Britton, 2000; Ávila *et al.*, 2009). The Azorean malacofauna is relatively well explored (see e.g., Ávila *et al.*, 1998; Ávila, 2000; Ávila *et al.*, 2000; Martins *et al.*, 2009; Ávila & Sigwart, 2013) with a high number of endemics especially among terrestrial snails (Martins, 2011). The diversity of aplacophoran molluscs in Azorean waters has been neglected, however, likely due to the fact that most species inhabit the deep sea and are therefore hard to access and due to common prejudices against their difficult taxonomy which usually requires histological sectioning and sclerite preparation for identification of specimens (Todt, 2013). According to a recent synopsis (García-Álvarez & Salvini-Plawen, 2007) only three species of Solenogastres are known from the deep sea regions surrounding the Azorean islands, inhabiting bathyal depths of 800 - 1200 meters: neomeniamorph *Hemimenia atlantica* Salvini-Plawen, 2006 and cavibelonian *Alexandromenia grimaldii* Leloup, 1946 and *Anamenia gorgonophila* (Kowalevsky, 1880). *H. atlantica* also occurs in the deep sea off Galicia, Spain (Salvini-Plawen, 2006) and *A. gorgonophila* is also recorded from the Western Mediterranean (Salvini-Plawen, 1972). In contrast to the few records of Solenogastres from the Azores, nearly 100 species are described from the Iberian Peninsula, accounting for nearly 30% of the known global diversity (García-Álvarez *et al.*, 2014). Like *H. atlantica* and *A. gorgonophila* other Iberian species might be distributed throughout the Atlantic, but most lineages are described from single localities only (García-Álvarez *et al.*, 2014). The Iberian Peninsula as global hot spot of solenogastran diversity is likely an artifact of more extensive aplacophoran research on Europe's continental coasts, probably other regions bear species diversities of a similar magnitude, which are simply still undiscovered.

During the World Congress of Malacology in July 2013, we sampled some intertidal and subtidal sands off São Miguel for molluscs inhabiting the interstices between sand grains. Few studies have investigated the interstitial fauna of the Azores so far and first exploratory studies indicated a sparse sedimentary infauna interpreted as due to sediment instability at the high-energy beaches (Bamber & Robbins, 2009). In samples of coarse sand collected subtidally partially sheltered between rocks, we discovered a surprisingly rich diversity of temporary meiofaunal but also entirely meiofaunal lineages (e.g., among gastropods representatives of caenogastropod *Caecum* or heterobranch *Rhodope* and *Hedylopsis*). Additionally, we extracted at least two different lineages of co-occurring minute pholidoskepid Solenogastres, an aplacophoran clade previously unknown to occur in Azorean waters and the first Solenogastres discovered in shallow water depth on the islands.

The present study describes one of the encountered lineages, the dondersiid *Dondersia* (?) *todtae* sp. nov., in full detail combining histological data from semithin section series with an ultrastructural investigation of the scleritome.

MATERIALS AND METHODS

Material

Material was collected on the Azorean island of São Miguel during the World Congress of Malacology in July 2013. Samples of coarse sand and shell gravel were taken from 26 m between rocks

via scuba-diving at the dive spot 'three houses' (37° 42.413' N, 25° 29.836' W) on the south coast.

Specimens were extracted from the sand using an anesthetization-decantation technique with magnesium chloride in seawater (see Jörger *et al.*, 2014). Seven individuals were fixed each in 96 % ethanol and in a 4 % solution of formaldehyde in seawater. All specimens are deposited at the Section Mollusca of the Bavarian State Collection of Zoology (Munich, Germany) Accession numbers: ZSM Mol 20150159-20150165.

Histology and 3D-reconstruction

Four formalin-fixed specimens were postfixed in 1 % OsO_4 buffered in 0.1 M sodium cacodylate (0.3 M NaCl, pH 7.2) for 3 h at room temperature and afterwards stained with a solution of 0.5 % Safranin in 80 % ethanol. Subsequently, they were decalcified overnight in 1 % ascorbic acid. Specimens were dehydrated via a graded acetone series, washed three times with sodium cacodylate and embedded in Epon resin. Two specimens were serially sectioned with a thickness of 0.75 and 1 µm respectively using a HistoJumbo diamond knife (Diatome, Biel, Switzerland) with contact cement applied to the lower cutting edge of the Epon block following the method of Ruthensteiner (2008). The ribbons were transferred to microscopic slides, stained with methylene blue/azure-II (Richardson *et al.*, 1960) and sealed in distyrene plasticizer Xylene (DPX). We took photographs of every section at 60x magnification with an Olympus SP 25 camera mounted on an Olympus CX 41 microscope and stack-processed (converted to 8-bit grayscale, white-balance, contrast enhanced, unsharp masked) them in Photoshop CS6 (Adobe Systems, Mountain View, CA, USA). After importing the images into the 3D-visualization software AMIRA 5.4.4 (Visage Imaging, Berlin, Germany), we aligned the slices and all major organ systems were reconstructed.

Scanning Electron Microscopy (SEM)

Four specimens were dehydrated in a graded acteone series and critical point dried in a Baltec CPD 030 (Leica, Microsystems) in carbon dioxide atmosphere. We successfully transferred two specimens to a SEM stub with self-adhesive carbon sticker (two individuals were unfortunately lost during preparation) and sputter coated with gold in a Polaron Sputter Coater (GaLa Gabler Labor Instrumente Handels GmbH) in an Argon atmosphere for a total of 285 seconds. We took SEM pictures with a LEO 1430 VP SEM at 7-15 kV and edited them in Photoshop CS6 (Adobe Systems, Mountain View, CA, USA).

Radula preparation

Two specimens were dissolved in potassium hydroxide and sodium hypochlorite respectively for radula preparation. Unfortunately, none of the minute radulae could be successfully transferred to a SEM stub.

Another specimen was slowly decalcified in 1 % ascorbic acid for 36 hours. Afterwards the entire specimen was placed on a microscopic slide, covered in Vectashield mounting media (Vector Laboratories Inc.) and sealed with a cover slip and clear nail polish. The radula was documented with 60x magnification with an Olympus SP 25 camera mounted on an Olympus CX 41 microscope.

SYSTEMATICS

(based on García-Álvarez & Salvini-Plawen, 2007)

Class Solesnogastres Gegenbaur, 1878
Supraorder Aplotegmentaria Salvini-Plawen, 1978
Order Pholidoskepia Salvini-Plawen, 1978

Family Dondersiidae Simroth, 1893
Genus *Dondersia* (?)

Dondersia (?) *todtae* sp. nov.

Type material – Holotype: one immature specimen, complete cross section series (sectioned at 0.75 μm) (ZSM Mol 20150159). Nine paratypes (all collected together with the holotype): one immature specimen, complete longitudinal section series (sectioned at 1 μm) (ZSM Mol 20150160), two specimens in Epon block (ZSM Mol 20150161 and ZSM Mol 20150162), two specimens on SEM-stub (dehydrated, sputtered with gold) (ZSM Mol 20150163), one whole-mount specimen (decalcified, mounted in Vectashield) (ZSM Mol 20150164), three entire specimens in 96 % Ethanol (ZSM Mol 20150165).

Type locality – Dive spot 'three houses' (37° 42,413' N, 25° 29,836' W), 26 m depth, south coast of the island of São Miguel, Azores, Atlantic Ocean.

Etymology – This species is named in honor of our friend and colleague Dr. Christiane Todt (University Museum of Bergen, Norway) who considerably promoted solenogastran research in the past years and was a great teacher to FSB and KMJ in introducing us to solenogastran taxonomy and microanatomy.

Distribution – the new species is currently only known from the type locality.

Zoobank - Dondersia todtae: urn:lsid: zoobank.org:act:7569C3B2-8131-474B-9E77-8BEF6BF18315

Diagnosis – based on immature specimens only. Elongate body slightly pointed at the posterior end, body diameter roundish. Thin cuticle with complex scleritome comprising a minimum of five different types of scales. Ovoid scales covering entire body, with prominent lanceolate spines towards the posterior. Mouth opening separate from atrium (= vestibulum), the latter without papillae. Monoserial radula. Ventrolateral foregut glands (type A) with conspicuous paired pouches with thin epithelium and unicellular gland cells located dorsally. Midgut without constrictions. With pedal commissural sac. No dorsoterminal sense organ detected.

DESCRIPTION

General morphology

Dondersia (?) *todtae* sp. nov. has an elongated worm shaped body (Figure 1C, D) with a total length of 0.3 - 0.4 mm, the cross body section measures up to 0.06 mm in diameter. Imbricated scales cover the whole body like a shiny white armour (Figure 1A). At least five different types of aragonitic scales and spines could be distinguished via SEM: the main body coverage is composed of large, oval overlapping scales (Figure 1G) with a width from 15 μm to 20 μm and 25 μm in length. They have a blunt and rounded tip and a thickened rim at their base (see Figure 1G). Furthermore, simple oval plates (Figure 1F, L) with half of the length and width are randomly distributed between aforementioned larger scales. Additionally, the scleritome bears two size classes of lanceolate spines (Figure 1H, I) starting from the anterior third of the body (Figure 1C). They differ in length and in the degree of projection away from the body, the latter might be an artifact of spines, which are broken at their base (see Figure 1C, D). The number and length of the larger lanceolate spines (up to 50 μm) increases continuously towards the posterior end (Figure 1C) and they seem to be denser on the lateral sides. In the smaller spines the oval basis is visible (Figure 1H). In the head region

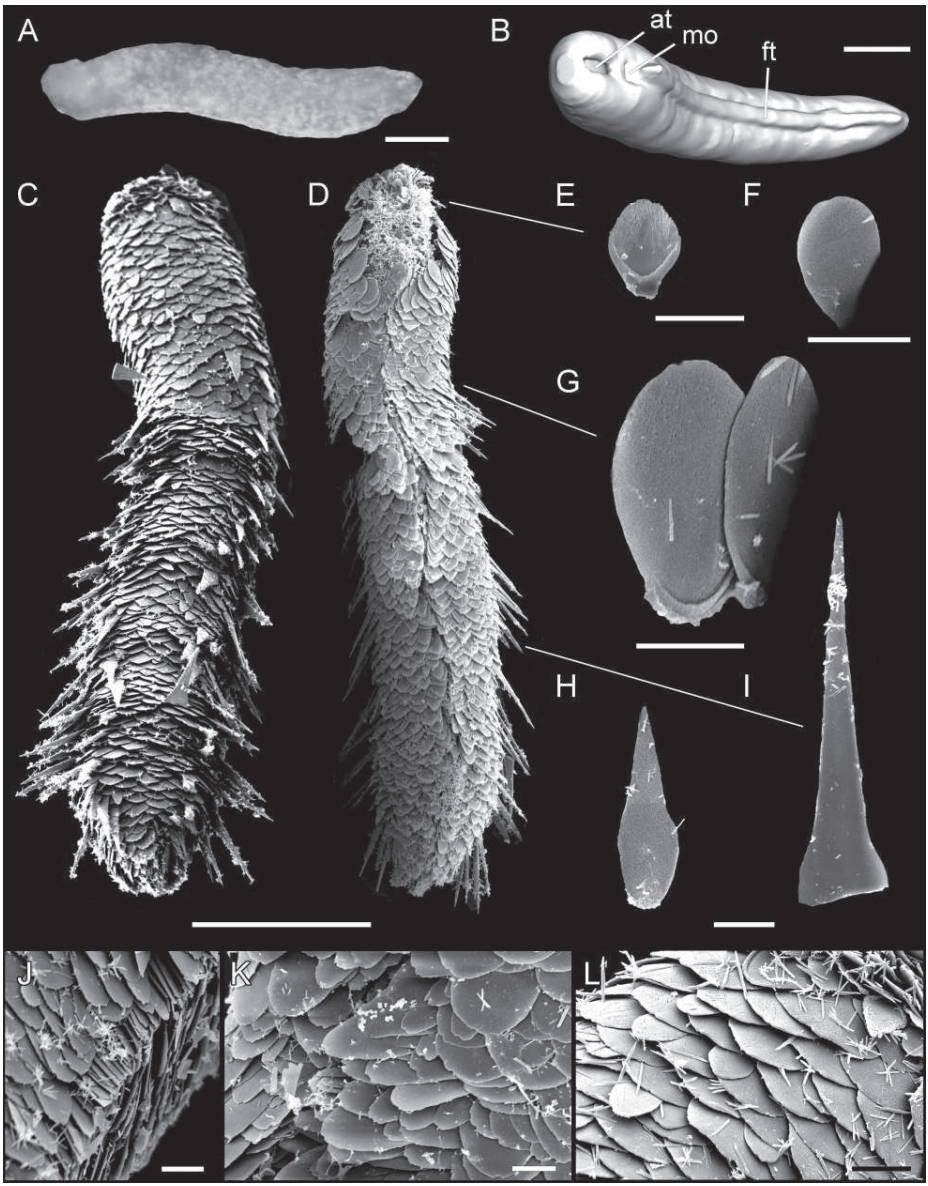

FIGURE 1. External morphology. **A.** Entire animal in natural color. **B.** 3D-reconstruction of body surface (lateroventral view) showing separated atrium and mouth opening. **C-L** SEM micrographs. **C.** Entire animal, dorsal view. **D.** Same individual as in C, ventral view. **E.** Scale of head region. **F.** Oval plate. **G.** Large main scales. **H.** Small and broader spine. **I.** Lanceolate spine. **J.** Densely packed foot scales, right view. **K.** Paddle shaped foot scales. **L.** Typical detail of the scleritome with main scales and oval plates. **at**, atrium; **ft**, foot; **mo**, mouth opening. Scale bars: A, C, D = 100 μm. B = 40 μm. E - H (H also valid for I), J, K = 10 μm. L = 20 μm.

around atrium and mouth opening there is one more type of smaller scales (approx. 12 μm), rhomboid in shape bearing a conspicuously thickened base (Figure 1E). The sclerites along the pedal groove are not clearly visible but seem to have a narrow paddle shape (Figure 1J, K). Abdominal copulatory spines are not developed in the investigated juveniles. We only discovered a small hole at the dorsal posterior end of one specimen, but since we were unable to detect any specific scales surrounding it, it was interpreted as an artifact rather than a dorsoterminal sense organ.

A ciliated foot (Figures 1B; 2A; 5) is located ventrally right behind the mouth opening and extends to the level of the anus. It is not retracted into a pedal groove in the histologically investigated specimens. Posterior of the mouth opening, the first cilia of the foot are conspicuously lengthend in relation to the remaining foot ciliation. A slight depression, which extends for approx. 15 μm was interpreted as pedal pit.

A paired pedal pit gland was found at the anterior edge of the foot (not shown). Each gland consists of a single drop-shaped cell opening via a small duct beside the foot. The content of the cells stained dark blue in histological sections. Close to the rectum exists a dark staining multi-cellular gland – interpreted as adhesive gland – which only could be found in the longitudinal section (Figure 2C).

The mouth opening is clearly separated from the anterior atrium (Figures 1B, 2A). The atrial opening appears as a longitudinal slit, whereas the mouth opening is a more vertical slit (Figure 1B). The atrial cavity is regularly spherical-shaped and nearly extends to the level of the cerebral ganglia (Figure 2A).

No pallial cavity is present in the investigated juveniles. The integument consists of an epidermis with homogeniously distributed glandular cells and a thin cuticle (Figure 2B, C). The cuticle shows noticeable pits where the scales are embedded into the integument.

Digestive system

The digestive system consists of the prominent midgut, a pharynx including the monoserial radula, two types of pharyngeal glands and a foregut gland complex (Figure 3). The mouth opening is positioned medioventral anteriorly to the foot (Figure 2A). It connects to a short oral tube leading into a muscular pharynx with ciliated epithelium (Figure 3A). The posterior part of the pharynx is surrounded by a dark staining, acellular sheath (see white arrowhead, Figure 4E). The monoserial radula measures 30 – 35 μm in length. It bears eight to nine single straight and pointed teeth, which each has a length between 6 – 10 μm (Figure 3 B, C, C′). In reconstructions based on histological sections each triangular tooth seems to bear a central, vertical depression (see Figure 3C). Light-microscopic investigation suggests a pair of denticles at the base of each tooth and 3D-reconstructions several small denticles. But SEM examination is needed in the future to confirm details on each radula tooth.

Two kinds of paired accumulated unicellular pharyngeal glands, one containing lighter staining vesicular secretions than the other, (Figures 2B; 3A, D; 5C) are located anterolaterally to the pharynx. The discharging canal of each cell of the more ventral gland seems to fuse with those of other cells and open jointly into the pharynx laterally of the radula. No ducts leading from the more dorsal glandular cells into the pharynx could be detected.

Paired multicellular foregut glands (Figure 3D, E) are positioned laterally of the posterior part of the pharynx and comprise

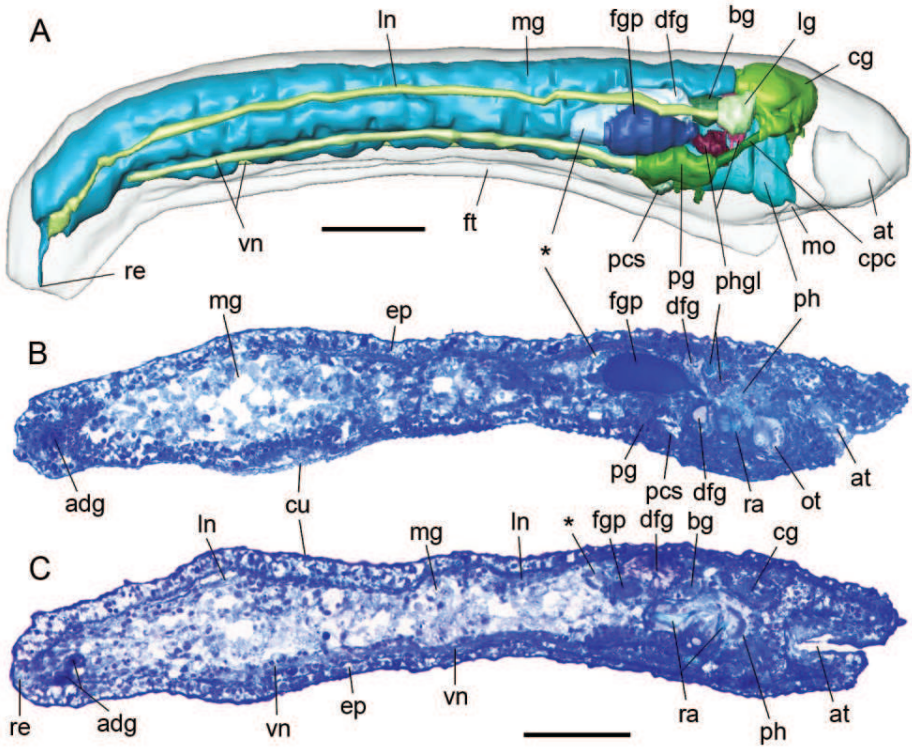

FIGURE 2. Overview of the microanatomy. **A.** Lateral right view of the 3D-reconstruction. **B, C.** Histological longitudinal sections. **B.** Openings of the foregut glands. **C.** Nerve cords. **adg**, adhesive gland; **at**, atrium; **bg**, buccal ganglion; **cg**, cerebral ganglion; **cpc**, cerebropedal connective; **cu**, cuticle; **dfg**, dorsal foregut gland; **ep**, epidermis; **fgp**, foregut gland pouches; **ft**, foot; **lg**, lateral ganglion; **ln**, lateral nerve cord; **mg**, midgut; **mo**, mouth opening; **ot**, oral tube; **pcs**, pedal commissural sac; **pg**, pedal ganglion; **ph**, pharynx; **phgl**, pharyngeal gland; **ra**, radula; **re**, rectum; **vn**, ventral nerve cord; *, large cells with comparatively enlarged nuclei. Scale bars (C also valid for B): 40 μm.

three histologically distinct structures: the most prominent components (ca. 30 μm length) of the foregut glandular complex are the paired foregut gland pouches (Figures 2A; 3A, D, E) filled with a dark bluish secretion (Figures 2B; 5A). Their epithelium is thin and non-glandular and non-muscular. No glandular cells with histologically similar content could be detected. The content of the pouches discharges into the pharynx posteriorly of the radula. The dorsal foregut glands are situated dorsally to the pouches extending posteriorly for approximately 20 μm. They consist of large glandular cells with light pinkish-staining secretion (Figures 2A, B; 4E; 5A, C) discharging their content via individual cellular ducts into the paired pouches and from the latter into the pharynx. Larger cells (approx. 15 μm) with comparatively enlarged nuclei (approx. 7 μm) (Figures 2; 3A, D, E; 5B) are directly

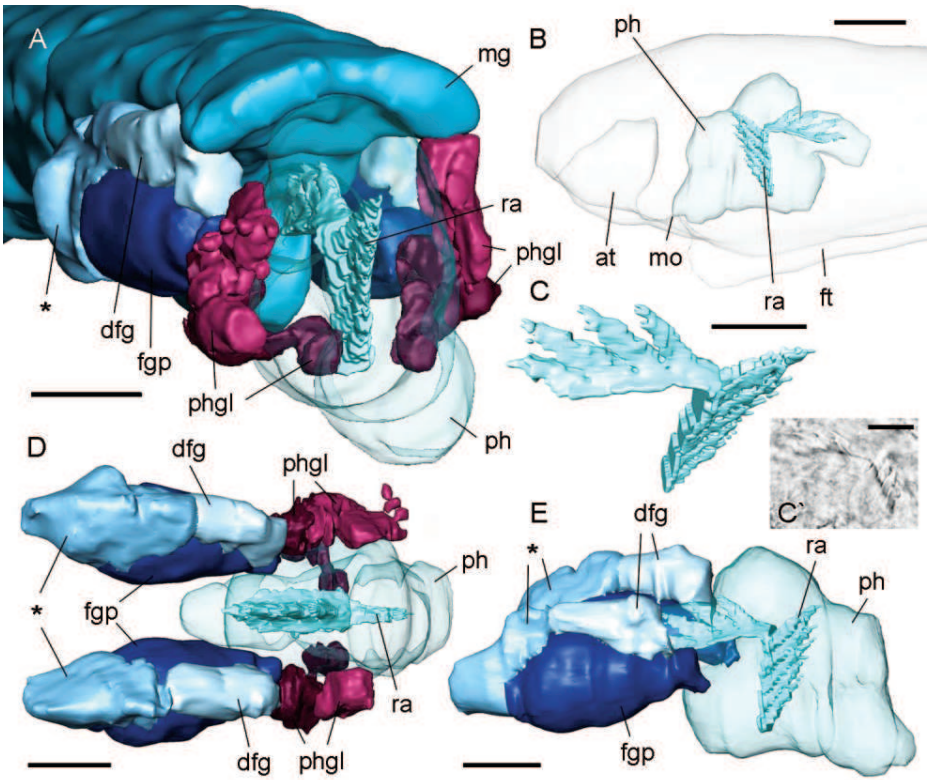

FIGURE 3. 3D-reconstruction of the digestive system and light-microscopy of the radula. **A.** Overview of all relevant structures. **B.** Position of pharynx and radula within the body, left view. **C.** Radula, dorsolateral, right view. **C'.** Radula, light-microscopy, lateral view. **D.** Dorsal view of the glands' position. **E.** Oblique right view without pharyngeal glands, showing the openings of the foregut glands. **at**, atrium; **dfg**, dorsal foregut gland; **fgp**, foregut gland pouches; **ft**, foot; **mg**, midgut; **mo**, mouth opening; **ph**, pharynx; **phgl**, pharyngeal glands; **ra**, radula; *, large cells with comparatively enlarged nuclei. Scale bars: A, D, E = 15 μm. B = 20 μm. C, C' = 10 μm.

attached posteriorly to the dorsal foregut glands, surrounding the posterior end of the pouches.

The large midgut fills the majority of the body cavity (Figure 2A) and is histologically uniform (Figures 2B, C; 4E; 5). We discerned no lateral constrictions. Anterodorsally of the pharynx, the midgut lengthens into an unpaired caecum (Figures 3A; 4E; 5C). It mainly consists of high columnar digestive cells for intracellular digestion. Those phagocytotic cells contain several minute globular to ovoid vesicles (Figure 5A, B, D). Partly, they resemble small bubbles due to their light staining content surrounded by a strong boundary. No typical cnidocytes were identified in the midgut. At the posterior end, the midgut narrows to a rectum (Figure 2A) which does not differ histologically from the remaining midgut.

Nervous system

The tetraneural nervous system consists of fused cerebral ganglia, paired lateral, buccal and pedal ganglia as well as paired ventral and lateral nerve cords (Figures 2A; 4C). All ganglia show a distinctive separation into central neuropil and a surrounding layer of perikarya (Figure 4D, E).

The cerebral ganglia (ca. 35 µm in length, 40 µm width at widest point) amount to approximately 5 % of the body mass. The ganglia are distinguishably separated into two structures posteriorly and fused anteriorly (see Figure 4A). One big nerve emerges anterolaterally from each cerebral ganglion and soon bifurcates (Figure 4F). Due to the compact stage of the tissue anterior to the cerebral ganglia, the nerves could not be traced to their destination. The cerebropedal connectives (Figure 4B) have their origin at the lateroposterior part of the cerebral ganglia and connect anteriorly to the pedal ganglia. The tissue anterior to the cerebral ganglia is presumably not fully developed, but basically shows a differentiation in spherical structures, however without clear boundaries (Figure 4D).

Paired lateral nerve cords (Figures 2A, C; 4; 5) originate posterolaterally from the cerebral ganglia. Swellings adjacent to the cerebral ganglia show a beginning differentiation into perikarya and neuropil. Therefore, they were identified as lateral ganglia (Figure 2A; 4A, B, F) (ca. 20 µm length and 10 µm width).

The ovoid buccal ganglia (Figure 2A, C; 4) (ca. 20 µm in length, 10 µm in width) show one dorsopharyngeal buccal commissure (Figure 5C) directly above the radula. They are located in between the lateral ganglia on the same level as the lateral nerve cords.

Ventrolaterally of the pharynx are the pedal ganglia (Figures 2; 4; 5A, C) (ca. 30 µm in length) with two ventral commissures and one thick commissure above the pedal commissural sac (pcs) (Figure 4 A, B, F). The pedal ganglia nearly touch posteriorely. The aforementioned pedal commissural sac is located ventrally between the second pedal commissure and nearly extends to the end of the pedal ganglia. The pcs is conically shaped and densly packed with several free-floating central cells (Figure 4E). They are largely vacuolized and measure 5 µm in diameter. It was not possible to count the free-floating cells.

Two ventral nerve cords (Figures 2A, C) emerge from the posterior end of the pedal ganglia, run until the last fifth of the body and disappear suddenly. Like the lateral nerve cords, they show symmetrical swellings on different spots.

As all investigated animals are immature, no gonads are yet developed. In addition, no heart or pericard could be distinguished. Histologically, we could detect no alternative site of ultrafiltration (i.e., protonephridia or rhogocytes) unambiguously. A minute paired structure shortly posterior to the mouth opening (not shown) requires ultrastructural re-investigation.

DISCUSSION

Systematics

Based on the predominant scaly scleritome this new solenogastran lineage from the Azores could be unambiguously identified as Pholidoskepia (for systematics and diagnoses of higher taxa see García-Álvarez & Salvini-Plawen, 2007). Based on the external morphology and details of the scleritome this new species closely resembles members of the family Meiomeniidae, which are characterized, however, by a distichous instead of monoserial radula (see e.g., Morse, 1979; Morse & Norenburg, 1992; Salvini-Plawen, 1985). A combination of the presence of a monoserial radula and

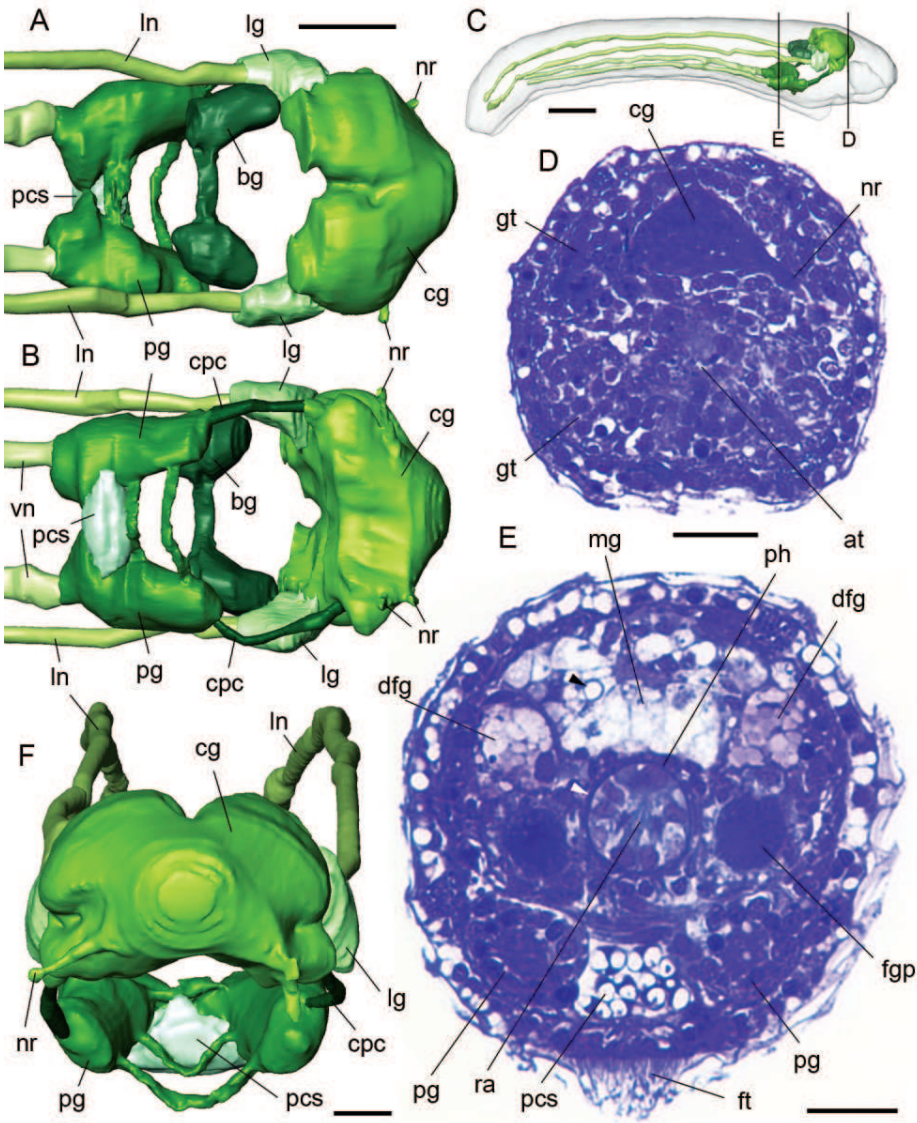

FIGURE 4. Nervous system. **A-C, F.** 3D-reconstruction. **D**, **E**. Histological cross sections. **A**, **B**. Anterior part of the nervous system, dorsal and ventral view, respectively. **C.** Position of the nervous system within the body, right view, vertical lines mark the position of the sections D and E. **D.** Undifferentiated ganglia-like tissue in front of the cerebral ganglion. **E.** Pedal commissural sac, black and white arrowheads mark midgut vesicle and pharyngeal sheath, respectively. **F.** Pedal commissural sac and surrounding commissures, frontal view. **at**, atrium; **bg**, buccal ganglion; **cg**, cerebral ganglion; **cpc**, cerebropedal connective; **dfg**, dorsal foregut gland; **fgp**, foregut gland pouches; **ft**, foot; **gt**, putative nervous tissue; **lg**, lateral ganglion; **ln**, lateral nerve cord; **mg**, midgut; **nr**, nerve root; **pcs**, pedal commissural sac; **pg**, pedal ganglion; **ph**, pharynx; **ra**, radula; **vn**, ventral nerve cord. Scale bars (A also valid for B): A, B, C = 20 μm. D- F = 10 μm.

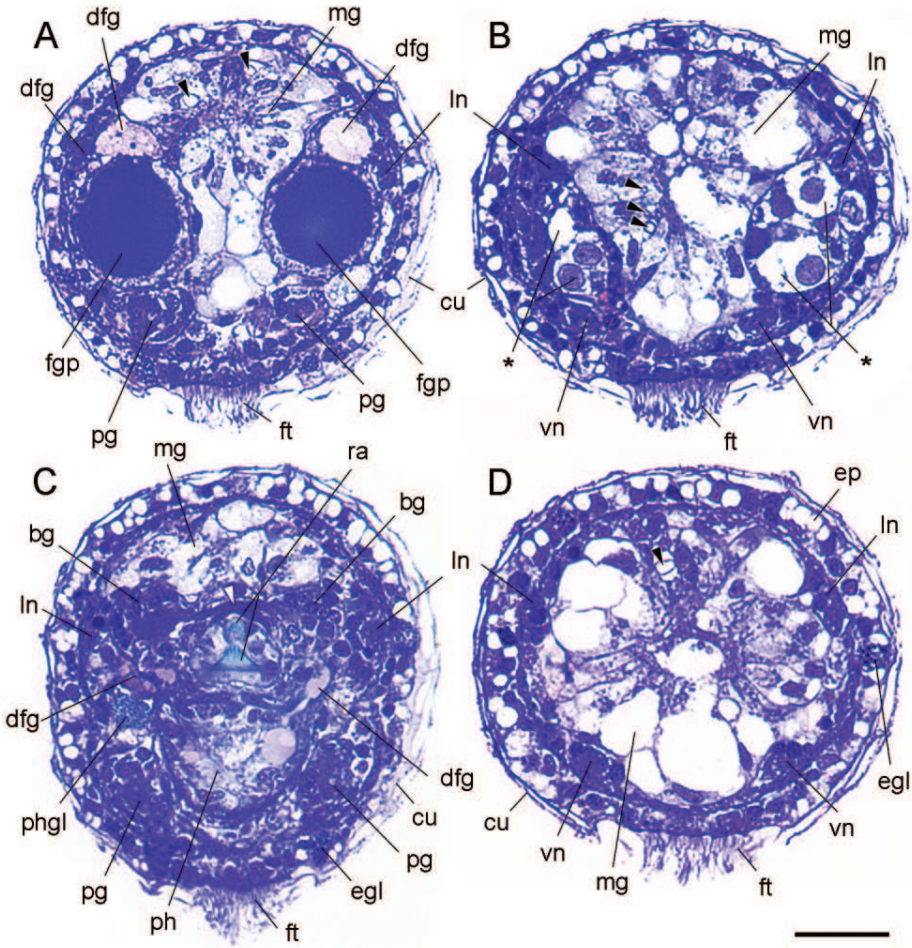

FIGURE 5. Histological cross sections of the different components of the digestive system, black arrowheads mark midgut vesicles. **A.** Foregut glands. **B.** Large cells posterior to fgp with comparatively enlarged nuclei (asterisks). **C.** Foregut glands opening into the pharynx, white arrowhead marks the buccal commissure. **D.** Midgut region. **bg**, buccal ganglion; **cu**, cuticle; **dfg**, dorsal foregut gland; **ep**, epidermis; **egl**, epidermal gland; **fgp**, foregut gland pouches; **ft**, foot; **ln**, lateral nerve cord; **mg**, midgut; **pg**, pedal ganglion; **ph**, pharynx; **phgl**, pharyngeal glands; **ra**, radula tooth; **vn**, ventral nerve cord. Scale bar (valid for A-D): 15 μm.

characters of the scleritome (i.e., presence of different types of scales and lack of nail-shaped sclerites as characteristic for Macellomeniidae) the herein described species can be best assigned to the family Dondersiidae. However, Dondersiidae is a poorly supported monophylum, lacking both synapomorphies as well as a unique mosaic of characters (Scheltema *et al.*, 2012). Our present study further adds to

the complexity and character variety of the family regarding the histology of the foregut glands and additionally reports a pedal commissural sac for the first time in the family.

García-Álvarez & Salvini-Plawen (2007) define the dondersiid foregut glandular organs as ventrally positioned type A with subepithelial gland cells. Scheltema *et al.* (2012) also include the possible occurrence of extraepithelial unicellular foregut gland 'follicles' to the diagnosis of the family. The latter might refer to the herein detected unicellular pharyngeal glands of *Dondersia* (?) *todtae* sp. nov., but the complex foregut gland system described herein including the prominent paired pouches is described – to our knowledge – for the first time (for more detailed discussion see below).

In *D. todtae* sp. nov. the pedal commissural sac is a conspicuous feature of the nervous system (see Figure 4E). Prior to this study, a pedal commissural sac (pcs) was only known from pholidoskepian Gymnomeniidae (Haszprunar, 1986), Meiomeniidae (Todt *et al.*, 2008; Bergmeier *et al.*, 2014) and enigmatically also for the cavibelonian *Scheltemaia mimus* (Scheltema & Schander, 2000) (see Scheltema & Schander, 2000, as *Eleutheromenia*) and was undescribed so far for any of the 32 dondersiid species. The pcs is well recognizable in semithin sections and it is surprising how it could have been overlooked in species descriptions with detailed records on the nervous system (e.g., in *Micromenia fodiens* (Schwabl, 1955) see Schwabl (1955)). Re-examination of dondersiid nervous systems is nevertheless urgently necessary to clarify the absence/presence of such a complex organ. The pcs is discussed as a movement receptor analogue to a statocyst organ (Haszprunar, 1986) and it is questionable why such a complex organ would be reduced either during ontogeny or independently in the evolution of different solenogastran lineages. An independent development in different lineages seems equally dubious at present, but comparative ultrastructural data is needed to reveal potential differences of the pedal commisural sac among different families of Pholidoskepia and especially of *S. mimus*.

Many other morphological characters are variable within the family Dondersiidae (e.g., body with or without posterior fingerlike projection, with or without copulatory spicules) (García-Álvarez & Salvini-Plawen, 2007; Scheltema *et al.*, 2012). Unfortunately, characters of the reproductive system such as e.g., copulatory spicules could not be investigated for *D. todtae* due to the immature stage of the investigated specimens. But among Dondersiidae, characters of the reproductive system are likely species specific and not useful for the discrimination of genera (Scheltema *et al.*, 2012).

The family Dondersiidae currently comprises nine genera (*Dondersia* Hubrecht, 1888, *Nematomenia* Simroth, 1893, *Ichthyomenia* Pilsbry, 1898, *Stylomenia* Pruvot, 1899, *Heathia* Thiele, 1913, *Micromenia* Leloup, 1948, *Lyratoherpia* Salvini-Plawen, 1978, *Helluoherpia* Handl and Büchinger, 1996 and *Squamatoherpia* Büchinger and Handl, 1996), which are mainly distinguished by characters of the scleritome, common vs. separate atrium and mouth opening, presence of constrictions in the midgut, presence or absence of a dorsoterminal sense organ and details of the monoserial radula (García-Álvarez & Salvini-Plawen, 2007). Unfortunately, we were unsuccessful in examining the minute radula of *D. todtae* sp. nov. via SEM and therefore lack ultrastructural detail. As investigated light-microscopically and reconstructed from histological sections, each tooth is elongated triangular in shape with a pointed tip (Figures 3C,

F). The central depression in each tooth of *D. todtae* sp. nov. (see Figure 3C) might refer to an incomplete, central fusion of the two elongated denticles as characteristic for the genus *Dondersia*, but also for *Ichthyomenia, Stylomenia* and *Micromenia* (Salvini-Plawen, 1978; García-Álvarez & Salvini-Plawen, 2007). Via SEM-examination, Scheltema *et al.* (2012) discovered that the fused central denticles of *Dondersia* are bordered by two additional outward curved denticles, very similar to the radula of *Lyratoherpia*. The fused status of the central denticles might not be well visible via light-microscopy, likely giving the radula tooth a three-denticle appearance as described for the dondersiid genera *Helluoherpia* and *Squamatoherpia* (Büchinger & Handl, 1996; Handl & Büchinger, 1996). Comparative SEM examination of the radula among the nine genera of Dondersiidae is urgently needed to reveal whether additional lateral denticles are also present in *Ichthyomenia, Stylomenia* and *Micromenia.* In fact, re-examination might reveal a very similar radula across the entire family (with the exception of lineages which entirely reduced the radula i.e. *Heathia* and some species of *Nematomenia*).

The foregut gland complex encountered in *D. todtae* sp. nov. most closely resembles that of *Squamatoherpia*, which is characterized by unicellular glands discharging into a common, prominent duct (Büchinger & Handl, 1996). However, this duct is described as muscular and no 'secretion' differing from the one of the unicellular glands is reported, as present in the pouches of *D. todtae* sp. nov.. At present, the only solenogaster with similar foregut pouches and associated enlarged cells as well as dorsal unicellular glands is a yet undescribed dondersiid from Bermuda (Bergmeier, unpublished data). However, the dorsal unicellular gland cells of the Bermudan and Azorean dondersiid also differ concerning the staining properties of the 'secretion'. The 'secretion' within the pouches remains mysterious as associated glandular cells are absent. TEM investigations are needed to clarify the origin and nature of the content within the pouches.

The absence of a dorsoterminal sense organ and the lack of constrictions in the midgut contradict a placement of the new solenogastran species from the Azores within the genus *Dondersia*. However, based on the scleritome it best fits within this genus and a placement within one of the other genera bears even more contradictions, the same accounts for the genera of Meiomeniidae. We refrain from establishing a new family respectively genus, however, until comparative re-examination of type material (ideally supported by molecular approaches) across all genera is conducted, which allows for critically re-evaluation of the diagnostic characters delineating dondersiid and meiomeniid genera. For example, separate vs. common atrio-buccal openings have been criticized as a highly variable and ambiguous character in other solenogastran lineages (see e.g. Zamarro *et al.*, 2013 on *Pruvotina*) and it seems to be poorly defined among dondersiid genera (for comparison see García-Álvarez & Salvini-Plawen, 2007; Scheltema *et al.*, 2012).

As described above, *D. todtae* sp. nov. can be clearly distinguished from all its congeners by the unique structure of the foregut glands. Moreover, it presents a unique and highly complex scleritome which differs considerably from the seven other known members of the genus *Dondersia*. In contrast to the herein described species, the scleritome of *D. festiva* Hubrecht, 1888 and *D. incali* (Scheltema, 1999) comprises shovel-shaped scales, *D. namibiensis* Scheltema, Schander & Kocot, 2012 more plectron-shaped scales and *D. annulata* Nierstrasz,

1902 is distinguished for minute heart-shaped scales. Almond-shaped to blade-shaped sclerites like they are present in *D. cnidevorans* Salvini-Plawen, 1978, *D. laminata* Salvini-Plawen, 1978 and its putative synonym *D. stylastericola* Salvini-Plawen, 1978 (Thiele, 1913; Salvini-Plawen, 1978; Scheltema *et al.*, 2012) do also not occur in the new species described herein. Scleritome characters were promoted with good cause as major taxonomic characters for Solenogastres (Scheltema & Schander, 2000) and at present, these characters seem to be most promising in species delineation within *Dondersia*. However, the degree of intraspecific variability in the scleritome has not yet been explored and requires to be tested against independent (e.g., molecular) markers for reliable species delineation in the future.

Habitat and distribution

A small percentage of the described solenogaster species lives epizoically on Cnidaria, the majority inhabits the surfaces of soft-sediments and only few lineages are known to lead an infaunal lifestyle (García-Álvarez *et al.*, 2000). So far only representatives of pholidoskepid Lepidomeniidae, Macellomenidae and Meiomeniidae and cavibelonian Simrothiellidae were reported as intersitial (García-Álvarez *et al.*, 2000; Kocot & Todt, 2014). This study is the first record of a mesopsammic representative of the family Dondersiidae. But the technique of extracting molluscs from sand samples does not allow for discrimination of epibenthic vs. infaunal representatives and an interstital lifestyle can only be inferred by either direct observations on e.g. burrowing behaviour (see Kocot & Todt, 2014) or indirectly by certain adaptations to the mesopsammic environment like e.g. the presence of a posterior adhesive gland (see e.g., Morse, 1979 on *Meiomenia swedmarki*). *D. todtae* sp. nov. bears a similar dark-staining gland which was herein interpreted as posterior adhesive gland analogous to the ones present in Meiomeniidae (Figures 2B, C), and we therefore conclude that *D. todtae* sp. nov. is at least temporary mesopsammic in its juvenile phase.

The genus *Dondersia* with its eight valid species (including *D. todtae* sp. nov.) shows a very patchy distribution with representatives in the Indo-Pacific, Eastern Pacific, Northern Atlantic and Mediterranean and three from Antarctic waters (see Scheltema *et al.*, 2012 for summary). This current dispersal pattern is likely due to a much higher still undiscovered global diversity and/or distribution range of known species than previously expected. Due to the insecure systematics of the novel species and unclear sister group relationships, a hypothesis of the directions in colonization of the Azores is impossible at present. The occurrence of two Eastern Atlantic species *Hemimenia atlantica* and *Anamenia gorgonophila* in Azorean waters (Salvini-Plawen, 1972; Salvini-Plawen, 2006) suggests shared faunal elements with the Eastern Atlantic analogous to the biogeographic affinities of the marine gastropod and bivalve fauna of the Azores (Ávila, 2000; Ávila *et al.*, 2009). Under the influence of the major modern current systems, i.e. the Gulf Steam passing the Azores from the Western Atlantic, these distribution patterns seem paradoxic for species dispersing via planktonic larvae. Additional recent colonization of Azorean waters by Solenogastres from the Western Atlantic triggered by these current systems remains to be explored.

Intense sampling for aplacophoran molluscs in shallow and deep waters surrounding the Azores is needed in the future to study their diversity to find out how many more solenogastran species stranded on these lonely islands in the mid Atlantic and to establish solid hypothesis on their biogeographic relationships.

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SHALLOW-WATER INTERSTITIAL MALACOFAUNA OF THE AZORES

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ABSTRACT

This study presents an illustrated checklist on the shallow-water interstitial malacofauna collected during one sampling trip in 2013 and the meiofauna workshop 'Meiozores2019' on São Miguel island, Azores. Aplacophoran Solenogastres were common with 54 specimens preliminarily assigned to 3-4 morphospecies in the order Pholidoskepia, but interstitial gastropods were extremely rare. In total, we only collected 22 specimens belonging to 5 species in the genera *Caecum* (Caecidae, Caenogastropoda), *Rhodope* (Rhodopemorpha, Heterobranchia) and *Hedylopsis* (Acochlidimorpha, Heterobranchia); one specimen of *Pseudovermis* (Nudibranchia, Heterobranchia) was additionally discovered on Santa Maria Island. Caecid microsnails show an association to the fauna of the continental coast of Europe and the Canary Islands, all other encountered microslugs are likely new to science and based on current knowledge can be considered as endemic to the Azores.

RESUMO

Este estudo apresenta uma checklist ilustrada sobre a malacofauna intersticial de águas pouco profundas recolhida durante uma campanha de amostragem feita em 2013 e sobre o workshop de meiofauna 'Meiozores2019' na ilha de São Miguel, Açores. Os Solenogastres Aplacophora foram o grupo mais comum com 54 espécimes atribuídos preliminarmente a 3-4 morfoespécies na Ordem Pholidoskepia, mas os gastrópodes intersticiais foram extremamente raros. No total, foram recolhidos apenas 22 espécimes pertencentes a 5 espécies nos géneros *Caecum* (Caecidae, Caenogastropoda), *Rhodope* (Rhodopemorpha, Heterobranchia) e *Hedylopsis* (Acochlidimorpha, Heterobranchia); adicionalmente foi descoberto um espécime de *Pseudovermis* (Nudibranchia, Heterobranchia) na Ilha de Santa Maria. Os micro-caracóis Caecid apresentam uma associação com a fauna da costa continental da Europa e das Ilhas Canárias, enquanto que todas as outras micro-lesmas que foram encontradas são provavelmente novas para a ciência e com base nos conhecimentos actuais podem ser consideradas endémicas dos Açores.

INTRODUCTION

Isolated in the middle of the Atlantic Ocean, the Azores archipelago attracts evolutionary biologists as a natural laboratory to study colonisation and specifically radiation patterns, which depends on reliable knowledge of the local biodiversity. A recent and ongoing inventory of Azorean biodiversity reports

8047 species to constitute the Azorean fauna and flora with currently 411 endemic species, which are mostly found among terrestrial arthropods and molluscs (Borges *et al.*, 2010). Looking back, considerable effort has been undertaken to document the local malacofauna of the Azores, since the first endemic land snail – the enid *Napaeus pruninus* (Gould, 1847) – was described in the early days of malacological research. By today, we are aware that this isolated archipelago bears a unique terrestrial malacofauna with a high rate of approximately 50 % endemic land snail species, which serve as models for evolutionary theory and island colonisation (Martins, 2011).

In comparison to the efforts undertaken to document the terrestrial fauna, the marine malacofauna of the Azores remained neglected until the 1990's when a series of marine malacological workshops conducted by the Sociedade Afonso Chaves and the University of the Azores systematically started exploration, which presented the major turning-point with 47 % of the total number of known shallow-water molluscs reported in the course of these inventories (Martins, 2009). Currently, there are 231 confirmed species of shallow-water marine molluscs recorded for the Azores including 18 endemic species (Ávila, 2000) and most have been beautifully illustrated in checklists of the workshops (see e.g., Martins *et al.*, 2009; Malaquias *et al.*, 2014). In particular, marine algae revealed high densities of molluscs; even species of microsnails like *Omalogyra*, which is rare to find in other parts of the world, occur in high densities in the Azores. The algae-associated mollusc species diversity also includes several endemic snails, e.g. among Rissoidae, which forms the best-represented family among the macroalgae of the Azores (e.g., Bullock *et al.*, 1990; Azevedo, 1992; Costa & Ávila, 2001). This is in sharp contrast with the relatively low density of molluscs in the sediments reported from Vila Franca do Campo, São Miguel, which is likely caused by the instability of sediment accumulations in these high energy marine areas with great sand mobility present even in rather sheltered lagoons (Wells, 1995). Despite previous reports on the impoverished infauna in marine sediments of the Azores (Wells, 1995; Bamber & Robbins, 2009), recent exploratory sampling targeting for micromolluscs hidden among sand grains added the 232nd species to the local species list of marine molluscs, reporting the first shallow-water Solenogastres from subtidal sediments of São Miguel Island (Klink *et al.*, 2015). Thus, even in light of the expected rarity of interstitial micromolluscs, resulting from the unfortunate combination of their biological attributes (e.g., low reproductive output and limited dispersal abilities) and the geographic and geological attributes of the archipelago (e.g., isolation from mainland, high energy beaches, rather young geological age of approx. 8 my), single findings might present valuable pieces in the puzzle towards understanding the evolution and biogeographic patterns in the different groups of interstitial micromolluscs.

Micromolluscs which permanently inhabit the interstices among sand grains, and are thus also referred to as 'mesopsammic', comprise representatives of aplacophoran molluscs (Solenogastres and Caudofoveata) (see Bergmeier & Jörger, 2020)

and representatives among eleven families of microsnails and -slugs with aberrant body plans and several biological peculiarities, challenging malacologists and meiobenthologists alike, e.g., most prominent the turbellarian-like Rhodopemorpha lacking all typical molluscan features in adult forms (see Jörger *et al.*, 2020 for an overview). So far only the family Caecidae (Caenogastropoda) – truncatelloid microsnails with unique tubular adult shells adapted to moving in interstitial habitats – have been reported with a total of four species from the Azores (Pizzini & Nofroni, 2001); none of the other ten families of interstitial gastropods, all classified among Heterobranchia, have been documented so far. We now took a first step towards filling this gap of knowledge of the Azorean malacofauna searching sediment samples for these rare and enigmatic micromolluscs. In the present study, we summarize the results of two sampling events, which took place alongside the World Congress for Malacology hosted by the University of the Azores in 2013 and a subsequent meiofauna workshop "*Meiozores2019*" in July 2019 both on São Miguel, Azores. We provide an illustrated checklist of the encountered diversity of micromolluscs including characterizations of the external morphology of the Azorean morphotypes and preliminary evaluations on the rate of endemism and potential routes of colonization.

MATERIAL AND METHODS

Sampling

We sampled intertidal and subtidal marine sediments around of São Miguel, Azores during the World Congress of Malacology in 2013 and during the meiofauna workshop "Meiozores2019 – exploring the diversity of the marine meiofauna of the Azores" in 2019 (see Figure 1). Additional samples were provided by Marco Curini-Galletti and Jon Norenburg from an exploratory sampling trip to Santa Maria Island directly after the Meiozores2019 workshop. Table 1 lists the respective stations of the two sampling events which we successfully sampled for interstitial molluscs. Intertidal sands and gravel were collected by hand off the beach, subtidal sediments were collected via snorkelling or SCUBA diving, always by sampling the upper oxygenated layer of the sediments only (see Jörger *et al.*, this volume for details).

Extraction techniques

Sediment samples were processed after 1-2 days resting period in which the sampling jars/ buckets remained untouched under dark and cool conditions with a minimum of seawater coverage, to force the molluscs to migrate to the sand surface. After this accumulation of meiofauna in the top layer, specimens were extracted via a standard isotonic $MgCl_2$-seawater decantation technique and collected in sieves with a 63-100 µm mesh size (for details see Jörger *et al.* (2014)). All extracts were thoroughly searched for the presence of interstitial molluscs under dissecting scopes.

Morphological investigation

Light-microscopy and fixation – All encountered molluscs were identified alive

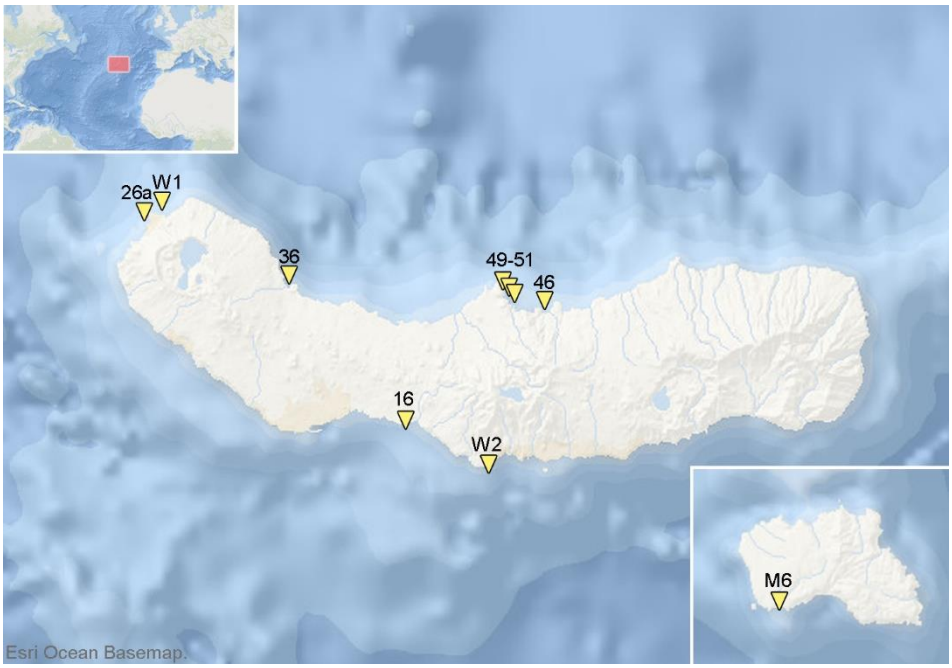

FIGURE 1: Map of São Miguel island (Santa Maria Island on lower right insert) indicating the sampling localities during the WCM2019 (W1 and W2) and the Meiozores2019 workshop (see Table 1 for details).

to the best taxonomic level possible and photographed individually under the light microscope. When appropriate, we carefully squeezed anaesthetized animals under a coverslip to analyse main taxonomic features and hard parts, such as sclerites, scales, spicules, and the radula. After photo documentation, solenogaster specimens of sufficient size were cut in half using a sterile razor blade. The anterior part was fixed in 4-5 % trialdehyde (10 ml 16 % PFA, 3 ml 25 % glutaraldehyde, 30 ml 0.1 M cacodylate buffer) for histological and ultrastructural investigations. The posterior part was preserved in 96 % EtOH or RNAlater for scanning electron microscopy (SEM) and subsequent molecular barcoding. Solenogastres too small to be cut in half were fixed in either 96 % EtOH or RNAlater with specimens in EtOH being suitable for SEM and subsequent barcoding and specimens in RNAlater being suitable for barcoding or transcriptome sequencing. Gastropods were either fixed (after prior relaxation with $MgCl_2$) in 96 % EtOH for molecular analyses or in 4-5 % trialdehyde, 4 % glutaraldehyde or 4 % formaldehyde in seawater for morphological analyses.

Scanning electron microscopy – The scleritome of the Solenogastres and the microstructure of the shells of the encountered Caecidae were analysed via

TABLE 1. Stations (**Sta**) on São Miguel (W1 and W2 during the WCM 2019, the remaining stations during Meiozores2019) and Santa Maria (only station M6), where we discovered interstitial molluscs.

Sta	Date	Locality	Habitat description	Depth (m)	Latitude	Longitude
W1	24.7.13	Ponta dos Mosteiros	shallow subtidal, in rock pools on rocky beach, gravel	0.2-0.5	37.9	-25.816666
W2	27.7.13	Off Caloura, dive spot 'three houses'	subtidal (SCUBA), coarse sand and shell gravel between rocks	26.0	37.706883	-25.497267
16	16.7.19	Piscinas Lagoa	subtidal (SCUBA), large sand plains with interspersed rocks, sand with ripple ridges from waves, medium-coarse sand	18.0	37.74	-25.57495
26a	16.7.19	Porto dos Mosteiros	subtidal (snorkelling), off rocky beach, gravel	2.0	37.893701	-25.822045
36	19.7.19	Capelas	subtidal (SCUBA), sandplain, seaward side of large boulders, medium-coarse sand	16.0	37.843563	-25.687351
46	20.7.19	Praia dos Moinhos	subtidal (snorkelling), small sand patches on rocky bottom	3.0	37.8242	-25.445744
49	22.7.19	Riberinha	subtidal (SCUBA), boulder field on sandy bottom, deposits of coarse sand	3.0	37.836074	-25.484111
50	22.7.19	Riberinha	subtidal (SCUBA), boulder field on sandy bottom, deposits of coarse sand	8.5	37.836171	-25.483941
51	22.7.19	Riberinha	subtidal (SCUBA), boulder field on sandy bottom, deposits of coarse sand	8.8	37.83636	-25.483757
M6	31.7.19	Farol da Maia, off the Lighthouse	channel among rocks, medium-coarse shelly sand	10	36.945338	-25.146045

SEM. Solenogastres of the Meiozores2019 workshop dedicated to DNA barcoding were brought back to the University of Alabama for imaging and extraction. Using a workflow combining external morphological characterization and barcoding (Bergmeier *et al.*, 2016), specimens were air-dried in a drop of 95 % EtOH and placed on a SEM stub and imaged on a Phenom Pro table-top SEM. After imaging, specimens were taken from the SEM stub and placed into 200 µL of TL Buffer (Omega Bio-Tek) and stored at -80 °C until DNA extraction. The material of the collected Caecidae was scanned and barcoded at the University of Munich/ Bavarian State Collection of Zoology. Microscopic debris on the shells was manually removed using an eyelash and the shells were subsequently rinsed in 96 % EtOH. Caecidae were dried by evaporation of the ethanol at 45 °C and were then transferred onto SEM stubs with self-adhesive carbon stickers. The snails were

oriented onto their lateral sides to allow for analyses of their mucros (= evagination of the closing septum at the distal end of the tubular, adult shell) and apertures. A sputter coater Polaron SC510 was used to coat samples with gold for 2.5 minutes with a current of 20 mA in a 10-2 mb vacuum argon atmosphere. SEM-micrographs were taken with a LEO SEM at a voltage of 15 kV.

Molecular barcoding

In support of our morphological identification, DNA was extracted from representatives of all encountered morphospecies. For Solenogastres, DNA was extracted from whole or partial specimens using the Omega Bio-Tek EZNA Microelute Genomic DNA kit. 16S rRNA barcodes were amplified using AMRESCO Hot Start PCR master mix using solenogaster specific primers (see Bergmeier *et al*., 2017). Additionally, the 16S sequence of *Dondersia todtae* was extracted from its transcriptome (KK unpublished data). Purified PCR products were sent to Genewiz in South Plainfield, New Jersey for Sanger sequencing.

Snails previously studied via SEM were mechanically crushed and DNA was extracted combining lysis via 2-mercaptoethanol in CTAB buffer, chloroform-isoamyl precipitation and recovery via columns with silica membrane (see Knebelsberger & Stöger (2012)). The DNA was eluted two times with 25 µl aliquots of pre-heated elution buffer to gain high yield. We amplified two mitochondrial markers partially via PCR: cytochrome *c* oxidase subunit I (COI) and 16S rRNA using standard primers (Folmer *et al*., 1994; Klussmann-Kolb *et al*., 2008) and the Phire™ polymerase (Thermo Fisher Scientific Inc., Waltham, USA) with the following protocol: 98 °C – 30 sec, 38 x (98 °C – 15 sec, 45-48 °C – 10 sec, 72 °C – 20-25 sec) 72 °C – 1 min. Successful PCR products were cleaned using a spin column purification kit (Zymo Research™, Irvine, USA). The cleaned PCR products were cycle sequenced on an ABI 3730 48 capillary sequencer (Applied Biosystems, Foster City, USA) using Big Dye 3.1™ (Thermo Fisher Scientific Inc., Waltham, USA) at the sequencing service of the Biocenter/ Ludwig-Maximilians-University Munich (http://www.gi.bio.lmu.de/sequencing).

Sequence analyses

Sequences were edited using Geneious Prime (vers. 11.02011, Bio-matters, Ltd., Auckland, New Zealand). We used the online web service BLAST to compare all sequences to the available public database NCBI GenBank (http://ncbi.nlm.nih.gov/genbank) and evaluated pairwise distances among our material and towards the identified closest relatives. We analysed the novel data from snails and slugs collected on the Azores within global datasets (own partially unpublished data) of the respective families or orders for a preliminary phylogenetic placement. In-depth phylogenetic analyses of the encountered material based on multiple markers are beyond the scope of the present study. COI and 16S sequences were aligned via MUSCLE as implemented in Geneious Prime with the standard settings, concatenated and analysed with the implemented RAxML-add on.

RESULTS

Overview on the interstitial malacofauna of the Azores

Overall, interstitial molluscs were rare and only encountered in 7 out of the totally 61 sampled stations of the Meiozores2019 workshop (see Jörger et al., this volume). Especially, interstitial gastropods were extremely scarce and, in total, we only collected 23 specimens belonging to six species, when summarizing the results of sampling 2013 and 2019. In comparison, meiofaunal Solenogastres were encountered more frequently with 54 specimens in total, preliminarily assigned to four morphospecies. However, only three of the encountered morphospecies are supported via preliminary molecular data, two might present an interesting case of intraspecific variation in the scleritome (see remarks below).

TAXONOMY

Class: SOLENOGASTRES Gegenbaur, 1878
Order: PHOLIDOSKEPIA Salvini-Plawen, 1978
Family: Dondersiidae, Simroth 1893

Dondersia todtae Klink, Bergmeier, Neusser & Jörger, 2015

Morphology: between 0.7 and 2 mm (when fully extended while crawling) long (Figure 2A). Five different types of sclerites (Figure 2B-E). Body covered mainly by variations of sclerite type 2 (ovate to round, with or without rim; occasionally with notch at the distal end - type 4), with interspersed elongated sclerites (type 3). Type 1 only found around the head region. Lanceolate sclerites (type 5) found along entire body. Stereocirri surrounding the vestibular cavity (Figure 2A), dorsoterminal sense organ and a pedal commissural sac were observed in several specimens via light microscopy. When observed under incident light, the foregut glands appear light blue.

Molecular barcodes: GenBank accession number 16S sequence. Based on our still preliminary analyses, the 16S sequences of *D. todtae* only shows 11.9–7.35 % genetic divergence to the co-occurring and externally highly similar second morphotype, below listed as Dondersiidae sp.

Remarks: During the present study, we found 23 individuals of *D. todtae*. Present at five stations, mostly in low numbers between two and four, but 11 animals were sampled at station W2 in 2013 (see Table 2). Collected from medium to coarse sands, depth range between 3 m and 26 m. At all four stations, they co-occur with a second morphotype characterized below. In-depth molecular analyses are still pending, but lack of genetic distance on the molecular barcoding marker already indicates that both morphotypes likely belong to the same species: *D. todtae*. The original description of *D. todtae* is based on juveniles only (collected at the type locality station W2), our recollection now allows us to supplement the description of the adult gonopericardial system. Further phylogenetic analyses in future research will hopefully allow for reliable classification of this unique meiofaunal species among pholidoskepid Solenogastres.

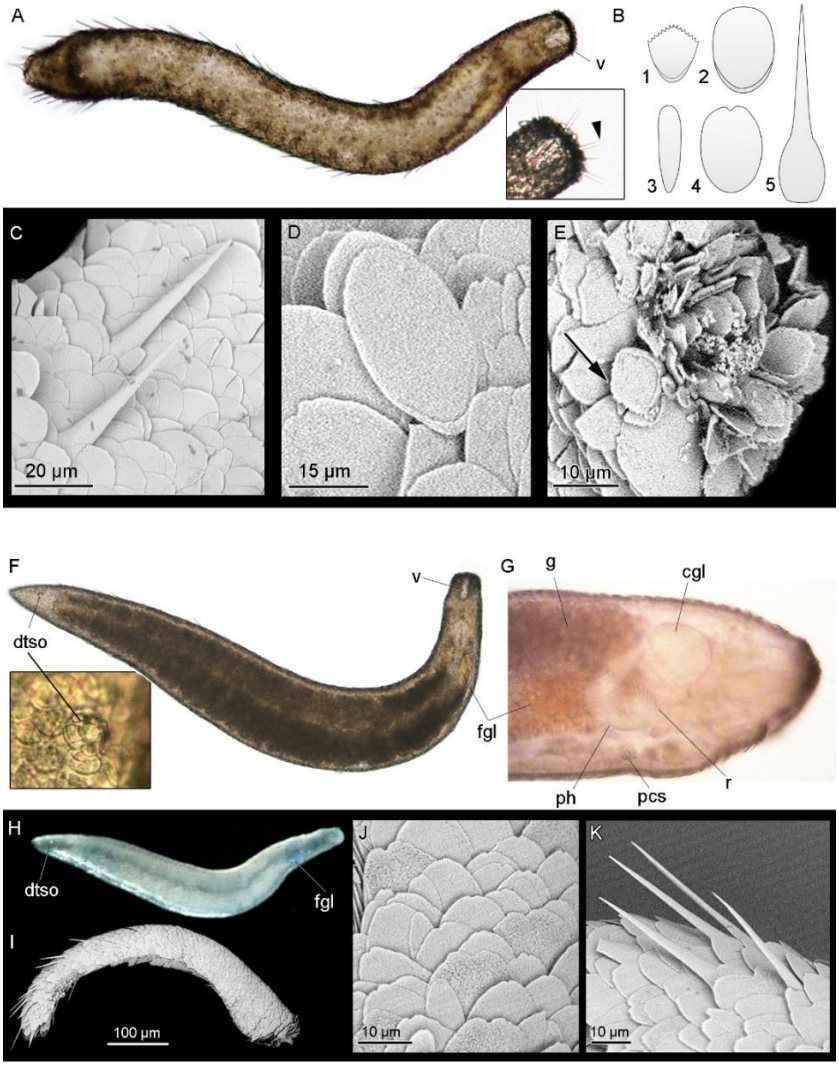

FIGURE 2. Interstitial Dondersiidae (Pholidoskepia, Solenogastres) from the Azores (heads to the right). **A-E**, *Dondersia todtae*. **A**, Light-microscope (**LM**) image of *D. todtae*. Arrowhead points to stereocirri surrounding the vestibular cavity; **B**, Sclerites of *D. todtae*. **C-E**: SEM micrographs showing details of the scleritome. Arrow in E points to a small scale with serrated proximal end found around the head area. **F-K**, Dondersiidae sp. **F**, LM image of Dondersiidae sp; **G**, Head region with structures of the digestive tract and nervous system; **H**, Incident light **LM** image. Note blue-coloured foregut glands. **I-K**, SEM micrographs including details of the scleritome. Abbreviations: **cgl**, cerebral ganglion; **dtso**, dorsoterminal sense organ; **fgl**, foregut glands; **g**, gut; **pcs**, pedal commissural sac; **ph**, pharynx; **r**, radula; **v**, vestibulum.

TABLE 2. Overview of interstitial species of shallow-water molluscs and number of specimens collected at the respective sampling sites on São Miguel (W1 and W2 during the WCM 2019, the remaining stations during Meiozores2019) and Santa Maria (only station M6).

Station / *Taxon*	W1	W2	16	26a	36	46	49	50	51	M6
Solenogastres:										
Dondersia todtae		11	2			3		2	4	
Dondersiidae sp.			8			10	1	2	4	2
Meiomeniidae sp.										3
Pholidoskepia sp.			1					1		
Gastropoda: Caenogastropoda										
Caecum wayae	1			1						
Caecum gofasi	3			1						
Gastropoda: Heterobranchia										
Rhodope sp.	1			1	1					
Pseudovermis sp.										1
Hedylopsis sp. 1	5								2	
Hedylopsis sp. 2		6								

Dondersiidae sp.

Morphology: up to approx. 1.5 mm when fully extended (Figure 2F). Sclerites similar to *D. todtae* (Figure 2J, K), but lanceolate sclerite type 5 only found in posterior region of body (Figure 2 I) and in lower numbers as in *D. todtae*. Overall appearance smoother than that of *D. todtae*. Stereocirri, dorsoterminal sense organ (Figure 2F, H), and pedal commissural sac (Figure 2G) observed in several specimens. Radula most likely monostichous, as several rows of triangular teeth (see Figure 2G, in lateral view). Foregut glands fluorescent blue under incident light (Figure 2H).

Molecular barcodes: GenBank accession number 16S sequence

Remarks: Most abundant solenogaster morphotype with 26 encountered specimens. Found at overall six stations, on São Miguel and Santa Maria. Between one and 10 individuals per station (see Table 2). Collected from medium to coarse sands, depth range between 3 and 16 m. Apart from *Dondersia todtae*, there are currently only two more interstitial species among Dondersiidae. Sclerites of these two *Micromenia* Leloup, 1948 species are different (drop- and rod-shaped) to the dondersiid reported herein. However, our preliminary 16S barcoding data does not show a considerable genetic distance to *D. todtae* described above, thus this morphotype likely presents variability in the scleritome of *D. todtae*, which is a highly valuable insight and first warning to not delineate solenogaster species based on slight differences in the scleritome only.

Family: Meiomeniidae Salvini-Plawen, 1985

Meiomeniidae sp.

Morphology: approx. up to 1.5 mm (Figure 3A), with eversed vestibulum (Figure 3A, B). Three types of sclerites: body covered in ovate adpressed scales (Figure 3D, G1) with lanceolate elements along the lateral sides of the body (Figure 3A, E, G2). Asymmetrical

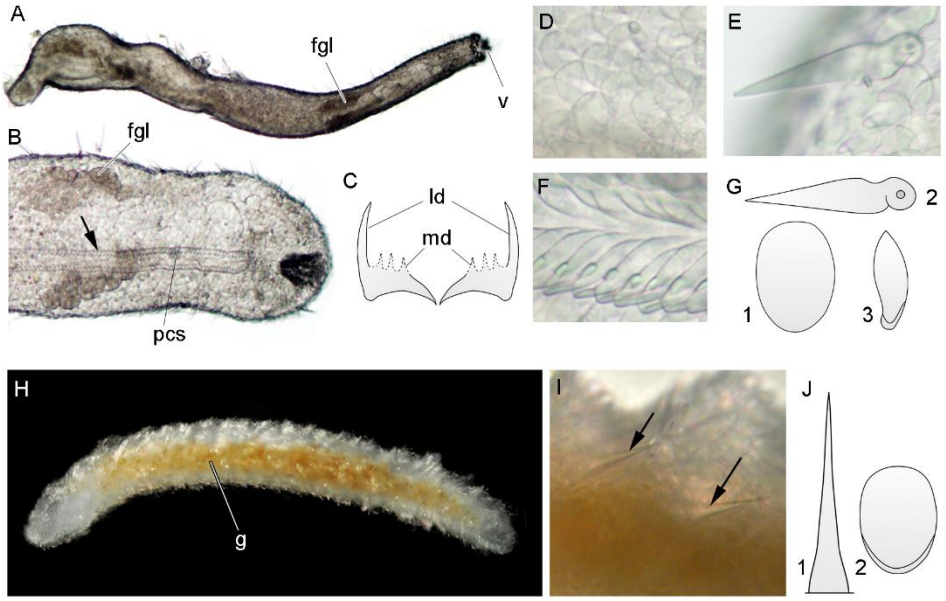

FIGURE 3. Interstitial Meiomeniidae sp. (**A-G**, LM photos courtesy of Jon Norenburg) and Pholidoskepia sp. (**H - J**) from the Azores. **A**, LM image of Meiomeniidae sp. (head to the right); **B**, Head region, ventral view; the arrow marks the foot groove; **C**, Distichous radula, as interpreted from LM whole mounts; the number of median denticles is unknown (dotted line); **D**, Ovate body scales; **E**, Lanceolate sclerite; **F**, Pedal scales bordering foot groove; **G**, Main sclerites of Meiomeniidae sp. as derived from LM images; **H**, LM image of Pholidoskepia sp. (head to the left); **I**, Lanceolate sclerites (arrow); **J**, Sclerites of Pholidoskepia sp., as derived from LM whole mounts. Abbreviations: **fgl**, foregut glands; **g**, gut; **pcs**, pedal commissural sac; **v**, vestibulum.

pedal scales (Figure 3F, G3). Presence/ absence of stereocirri and dorsoterminal sense organ unknown; pedal commissural sac present (Figure 3B). Radula distichous, each tooth with a large lateral denticle, number of median denticles cannot be ascertained from LM images (see Figure 3C). Molecular barcodes are still pending for further analyses.

Remarks: Only three individuals found at a single station on Santa Maria (Table 2). Collected from medium to coarse, shelly sand at 10 m depth. Meiomeniidae consists of two genera (*Meioherpia* Salvini-Plawen, 1985 and *Meiomenia* Morse, 1979) with two species each, and so far only interstitial lineages have been described for this family. *Meioherpia atlantica* Salvini-Plawen & Sterrer, 1985 and *M. stygalis* Salvini-Plawen & Sterrer, 1985 have both been collected from the shallow waters of Bermuda, while *M. atlantica* and *Meiomenia arenicola* Salvini-Plawen & Sterrer, 1985 in Salvini-Plawen (1985) have also been reported from the Western Atlantic coast of North Carolina and Florida (*M. arenicola* only). *Meiomenia swedmarki* Morse, 1979 is

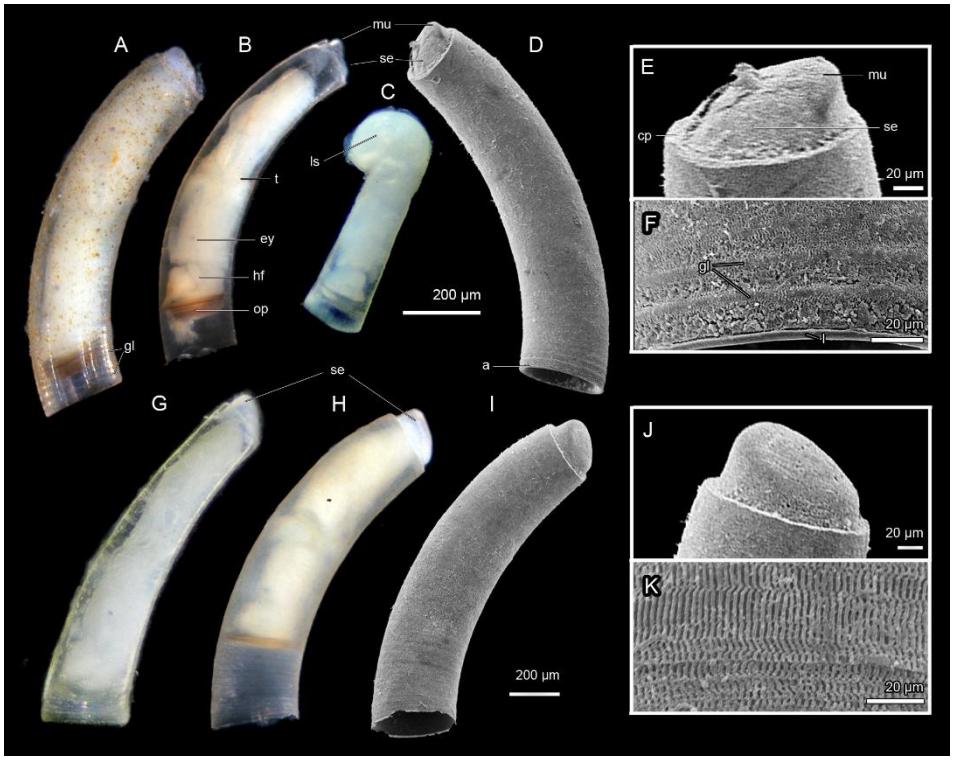

FIGURE 4. *Caecum gofasi* and *Caecum wayae* highlighting morphology and important shell features. **A-F**: *C. gofasi*. **A**, Specimen ZSM-Mol-20130966a; **B**, Juvenile specimen ZSM-Mol-20130971a; **C**, Juvenile specimen with the larval shell still attached, station 26a, ZSM Mol 20202410; **D**, SEM scan; **E**, SEM close-up of septum and mucro; **F**, SEM of microsculpture of specimen ZSM-Mol-20130966a; **G-K**, *C. wayae*. **G**, Juvenile specimen, station 26a, ZSM Mol 20202409; **H-K**, Juvenile specimen close to an adult, ZSM-Mol-20130971c; **I**, SEM scan; **J**, SEM of septum and aperture; **K**, SEM close-up of microsculpture. Abbreviations: **a**, aperture; **cp**, cutting-plane; **ey**, eye; **gl**, growth-lines; **hf**, retracted head and foot; **l**, lip; **ls**, larval shell; **mu**, mucro; **op**, operculum; **se**, septum; **t**, tube.

only known from the Northeast Pacific (San Juan Island, Washington). Known anatomical differences between the genera are based on the location of the mouth opening (within the vestibulum in *Meiomenia*, posterior to the vestibulum in *Meioherpia*) and potentially the presence of copulatory stylets in *Meiomenia* (but see Morse, 1994) with scleritomes varying only slightly (see figures 7 to 10 in Salvini-Plawen (1985)). Meiomeniidae sp. from the Azores cannot be unambiguously assigned to one of the two genera based on its hard parts: the three types of sclerites of Meiomeniidae sp. (see Figure 3G) resemble body sclerites of *Meiomenia arenicola* (see Figure 7B, C, D in Salvini-Plawen & Sterrer (1985)), while the radula is similar to the

Meioherpia radula due to the presence of a large lateral denticle (see figures. 9H, 10J in Salvini-Plawen (1985)).

Family: indet.

Pholidoskepia sp.

Morphology: approx. 1 mm in size, conspicuous orange colouration due to gut contents (Figure 3 H). Scleritome combination of ovate scales with rimmed base (Figure 3 J2) and interspersed, short lanceolate elements (Figure 3 J1). Rough appearance. Presence/ absence of dorsoterminal sense organ, pedal commissural sac, and radula unknown.

Remarks: rare in samples investigated during this study; only two individuals from two stations (Table 2) at São Miguel. Found in medium-coarse to coarse sands; depth range between 8.5 m and 18 m. With a combination of solid lanceolate elements and scales (Figure 4I, J), the scleritome is typical for Pholidoskepia, but reliable classification among the families of the order is impossible without further microanatomical and histological analyses. No externally similar morphospecies known from shallow-water mesopsammic environments, thus, likely new to science.

Class: GASTROPODA Cuvier, 1797
Subclass: CAENOGASTROPODA
Family: Caecidae Gray, 1850

Caecum gofasi Pizzini & Nofroni, 2001
Pizzini & Nofrini, 2001, p. 19, 20 figures 1-7

Morphology: Shell translucent, glossy. Body white (Figure 4A adult; B, C juveniles). Operculum brownish. Adult size 1.5 mm long, 0.2 mm wide. Tube curved regularly and evenly wide over whole length or slightly cylindrical. Aperture straight, lip present in adults (Figure 4D, F). Septum slightly domed. Mucro triangular, appears more roundish from lateral view. (Figure 4E). No shell sculpture present, appears completely smooth using light microscopy except fine growth lines close to the aperture (Figure 4A, B, F). Microsculpture consisting of fine longitudinal wavy striae (Figure 4F).

Molecular barcodes: COI/ 16S sequences [GenBank accession numbers]

Remarks: Rare in sand samples investigated in the present study (four specimens, depth range 0.1–2.0 m). Previously reported as common in deeper samples (57–171 m) (Martins *et al.*, 2009), considered as endemic to the Azores. This is the first description of living specimens. The original description (Pizzini & Nofroni, 2001) is based on empty shells solely, which are described as opaque of dirty white colour in contrast to our living specimens which are all highly translucent. Slight differences in shell morphology, especially for shape of septum and mucro can consequently be attributed to the "worn off" condition of the dead shells. Morphologically with similarities to *C. swinneni* from the Canary Islands and the widespread European *C. clarkii* (also reported from the Azores, see Discussion).

Caecum wayae Pizzini & Nofroni, 2001
Pizzini & Nofroni (2001) p. 21-23 figures 8-14

Morphology: Shell translucent, slightly opaque whitish when older (Figure 4G

juvenile and H nearly fully grown). Size 1.2 mm long, 0.3 mm wide. Tube gradually narrowing towards posterior end in juvenile (Figure 4G) but evenly wide in adult form (Figure 4H). Septum and mucro indistinguishable, one merging in the other. Both first protruding straight from the cutting plane then wedge-shaped (Figure 4J). Aperture fringed in all investigated specimen. Shell surface smooth, no sculpture visible apart from extremely fine growth lines. Microstructure consists of flattened furrows, shifted against each other at irregular distances (crossing growth lines).

Molecular barcodes: COI/ 16S sequences [GenBank accession numbers]

Remarks: Rare in sand samples, only two individuals encountered at one locality (depth range low intertidal 0.5 m – shallow subtidal 2 m). Previously reported as common in deeper samples (57-171 m) (Martins *et al.*, 2009). Both specimens are late juvenile stages, based on the still fringed aperture (Figure 4H-K). Pizzini & Nofroni (2001) further figure the adult aperture as simple without remarkable lip. We can confirm the wax-like appearance of the shells; however, our specimens did not show any brown colour patterns as described in Pizzini & Nofroni (2001). These differences in colouration might be attributed to different sandy habitats.

Two additional species belonging to the family Caecidae have been previously recorded from the Azores, but not recollected during the present survey: *C. clarkii* Carpenter, 1859 and *C. armoricum* de Folin, 1869, both with putative wide distribution ranges along the European Coast and recorded also from the Canary Islands (see Discussion).

Subclass: HETEROBRANCHIA
Infraclass: "LOWER HETEROBRANCHIA"
Order: RHODOPEMORPHA
Family: Rhodopidae Ihering, 1876
Genus: *Rhodope* Kölliker, 1847

Rhodope sp.

Morphology: Body vermiform lacking any body appendages and variable in shape depending on the degree of contraction. Body length approx. 1 mm, body colour whitish-translucent, the present species lacking any colouration (i.e., orange, red or purple bars) as frequently found in *Rhodope*. Eyes visible dorsally and laterally (see Figure 5A, B). Spicules thick (length-width ration approximately 5:1), monoaxone, of 40-70 µm length with shallow central indentation on convex side (Figure 5C); evenly distributed throughout body.

Molecular barcodes: COI/ 16S sequences [GenBank accession numbers]

Remarks: Rare, only three specimens collected on three sites on São Miguel (see Table 2) during both sampling events. Shallow subtidal (St. W1 and 26a) to subtidal (16 m, St. 36) – conspecificity of morphologically similar shallow and deeper specimens needs to be confirmed via molecular data in future studies. This species of *Rhodope* morphologically resembles other colourless *Rhodope*, such as *R. marcusi* Salvini-Plawen, 1991 from Southern Brazil (Western Atlantic), *R. placozophagus* Cuervo-González, 2017 from the Gulf of Mexico (Western Atlantic) and a

so far undescribed species from the Eastern Atlantic Coast of Brittany (Salvini-Plawen, 1991; Cuervo-González, 2017; own unpublished data). Our specimens can be morphologically distinguished from *R. placozophagus*, however, based on distinct spicules, which are considerably thinner in the latter (length-width ratio approximately 10:1). Moreover, our specimens only show a genetic similarity of < 80 % with *R. marcusi* on COI. This is similar to the genetic similarity with Mediterranean *R. veranii* Kölliker, 1847, which can be morphologically clearly distinguished based on the orange colouration. Thus, we consider this Azorean species new to science awaiting formal description.

Superorder: NUDIPLEURA
Order: NUDIBRANCHIA
Family: Pseudovermidae Thiele, 1931
Genus: *Pseudovermis* Pereyaslavtzeva, 1891

Pseudovermis sp.

Morphology: Body vermiform, whitish translucent with seven rather short digitiform cerata in dorsolateral position (Figure 5D), each containing one cnidosac filled with cnidocysts of the prey. First pair of cerata situated vis-à-vis at the level of the stomach, cerata more posteriorly are alternating. Head 'acorn'-shaped lacking appendages. Pharynx with a pair of cuticular jaws and the radula with a rhachidian tooth bearing approx. 5-7 lateral denticles (Figure 5E). No eyes visible, one statocyst on each pedal ganglion (Figure 5F). The only collected specimen is an adult with male organs fully developed; no mature oocytes present yet. Stomach and digestive gland filled with nematocytes, indicating recent feeding.

Remarks: Rare, only one specimen collected on Santa Maria, none found on São Miguel. This Azorean *Pseudovermis* externally closely resembles other '*Pseudovermis papillifer*-forms' (i.e. the morphotype of *Pseudovermis* which bears digitiform cerata), which is globally distributed and includes *P. papillifer* Kowalevsky, 1901 from the Black Sea and the Mediterranean and *P. artabrensis* Urgorri, Cobo & Besteiro, 1991 from the European Atlantic Coast, *P. salamandrops* Marcus, 1953 from the Western Atlantic, *P. soleatus* Salvini-Plawen & Rao, 1973 and *P. indicus* Salvini-Plawen & Rao, 1973 from the Indian Ocean, *P. chinensis* Hughes, 1991, *P. japonicus* Hamatani & Nunomura, 1973, *P. hancocki* Challis, 1969, and *P. mortoni* Challis, 1969 from the Pacific (see Jörger *et al.*, 2020). Molecular analyses are needed for species delimitation. Our initial barcoding indicates no close genetic relationship with the Western Atlantic forms, but this should be repeated due to the low quality of the sequence. Also, comparative molecular data from European species are lacking. Based on previously known restricted distribution ranges of *Pseudovermis*, the specimen encountered on Santa Maria likely represents a species new to science.

Superorder: ACOCHLIDIMORPHA
Order: HEDYLOPSACEA Wawra, 1987
Family: Hedylopsidae Odhner, 1952
Genus: *Hedylopsis* Thiele, 1931

Hedylopsis sp. 1 (Figure 6A, B, C)

Morphology: Body divided into an anterior head-foot complex and an elongated sac-like visceral hump, within which the head can be retracted. Body size 3-4 mm. Body colour whitish-translucent with yellowish-

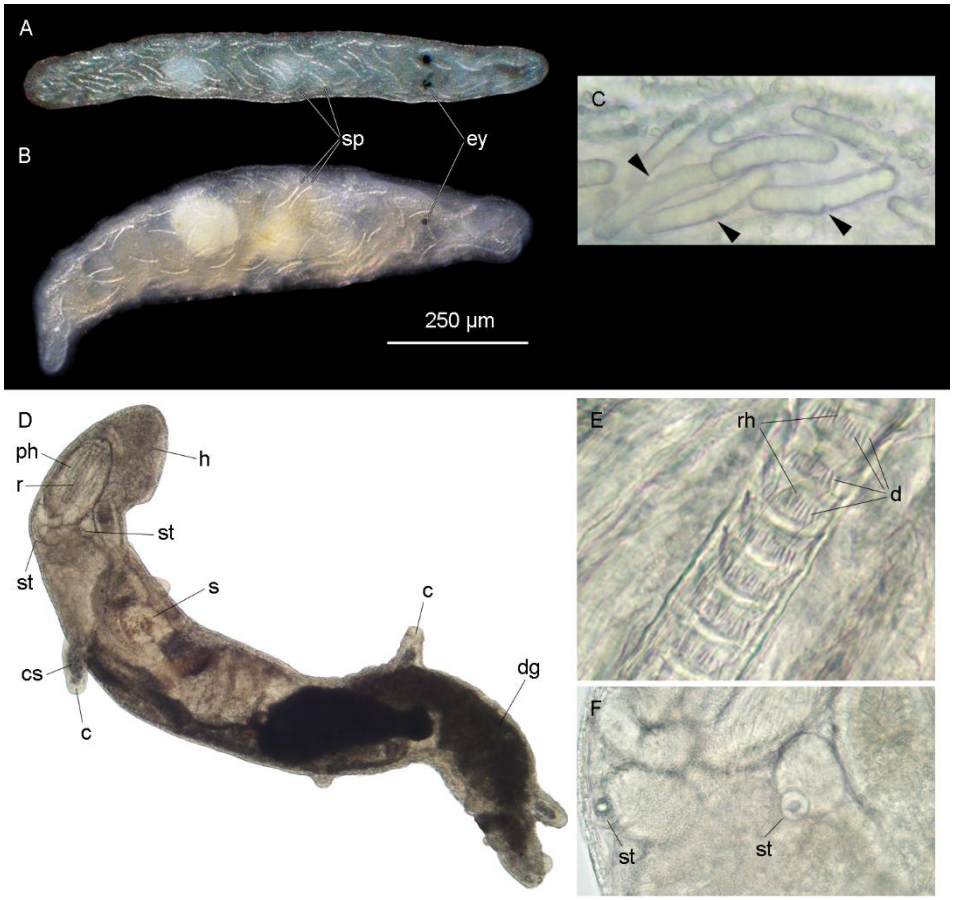

FIGURE 5. Light-microscope images of living *Rhodope* sp. (**A-C**) and *Pseudovermis* sp. (**D-F**). **A**, *Rhodope* sp., station 36, ZSM Mol 20202397 (dorsal view); **B**, *Rhodope* sp., station 26a, ZSM Mol 20202396 (left view); **C**, Monoaxone spicules of *Rhodope* sp. shown in **A**, between 40 and 70 µm, note notch on the convex side (arrowheads); **D**, *Pseudovermis* sp., DNA extracted at Smithsonian National Museum of Natural History (ventrolateral view); **E**, Radula with one rhachidian tooth and lateral denticles; **F**, Statocysts attached to the pedal ganglion. Abbreviations: **cs**, cnidosac; **c**, ceras; **d**, denticle; **dg**, digestive gland; **ey**, eye; **h**, head; **ph**, pharynx; **r**, radula; **rh**, rhachidian tooth; **s**, stomach; **sp**, spicule; **st**, statocyst. Scale bar valid for **A** and **B**.

brownish digestive gland shining through the epidermis. Two pairs of head appendages: broad, flattened labial tentacles and thinner, cylindrical rhinophores. Paired eyes situated far posterior to rhinophores approx. half-way along with the head-foot complex. Foot slightly broader than head. Free posterior

part of the foot tapering to the end and extending to three-fourths of the visceral hump. Monoaxone spicules distributed all over the body, in the visceral hump spicules are more densely arranged and larger.

Molecular barcodes: COI/ 16S sequences [GenBank accession numbers]

Remarks: In comparison to the mitochondrial COI and 16S rRNA gene markers, the intertidal (sp. 1) and subtidal (sp. 2, see below) species of *Hedylopsis* collected from the Azores show a remarkable genetic distance of 16 % and 19 % (on COI and 16S rRNA, respectively). These genetic distances together with minor morphological differences and differences in the choice of habitat support our suspicion that we are dealing with two distinct species. In preliminary phylogenetic analyses, intertidal *Hedylopsis* sp. 1 from the Azores clusters with other *Hedylopsis* from temperate and tropical zones; however, it is not sister to the Eastern Atlantic and Mediterranean *H. spiculifera* (Kowalevsky, 1901), but more closely related to an undescribed *Hedylopsis* sp. from South-Pacific Moorea and *H. ballantinei* Sommerfeld & Schrödl, 2005 from the Red Sea. Based on current knowledge *Hedylopsis* sp. 1 represents a species new to science endemic to the Azores.

Hedylopsis sp. 2
(Figure 6D, E)

Morphology: Similar to *Hedylopsis* sp. 1 characterized above, but more robust in appearance and oral tentacles larger and more lobe-like (Figure 6D). Body length 4-5 mm. Foot almost twice as broad as head. Eyes larger and positioned right posterior to rhinophores. The head-foot complex can be retracted completely into the visceral hump (no tentacles visible).

Molecular barcodes: COI/ 16S sequences [GenBank accession numbers]

Remarks: In preliminary phylogenetic analyses, *Hedylopsis* sp. 2 is the sister taxon to all other *Hedylopsis* species or alternatively at the base of a higher-rank clade uniting various Hedylopsacea with mesopsammic or benthic lifestyle. Based on the available sequence data and slight differences in external morphology, it can be considered a species new to science at the present state of knowledge endemic to the Azores. Due to its potentially basal phylogenetic position, it is of major interest for understanding the evolution of acochlidimorph Hedylopsacea which involves several habitat shifts including next to marine mesopsammic species in the genus *Hedylopsis*, marine and brackish Pseudunelidae, freshwater benthic Acochlidiidae and interstitial Tantulidae and (semi-) terrestrial Aitengidae (see e.g., Neusser *et al.*, 2011a, b). Marine benthic Hedylopsacea is only known from the deep-sea Bathyhedylidae (Neusser *et al.*, 2016). With its broad foot and large eyes, *Hedylopsis* sp. 2 might not be truly interstitial but rather live epibenthically on the surface of the sediments and maybe only occasionally hiding between the interstices of sand grains, potentially representing a missing link to study the adaptations in shifting from an epibenthic to infaunal lifestyle (or vice versa).

FIGURE 6. External morphology of living acochlidimorph specimens. **A**. *Hedylopsis* sp. 1, station W1, ZSM 20130969 or 20130982 (juvenile, dorsal view); **B**, Same as **A** (ventral view); **C**, *Hedylopsis* sp. 1, station 51 (adult, dorsal view, photo courtesy of Duarte Frade); **D**, *Hedylopsis* sp. 2, station W2, ZSM 20130993 (adult, dorsal view); **E**, Same as **D** (ventral view). Abbreviations: **dg**, digestive gland; **ey**, eye; **f**, foot**; lt**, labial tentacle; **rh**, rhinophore; **sp**, spicule; **vh**, visceral hump. Scale bars: **A, B**, 0.5 mm; **C, D, E**, 1 mm.

DISCUSSION

Interstitial malacofauna – faunal overlap and degree of endemism

Interstitial molluscs are usually considered to have rather restricted distribution ranges commonly attributed to their (assumed) poor dispersal abilities with no free-swimming larval stage or only few veliger larvae (Jörger *et al.*, 2020). These larvae usually do not enter the water column but remain in interstitial waters between sand grains (Swedmark, 1968). In regions with a continuous coastline (such as e.g., the European Atlantic Coast, Mediterranean and the Black Sea) some interstitial gastropods show wide distribution ranges, which was confirmed via molecular data (see e.g., Eder *et al.*, 2011). But no data is available so far on these micromolluscs sharing a common gene pool among populations on Oceanic Islands with source populations on the continental coast. Based on morphologically-driven hypotheses, mesopsammic microsnails in the family Caecidae include two species – *Caecum armoricum* and *C. clarkii* – which are recorded from the Canary Islands, the Azores, as well as the Mediterranean and North-Atlantic Coast. However, the conspecificity of the distant populations of "*C. clarkii*" is debated based on morphological data (van Aartsen & Fehr-de Wal, 1975; Pizzini & Nofroni, 2001) and molecular markers are still pending to confirm the hypothesis. In comparison to heterobranch microslugs, Caecidae likely have the best dispersal abilities with larval shells of some species indicating a planktotrophic lifestyle, which might allow for these wide-ranged distributions including oceanic archipelagos, but this generalization is tenuous as there are also direct developers known among Caecidae (Bandel, 1996). Both potentially endemic *Caecum* species known from the Azores – *C. gofasi* and *C. wayae* – show high morphological similarities with species described from the Canary Islands (*C. swinneni* Nofroni, Pizzini & Oliverio, 1997 and *C. pollicare* Carpenter, 1859 respectively) (Nofroni *et al.*, 1997; Pizzini & Nofroni, 2001). In contrast, preliminary analyses of the Azorean *C. gofasi* and *C. wayae* within a global phylogeny of the family Caecidae place them each in different sister group relationships with Western Atlantic species (Egger *et al.*, 2020, own unpublished data). But these preliminary results are likely biased by the underrepresentation of European species (and lack of Canary Islands species) and await further re-analyses based on an expanded taxon sampling. At present, the faunal overlap in Caecidae between the Azores and the Canary Islands, as well as the North-Eastern Atlantic, suggests a colonisation route from the European mainland via the Canary Islands as a most likely scenario based on the available data. This route of colonisation might be counterintuitive at first sight, given the overall eastward oceanic currents in the North Atlantic predicting the American East Coast and Western Atlantic as a source for colonisation of the Azores by marine organisms (Morton & Britton, 2000), but it is in line with the common pattern in marine molluscs (e.g., Gofas, 1990; Ávila, 2000; Ávila *et al.*, 2009; Martins *et al.*, 2009). Before the closure of the Isthmus of Panama and the formation of

the Gulf Stream, the ocean currents likely had an east to west direction, which might explain the dominance of European species in the marine environment in the Azores (Ávila *et al.*, 2009).

No faunal overlap can be reported for all heterobranch slugs encountered during the two sampling trips. All three genera (*Rhodope*, *Pseudovermis* and *Hedylopsis*) show a global distribution in temperate and tropical zones. The encountered species are likely new to science with phylogenetic relationships still requesting thorough analyses based on global datasets before biogeographic scenarios can be reliably proposed. Nevertheless, some observations on the faunal composition on the Azores are striking in comparison to other regions: the most common interstitial microslugs (microhedylid acochlidimorphs) of the continental Atlantic Coasts (Western as well as Eastern Atlantic) have not been encountered during our surveys on the Azores and were already extremely rare or also absent on the Canary Islands (see Martínez *et al.*, 2019). Especially remarkable is the absence of the microhedylid acochlidimorphs *Pontohedyle*, *Microhedyle* and *Asperspina*, which are usually frequently encountered or even locally common on both sides of the Atlantic (Jörger *et al.*, 2008, 2012; Neusser *et al.*, 2009; Eder *et al.*, 2011) and also the cephalaspidean Philinoglossidae (Salvini-Plawen, 1973). This encountered species composition cannot be explained by a gradient of poor vs. slightly better dispersal abilities: *Rhodope* encountered on the Azores presumably lacks a free-swimming dispersal stage and is thus likely limited to rafting, phoresy or human-aided transport. *Hedylopsis* spp. collected in this study can be considered to have a similar larval ecology as its acochlidimorph relatives, which are more common on the continental coast, but absent (i.e., not yet recorded) on the Azores. We believe that this absence in our samples is likely related to the lack of suitable, well-sorted sediments of biogenic calcareous origin. The latter might be of higher importance for truly permanently mesopsammic forms highly adapted to the infaunal lifestyle than for *Hedylopsis* which still shows typical features of an epibenthic lifestyle. The finding of *Pseudovermis* on Santa Maria during a short exploratory sampling trip by Marco Curini-Galetti and Jon Norenburg indicates that our sampling focused on São Miguel might not be representative for the entire archipelago. Suitable sands on Santa Maria should be targeted in future studies followed by exploratory trips to the western islands of the Azores.

Interestingly, of all interstitial micromolluscs, the one with presumably the poorest dispersal abilities successfully colonized São Miguel island. *Rhodope* lacks a free-swimming veliger larva and develops directly with a 'crawl-away larva' (Riedl, 1960) and one would thus expect them to be less widespread in contrast to species with planktonic larval stages. However, *Rhodope* has been previously found on oceanic islands like Madeira, the Galapagos archipelago and Hawaii (Graff, 1883; Haszprunar & Heß, 2005; Brenzinger *et al.*, 2011; own unpublished data). This paradox has been reported previously also from littoral snails when comparing the distribution ranges of closely related species with brooding and direct development vs. planktonic larvae, in

which the former colonised the isolated island of Rockall (Johannesson, 1988). *Rhodope* frequently occurs associated with algae. The transport of specimens by rafting on marine macroalgae might be rare and dangerous for a minute slug. However, once a founder group with direct development (such as *Rhodope*) reaches an Oceanic archipelago, it has a higher chance to locally establish a population in comparison to species with veliger larvae living in the mesopsammon. The latter might be washed away in unstable sediments of the Azores thus hampering successful colonisation. This might also explain at least partially the high density of microsnails on littoral algae in the Azores.

Our knowledge on the global distribution of shallow-water aplacophoran molluscs is still highly fragmentary and lacks a comprehensive phylogenetic framework, which currently hampers discussions on the origin and relationships of the encountered Solenogastres on São Miguel. In contrast to interstitial gastropods, mesopsammic Solenogastres were not strikingly rare in our samples and their species diversity and overall abundance show similar levels when comparing our own field experience of Azores and Hawaii with sampling trips along the Eastern and Western Atlantic mainland. This allows for two cautious hypotheses: 1) Solenogastres successfully inhabit sediment of volcanic origin and are less restricted to well-sorted calcareous sediments, than mesopsammic gastropods which are seldomly found in the former. 2) Solenogastres either have advantageous reproductive patterns to colonize isolated islands (some might be brooders with direct development, see discussion above) or have source populations in deeper waters adapted to life in the deep-sea and distribution along the deep-sea floor. Especially in Solenogastres, which show the highest diversity in the deep-sea, the deep-sea floor might function as an evolutionary reservoir from which newly forming islands and shallow-water habitats are colonised added by the characteristic upwelling zones on Oceanic Islands.

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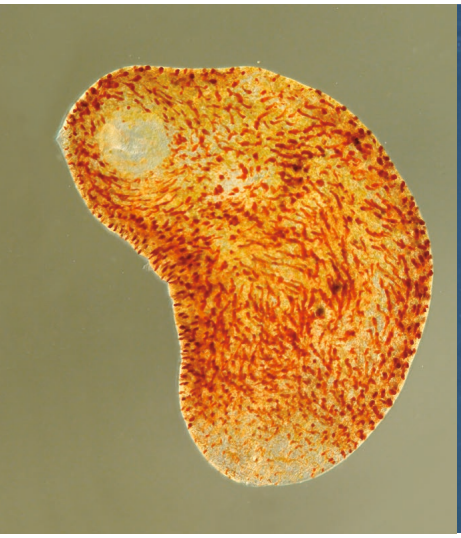

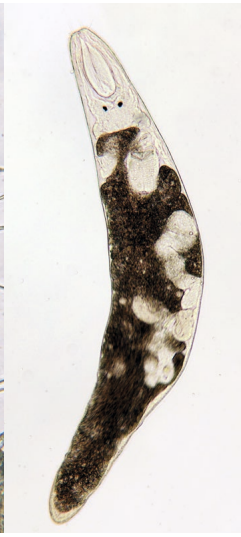

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16 Aplacophoran molluscs: Solenogastres and Caudofoveata

Franziska S. Bergmeier[1] **and Katharina M. Jörger**[1]

Introduction

Aplacophoran molluscs traditionally unite the two exclusively marine classes of worm-shaped, shell-less molluscs known as Caudofoveata (= Chaetodermomorpha) and Solenogastres (= Neomeniomorpha). Based on current knowledge, most of the approx. 125 species of Caudofoveata and approx. 300 species of Solenogastres occur in the deep sea beyond the 200 m shelf area, but some lineages are also found in shallow waters. In general, aplacophoran molluscs range in size from less than one mm to 40 cm; the majority of species is smaller than a few mm. For the present chapter, we focus on the shallow-water lineages reported to occur in sediments of the continental shelf above 100 m of depth. Due to their flexible worm-shaped body, they easily pass a 1 mm mesh size, we thus also included species up to a body length of 2–3 mm described in the course of marine meiofauna surveys. This restricts the records of meiofaunal shallow-water species of Caudofoveata to five species within the family Prochaetodermatidae. Some representatives of the two other chaetodermomorph families (Chaetodermatidae and Limifossoridae) can also occur shallower than 100 m of depth, but usually exceed meiofaunal size limits as adults (but see note on the chaetodermatid *Falcidens sterreri* (Salvini-Plawen, 1967) below). In Solenogastres, 23 species in nine families fall within the definition above (excluding minute, but epizoic (i. e. living on cnidarians) shallow-water Solenogastres). The diversity of aplacophoran molluscs is poorly explored, however, and new lineages, even at higher taxonomic ranks, are to be expected in future surveys.

Aplacophoran molluscs have a vermiform body, which is covered by a cuticle bearing various types of calcareous sclerites (spicules and scales). The two aplacophoran classes can be distinguished externally by a narrow ventral foot (located in a central foot groove that emerges from an anterior pedal pit and extends over the entire body length), which is present in Solenogastres (Fig. 16.1), but absent in Caudofoveata. Caudofoveates are characterized externally by an oral shield (= head shield) (see Fig. 16.2A), lacking in Solenogastres, the latter bear a rounded head occasionally equipped with sensory bristles and an eversible vestibulum. The body of Caudofoveata can show subdivisions into three main body regions (anterium, trunk and posterium, see Fig. 16.2B). The aplacophoran body has a roundish or laterally slightly compressed diameter (sometimes with a dorsal keel); the posterior end can be also roundish, pointed or truncated. In prochaetodermatid Caudofoveata it is formed by a narrow shank with a terminal knob fringed by elongated sclerites (Fig. 16.2B). Some lineages of aplacophoran molluscs bear a dorsoterminal sense organ (DTSO), which is externally visible as a small pit or protuberance lined with scales differing from the remaining body scleritome. The radula can be present or absent in Solenogastres and show different configurations (in meiofaunal forms: monostichous, biserial or distichous, see Fig. 16.3). In Caudofoveata it is usually distichous or reduced to only a single pair of tweezer-like teeth attached to a central cone-shaped basis; prochaetodermatid Caudofoveata additionally bear a pair of spatulate jaws (Fig. 16.2C). The midgut of Caudofoveata is divided into a dorsal duct ("stomach") and a ventral midgut sac ("digestive diverticulum"), while the midgut of Solenogastres is undivided, apart from lateral constrictions which occur in some lineages. In the posterior mantle cavity, caudofoveates bear a pair of ctenidia, which is absent in Solenogastres. Larger forms of the latter have respiratory folds in the mantle cavity, meiofaunal forms lack specialized respiratory organs. Solenogastres are hermaphrodites with internal fertilization, while caudofoveates have separate sexes and reproduc-

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Marine meiofauna, the community of smallest animals in marine sediments, is a fascinating and important part of the marine ecosystem. In this book, 53 authors introduce the 32 animal groups that occur in the meiofauna. Chapters contain information on where and how to sample meiofaunal animals as well as how to identify them. Keys help in the identification.

This book complements existing literature on meiofauna and may serve as a fascinating introduction into meiofaunal taxa as well as a practical guide to work with meiofauna in the field or in the laboratory.

The rich illustration of the book and the expertise of specialists make this book suitable for beginners as well as experienced researchers on meiofauna.

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Contents

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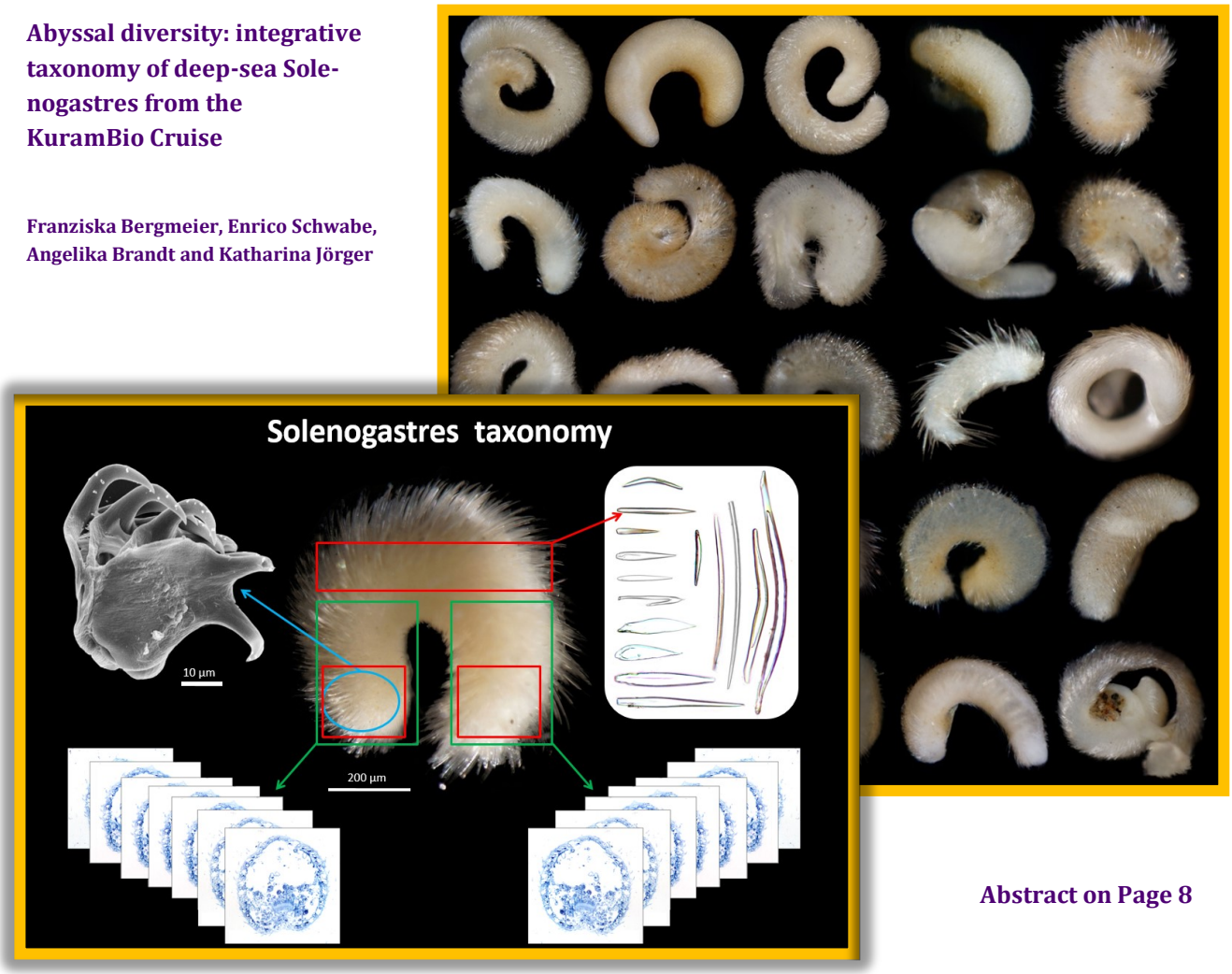
Abyssal diversity: integrative taxonomy of deep-sea Solenogastres from the KuramBio Cruise

Franziska Bergmeier, Enrico Schwabe, Angelika Brandt and Katharina Jörger



Abstract on Page 8

The Malacological Society of London was founded in 1893 and registered as a charity in 1978
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RESEARCH GRANT REPORT

Getting the most out of minute singletons: molecular data from SEM-samples in Solenogastres (Mollusca)

Franziska Bergmeier[1], Roland Melzer[2], Gerhard Haszprunar[1,2], Katharina Jörger[1,2]

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Introduction

Identification of Solenogastres, a small clade of marine aplacophoran molluscs, requires time-consuming investigations of various character sets (i.e. scleritome, radula, and histology of the foregut glands and reproductive system), resulting in a broad neglect of these species in biodiversity surveys (Todt 2013). Molecular barcodes can facilitate re-identification of Solenogastres in future research, but need to be unequivocally connected to actual taxonomic names. Ideally, a single specimen can be subdivided for sacrificing e.g., the mid-body region with few morphological characters for molecular analyses. However, many Solenogastres are minute (i.e., <2mm), which hampers division for molecular and morphological approaches. Moreover, combining characters investigated on multiple individuals might create chimera as cryptic species co-occurri(Bergmeier *et al.* 2016). The present project aims to combine examination via scanning electron microscopy (SEM) from all body regions of singletons with subsequent DNA-extraction and amplification of molecular barcodes. We test different drying and extraction protocols to establish a workflow of this technique for molluscs, which has so far only been successfully applied to tiny insects (i.e., Thysanoptera; Kumar *et al.* 2014).

Material and Methods

We used three different morphospecies of cavibelonian and pholidoskepian Solenogastres, each with multiple individuals (nine per species, body sizes between 0.7 mm and 2.5 mm), for a critical evaluation of the various methodologies. Specimens were collected between 2009 and 2012 and were fixed and stored in 96% ethanol.
We combined three different SEM-drying protocols (critical point (CP), hexamethyldisilazane (HMDS) and 100% ethanol (EtOH)) with three standard DNA-extraction methods (spin columns (SC), CTAB buffer and a 'quick-and-dirty' approach via boiling in extraction buffer (EB) adapted from Kumar *et al*. (2014)) on the 27 specimens, so that each drying protocol was combined with each extraction method on one specimen of each morphospecies. We then extracted DNA from the SEM-samples and compared the DNA concentration and COI sequence quality. For a schematic depiction of the workflow, see Fig. 1.

Step 1: Drying of specimens for SEM

(i) Critical Point: Specimens rehydrated in 80%, 70% ethanol, 15 min each; followed by dehydration in graded acetone series (70%, 80%, 90%, 100%, 15 min each; 2x 100%, 30 min). Dried in a Baltec CPD 030 (Leica Microsystems) in CO_2;

(ii) HMDS: Specimens transferred to 100% EtOH, exchanged twice (15 min each); exchanged for 1:3 solution of HMDS and 100% EtOH followed by 1:2 solution of HMDS and 100% EtOH (30 min each); exchanged for 100% HMDS and slowly evaporated overnight;

(iii) 100% EtOH: Specimens transferred to 100% EtOH, exchanged twice for 100% dehydrated EtOH (15 min each);

Step 2: Mounting for 360°-SEM

We mounted the dried specimens on 'carousels' to enable 360° SEM-investigations of the scleritome characters from all body regions. We used colloidal silver G 302 (Plano GmbH) to mount them at the tips of short snips of tungsten or silver wire (Fig. 2b) and stuck these into lumps of Leit-C-Plast plastic conductive carbon cement (Neubauer Chemikalien), placed on self-adhesive carbon stickers on the SEM-stubs. Afterwards we coated them with gold (Fig. 2c) in an Argon atmosphere for 240 sec in a Polaron sputter coater (GaLa Gabler Labor Instrumente Handels GmbH).

Step 3: Documentation of scleritome via SEM

Specimens were investigated under a LEO 1430 VP SEM (10-15kV) (Zeiss). If necessary, specimens were repositioned by turning the wires in a different angle in the central plastic conductive carbon cement.

Step 4: DNA-extraction of SEM samples

We transferred the sputtered specimens into tubes, and ground them in the respective buffers using plastic pestles. We applied three protocols of DNA-extractions:

- **Spin column**: using the DNeasy Blood & Tissue Kit (QIAGEN), following the manufacturer's protocol.
- **CTAB**: standard precipitation method using a CTAB buffer and ß-mercaptoethanol followed by chloroform and isoamyl alcohol.
- **Extraction buffer**: boiling in DNA lysis buffer containing KCl, Tris-HCL, Tween20 and NP40 (see and Dickey *et al.* 2012 and Kumar et al. 2014). After DNA-extraction we measured the DNA concentration using a Qubit Reader 2.0 (ThermoFischer Scientific).

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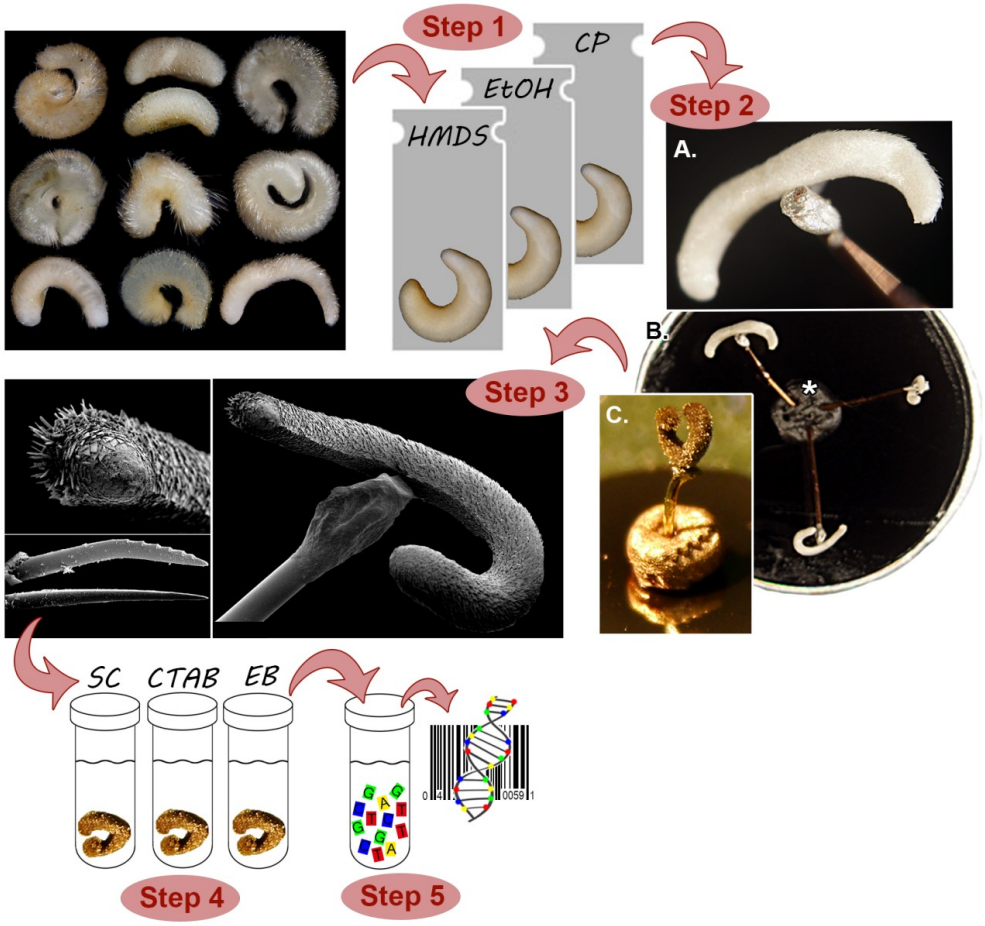

Fig. 1: Workflow on how to combine SEM with molecular barcoding. A. Specimen mounted on tungsten wire using colloidal silver. B. 'Solenogaster carousel' with three specimens, asterisk marks plastic conductive carbon cement. C. Single specimen mounted for rSEM, after sputter coating with gold.

Step 5: Barcode amplification

We amplified cytochrome *c* oxidase subunit I (COI) with the HCO-LCO primer set (Folmer *et al.* 1994) using the Phire Hot Start II polymerase (ThermoFischer Scientific) in PCR protocols with varying annealing temperatures (initial step at 98°C for 30 sec, (denaturation at 98°C for 5 sec, annealing at 51°C/52°C for 5 sec, elongation at 72°C for 20 sec) x 35, final elongation at 72°C for 1 min, cooling at 4°C). We purified PCR products using the DNA purification kit NucleoSpin Gel and PCR Clean-Up (Machery-Nagel) following the manufacturer's protocol. Cleaned PCR products were sent off for cycle sequencing (Big Dye 3.1) and sequencing on an ABI 3730 capillar sequencer by the Genomic Service Unit of the Ludwig-Maximilians-University, Munich.

Results and Discussion

SEM

The 'solenogaster carousels' are advantageous for regular mounting: the carousel allows turning of specimens without damaging them, thus enabling coverage of all taxonomically important body regions of a single individual (i.e., spicules of mouth and atrium, body scleritome, foot groove, dorso-terminal sense organ, copulatory or abdominal spicules) and the production of rotational scanning electron micrographs (rSEM, see Cheung *et al.* 2013). Due to the carousel construction we were partially struggling with strong charging artifacts, and 6 of 27 specimens were lost during preparation and SEM examination. At least for Solenogastres with a scaly scleritome, direct mounting on self-adhesive carbon stickers with subsequent flipping was a more secure and faster method.

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Molecular data

After SEM investigations, the remaining 21 specimens were used for testing the three different extraction methods. We boiled 9 specimens in extraction buffer following the protocol of Kumar *et al.* (2014), which resulted in the consistently highest DNA concentrations of the three methods ranging from 7.54 ng/µl to 45.8 ng/µl. CTAB extraction on five specimens yielded DNA concentrations between <0.5 ng/ml (in one specimen) and 61.6 ng/µl. Spin column extraction was used on seven specimens and resulted in DNA concentrations of <0.5 ng/µl (in three specimens) to 10.7 ng/µl. Despite the high DNA measurements for the EB-protocol,we were unable to amplify COI from any of the EB-samples, indicating that this 'quick-and-dirty' approach successfully used for insects investigations might not lead to clean enough DNA for amplification in molluscs. From the five CTAB-extracted specimens, four COI barcodes were successfully sequenced. Of the seven spin column extracted specimens, only three yielded COI sequences (failure corresponds to the ones with lowest DNA concentration <0.5 ng/µl, indicating that DNA-extraction might have failed in these cases). The comparably low success rate in COI amplification corresponds to our experience when handling non-SEM treated Solenogastres and therefore rather points to a general problem with COI primers and PCR protocols rather than an influence from the SEM investigation.

Conclusion and outlook

Due to the low numbers of Solenogastres available to the present project, our initial exploration of methods does not allow for a sound statistical evaluation. Nevertheless, our study provides some experience to other researchers aiming to combine molecular approaches with SEM examination in minute molluscs:

We were able to extract DNA and amplify COI independently from the applied drying protocol, suggesting that critical point drying (with previous dehydration in acetone) and drying via HMDS and 100% EtOH are equally suitable for subsequent molecular analyses. However, simple EtOH evaporation caused strong shrinkage artifacts, thus it should be avoided in cases like Solenogastres, which have a thin cuticle.

The EB-protocol established for insects (Kumar *et al.* 2014) failed on our material, whereas standard CTAB precipitation and spin-column extraction both successfully yielded DNA with subsequent amplification success. Spin-columns had a higher total failure in DNA-extraction that CTAB but more data is needed to compare both methods reliably.

In conclusion, generating molecular sequence data from specimens previously dried, sputter-coated with gold and examined via SEM can be conducted without any adjustment of standard lab. routines. We are currently working on a broader application of this method to Solenogastres collected during the Kurile-Kamchatka Biodiversity (KuramBio) Expedition, where numerous specimens were sampled from abyssal depths. Currently 18 morphospecies have been identified using SEM, including many lineages new to science and only represented by singletons. Using CTAB extractions, DNA has been successfully extracted from most individuals to provide molecular barcodes for easier identification in future research.

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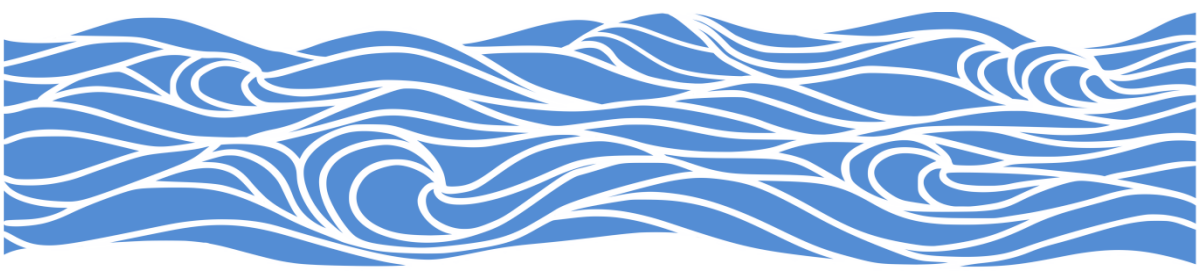
Part II. Integrative taxonomy to address diversity, biogeography and systematics of deep-sea Solenogastres from the Northwest Pacific



Chapter 6. Bergmeier FS, Brandt A., Schwabe E & Jörger KM (2017) **Abyssal Solenogastres (Mollusca, Aplacophora) from the Northwest Pacific: Scratching the Surface of Deep-Sea Diversity Using Integrative Taxonomy**. *Frontiers in Marine Science*, 4, 410: 1-22.

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Abyssal Solenogastres (Mollusca, Aplacophora) from the Northwest Pacific: Scratching the Surface of Deep-Sea Diversity Using Integrative Taxonomy

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Solenogastres (Aplacophora) is a small clade of marine, shell-less worm-molluscs with close to 300 valid species. Their distribution ranges across all oceans, and whereas the vast majority of species has been collected and described from the continental shelf and slope, only few species are known from depths below 4,000 m. Following traditional taxonomy, identification of specimens to species level is complex and time-consuming and requires detailed investigations of morphology and anatomy—often resulting in the exclusion of the clade in biodiversity or biogeographic studies. During the KuramBio expedition (Kuril-Kamchatka Biodiversity Studies) to the abyssal plain of the Northwest Pacific and the Kuril-Kamchatka Trench, 33 solenogaster specimens were sampled from 4,830 m to 5,397 m. Within this study we present an efficient workflow to address solenogaster diversity, even when confronted with a high degree of singletons and minute body sizes, hampering the use of single individuals for multiple morphological and molecular approaches. We combine analyses of external characters and scleritome with molecular barcoding based on a self-designed solenogaster specific set of mitochondrial primers. Overall we were able to delineate at least 19 solenogaster lineages and identify 15 species to family level and beyond. Based on our approach we identified three key lineages from the two regionally most species-rich families (Acanthomeniidae and Pruvotinidae) for deeper taxonomic investigations and describe the novel abyssal species *Amboherpia abyssokurilensis* sp. nov. (Cavibelonia, Acanthomeniidae) using microanatomical 3D-reconstructions. Our study more than doubles the previous records of solenogaster species from the Northwest Pacific and its marginal seas. Almost all lineages are reported for the first time from the region of the (Northwest) Pacific, vastly expanding distribution ranges of the respective clades. Moreover it doubles the number of Solenogastres collected from abyssal depths on a global scale and underlines the lack of exploratory α-diversity work in the abyssal zone for reliable species estimates in marine biodiversity.

Keywords: biodiversity, molecular barcoding, species delineation, Aculifera, Neomeniomorpha, worm-mollusc

INTRODUCTION

Two-thirds of the world's surface are constituted by the deep sea, providing one of the largest biomes of our planet (Danovaro et al., 2010). A plethora of different habitats and ecosystems such as plains, canyons, seamounts, hydrothermal vents, and cold seeps harbors an unknown magnitude of diversity, as large parts of the oceans' depths are still unexplored (Ramirez-Llodra et al., 2010). Efforts to increase knowledge on the deep sea—in terms of ecology, biodiversity and bathymetry—have triggered numerous expeditions to explore its inhabitants and ecosystems and consequently led to the discovery of hundreds of new species (e.g., Brandt et al., 2007). These discoveries emphasize the richness of yet unknown diversity in the deep sea and the need for basic α-taxonomic work to resolve what has been identified as the key shortfall of current biodiversity data. The lack of species taxonomy ("Linnean Shortfall" of biodiversity knowledge; Hortal et al., 2015), describing biodiversity at species level, affects and limits all approaches to understand distribution, abundance and evolutionary processes of deep-sea fauna.

Many paradigms in deep-sea research (e.g., on diversity gradients, bathymetric ranges, dispersal barriers, or the connectivity of populations) have been proposed based on a limited number of organisms linked to their specific biological traits and their general applicability still needs to be tested when broader data sets are available (see, e.g., Gage and Tyler, 1991; Rex et al., 2005, 2006; McClain and Hardy, 2010; Rex and Etter, 2010). In fact, recent studies have revealed taxon-specific patterns of bathymetric and geographic distribution, therein highlighting the potential pitfalls of generalizing single-taxon studies (McClain and Hardy, 2010). Comparative data from multiple taxonomic groups is needed to evaluate the role of specific biological traits and different evolutionary histories.

Frequently, available data on biodiversity is taxonomically biased toward larger, easily identifiable and well-known clades (Hortal et al., 2015) and with regard to molluscan deep-sea fauna research has largely focused on gastropods and bivalves (see, e.g., Rex, 1973; Bouchet and Warén, 1980; Zardus et al., 2006; Allen, 2008; Schrödl et al., 2011; Brault et al., 2013; Jörger et al., 2014). In faunistic analyses and biodiversity studies of benthic deep-sea communities the two classes of worm-molluscs are often lumped together in the generalizing supraclass term "Aplacophora" (see, e.g., Girard et al., 2016; Gutt et al., 2016; Román et al., 2016). The diversity within Caudofoveata (or Chaetodermomorpha) and Solenogastres (or Neomeniomorpha) is largely ignored, presenting a significant gap in detailed and comparative biodiversity information. To date, nearly 300 species of the small clade of Solenogastres are formally described, but estimates propose that its true diversity is at least 10-fold and many more novel species are routinely collected in the framework of e.g., biodiversity or monitoring surveys (Glaubrecht et al., 2005; Todt, 2013).

The lack of α-taxonomic work on Solenogastres is partially owed to their time-consuming and challenging taxonomic assignment, which requires studying a complex set of characters via various methods to reach suprageneric identification (Todt, 2013). Externally, Solenogastres can be differentiated based on aragonitic sclerites covering their entire body surface. This "scleritome" is unique to aplacophorans and in Solenogastres it consists of combinations of hollow or solid needles and scales of diverse shapes, arranged in single or several layers. Further important taxonomic characters are the radula and several anatomical and histological features of the digestive system (especially the foregut glands) and the gonopericardial system (Salvini-Plawen, 1978a,b; García-Álvarez and Salvini-Plawen, 2007). Identification to family level and beyond thus requires external investigations via light (LM) or scanning electron microscopy (SEM) and at least partial histological sectioning of the anterior and posterior part of the animal. The difficulty of solenogaster taxonomy is augmented by (1) the rarity of many lineages, (2) the small size of the majority of species, hampering the extraction of all necessary taxonomic characters from single individuals and (3) the reported co-occurrence of externally cryptic species (Bergmeier et al., 2016a). As a first step toward integrating solenogaster diversity in overall biodiversity assessments of deep-sea fauna we need an efficient and fast workflow able to reliably address species-level diversity collected in a certain region. This shall guarantee that the entire discovered diversity is initially characterized, providing the baseline for full taxonomic descriptions and further ecological or evolutionary approaches.

As in most marine invertebrates, solenogaster taxonomy and systematics show a strong geographical bias in biodiversity assessment because of historical collecting efforts. Due to the focus of taxonomic research on atlantic, mediterranean and antarctic Solenogastres almost two thirds of the known species diversity is described from these regions (see e.g., work by Salvini-Plawen, 1978a,b; García-Álvarez et al., 2014; Pedrouzo et al., 2014). In terms of their bathymetric distribution the majority of species has been described from the lower region of the continental shelf, around 22% (64 species) from bathyal depths and only 8% (18 species) are described from abyssal plains (extending between 4,000 and 6,000 m, as defined by Gage and Tyler, 1991). Currently, the majority of abyssal species belongs to the order Cavibelonia and is classified within five families, but two novel, undescribed species of the family Dondersiidae (order Pholidoskepia) have been recently reported from similar depths (Cobo et al., 2013). These species have all been described from either the Southern Atlantic (e.g., Gil-Mansilla et al., 2008) or Antarctica (Salvini-Plawen, 1978a,b). Only a single species has been collected below 4,000 m in the Pacific: *Pachymenia abyssorum* Heath, 1911 (Amphimeniidae) off the Californian coast (USA) in the beginning of the twentieth century.

The aim of the present study is to characterize the morphological and molecular diversity of abyssal Solenogastres, which were collected during the German-Russian joint "KuramBio (Kuril-Kamchatka Biodiversity Studies) Expedition" on board of R/V *Sonne* (SO 223) to the Northwest Pacific (NWP). The sampled abyssal Northwest Pacific Plain biogeographically belongs to the North Pacific Abyssal Province (Watling et al., 2013) and is situated in an eutrophic area of increased primary production, with uniform bathymetry and a mean depth of 5,000 m (Zenkevitch, 1963). To the west of the abyssal plain lies the Kuril-Kamchatka Trench, reaching down to 9,500 m.

The cold East Kamchatka Current, originating from the Bering Sea, influences especially the northern part of the trench and permeates into the semi-isolated Sea of Okhotsk (Qiu, 2001), which is connected to the open Northwest Pacific and its abyssal plain via two bathyal straits (Krusenstern and Bussol Strait). Following the routes of the Russian R/V *Vityaz* cruises from the mid twentieth century (Zenkevitch, 1963; Belyaev, 1983) the "KuramBio" expedition applied standardized deployments of state-of-the-art sampling gear to investigate 12 abyssal stations on the NWP Plain and close to the Kuril-Kamchatka Trench (see **Figure 1** for a station map of the expedition) (Brandt and Malyutina, 2012).

In summary, the present study has a two-fold focus: (1) develop an efficient workflow applicable also by non-specialists to initially characterize solenogaster diversity to make this neglected molluscan clade easier accessible to deep-sea research and (2) provide first insights into the still completely unknown abyssal solenogaster fauna from the NWP by using the proposed workflow. Despite being confronted with a sample dominated by singletons and hampered by incomplete datasets, we characterize the full diversity of abyssal Solenogastres collected during the KuramBio expedition. We identify three key lineages from the regionally most diverse families for further in depth taxonomic analyses and describe the novel abyssal species *Amboherpia abyssokurilensis* sp. nov. from the deep-sea family Acanthomeniidae based on 3D-microanatomical data.

MATERIALS AND METHODS

Sampling and Fixation

The KuramBio expedition on board of R/V *Sonne* set out to explore the benthos of the slope of the Kuril-Kamchatka Trench and the adjacent abyssal plain in the Northwest Pacific, east of the Kuril Island chain, at overall 12 stations (see **Figure 1**, **Table 1**). This study is based on the Solenogastres collected with a camera-equipped epibenthic sledge (C-EBS; Brandt et al., 2013) during this cruise. On deck, the complete C-EBS samples were transferred to pre-chilled (−20°C) 96% ethanol and stored in a −20°C freezer for at least 48 h for molecular analyses. Alternatively, 3.6% formalin-seawater was used as a fixative for some hauls. In the laboratories of R/V *Sonne* the samples were then identified and sorted on ice into metazoan phyla. At eight cruise stations (10 C-EBS hauls), from depths between 4,830 and 5,397 m, a total of 33 solenogaster specimens was collected. Thirty were fixed and stored in 96% ethanol and three (ZSM Mol20170086, −87, −88) fixed in 3.6% formalin-seawater. All specimens

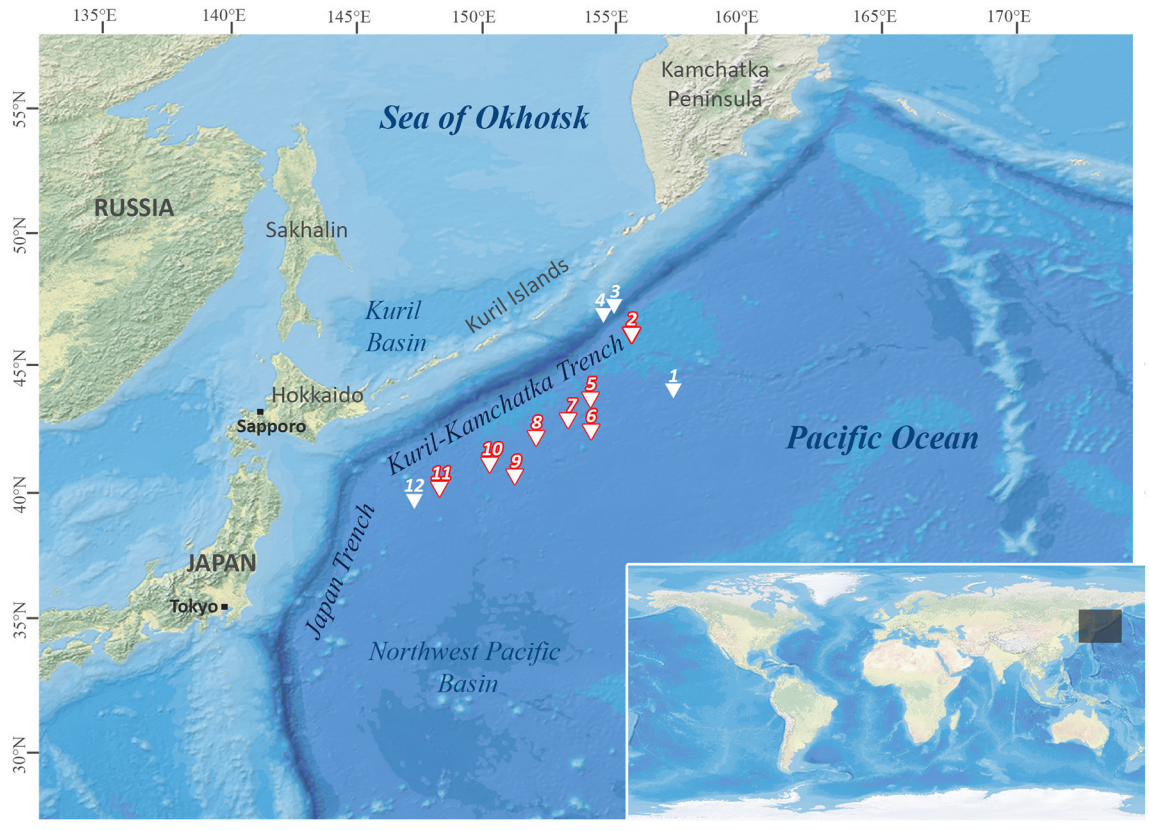

FIGURE 1 | Station map of the Kuril-Kamchatka-Biodiversity Studies (KuramBio) expedition. Stations emphasized in red: sampling sites where Solenogastres were collected. Map created with DivaGis.

TABLE 1 | Station table of the KuramBio expedition.

Station	Coordinates (Decimal Degree)	Depth (m)	Number of individuals
1	43.9710°N–43.9722°N; 157.3278°E–157.2995°E	5,412–5,429	0
2–09	46.2268°N–46.2487°N; 155.5567°E–155.5428°E	4,830–4,864	2
3	47.2307°N–47.2477°N; 154.6982°E–154.7197°E	4,859–4,863	0
4	46.9640°N–46.9747°N; 154.5398°E–154.5565°E	5,681–5,780	0
5–09	43.5913°N–43.5717°N; 153.9647°E–153.9693°E	5,376–5,379	3
6–12	42.4915°N–42.4704°N; 153.9989°E–153.9953°E	5,291–5,307	5
7–09	43.0437°N–43.0248°N; 152.9905°E–152.9727°E	5,216–5,223	3
7–10	43.0463°N–43.0276°N; 152.9882°E–152.9743°E	5,218–5,221	3
8–09	42.2447°N–42.2378°N; 151.7351°E–151.7082°E	5,125–5,140	4
8–12	42.2453°N–42.2387°N; 151.7391°E–151.7157°E	5,115–5,124	6
9–12	40.5918°N–40.5713°N; 150.9976°E–150.9864°E	5,392–5,397	5
10–12	41.1939 °N–41.2169°N; 150.0928°E–150.0942°E	5,249–5,262	1
11–12	40.2184°N–40.2018°N; 148.1088°E–148.0923°E	5,348–5,350	1
12	39.7300°N–39.70821°N; 147.18131°E–147.15621°E	5,215–5,228	0

Epibenthic sledge (C-EBS) sampling sites where Solenogastres were collected are highlighted in bold and also state the respective C-EBS haul number.

are deposited at the Bavarian State Collection of Zoology (ZSM) in Munich, Germany (see **Table 2** for overview of material).

Hard-Part Morphology via Light and Scanning Electron Microscopy

We took overview photographs of all specimens using a Leica camera mounted on a Leica Z16 APO compound microscope. Body length was measured after fixation in ethanol. Hard parts were analyzed via light (LM) and/or scanning electron microscopy (SEM) (see **Table 2**).

Specimen dehydration for SEM was achieved by slow, overnight evaporation of 100% dehydrated ethanol from glass vials containing the specimens. Each animal was mounted on a self-adhesive carbon sticker on a SEM-stub. We coated them with gold in Argon atmosphere for 240 s in a Polaron sputter coater (GaLaGabler Labor Instrumente Handels GmbH). SEM micrographs were taken with a LEO 1430 VP SEM (Zeiss). SEM specimens were then directly used for DNA extractions (see Bergmeier et al., 2016b). Specimens with sufficient body size (i.e., >2 mm) were divided using a razorblade. The posterior part was used for DNA extraction and the anterior part for manual preparation of the radula. Soft tissue was dissolved in a 3:1 solution of distilled water and household bleach and radula (if preparation was successful) and sclerites were documented via LM and SEM.

Histology and 3D-Reconstruction

One 3.6% formalin fixed specimen (ZSM Mol20170088) and two specimens fixed in 96% ethanol (ZSM Mol20170093 and 20170077) were selected for histological investigations. ZSM Mol20170077 was tri-sectioned. The midsection was used for DNA extraction (see below) and the anterior and posterior part processed for anatomical investigations: specimens were decalcified in 1% ascorbic acid over night, post-fixed with osmium tetroxide and embedded in Spurr's low viscosity resin (Spurr, 1969). Serial sections of 1 µm thickness were cut using a diamond knife on a RMC MT 7000 microtome (Leica AG). Contact cement on the lower cutting edge of the resin blocks ensured the formation of ribbons (Ruthensteiner, 2008). Ribbons were collected on microscopic slides, stained with azure II/methylene blue (Richardson et al., 1960) and sealed with cover slips. All sections were semi-automatically digitalized with a BX16VS Olympus microscope (20x magnification) in conjunction with the software DotSlide vs-ASW FL by Olympus. Digital files were exported as ".vsi" files and ".tif" images generated with the Olympus software OlyVia, using 16.5x magnification. All digital photos were converted to 8-bit grayscale, contrast enhanced and unsharp masked with Photoshop CS6 (Adobe Systems Software). The digitalized histological section series was imported into the 3D-vizualization software Amira 5.3.3 (Visage Imaging, FEI) and a computer based 3D-reconstruction of all major organ systems was carried out for specimen ZSM Mol20170088. Anatomy of specimens ZSM Mol20170093 and 20170077 was studied using Amira and schematic drawings were done with the freeware Inkscape (www.inkscape.org).

DNA Extraction, Barcode Amplification, and Sequencing

DNA extraction was done either by standard CTAB extraction, or by combining DNA extraction via CTAB with recovery of the DNA using spin-columns (Machery-Nagel Blood and Tissue Set). Dried and sputter-coated samples previously used for SEM investigations were ground up using pistils before tissue lyses (see Bergmeier et al., 2016b). We amplified mitochondrial 16S rRNA via PCR using the Phire II Hotstart polymerase (ThermoFischer) with the supplied 5x reaction buffer containing

TABLE 2 | Overview of all morphospecies of Solenogastres collected during the KuramBio cruise and the data generated from the respective individuals.

Species ID	ZSM Number Mol.	Station	Habitus	Approx. length (mm)	Scleritome			Radula	Barcode
					# Types of acicular sclerites	Types of scales	Foot scales		
Cavibelonia sp. 1	20170101	10–12	spiny	3	?	?	?	?	MG524989
Cavibelonia sp. 2	20170102	2–09	fuzzy	5	3○	1	b-s + bulge	?	MG524988
Cavibelonia sp. 3	20170103	6–12	furry	2.5	2○	?	?	?	?
Cavibelonia sp. 4	20170104	6–12	v. spiny	1	1○1●	3	b-s + bulge	?	?
Proneomeniidae sp. 1	20170100	8–09	v. smooth	3.5	1○	0	b-shape	?	MG524985
Halomeniinae sp. 1	20170076	5–09	fuzzy	2.5	2○	1	b-s + bulge	distichous	MG524986
	20170077*			1.7				distichous	MG524987
Pruvotinidae sp. 1	20170078	2–09	fuzzy	2	3○ + hooks	0	?	?	MG524984
	20170079	5–09	spiny	2	3○ + hooks	1	b-s	?	MG524982
Pruvotinidae sp. 2	20170080	7–09		2					MG524980
	20170081	7–10		2	3○ + hooks	1	b-s		MG524983
	20170082	8–09		2	3○ + hooks	1	b-s		MG524981
Pruvotinidae sp. 3	20170083	6–12	spiny	1.5	1○ + hooks	0	?	distichous	?
Simrothiellidae sp. 1	20170097	8–09	rough	1.2	3○	0	l-s	?	MG524979
Simrothiellidae sp. 2	20170098	8–09	rough	1.5	2○	1	?	?	?
Spiomenia sp.	20170099	6–12	v. spiny	2	3○	0	?	?	?
	20170089	7–10		3.5	4○	5	b-s + bulge	not present	MG524978
	20170090	7–10		3	4○	5	b-s + bulge	not present	MG524977
Veromenia cf. *singula*	20170091	8–12	fuzzy	2.2	4○	5	b-s + bulge	not present	MG524976
	20170092	8–12		2	4○	5	b-s + bulge	not present	MG524975
	20170093*	8–12		2.5				not present	
	20170084			3.5	3○	2	b-s + bulge		
Amboherpia	20170085			4.5	3○	2	b-s + bulge		
abyssokurilensis sp.	20170086	9–12	smooth	5.5	3○	2	b-s + bulge	monoserial	?
nov.	20170087			3	3○	2	b-s + bulge	monoserial	
	20170088* +			3.6				monoserial	
Acanthomeniidae sp. 1?	20170095	7–09	smooth to rough	2	2○2●	1	?	?	?
	20170096	8–12		2.5	2○2●	1			
Acanthomeniidae sp. 2	20170094	8–12	smooth	6.5	2○	5	b-s + bulge	?	?
Sterrofustia sp. 1	20170108	11–12	furry	2.7	2●	1	b-s	?	?
Dondersiidae sp. 1	20170106	6–12	velvety	3	1●	1	?	?	?
Dondersiidae sp. 2	20170107	8–12	smooth	3.5	2●	4	?	?	?
Dondersiidae sp. 3	20170105	7–09	spiny	1	1○1?	3	?	?	?

*White circle, hollow acicular elements. Black circle, solid acicular elements. ?, no data available for the entire clade, blanks indicate that this character set was not studied in the respective individual. *, histological section series available. +, holotype. b- or l-s, blade- or leaf-shaped; V., very.*

$MgCl_2$ as an additive. We used two different primer sets: 16S-S2 (Schwenk et al., 1998) and 16S-a (Simon et al., 1994) resulted in a sequence length of the amplified part of ∼470 base pairs. In addition, a pair of self-designed, solenogaster specific internal 16S rRNA primers (designed using Primer3; Untergasser et al., 2012) resulted in a sequence length of ∼360 base pairs: 16Soleno-r (5′-YYTAATCCAACATCGAGGTC-3′) and 16Soleno-f (5′-RRGAGTWAGRCCTGCCCAGT-3′). The following PCR specifics were used for amplification: 30 s at 98°C and (5 s at 98°C, 5 s at 47–50°C, 20 s at 72°C) × 35–37 and 60 s final elongation at 72°C. PCR products were cleaned up using the DNA Clean & Concentrator-5 (Zymo Research) and sent off for cycle sequencing (using Big Dye 3.1) on an ABI 3730 capillar sequencer at the Genomics Service Unit of the LMU Munich.

Molecular Sequence Data and Analyses

Overall we obtained 15 16S rRNA sequences. These were edited manually using the software Geneious 6.1 (Biomatters Ltd.) and a BLAST search against the existing databank was performed to check for any contaminated sequences. Sequences were aligned using the MUSCLE algorithm as implemented in Geneious, and ambiguous regions within the alignment were masked using GBlocks with options for less stringent selection (Castresana, 2000). We performed a maximum likelihood analysis using RAxML (Stamatakis, 2014) with the nucleotide substitution model GTR+G determined via jModelTest (Posada, 2008). In a pre-analysis we used a Caudofoveata 16S sequence from GenBank (AY340451) as outgroup to determine the basal lineage within our dataset. All sequence analyses were conducted using the CIPRES gateway (Miller et al., 2010).

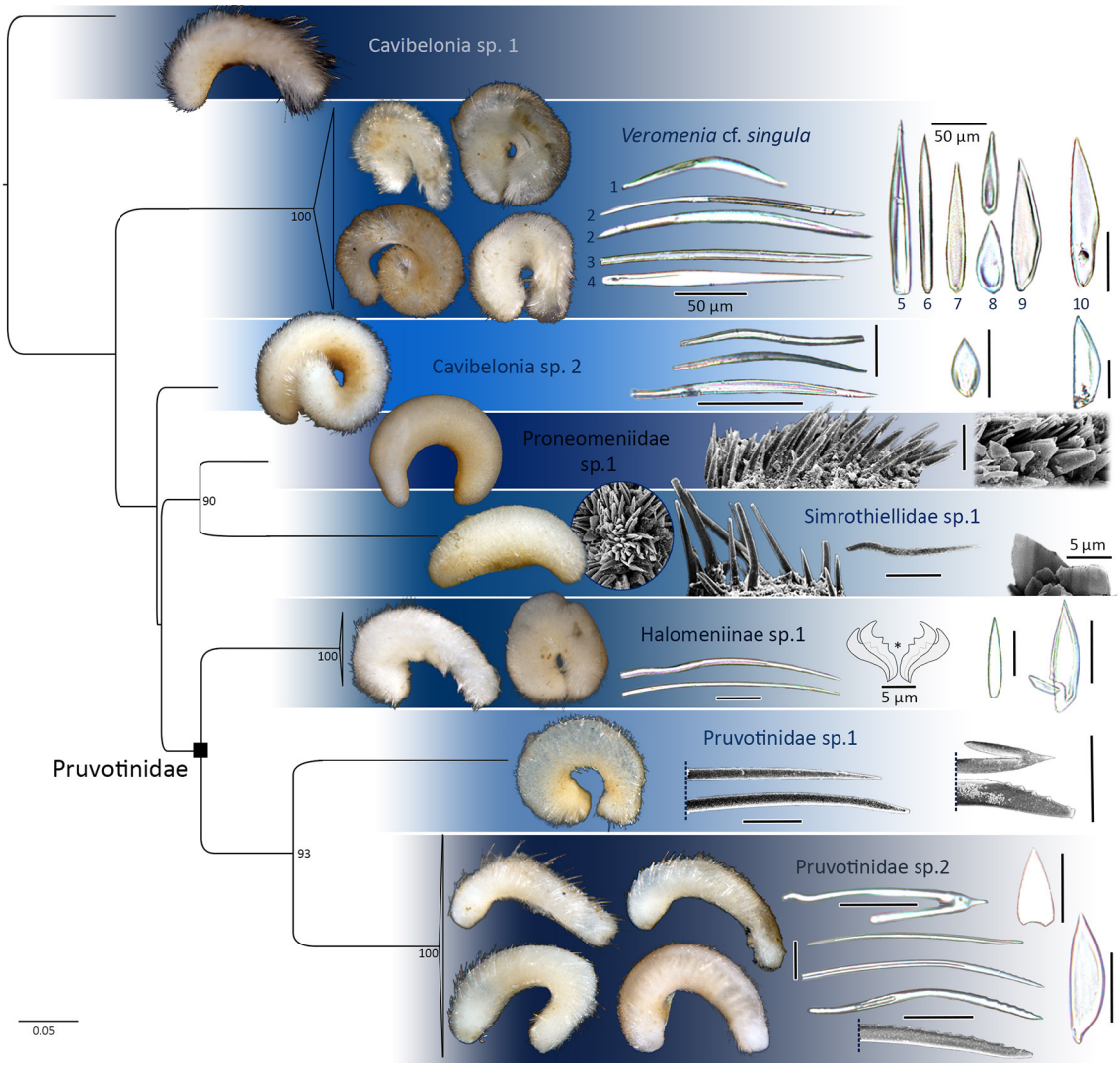

FIGURE 2 | 16S rRNA single gene maximum likelihood tree reconstructed with RAxML, bootstrap values of >85 plotted. Distinct lineages of Cavibelonia. Hard parts of respective lineages plotted. Asterisk in Halomeniinae sp. 1 marks radula. Foot scales always to the right. If not stated otherwise, all scleritome scale bars 25 µm.

For GenBank accession numbers of the generated barcodes see **Table 2**.

RESULTS

Diversity and Distribution

Based on molecular sequence data, morphology, and additional anatomical characters we were able to confidently differentiate 19 lineages of Solenogastres in our material, representing at least five different families. One potential additional lineage (referred to as Acanthomeniidae sp. 1?) cannot be delineated with certainty, due to lack of some important characters—see section Discussion. Of the 19 definite lineages present in our dataset eight are robustly supported by a combination of molecular and morphological data (see **Figure 2**). The delineation of the remaining 11 (or 12, including Acanthomeniidae sp. 1?) (morpho-) species is based on detailed hard-part features, i.e., scleritome and radula (**Figures 3–5**) and in three cases on additional anatomical data derived from histological serial sections (**Figures 6–10**). Identification to family level and beyond was possible for 11 species, respectively three species. We were not able to identify four lineages by comparing their scleritome with literature data of any of the currently existing families, and for now they remain identified to order level only. Cavibelonia sp. 1 (**Figure 2**) unfortunately lacks scanning electron or light microscopic scleritome data (see **Table 2**) since the specimen was lost during preparation.

With the exception of four individuals, all other collected specimens (29 individuals) belong to the order Cavibelonia. Within the cavibelonian lineages identified to family level and beyond, four families are represented by 12 species.

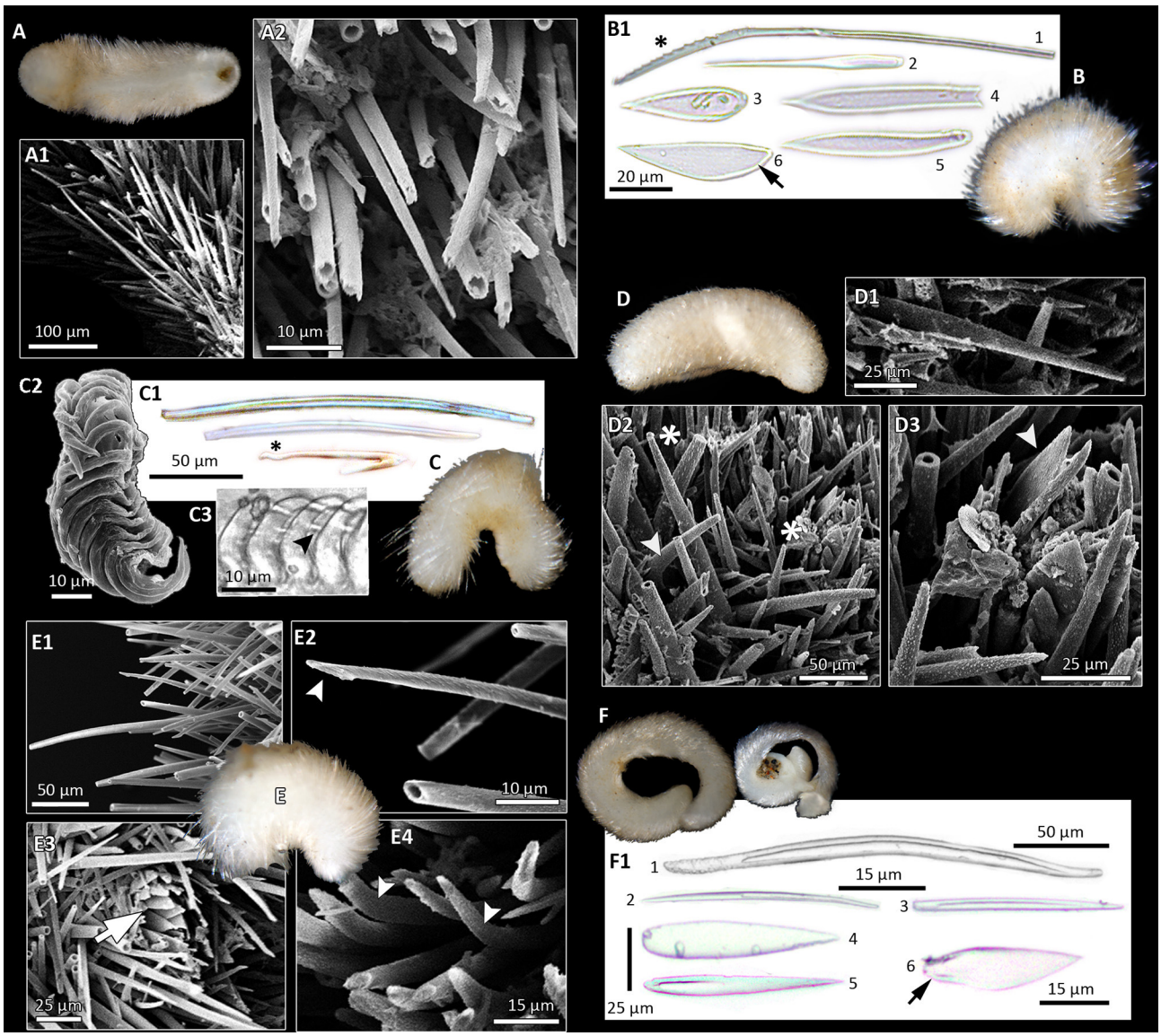

FIGURE 3 | Morphological diversity of Solenogastres, based on light (**B1,C1,C3,F1**; foot scales indicated by black arrows) and scanning electron microscopy of hard parts. Photographs of animals all taken after fixation in ethanol. **(A)** Cavibelonia sp. 3, covered in acicular, hollow needles **(A1)** of varying length, both straight and bent **(A2)**. **(B)** Cavibelonia sp. 4 with hollow and solid acicular elements and scales **(B1)**. Note proximal serrations (asterisk). **(C)** Pruvotinidae sp. 3 with acicular elements [straight and hook-shaped (asterisk)] **(C1)** and distichous radula (**C2,C3**—black arrowhead points to radula denticle). **(D)** Simrothiellidae sp. 2 with straight (**D1,D2** asterisk) and curved (**D2**, arrowhead) acicular hollow elements and scales (**D3**, arrowhead). **(E)** *Spiomenia* sp. with long acicular elements **(E1)** with captate distal ends (**E2**, arrowhead), scales surrounding the dorsoterminal sense organ (**E3**, arrow) and short, slightly curved elements (**E4**, arrowheads). **(F)** *Amboherpia abyssokurilensis* sp. nov. with **(F1)** hollow, acicular sclerites (top), and scales (bottom).

The non-cavibelonian individuals represent three morphospecies of the order Pholidoskepia and one of Sterrofustia. No lineages of the small order Neomeniamorpha were found.

Fifteen of the 19 distinct lineages were collected as singletons. With almost 40% of all collected individuals, Acanthomeniidae is the most common family, represented by at least three lineages found at Sts. 7, 8, and 9. Whereas less individuals of Pruvotinidae (eight specimens) were collected, representatives of the family occurred slightly more widespread at Sts. 2, 5, 6, 7, and 8 (see **Figure 1** and **Table 1**). Solenogastres were most abundant and diverse at St. 8, with overall 10 individuals belonging to seven species (excluding Acanthomeniidae sp. 1?), collected during two C-EBS hauls. At St. 7, six individuals were found and at Sts. 6 and 9 five individuals each. At St. 5 three individuals were found. At Sts. 10 and 11 only one singleton each was collected. We collected no Solenogastres from the two deepest stations (St. 3 and 4) from the slope of the Kuril-Kamchatka Trench.

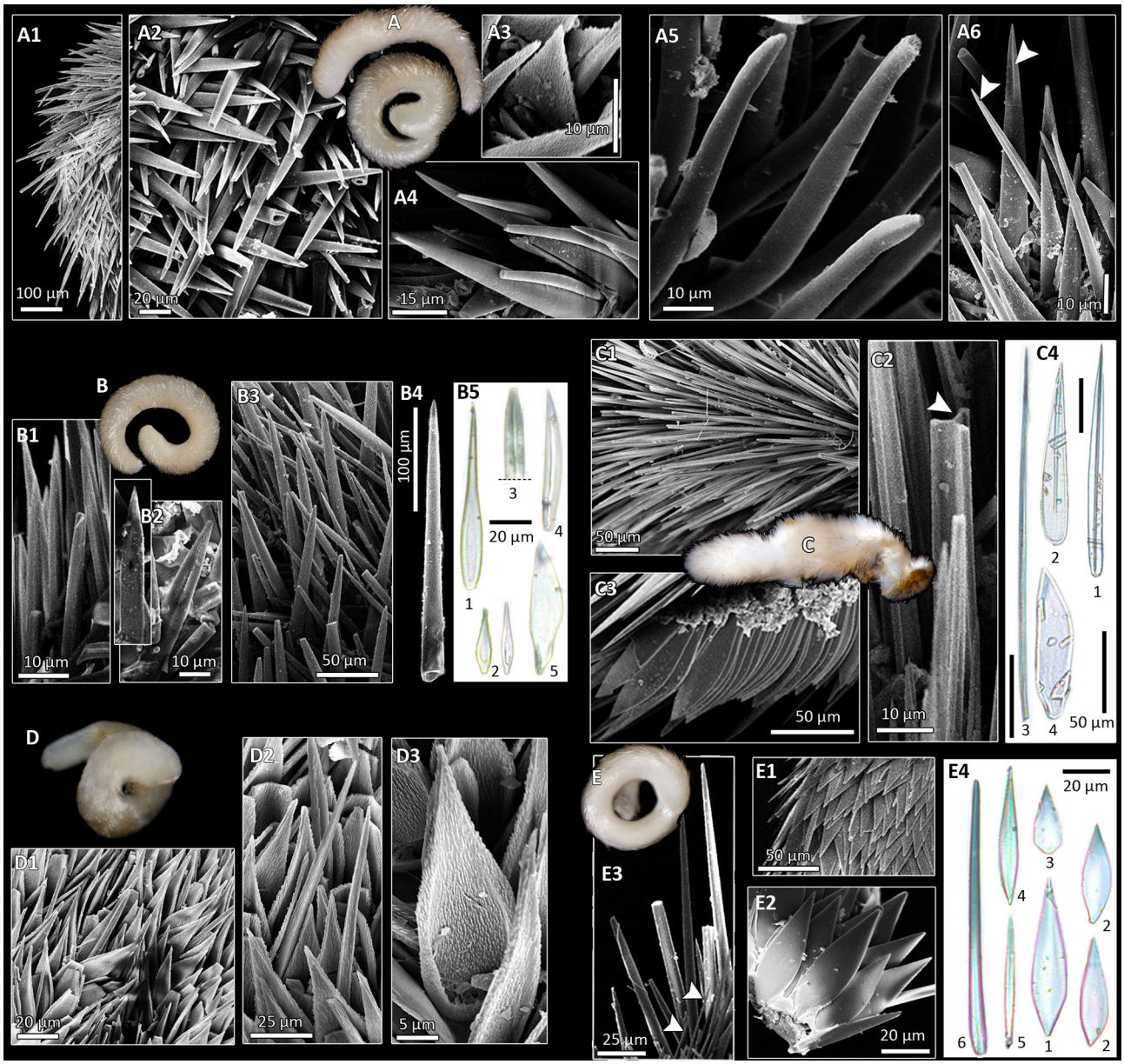

FIGURE 4 | Morphological diversity of Solenogastres, based on light **(B5,C4,E4)** and scanning electron microscopy of hard parts. **(A)** Acanthomeniidae sp. 1? **(A1)** Dorsal body with acicular spicules. **(A2)** Intercrossing hollow, needles. **(A3)** Leaf-shaped scale. **(A4)** Large and small needles. **(A5)** Sclerites with slight bend and distally thickened. **(A6)** Needles with distal keel (white arrowheads). **(B)** Acanthomeniidae sp. 1. **(B1)** Acicular, concave sclerite. **(B2)** Sclerites with thick lateral rims and flat back. **(B3)** Acicular sclerites of varying thickness and length *in situ* **(B3)** and one isolated **(B4)**. **(B5)** LM picture of different types of scales [foot scale (5)]. **(C)** Sterrofustia sp. 1 with a scleritome consisting of solid needles (**C1,C2** white arrowhead, **C4**) and blade-shaped foot scales [**C3,C4** (4)]. **(D)** Dondersiidae sp. 1 with a combination of needles and scales **(D1)**. **(D2)** Detail of short needles. **(D3)** Scale with rimmed base. Note hair-like ultrastructure of sclerite surfaces. **(E)** Dondersiidae sp. 2 covered with scales **(E1,E2)**. **(E3)** Long and thick as well as thin and short (white arrowheads) needles. **(E4)** LM images of sclerites.

Delimitation and Identification of Abyssal Solenogaster Lineages

The following species delimitation is based on external morphology of each lineage (remarks on coloration refer to the animal after fixation in ethanol), scleritome data (terminology mainly following García-Álvarez and Salvini-Plawen, 2007), radula (if radula extraction was successful) as well as genetic distances between sister clades in our one marker maximum likelihood analyses. For each lineage we differentiate between scales covering the body vs. scales surrounding the foot groove or dorsoterminal sense organ, since the latter two structures are always associated with scales. The term "sclerite" describes any element of the scleritome, whereas "spicule" refers to an elongated and needle-like structure in

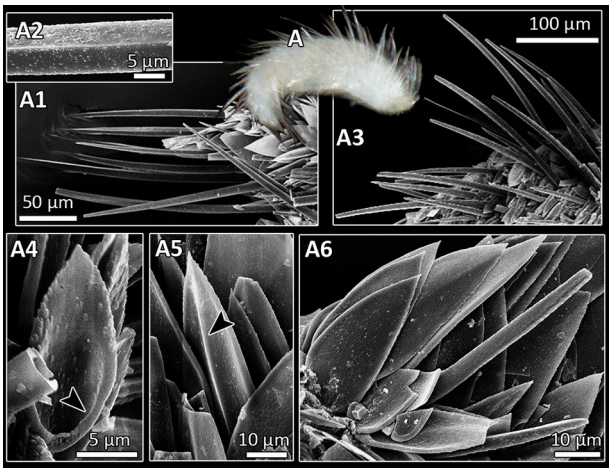

FIGURE 5 | Entire specimen **(A)** and SEM micrographs **(A1–A6)** of Dondersiidae sp. 3 **(A1)** Long spicules, straight and with keel **(A2)** or with slight bend **(A3). (A4)** Scale with rimmed base (arrowhead). **(A5)** Scale with median keel (arrowhead). **(A6)** Flat scale and short sclerite.

contrast to "scales". Foot scales are leaf- or blade-shaped, and the latter can be either of evenly convex shape [see, e.g., **Figure 2** (Pruvotinidae sp. 2)] or with a slight [**Figure 3B1** (6)] to distinct bulge (see, e.g., **Figure 2** *Veromenia* cf *singula*, Cavibelonia sp. 2).

See **Table 2** for a summary of all characters available for each clade and its individuals.

CAVIBELONIA Salvini-Plawen, 1978

Species belonging to this order in general exhibit a scleritome characterized by the presence of hollow, acicular needles ("spicules"). These can be combined with different types of scales, resulting in highly diverse scleritomes.

Cavibelonia sp. 1 (Figure 2):
Material: single specimen (ZSM Mol20170101).
Distribution: St. 10–12, 5,249–5,262 m.
Habitus: spiny, ∼3 mm in length; white coloration.
Scleritome: no scleritome data available, animal was lost during preparation.
Radula: unknown.
GenBank Accession Number: MG524989.
Interspecific genetic distance to sister clade based on 16S rRNA analyses: 23.1–37.0%.

Cavibelonia sp. 2 (Figure 2):
Material: single specimen (ZSM Mol20170102).
Distribution: St. 2–09, 4,830–4,864 m.
Habitus: fuzzy appearance, ∼5 mm in length; light yellow coloration.
Scleritome: dominated by three types of hollow, acicular sclerites of sigmoid shape, curved, and with broad mid-region. Leaf-shaped scales only. Foot scales blade-shaped with bulge.
Radula: unknown.

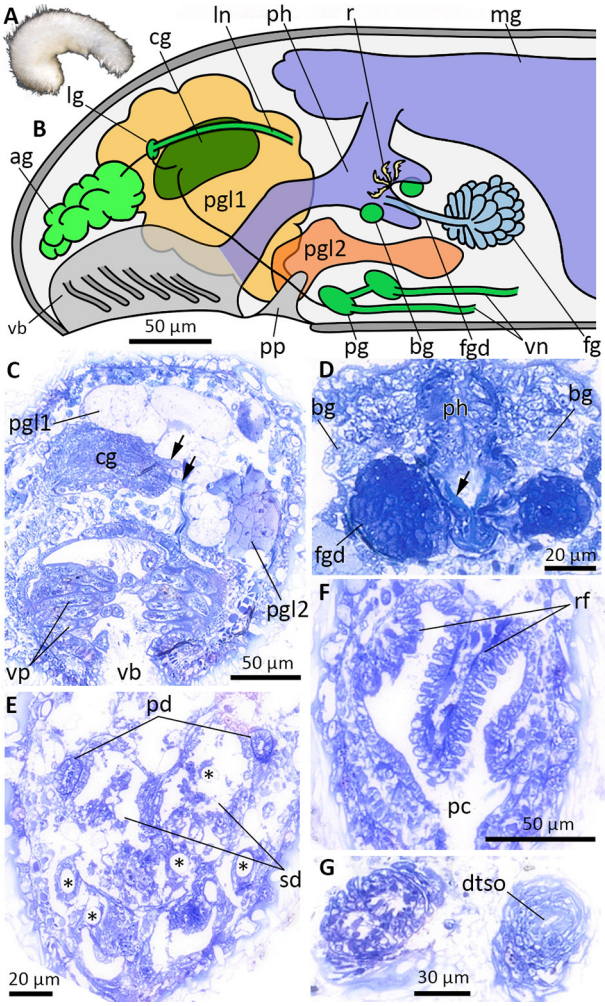

FIGURE 6 | Anatomy and histology of Halomeniinae sp. 1, highlighting the main taxonomic features. **(A)** Habitus. **(B)** Schematic illustration of the anterior body. **(C)** Cross-section at the level of the vestibular-buccal cavity. Arrows point to cerebrolateral (upper arrow) and cerebropedal (lower arrow) connectives. **(D)** Histology of the foregut gland ducts. Arrow points to radula tooth. **(E)** Cross-section through the posterior reproductive system at the transition of pericardioducts into paired spawning duct. Asterisks indicate hollows of dissolved body sclerites. **(F)** Respiratory folds in pallial cavity. **(G)** Section through the posteriormost part of the animal, before dorsoterminal sense organ (dtso) opens to the exterior. ag, accessory ganglia mass; bg, buccal ganglia; cg, cerebral ganglion; dtso, dorsoterminal sense organ; fg, ventrolateral foregut gland; fgd, duct of foregut gland; lg, lateral ganglion; ln, lateral nerve cord; mg, midgut; pc, pallial cavity; pd, pericardioduct; pg, pedal ganglion; pgl1/pgl2, type 1 and 2 of pedal glands; ph, pharynx; pp, pedal pit; rf, respiratory folds; sd, spawning duct; vb, vestibular-buccal cavity; vn, ventral nerve cord; vp, vestibular papillae.

GenBank Accession Number: MG524988.
Interspecific genetic distance to sister clade: 15.6–24.2%.

Cavibelonia sp. 3 (Figure 3A):
Material: single specimen (ZSM Mol20170103).
Distribution: St. 6–12, 5,291–5,307 m.
Habitus: furry appearance due to perpendicular projecting

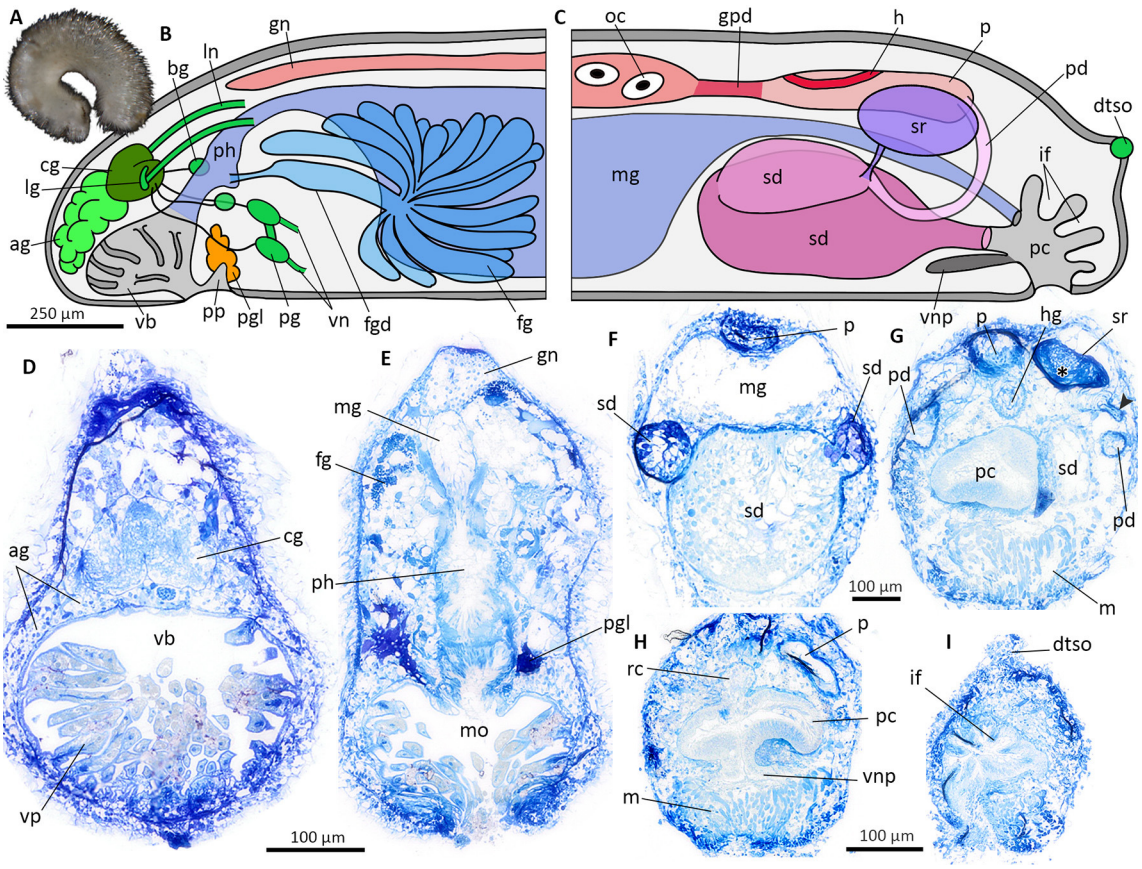

FIGURE 7 | *Veromenia* cf. *singula* **(A)** Habitus. Schematic illustration of the general anatomy **(B,C)**, histological cross sections **(D–I)**. **(B)** Anterior body. **(C)** Posterior body. **(D)** Vestibular-buccal cavity with cerebral ganglion and "accessory ganglia". **(E)** Anterior digestive tract with pharynx and midgut. **(F)** Reproductive system at the transition of pericardioducts and spawning duct. **(G)** Anterior part of pallial cavity and seminal receptacle filled with sperm (asterisk). Arrowhead points to duct connecting seminal receptacle and pericardioduct. **(H)** Pallial cavity with ventral pouch. **(I)** Dorsoterminal sense organ and epidermal infoldings of posterior pallial cavity. ag, accessory ganglia mass; bg, buccal ganglion; cg, cerebral ganglion; dtso, dorsoterminal sense organ; fg, ventrolateral foregut gland; fgd, duct of foregut gland; gn, gonad; gpd, gonopericardioduct; h, heart; hg, hindgut; if, epidermal infoldings; lg, lateral ganglion; ln, lateral nerve cord; m, muscle bundles; mg, midgut; mo, mouth opening; oc, oocyte; p, pericardium; pc, pallial cavity; pd, pericardioduct; pg, pedal ganglion; pgl, pedal gland; ph, pharynx; pp, pedal pit; rc, rectum; sd, spawning duct; sr, seminal receptacle; vb, vestibular-buccal cavity; vn, ventral nerve cord; vnp, ventral pouch; vp, vestibular papillae.

sclerites of equal length along the lateral sides (**Figures 3A,A1**), ∼2.5 mm in length; white coloration.
Scleritome: sclerites damaged, majority with broken tips. Two types of hollow, acicular elements discernible: one straight with pointed tip; second type with slight bend (**Figure 3A2**). No scales or foot scales observed.
Radula: unknown. No molecular data available.

Cavibelonia sp. 4 (Figure 3B):
Material: single specimen (ZSM Mol20170104).
Distribution: St. 6-12, 5,291–5,307 m.
Habitus: very spiny appearance, ∼1 mm in length; light yellow coloration.
Scleritome (**Figure 3B1**): two types of acicular elements: one hollow, distally bent with serrated end (**Figure 3B1**, asterisk) (200 µm length) (1), the other one solid with broadened proximal end (75 µm) (2). Three types of scales: leaf-shaped scales (50 µm) (3), groove-shaped sclerites with thickened lateral rims (up to 170 µm in length) (4) and slender elongated scales (60 µm in length) (5). Foot scales blade-shaped with bulge (50 µm length) (6).
Radula: unknown. No molecular data available.

Proneomeniidae Simroth, 1893
Representatives of this family are characterized by several layers of acicular sclerites of various shapes. Proneomeniidae sp. 1 was assigned to this family based on its very smooth appearance due to flatly arranged body sclerites, the slightly narrowed and flattened posterior end as well as the distinct yellow coloration after fixation in ethanol (see García-Álvarez et al., 1998).

Proneomeniidae sp. 1 (Figure 2):
Material: single specimen (ZSM Mol20170100).
Distribution: St. 8–09, 5,125–5,140 m.
Habitus: smooth appearance, ∼3.5 mm in length; body stout and thick, posterior tapering; strong yellow coloration.

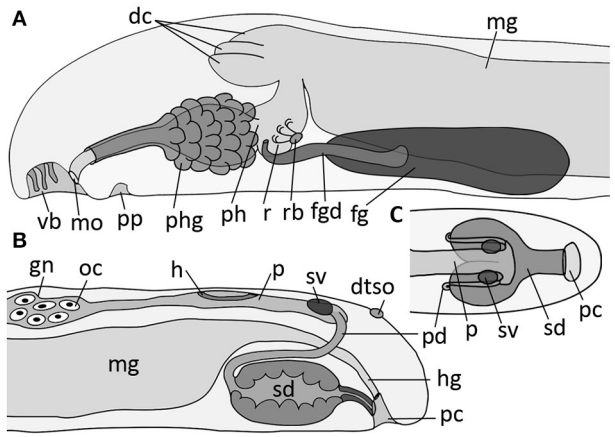

FIGURE 8 | Schematic illustration of main taxonomic characters of *Amboherpia abyssokurilensis* sp. nov. **(A)** Anterior digestive tract and **(B)** gonopericardial system, both in lateral view. **(C)** Posterior gonopericardial system, dorsal view. dc, dorsal caecum; dtso, dorsoterminal sense organ; fgd, duct of foregut gland; fg, ventrolateral foregut gland; gn, gonad; h, heart; hg, hindgut; mg, midgut; mo, mouth opening; oc, oocyte; p, pericardium; pc, pallial cavity; pd, pericardioduct; ph, pharynx; phg, pharyngeal gland; pp, pedal pit; r, radula; rb, radula bolster; sd, spawning duct; sv, seminal vesicle; vb, vestibular-buccal cavity.

Scleritome: specimen shrunk during drying process for SEM. Only one type of slightly curved hollow, acicular elements observed, projecting up to 40 μm from shrunken cuticle. Foot scales blade-shaped.
Radula: unknown.
GenBank Accession Number: MG524985.
Interspecific genetic distance to sister clade Simrothiellidae sp. 1: 26.7%.

Pruvotinidae Heath, 1911

The scleritome of Pruvotinidae is characterized by hollow acicular needles with occasionally serrated end, and the occurrence of hook-shaped elements in certain subfamilies.

With four species belonging to at least two subfamilies, Pruvotinidae is the most diverse family collected during the cruise. All of the pruvotinid species have hollow acicular elements in common, but vary in the presence of additional sclerites (e.g., hook-shaped elements are missing in the subfamily Halomeniinae - see below) and in the overall composition of their scleritome.

Halomeniinae sp. 1 (Figures 2, 6)

Material: two specimens (ZSM Mol20170076, 20170077).
Distribution: St. 5–09, 5,376–5,379 m.
Habitus: fuzzy appearance, ∼1.7 and 2.5 mm in length; white to light yellow coloration.
Scleritome: dominated by two types of hollow acicular elements, straight and with slight sigmoid shape (up to 120 μm long). One type of elongated scales, up to 75 μm in length. Foot scales blade-shaped (∼60 μm length).
Radula: distichous, each tooth (∼25 μm in length) with large lateral denticle and two smaller median denticles.
GenBank Accession Number: MG524986, −87.
Intraspecific genetic variability: 1.5%.
Interspecific genetic distance to sister clade Pruvotinidae sp. 1 and 2: 21.5–23.2%.

Anatomy of Halomeniinae sp. 1 *(Figure 6)*

Based on histological serial sections of ZSM Mol20170077 (**Figure 6A**, fixed specimen). Vestibular-buccal cavity common, with numerous papillae (**Figures 6B,C**), followed by conspicuous pedal pit with two types of pedal glands (**Figure 6B**). Pedal gland 1 large and extending far into head region, no particular staining properties. Pedal gland 2 smaller and extending posterior, staining light-purple (**Figure 6C**). Cerebral ganglion with two laterally emerging connectives leading to paired lateral and pedal ganglia (**Figures 6B,C**). From lateral ganglion, nerve exits toward "accessory ganglia" (i.e., pre-cerebral ganglia) dorsal to vestibulum; posterior the lateral nerve cords emerge on each side. Paired pedal ganglia with commissure; ganglia giving rise to ventral nerve cords. Small paired buccal ganglia present on both sides of radula (**Figure 6D**). Dorsoterminal sense organ present (**Figure 6G**). Mouth opening located in posterior part of vestibular-buccal cavity. Pharynx ciliated, with distichous radula (**Figures 2**, **6D** arrow). Foregut glands comprised of ducts with surrounding muscle layer (**Figure 6D**), posterior surrounded by gland cells (Type *Pararrhopalia* = Type A). Midgut with dorsal caecum, cnidocysts in midgut indicate cnidarivory of species. Hindgut opens into dorsoanterior part of pallial cavity. Gonads not present on section series of posterior third of body - either not developed due to immaturity or lost during tri-partitioning of specimen for DNA extraction. Paired pericardioducts opening into paired spawning duct (**Figure 6E**). Spawning duct fuses, but transition into pallial cavity (secondary genital opening) not detectable. Large hollows of dissolved spicules in ventroposterior part of animal most likely from copulatory spicules. Additional hollows indicate that body spicules reach from dorsal part inside of the animal to the ventral side and exit through cuticle (**Figure 6E**, asterisks). Pallial cavity with three respiratory folds (**Figure 6F**).

Taxonomic remarks

The posterior body, especially the entire reproductive system, could not be unambiguously reconstructed due to poorly preserved and for some parts destroyed histology. The combination of hard-part and anatomical features (no hook-shaped sclerites, foregut glands of Type *Pararrhopalia*, lack of pharyngeal glands, presence of respiratory folds) suggest the placement of this lineage within the subfamily of Halomeniinae. The single genus *Halomenia* Heath, 1911 is characterized by separated vestibulum (=atrium) and mouth opening, unpaired secondary genital openings (=unpaired opening of the spawning duct into the pallial cavity), the lack of copulatory spicules and the presence of a dorsoterminal sense organ and respiratory folds. Whereas the latter two features are present in our lineage, vestibulum and mouth opening share a common cavity (vestibular-buccal cavity) and copulatory spicules are present, but the state of the secondary genital opening cannot be

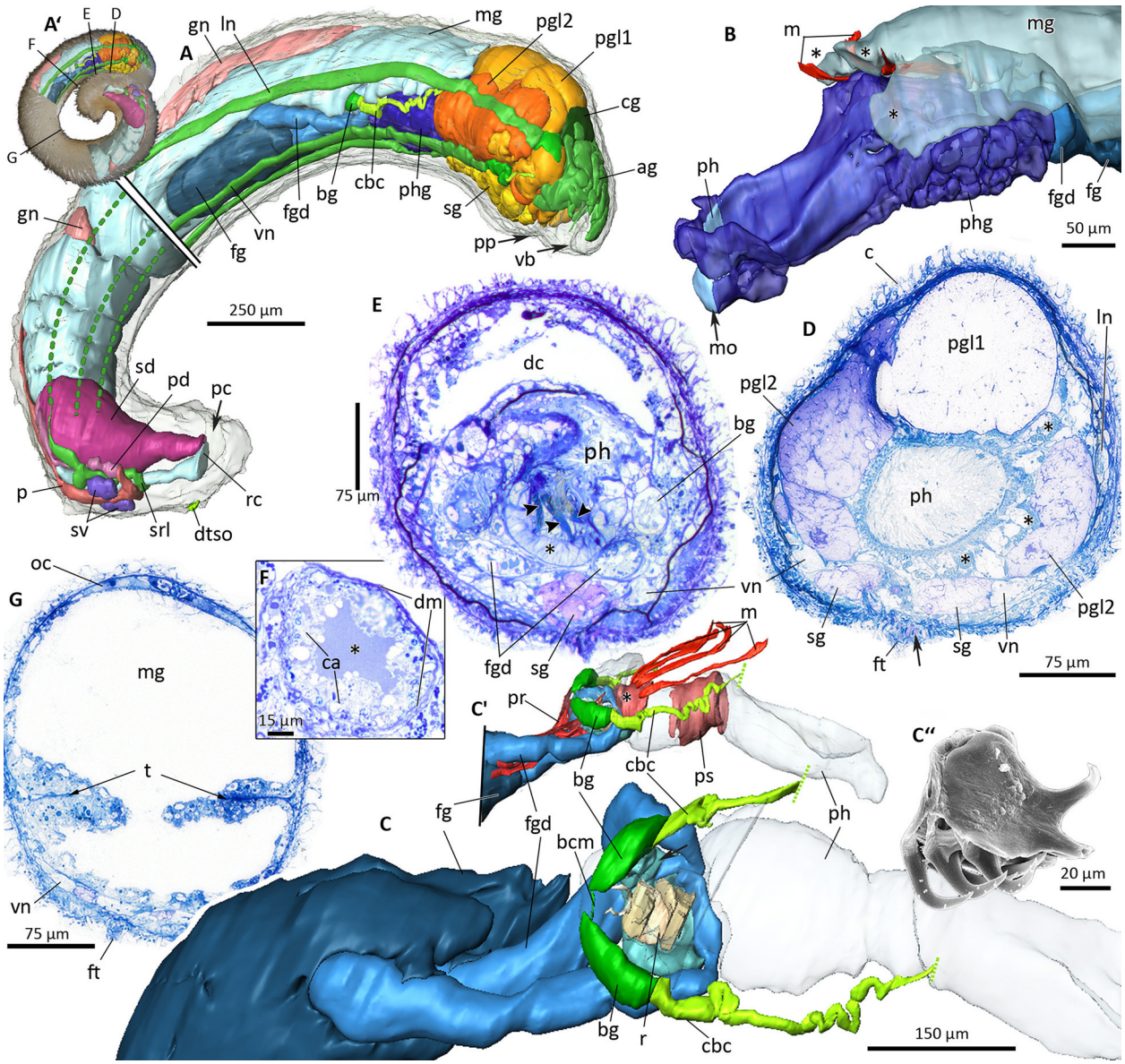

FIGURE 9 | 3D-microanatomy of *Amboherpia abyssokurilensis* sp. nov. **(A–C′)** with histological details of the digestive tract **(D–G)**. **(A′)** Habitus prior to sectioning, showing the regions that were used for the 3D-reconstructions. Black labeled lines indicate levels of respective histological sections. **(A)** 3D-reconstruction of all major organ systems, lateral view. Lateral and ventral nerve cords only partially reconstructed in the posterior half, as indicated by dotted lines. **(B)** Pharynx with surrounding pharyngeal glands and tripartite midgut caecum (asterisks), anterolateral view. **(C)** Anterior digestive tract showing the position of the radula within the pharynx (pharynx transparent, midgut, and pharyngeal glands omitted), dorsolateral view. **(C′)** Pre-radula sphincter (asterisk) and muscle fibers associated with the structures of the anterior alimentary tract, lateral view. **(C″)** SEM micrograph of radula, orientation as in living animal. **(D)** Histology of pharynx and pharyngeal glands (asterisks), pedal gland type 1 and 2. Arrow points to opening of sole glands. **(E)** Digestive tract at the level of radula (arrowheads) and radula bolster (asterisk). **(F)** Detail of the foregut gland duct with its lumen (asterisk), apices of gland cells and surrounding muscle layer. **(G)** Midregion of the body, showing typhlosolis formed by infoldings of the gut epithelium. ag, accessory ganglia mass; bcm, buccal commissure; bg, buccal ganglion; c, cuticle; ca, apical part of gland cells; cbc, cerebrobuccal connective; cg, cerebral ganglion; dc, dorsal caecum; dm, muscle layer surrounding duct; dtso, dorsoterminal sense organ; fg, ventrolateral foregut gland; fgd, ducts of foregut glands; ft, foot; gn, gonad; ln, lateral nerve cord; m, dorsopharyngeal muscle; mg, midgut; mo, mouth opening; oc, oocyte; p, pericardium; pc, pallial cavity; pd, pericardioduct; pgl1, pedal gland type 1; pgl2, pedal gland type 2; ph, pharynx; phg, pharyngeal gland; pp, pedal pit; pr, pharynx retractor muscle; ps, pharyngeal muscle sheath; r, radula; rc, rectum; sd, spawning duct; sg, sole gland; srl, suprarectal loop; sv, seminal vesicle; t, typhlosolis; vb, vestibular-buccal cavity; vn, ventral nerve cord.

ascertained from our material. Due to the problematic fixation and preservation of the material for histological purposes and the lack of complete information on the reproductive system, we refrain from establishing a novel genus within Halomeniinae. We postpone the formal description of this species, with the possibility of linking individuals collected in the

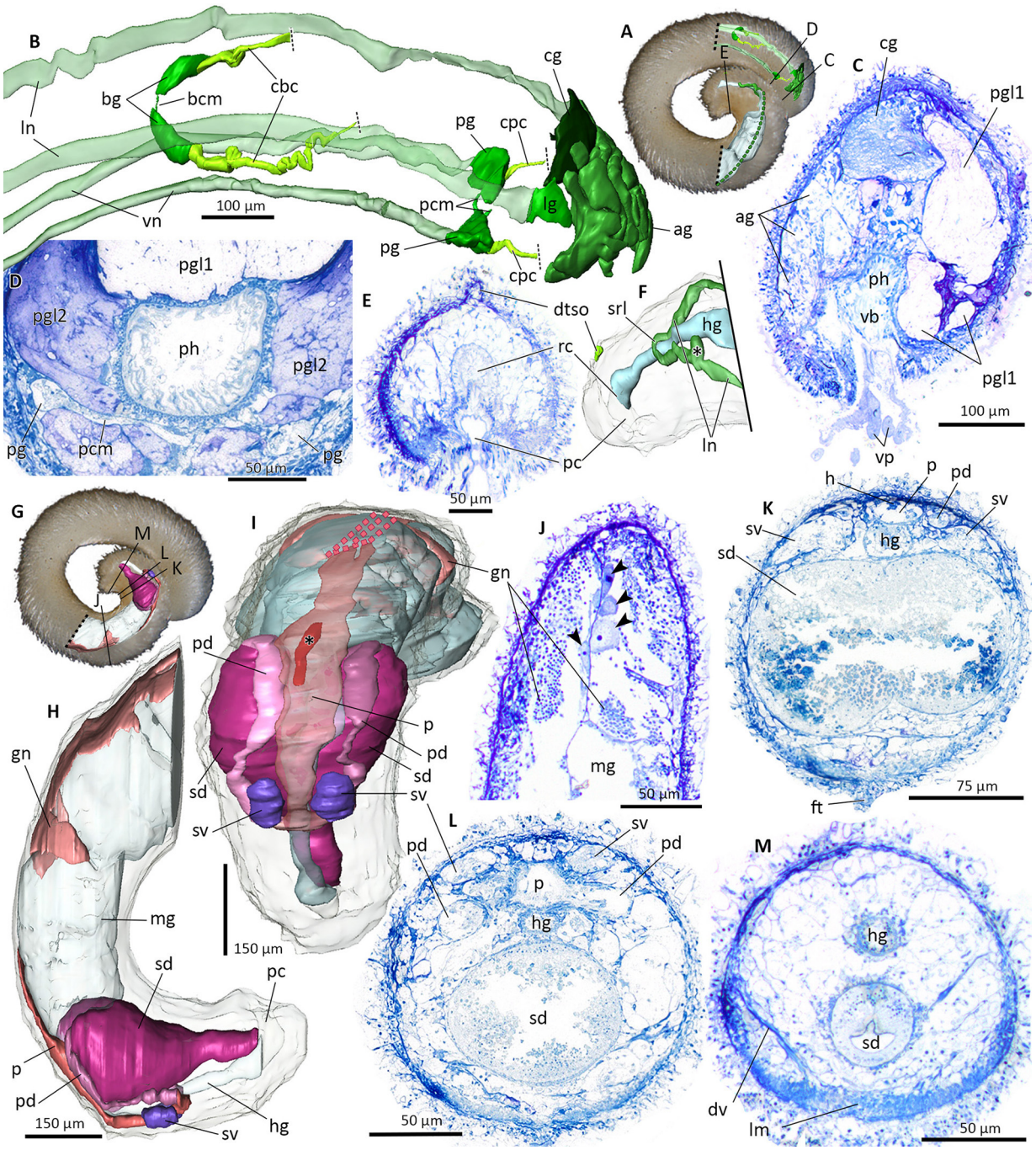

FIGURE 10 | Nervous system **(A–F)** and reproductive system **(G–M)** of *Amboherpia abyssokurilensis* sp. nov. in 3D **(A,B,F,H,I**–dotted lines indicate areas which could not be reconstructed) and histology from cross-sections **(C–E, J–M)**. **(A)** Position of reconstructed nervous structures within animal. Black labeled lines indicate level of histological sections. **(B)** Anterior nervous system. Lateral and ventral nerve cords transparent. Dorsolateral view. **(C)** Atrium at the transition into the pharynx. **(D)** Pedal ganglia and pedal commissure. **(E)** Dorsoterminal sense organ. **(F)** Posterior nervous system, body transparent. Dorsolateral view. Asterisk marks ganglia-like swelling of lateral nerve cord. **(G)** Position of reconstructed reproductive organs within animal, black labeled lines indicate levels of respective histological sections. **(H)** Lateral view of the reproductive system. Gut and body transparent. **(I)** Dorsal view of the reproductive system. Pericardium (transparent) with heart (asterisk). **(J)** Histology of the gonad, showing oocytes (arrow heads) along the median septum and spermatocytes. **(K)** Anterior part of the spawning duct, still bilobed, filled with secretion. **(L)** Fused mid part of spawning duct. **(M)** Posterior part of the spawning duct. ag, accessory ganglia mass; bcm, buccal commissure; bg, buccal ganglion; cbc, cerebrobuccal connective; cg, cerebral ganglion; cpc, cerebropedal connective; dtso, dorsoterminal sense organ; dv, dorsoventral musculature; ft, foot; gn, gonad; h, heart; hg, hindgut; lg, lateral ganglion; lm, longitudinal musculature; ln, lateral nerve cord; mg, midgut; p, pericardium; pc, pallial cavity; pcm, pedal commissure; pd, pericardioduct; pg, pedal ganglia; pgl1, pedal gland type 1; pgl2, pedal gland type 2; ph, pharynx; rc, rectum; sd, spawning duct; srl, suprarectal loop; sv, seminal vesicle; vb, vestibular-buccal opening; vn, ventral nerve cord; vp, vestibular papillae.

future with this lineage via the provided barcode and hopefully delivering all necessary features for a detailed integrative species description.

Pruvotinidae sp. 1 (Figure 2):
Material: single specimen (ZSM Mol20170078).
Distribution: St. 2–09, 4,830–4,863 m.
Habitus: fuzzy appearance, ∼2 mm in length, light yellow coloration with thin and transparent cuticle.
Scleritome: dominated by three types of hollow acicular spicules, straight with either pointed or serrated ends and one type curved (both up to 200 μm). Interspersed hook-shaped elements with pointed curvature, present along the dorsal side of the body. No further sclerites discernible due to shrinkage of animal during SEM preparation.
Radula: unknown.
GenBank Accession Number: MG524984.
Interspecific genetic distance to sister clade Pruvotinidae sp. 2: 21.5–23.9%.

Pruvotinidae sp. 2 (Figure 2):
Material: four specimens (ZSM Mol20170079, −80, −81, −82).
Distribution: Sts. 5–09, 8–09, 7–09, 7–10, 5,125–5,379 m.
Habitus: spiny appearance with several sclerites conspicuously longer than rest of scleritome; ∼2 mm in length, head region slightly enlarged in comparison to rest of body; white coloration.
Scleritome: dominated by three types of hollow, acicular sclerites: slightly curved with either pointed or serrated distal end (up to 400 μm) or of sigmoid shape with serrated distal ends (190 μm). Hook-shaped elements present with curve at proximal base, pointed at distal curvature (length 50 μm). One type of triangular scales (35 μm length). Foot scales: blade-shaped (45 μm).
Radula: unknown.
GenBank Accession Number: MG524980, −81, −82, −83.
Intraspecific variation: 0.4–1.0%.
Interspecific genetic distance to sister clade Pruvotinidae sp. 1: 21.5–23.9%.

Pruvotinidae sp. 3 (Figure 3C)
Material: single specimen (ZSM Mol20170083).
Distribution: St. 6–12, 5,291–5,307 m.
Habitus: spiny, several sclerites conspicuously larger than rest of scleritome (**Figure 3C**); ∼1.5 mm in length; white to light yellow coloration.
Scleritome: dominated by one type of hollow, acicular sclerites curved with slight bend, no serrated distal end (up to 250 μm length). Hook-shaped elements present, curved at proximal base, pointed at distal curvature (length 75 μm) (**Figure 3C1**). Type of foot scales unknown.
Radula: distichous, each tooth with one large lateral denticle and at least two smaller median denticles (**Figures 3C2,C3**). No molecular data available.

Simrothiellidae Salvini-Plawen, 1978
The scleritomes within this family are highly variable and can reach from hollow or solid acicular needles to more scaly sclerites arranged in up to several layers. We identified three different simrothiellid species, all collected as singletons. Habitus and scleritome of Simrothiellidae sp. 1 and sp. 2 are highly similar, but the two species differ in the presence (sp. 1) respectively absence (sp. 2) of a dorsoterminal sense organ (DTSO), observed using SEM. Due to the presence of captate sclerites and a dorsoterminal sense organ, the identification of the third lineage to genus level (*Spiomenia* sp.) is possible without additional anatomical characters.

Simrothiellidae sp. 1 (Figure 2):
Material: single specimen (ZSM Mol20170097).
Distribution: St. 8–09, 5,125–5,140 m.
Habitus: rough appearance, ∼1.2 mm in length, stout body; yellow coloration.
Scleritome: dominated by three types of hollow, acicular elements: straight with pointed distal end, one curved, one of sigmoid-shape (about 60 μm in length). Leaf-shaped scales visible surrounding a dorsoterminal sense organ. Foot scales leaf-shaped.
Taxonomic remarks: curved and sigmoid hollow spicules similar to the spicules of this species have been described for e.g., *Simrothiella comorensis* (see Figure 4A in Todt and Salvini-Plawen, 2003).
However, Simrothiellidae sp. 1 additionally has straight spicules which are lacking in *S. comorensis*.
Radula: unknown.
GenBank Accession Number: MG524979.
Interspecific genetic distance to sister clade Proneomeniidae sp. 1: 26.7%.

Simrothiellidae sp. 2 (Figure 3D):
Material: single specimen (ZSM Mol20170098).
Distribution: St. 8–09, 5,125–5,140 m.
Habitus: rough appearance, ∼1.5 mm in length, stout body; yellow coloration.
Scleritome: two types of hollow, acicular sclerites (up to 100 μm): straight with pointed distal end (**Figures 3D1,D2** asterisks) and curved sclerites (**Figure 3D2,** white arrowhead). No dorsoterminal sense organ. Elongated leaf-shaped scales of one type only (**Figure 3D3**, white arrowhead). No information on foot scales.
Radula: unknown. No molecular data available.

Spiomenia sp. (Figure 3E):
Material: single specimen (ZSM Mol20170099).
Distribution: St. 6–12; 5,291–5,307 m.
Habitus: very spiny appearance, stout body, 2 mm length; white coloration.
Scleritome: dominated by three types of hollow acicular sclerites, majority with pointed distal ends (**Figure 3E1**) or captate (distal end asymmetrically enlarged, **Figure 3E2**, white arrowhead), others with curved distal portion (**Figure 3E4**, white arrow head). Scales surrounding dorsoterminal sense organ only (**Figure 3E3**, white arrow). No information on foot scales. Radula: unknown. No molecular data available.

Acanthomeniidae Salvini-Plawen, 1978
Solenogastres with thin cuticle, hollow acicular sclerites and scales. Radula either monoserial or absent. Ventrolateral foregut glands of Type *Acanthomenia* (Type A sensu Salvini-Plawen, 1978a; Gil-Mansilla et al., 2008).

Veromenia *cf.* singula *(Figures 2, 7):*
Material: five specimens (ZSM Mol20170089, −90, −91, −92, −93).
Distribution: Sts. 7–10 and 8–12, 5,115–5,221 m.
Habitus: fuzzy appearance, ∼2–3.5 mm in length; coloration ranging from white to light orange (**Figure 2**).
Scleritome (**Figure 2** *Veromenia* cf. *singula* 1-10): dominated by four types of hollow, acicular elements: short and strongly curved (250 μm) (1), slightly curved, with varying thickness (2), straight (3), straight with widened mid-region and pointed distal end (4); (2)–(4) all up to 300 μm in length. Five types of scales: with folded lateral edges (5), rod-shaped with pointed distal end (6) rimmed and elongated (7), rimmed and leaf-shaped of varying length (8), blade-shaped with rim (9). Foot scales (10): blade-shaped with rimmed base and bulge. Without radula.
GenBank Accession Number: MG524975, −76, −77, −78.
Intraspecific genetic variability: 1.6–3.8%.
Interspecific genetic distance to sister clade: 21.4–33.4%.

Anatomy of Veromenia cf. singula
The anatomy of *Veromenia* cf. *singula* is based on histological serial sections of ZSM Mol 20170093 (**Figure 7A**, animal after fixation in ethanol). Vestibular-buccal cavity with papillae (**Figures 7B,D**); pedal pit small, with one type of pedal gland (**Figures 7B,E**). Cerebral ganglion fused with four nerve cords along lateral and ventral body wall (**Figures 7B,D**). "Accessory ganglia" mass (=pre-cerebral ganglia), paired buccal ganglia, and paired pedal ganglia present (**Figure 7B,D**). Dorsoterminal sense organ located dorsoposterior to pallial cavity (**Figures 7C,I**). Mouth opening located in dorsoposterior region of vestibular-buccal cavity. No radula present, pharynx leads directly into midgut (**Figure 7E**); no dorsal midgut caecum, no lateral constrictions. Hindgut opens dorsal into pallial cavity (**Figures 7C,H**). Ventrolateral foregut glands of Type *Acanthomenia* (**Figures 7B,E**) (definition according to Handl and Todt, 2005). Gonad extending far anterior (**Figures 7B,E**). Connected to pericardium via gonopericardioducts; paired ciliated pericardioducts leading into paired spawning ducts (**Figures 7C,F**). Seminal receptacle with sperm connected to dorsoanterior part of spawning duct (**Figures 7C,G**). Pallial cavity with infoldings (**Figures 7C,I**), but no true respiratory folds (compare **Figure 6F** for respiratory folds); ventral pouch connected to anterior part of pallial cavity (**Figures 7C,H**) with underlying conspicuous muscle layer (**Figures 7G,H**).

Taxonomic remarks
Anatomy and histology of the encountered lineage hardly differ from the holotype of the species (Gil-Mansilla et al., 2008), but the scleritome of the present material is more diverse than shown in the original species description (**Figure 2** sclerites 1, 3, 4, 6, 7, 9 not depicted in original description). Since intraspecific variation of scleritomes and distribution ranges in Solenogastres are still largely unknown, our species assignment is still tentative.

Amboherpia abyssokurilensis *sp. nov. (Figures 3F, 8–10)*
Material examined: Holotype (ZSM Mol20170088, histological section series), paratypes1-4 (ZSM Mol20179984-87) used for examination of the scleritome and preparation of the radula (ZSM Mol20170086, radula SEM mounted).
ZooBank registration: urn:lsid:zoobank.org:pub:A85AFE00-14C8-469D-A797-594C7DB3DB7A.
Type locality: Northwest Pacific, near Kuril-Kamchatka Trench, KuramBio Station 9-12 (40.5918°N–40.5713°N; 150.9976°E–150.9864°E), 5,392–5,397 m depth.
Etymology: Referring to the type locality of the species on the abyssal plain close to the Kuril-Kamchatka Trench.
Diagnosis: Hollow, acicular sclerites and two different types of solid body scales. Pharynx with pharyngeal glands, midgut without constrictions, but lateral typhlosolis. Radula teeth up to 50 μm at widest part of base. No esophagus. No sphincter muscle surrounding secondary genital opening (=opening of spawning duct).

Description
Smooth appearance; body round in diameter, ∼3.6 mm in length. Body white in fixed condition (**Figures 3F**, **9A′**). Cuticle up to 25 μm thick, no epidermal papillae. Scleritome (**Figure 3F1**): three types of hollow, acicular elements; most common one curved with flattened distal end (250 μm length) (1); slightly curved (ca. 50 μm) (2) and straight (40 μm) (3). Two types of body scales: flat (3) and with rim at proximal base (4). Foot scales blade-shaped with slight bulge (5). Vestibulum (=atrium) and buccal opening fused into single vestibular-buccal cavity (**Figure 8A**), with at least three discernible, unbranched vestibular papillae protruding from dorsoanterior wall. Foot emerges posterior to vestibular-buccal opening from inconspicuous pedal pit and terminates anterior to small pallial cavity. Two types of pedal glands open into the pedal pit. Type 1 fills most of the head region (**Figures 9A,D**), and stains whitish to very light pink. Type 2 is smaller, present on both sides of pedal pit and stains in a slightly darker pink compared to Type 1 (**Figure 9D**). Both of diffuse appearance, but clearly delimited by thin layer of connective tissue. Both discharge secretions directly into pedal pit. Sole glands as unicellular glands distributed evenly along both sides of foot groove (**Figure 9A**, shown only in the anterior part of the reconstruction). Light-purple staining secretions are discharged by each cell via minute outlets along the foot (**Figure 9D**, arrow).

Digestive system
The digestive system consists of pharynx, midgut, and the associated glands (**Figures 8A**, **9**). Mouth opening located in dorsoposterior region of vestibular-buccal opening (**Figures 8A**, **9A**). Pharynx (up to level of radula) surrounded by unicellular pharyngeal glands, each discharging light-purple staining secretions into pharyngeal lumen (**Figures 9B,D** asterisks). Pharynx divided into two histologically distinct parts: first part ciliated (**Figure 9D**), after pharyngeal sphincter

(**Figure 9C′**) lined with pseudostratified, glandular epithelium. After pharyngeal muscle sheath (**Figure 9C′**, asterisk), pharynx contains radula, which rests on several muscular radula bolsters (as defined by Handl and Salvini-Plawen, 2002) (**Figure 9E,** asterisk). Radula monoserial: five rows of teeth (**Figures 9C,C″,E**) discernible in serial section (only three shown in reconstruction); base of tooth almost 50 μm wide, two hook-shaped hollow denticles (∼45 μm long) bend dorsal from each tooth (**Figure 9C″**). Paired ventrolateral foregut glands of Type *Acanthomenia* (Handl and Todt, 2005) open into pharynx on each side laterally to radula (**Figures 8A**, **9C**). Each duct ∼300 μm long, comprising supporting cells and cell necks of glandular cells (cell apices) surrounded by muscular sheath (**Figure 9F**). Ducts posterior surrounded by large mass of somata of glandular cells, which produce dark-purple staining secretions. Secretions released into duct lumen via cell apices (**Figure 9F**). Pharynx connected almost vertically with midgut (**Figure 8A**); anterior midgut ceacum present, divided into three parts by muscles connecting pre-radula sphincter with dorsal body wall [**Figures 8A**, **9B,C′** (asterisks)]. In the mid region of animal, lateral walls of midgut epithelium form a typhlosole-like horizontal fold (**Figure 9G**). Midgut narrows into ciliated hindgut, opening into dorsoanterior region of pallial cavity.

Nervous system and sensory structures

Anterior central nervous system consists of cerebral ganglion, mass of pre-cerebral "accessory ganglia," paired buccal and pedal ganglia and four nerve cords (**Figures 10A,B**). Cerebral ganglion without median sulcus, at least two anterior cerebral nerves connect it to "accessory ganglia" mass; this mass is an interconnected accumulation of spherical bodies presumably comprised of neural tissue; clearly delimited by connective tissue and with random distribution of neuropil and perikarya (**Figure 10C**). At least four nerves run from accessory ganglia toward vestibulum. A short cerebrolateral connective emerges posteriorlaterally from each side of cerebral ganglion. Lateral nerve cords emerge from lateral ganglia and extend along lateral body wall toward posterior end of the animal (**Figure 10A**). Single nerve from left lateral ganglion innervates vestibular papillae. Paired buccal ganglia located dorsoposterior to radula, interconnected via thin and short buccal commissure (**Figures 9C**, **10A**).

Origin of cerebropedal and -buccal connectives not identifiable on histological sections, but nerves found to run in direction of cerebral ganglion from each pedal and buccal ganglion, presumably constituting missing connectives (**Figure 10B**). Pedal ganglia flattened; located ∼150 μm posterior to cerebral ganglion on both sides of foot groove (**Figures 10B,D**). Single nerve exits each ganglion medially in direction of the foot, most likely forming pedal commissure (**Figure 10D**). In posterior part of nervous system, only lateral nerve cords could be reconstructed (**Figures 10A,F**). Right lateral nerve cord thickens into swelling (**Figure 10F**, asterisk) and merges with left lateral nerve cord in suprarectal loop (**Figure 10F**).

Dorsal to pallial cavity, cuticle and underlying epidermis form protuberance (**Figures 10E,F**). Based on form, position and proximity to suprarectal loop thus interpreted as dorsoterminal sense organ, but innervation could not be detected.

Gonopericardial system

Paired gonads connected to pericardium, paired pericardioducts, a partially fused spawning duct and seminal vesicles form the reproductive system (**Figures 8B,C**, **10G–M**). Tubular, hermaphroditic gonads enclosed between midgut and dorsal body wall. Anterior region filled with oocytes (**Figures 9G**, **10J**), spermatozoids present in posterior region. Initially paired gonads fuse medially in posterior region. Connection to pericardium (=gonopericardioducts) could not be detected on histological sections (see **Figure 10I**, dotted lines). Pericardium as a flat and elongated sac delimited by thin epithelium; contains tubular heart of ∼110 μm in length (**Figures 10I** (asterisk), **K**). Posterior, pericardium widens and divides into two pericardioducts (**Figure 10L**). Two vesicles present at transition of pericardium into pericardioducts (**Figures 8B,C**, **10H,I,K**); connection between vesicles and other reproductive structures not detectable, but based on their position herein interpreted as seminal vesicles. Pericardioducts loop ventroanterior, then transition into paired spawning ducts each filled with glandular secretions (**Figures 8B,C**, **9H,I**). Spawning ducts initially paired in first half, then fusing into single duct with high columnar glandular epithelium before opening ventroanterior to hindgut into pallial cavity (**Figure 8B**).

Taxonomic remarks

Amboherpia abyssokurilensis sp. nov. is placed within Acanthomeniidae Salvini-Plawen, 1978 due to its scleritome consisting of hollow acicular needles and scale-like sclerites as well as the presence of ventrolateral foregut glands of Type *Acanthomenia* (Handl and Todt, 2005) sensu Type A (according to Salvini-Plawen, 1978a). Based on the presence of a fused vestibular-buccal cavity, radula and dorsoterminal sense organ as well as the absence of respiratory folds in the pallial cavity, the new species is assigned to the genus *Amboherpia*. *A. abyssokurilensis* sp. nov. is the third species within the genus and exhibits a combination of characters found in *A. heterotecta* Handl & Salvini-Plawen, 2002 (collected in 250–610 m in a Norwegian fjord) and *A. dolicopharyngeata* Gil-Mansilla, García-Álvarez & Urgorri, 2008 (collected in 5,389–5,415 m from the Angola Basin). Scleritome and radula resemble *A. dolicopharyngeata*, but *A. abyssokurilensis* sp. nov. differs from this species in lacking a well-developed esophagus and sphincter muscle around the spawning duct, the presence of conspicuous unicellular pharyngeal glands and a midgut with internal folds (typhlosolis) as well as the size of the base of the radula teeth.

At Stations 7–09 and 8–12 two additional specimens of an acanthomeniid lineage were collected. However, based on the available scleritome data of this lineage (**Figure 4A**) we were unable to reliably discriminate it from *A. abyssokurilensis* sp. nov. (**Figure 3F**) and we thus tentatively refer to it as Acanthomeniidae sp. 1? (see below).

Acanthomeniidae sp. 1? (Figure 4A)
Material: two specimens (ZSM Mol20170095, −96).
Distribution: Sts. 7–09, 8–12; 5,115–5,223 m.
Habitus: smooth to slightly rough appearance, ∼2 and 2.5 mm in length. Body slender, white coloration.
Scleritome: dominated by four types of acicular elements: hollow, straight with pointed distal end (**Figures 4A2,A4**) or curved with flattened distal end (**Figure 4A5**). Additionally with short, solid (?) curved needles and straight needles with ridge (**Figure 4A6**, white arrowheads). Only one type of scale visible: leaf-shaped, proximal base morphology (e.g., presence of rims) unknown (**Figure 4A3**).
Foot scales not visible.
Radula: unknown. No molecular data available.

Acanthomeniidae sp. 2 (Figure 4B):
Material: single specimen (ZSM Mol20170094).
Distribution: St. 8–12, 5,115–5,124 m.
Habitus: smooth appearance, 6.5 mm in size; body slender, yellow coloration (**Figure 4B**).
Scleritome: dominated by two types of hollow, acicular elements: curved with pointed distal end (**Figure 4B3**) and straight (**Figure 4B4**). Five types of scales: slightly concave, with pointed distal end (**Figure 4B1**), excavated scales with drawn-out, sharp distal end (75 μm) [**Figure 4B5** (1)], scales with small stalk and rim at proximal base of (25–35 μm) [**Figure 4B5** (2)], pointed rod-shaped scales with strongly thickened lateral ridges and flat back (25 μm) [**Figures 4B2, B5** (3)], excavated blade-shaped scales [**Figure 4B5** (4)]. Foot scales blade-shaped with bulge (60 μm length) [**Figure 4B** (5)].
Taxonomic remarks: this lineages is similar to the other acanthomeniids in its habitus (compare **Figures 4A,B**) as well as in some distinct scleritome characters, such as the presence of hollow, straight needles (**Figures 4A2,B3**) and scales with a small stalk and rimmed proximal base [compare **Figure 4B5** (1, 2) with Figure 2D (14–16, 19) in Scheltema (1999) and Figure 2 in Handl and Salvini-Plawen (2002)]. Radula: unknown. No molecular data available.

STERROFUSTIA Salvini-Plawen, 1978

Order characterized by the presence of only solid acicular sclerites, which can be combined with various types of solid scales.

Sterrofustia sp. 1 (Figure 4C):
Material: single specimen (ZSM Mol20170108).
Distribution: St. 11–12, 5,348–5,350 m.
Habitus: furry appearance due to perpendicularly projecting sclerites, 2.7 mm long; white coloration (**Figure 4C**). Scleritome: dominated by solid acicular elements of varying length (150–250 μm) distributed evenly across the body [**Figures 4C1,C4** (1)]; cross-section like a three-point star (**Figure 4C2**, white arrowhead), second type of solid elements as interspersed straight needles [**Figure 4C4** (3). Keeled scales up to 150 μm in length (**Figure 4C4** (2)]. Foot scales blade-shaped (75 μm length) [**Figure 4C4** (4)].
Radula: unknown. No molecular data available.

PHOLIDOSKEPIA Salvini-Plawen, 1978

This order is characterized by almost exclusively scaly sclerites and only occasional acicular elements. Only one family of this order was represented in our material by three distinct lineages.

Dondersiidae Simroth, 1893
The scleritome of dondersiids consists of various types of scales, occasionally combined with solid acicular elements. Based on this combination of scleritome elements (scales together with solid needles) the following three lineages were assigned to Dondersiidae.

Dondersiidae sp. 1 (Figure 4D):
Material: single specimen (ZSM Mol20170106).
Distribution: St. 6–12, 5,291–5,307 m.
Habitus: velvety, with sclerites arranged flat against body surface; ∼3 mm in length; yellow coloration (**Figure 4D**).
Scleritome: dominated by leaf-shaped scales with rimmed base (up to 30 μm) (**Figures 4D1,D3**).
Acicular elements interspersed, solid short needles (up to 60 μm) (**Figures 4D1,D2**). No information on foot scales. Surface of all sclerites with hair-like ultrastructure (**Figure 4D3**).
Radula: unknown. No molecular data available.

Dondersiidae sp. 2 (Figure 4E):
Material: single specimen (ZSM Mol20170107).
Distribution: St. 8–12, 5,115–5,124 m.
Habitus: smooth, ∼3.5 mm in length; white coloration (**Figure 4E**).
Scleritome: dominated by scales in leaf-shaped form [**Figures 4E2,E4** (1)]; leaf-shaped scales with short stalk [**Figure 4E4** (2)]; scales with diamond shape [**Figure 4E4** (3)]; elongated, slender scales with stalk [**Figure 4E4**, (4)]. Interspersed solid short [**Figure 4E3** white arrowheads, **Figure 4E4** (5)] and long needles (up to 100 μm in length) [**Figures 4E3,E4** (6)]. No information on foot scales.
Taxonomic remarks: the scleritome of Dondersiidae sp. 2, especially the stalked elements [**Figure 4E4** (2, 4)], is highly similar to the Antarctic *Nematomenia* (?) *squamosa* (Thiele, 1913) (see Figure 29 in Salvini-Plawen, 1978a).
Radula: unknown. No molecular data available.

Dondersiidae sp. 3 (Figure 5):
Material: single specimen (ZSM Mol20170105).
Distribution: St. 7–09, 5,216–5,223m.
Habitus: spiny, with several conspicuously longer acicular elements; ca. 780 μm in size; white coloration (**Figure 5A**).
Scleritome: three types of scale-like, solid elements. Main type: symmetrically leaf-shaped, with median keel (∼40 μm length) (**Figure 5A5**), second type only found anterior, leaf-shaped but with strongly rimmed base and lateral ridge (12 μm) (**Figure 5A4**), and flat leaf-shaped scales (40 μm) (**Figure 5A6**). Two types of hollow, needle-like elements interspersed between scales at lateral and dorsal sides. Curved ones with circular diameter (up to 200 μm length) (**Figure 5A3**). At posterior end: second type of acicular sclerites with strong median keel; length: up to 200 μm (**Figures 5A1,A2**); unknown whether solid or

hollow. No information on foot scales. Radula: unknown. No molecular data available.

Taxonomic remarks: within Dondersiidae the encountered lineage bears some similarities to *Helluoherpia aegiri* Handl & Büchinger, 1996 in the presence of distinctively rimmed and flat scales as well as solid needles. However, there are some distinct differences i.e., the presence of medially keeled scales in Dondersiidae sp. 3 (**Figure 5A5**) which are lacking in *H. aegiri* (Handl and Büchinger, 1996).

DISCUSSION

Combining Scleritome Morphology and Barcodes: A Fast and Efficient Approach to Assess Solenogaster Diversity

Solenogastres are usually excluded from biodiversity assessments due to the complex and time-consuming methods necessary for their taxonomic identification. While most solenogaster taxonomists have focused on species delineation based on microanatomical and histological characters (Nierstrasz, 1902; Heath, 1911; Salvini-Plawen, 1978a,b), Scheltema and Schander (2000) already highlighted the value of habitus and scleritome for species delineation and phylogeny. Solenogaster external morphology provides a character set which is easily accessible via light microscopy, and since its analysis does not require more taxonomic training than morphological species delineation in other clades, it is also feasible for non-specialists.

Our study identifies at least 19 morphospecies, which can be distinctively delineated based on the differences in their scleritomes. Methodologically, light microscopy is sufficient to characterize the overall diversity of the scleritome. Scanning electron microscopy (SEM) on entire specimens, however, provides a better overview on the distribution of sclerites as well as additional ultrastructural information (see, e.g., **Figure 4A6**). However, SEM of entire individuals needs to be combined with investigation of isolated sclerites, as their base might also provide important taxonomic characters. Scleritome-based species delineation might become limited, however, with denser sampling and when dealing with closely related lineages. We were unable, for example, to reliably discriminate Acanthomeniidae sp. 1? (**Figure 4A**) from *A. abyssokurilensis* sp. nov. (**Figure 3F**) based on the available scleritome data. Knowledge on the intraspecific variation of the scleritome is generally scarce, but in some families, as e.g., Epimeniidae (Cavibelonia), the slight interspecific variability is overlapped by intraspecific variability (Salvini-Plawen, 1997a). So far, species boundaries in Solenogastres have never been tested via molecular markers. Especially variability of sclerite size is difficult to evaluate with regard to its taxonomic value, without knowledge on the maturity of the individual, as can only be derived from histological sectioning. Moreover, in minute mesopsammic Solenogastres externally cryptic and co-occurring species have been documented (Bergmeier et al., 2016a), and crypsis might be a common phenomenon also among deep-sea Solenogastres. Thus, scleritome-based species delineation needs to be combined with molecular approaches.

Nuclear genes of Solenogastres are notoriously hard to amplify due to complex secondary structures, which inhibit amplification via standard PCR (Meyer et al., 2010). Using the mitochondrial standard markers for cytochrome *c* oxidase subunit 1 and 16 S rRNA broadly applied on various clades of molluscs (e.g., Klussmann-Kolb et al., 2008; Wilson et al., 2010; Stöger et al., 2013; Kano et al., 2016) resulted in low amplification success. In general, successful DNA amplification of these deep-sea molluscs is dependent on fast processing of the material. After 2.5 years of storage in ethanol, the success rate dropped considerably in comparison to fresh material (own observations). Using a solenogaster-specific pair of self-designed primers ("16Soleno-f" and "–r," see section Materials and Methods) increased our amplification success of mitochondrial 16S rRNA, and we were at least able to generate sequence data for ∼50% of our material.

Our molecular dataset (in conjunction with the provided scleritome data) will allow to reliably assign novel material collected in the region to our herein preliminary characterized morphospecies. The comparably high (i.e., >16%) genetic distances on the 16S rRNA sequences between the identified morphospecies at present supports the hypothesis of them being independently evolving lineages. Due to the low number of sequences of genetically distant lineages within this worm-mollusc clade with hypothesized Paleozoic origin (Vinther et al., 2012), our barcode dataset currently does not allow for meaningful molecular species delineation. Tree-based approaches (as, e.g., GMYC; Pons et al., 2006; Monaghan et al., 2009) analyzing the transition point between the speciation and coalescent processes on an ultrametric gene tree are largely hampered by the inclusion of singletons (80% in our dataset). Bayesian species delineations (Yang and Rannala, 2010; Zhang et al., 2011) rely on multiple loci, but have nevertheless been criticized for reconstructing structure in a dataset but not necessarily species, unable to discriminate between population and speciation processes (Sukumaran and Knowles, 2017). A denser sampling of abyssal Solenogastres and a multi marker molecular approach is needed to investigate the species boundaries between deep-sea Solenogastres.

An advantage of our workflow is the efficient and fast assessment of operational taxonomic units and therein providing valuable information on α-diversity. The generated molecular barcodes (though unfortunately incomplete) allow for fast and reliable assignment of future material to the identified lineages. Low success rates in direct amplification via PCR on older material might be circumvented by high throughput sequencing approaches resulting in more significant datasets for molecular species delineation. External morphology links amplified barcodes to the established taxonomic system of the clade: ∼75% of lineages in our study could be assigned to family level and beyond. For four lineages [Cavibelonia sp. 1, sp. 2 (both **Figure 2**), sp. 3 (**Figure 3A**) and sp. 4 (**Figure 3B**) and Sterrofustia sp. 1 (**Figure 4C**)] taxonomic placement was impossible, despite detailed documentation of their scleritomes. Unique and conspicuous sclerites, such as the three-point star needles in Sterrofustia sp. 1 (**Figure 4C2**), never documented before in Solenogastres, suggest that these lineages are new to science and thus currently cannot be integrated into the existing

classificatory system. Singletons like Sterrofustia sp. 1 could be easily excluded from traditional approaches, underlining the value of an overall characterization of α-diversity, which helps to identify key lineages of major evolutionary importance for future work and at the same time provides full formal species descriptions in common lineages.

Systematics and Diversity of Abyssal Solenogastres in the Northwest Pacific

The current state of knowledge regarding solenogaster species diversity in the region is generally scarce with hitherto only 11 known species prior to this study. Nine have been described and reported from the coastal waters off Japan, one of them—*Alexandromenia marisjaponica* Saito & Salvini-Plawen, 2014 (Amphimeniidae)—from the Sea of Japan and the others from the Pacific coast [*Neomenia yamamotoi* Baba, 1975 (Neomeniidae), *Epimenia ohshimai* Baba, 1940 and *E. babai* Salvini-Plawen, 1997 (Epimeniidae), *Anamenia triangularis* (Heath, 1911), *A. amabilis* Saito & Salvini-Plawen, 2010 and *A. farcimen* (Heath, 1911) and *Strophomenia ophidiana* Heath, 1911 (all Strophomeniidae), and *Driomenia pacifica* Heath, 1911 (Rhopalomeniidae)] (see Baba, 1940, 1975; Saito and Salvini-Plawen, 2010, 2014). Two species are known from the Sea of Okhotsk (*Neomenia yamamotoi* and *Halomenia gravida* Heath, 1911) and one from the Bering Sea [*Nematomenia platypoda* (Heath, 1911), Dondersiidae] (García-Álvarez and Salvini-Plawen, 2007; Sirenko, 2013). All of these species were collected from 40 to 1,500 m, while the Northwest Pacific lower bathyal and beyond remains largely unexplored with regard to solenogaster fauna. Our sampling of the abyssal Northwest Pacific (NWP) Plain, close to the Kuril-Kamchatka Trench, revealed 19 distinct solenogaster lineages (excluding Acanthomeniidae sp. 1?), which were delimited based on a combination of external morphology, molecular sequence data and microanatomy. Of the five families identified in our study, only Dondersiidae and Pruvotinidae have already been reported from the NWP region (*N. platypoda* from the Bering Sea and *H. gravida*). Based on distinct differences in habitus (*N. platypoda* has a distinct dorsal keel, see plate 1, Figure 4 in Heath, 1911), scleritome (and in case of Halomeniinae sp. 1 also anatomy) as well as bathymetric differences of more than 4,000 m, conspecifity between *N. platypoda*, *H. gravida* and any of the five pruvotinid and dondersiid lineages uncovered in this study can be excluded, and we assume that all 19 abyssal lineages present novel records for this region. The diversity of Solenogastres in the Northwest Pacific and its marginal seas is thus almost tripled to 30 recorded species.

The only sterrofustian lineage Sterrofustia sp. 1 found during the KuramBio cruise presents the first record of this order in the Northwest Pacific. For the discovered cavibelonian lineages (except Pruvotinidae, see above), the geographically closest records of Proneomeniidae are from Hawaii and the Indonesian Sunda Sea (Heath, 1911), and Simrothiellidae have been described from the Atacama Trench and hot-vent sites on the East Pacific Rise (Scheltema and Kuzirian, 1991; Scheltema, 2000; Salvini-Plawen, 2008). Proneomeniidae sp. 1, Simrothiellidae sp. 1 and sp. 2 expand the potential distribution ranges of the two families to the Northwest Pacific, and besides the West Indian Ocean, the simrothiellid genus *Spiomenia* is for the first time reported from the Pacific. The known distribution range of this family now covers the North and South Atlantic and the Antarctic Davis Straight as well as the Northwest Pacific. The discovery of *A. abyssokurilensis* sp. nov. expands the known distribution range of the genus from the abyssal Angola Basin (5,300–5,500 m) in the Southern Atlantic and Norwegian fjords (250–610 m) to the abyssal plain of the Northwest Pacific (5,390–5,400 m; Handl and Salvini-Plawen, 2002; Gil-Mansilla et al., 2008).

Little is known about vertical and horizontal distribution ranges of Solenogastres. While some pruvotinid and proneomeniid Solenogastres are brooders (Heath, 1911; Salvini-Plawen, 1978a; Todt and Kocot, 2014) with low distribution ranges, the majority of species develops via lecitotrophic, planktonic larvae. Between 5 and 11 days of swimming behavior from hatching to settlement of larvae have been observed (Okusu, 2002; Todt and Wanninger, 2010) for some clades and longer larval survival periods can be assumed for deep-sea lineages in cold waters. Due to the different modes in development, the dispersal abilities between the different clades might be highly variable and wide distribution ranges as commonly found in many deep-sea taxa cannot be excluded.

The majority of the discovered lineages from the Northwest Pacific presents unique scleritome characters distinguishable from other described abyssal lineages, and likely are species new to science. Only the acanthomeniid *Veromenia* cf. *singula* was identified in our material based on external similarities and the lack of any anatomical features distinguishing it from the individuals described from the abyssal Angola Basin (see Figures 4E,F, 5 in Gil-Mansilla et al., 2008). Comparative molecular data from the Atlantic material is needed to clarify the relationship between the Atlantic and Pacific *Veromenia* lineages and to gain valuable insights on the distribution patterns of abyssal Solenogastres. Currently, available data is sufficient to present the discovered diversity of abyssal Solenogastres from the NWP plain; however, the collected material and thereof retrieved data is insufficient to provide formal species descriptions within the present classificatory system on all discovered lineages. A combination of scleritome and molecular characters will likely be most suitable for rapid species description in the future, this is still hampered at present, however, by a largely histology-based classificatory system. Molecular data from type material of the established lineages is needed to avoid creating a parallel taxonomic system for Solenogastres (Jörger, 2015).

A First Glimpse into the Abyssal Solenogaster Fauna—All Lost Loners?

The Mediterranean and the Eastern Atlantic off the European coast constitute relatively well-studied regions in terms of solenogaster fauna, harboring nearly 30% of the global solenogaster diversity (Salvini-Plawen, 1997b; García-Álvarez et al., 2014; Pedrouzo et al., 2014). Intensive taxonomic work has also been conducted around Antarctica (Salvini-Plawen, 1978a,b; García-Álvarez et al., 1998, 2009; Zamarro et al., 2012), resulting

in close to 45% of the described global species diversity. In general, sampling has so far largely focused on bathyal depths and the only comparable study on Solenogastres from similar depths has been carried out in the South Atlantic Angola Basin (DIVA-1 expedition, Arbizu and Schminke, 2005): half of the globally known 18 species (classified in five families and 11 genera) described from below 4,000 m were collected here. While almost all previously known lineages belong to the order Cavibelonia (except for two), our study adds the first record of an abyssal sterrofustian lineage and doubles the global number of solenogaster lineages discovered in the abyss.

The solenogaster fauna of the abyssal Angola Basin and the Northwest Pacific Plain are dominated by cavibelonian Simrothiellidae, Acanthomeniidae and Pruvotinidae—with three identified genera (*Spiomenia*, *Amboherpia,* and *Veromenia*) present in both regions. From the NWP plain we collected 33 specimens, after sampling 53,708 m^2 of sea-floor with the C-EBS (Brandt et al., 2015) resulting in 19 morphospecies assigned to at least five different families, with a high number of singletons (74%). EBS sampling of only half the area of sea-floor in the Angola Basin (27,765 m^2, see Brandt et al., 2005) yielded twice as many specimens (64 individuals) and 50% more species (30 spp.) (Gil-Mansilla, 2008; Gil-Mansilla et al., 2008, 2009, 2012), indicating an overall higher diversity and abundance of Solenogastres in the region. The spatial distribution of Solenogastres is patchy in both regions. This patchiness seems to be more pronounced in the Angola Basin, where either only single specimens or between 10 and 23 individuals were sampled (Gil-Mansilla, 2008). In the NWP individual numbers from neighboring C-EBS hauls are continuously low (see **Table 1**). Distributional patchiness has been observed for several deep-sea taxa including molluscs (Schwabe et al., 2007; Jörger et al., 2014), and is generally linked to the heterogeneity of habitats and the availability of food sources (Rex and Etter, 2010; McClain et al., 2011). The majority of Solenogastres are predators of anthozoan or hydrozoan cnidarians (Salvini-Plawen, 1981), and their occurrence might thus be connected to the presence of their preferred prey.

The observed rarity of the discovered abyssal Solenogastres might be an indication for source-sink mechanics, in which the low density of individuals prevents the sustainability of populations of sexually reproducing organisms and the impoverished abyssal fauna is dependent on larval influx of bathyal populations (Rex et al., 2005). Preliminary diversity assessments indicate differences in the taxonomic composition of the shallower Kuril basin in the semi-isolated Sea of Okhotsk and the abyssal fauna investigated herein with only few conspecifics (Ostermair et al., accepted). Comparative analyses of the bathyal solenogaster diversity of the eastern slopes of the Kuril Islands and intensified sampling in the area are necessary to evaluate the origin of the abyssal NWP fauna, whether they present lost loners in the abyss or belong to reproducing abyssal populations.

AUTHOR CONTRIBUTIONS

FB: conducted the morphological and molecular analyses and drafted the manuscript; AB: organized the KuramBio expedition; ES: collected and sorted the KuramBio material; KJ: designed the study. All authors contributed to and approved the final version of the manuscript.

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First insights into the solenogaster diversity of the Sea of Okhotsk with the description of a new species of *Kruppomenia* (Simrothiellidae, Cavibelonia)

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ABSTRACT

Solenogastres form a small clade of worm-shaped molluscs distributed world-wide with most of the approx. 300 species occurring in the deep sea. A recent diversity study from the abyssal plain in the Northwest Pacific has revealed a wealth of new abyssal solenogaster lineages highlighting the need for further alpha-taxonomic work. During the 'Sea of Okhotsk Biodiversity Studies' (SokhoBio) expedition, 93 specimens were collected at overall eight stations ranging from depths of 1696–3377 m. Preliminary investigations revealed twelve clearly distinguishable morphospecies, including one relatively common species present at six of eleven stations. All morphospecies are characterized externally via light microscopy and identified to the best possible taxonomic level via scleritome characters. Molecular barcodes (16S rRNA) are provided as a first step towards establishment of a barcoding library to enable fast and accurate re-identification of these taxonomically challenging molluscs in future studies. The most wide-spread and abundant cavibelonian Solenogastres *Kruppomenia genslerae* sp. nov. (Simrothiellidae) is exemplarily described in full microanatomical detail based on 3D-reconstructions from histological semithin section series and scanning electron microscopy. We discuss our results in comparison to the solenogaster diversity of the neighbouring abyssal plain of the Northwest Pacific, which is connected with the Kuril Basin via two straits. Our initial characterization of the diversity boosts the hitherto poorly known diversity of Solenogastres in the Far Eastern Seas, but also underlines the taxonomic impediment for proper taxonomic descriptions of the wealth of new discovered material.

1. Introduction

Currently, the World's Register of Marine Species (WoRMS) records 226,000 valid marine species of eukaryotes, and listed another 40% of nominal species considered as synonyms (Appeltans et al., 2012). Estimates based on the number of species still to be discovered in the world's oceans vary considerably between approximately 300,000 based on description rates (Costello et al., 2012), 480,000–740,000 based on expert opinions (Appeltans et al., 2012) and up to 2.2 million (0.18 million standard error) based on extrapolations of the rate of discovery (Mora et al., 2011). Besides various approaches to retrieve biodiversity estimates, numbers remain highly speculative especially in the marine environment due to the poor overall exploration. In particular, the deep sea remains vastly unexplored with less than 1% of the deep-sea floors sampled and analysed (Ramirez-Llodra et al., 2010). Consequently, knowledge on the alpha-diversity of deep-sea organisms remains scarce and hampers broad scale beta-diversity approaches between regions as well as conservation attempts to protect this threatened habitat.

Molluscs form one of the dominant groups in the marine environment accounting for about 20% of the overall species-level diversity (Bouchet, 2006; Rosenberg, 2014) and the state-of-knowledge index on their diversity is comparably high in relation to other marine invertebrates (Costello et al., 2010). However, especially in the deep sea, biodiversity surveys have largely focused on the two major clades – bivalves and gastropods – which also provide comparably straightforward species delineation of morphospecies based on shell characters. This has enabled initial beta-diversity comparisons and the evaluation of hypotheses on source-sink mechanism and longitudinal gradients (e.g., Aldea et al., 2008; Brault et al., 2013a, 2013b; Jennings et al., 2013; McClain et al., 2012; Schrödl et al., 2011), which revealed taxon-specific patterns and underlined the risk of extrapolating conclusions based on single taxa to global scale deep-sea biodiversity patterns (McClain and Hardy, 2010).

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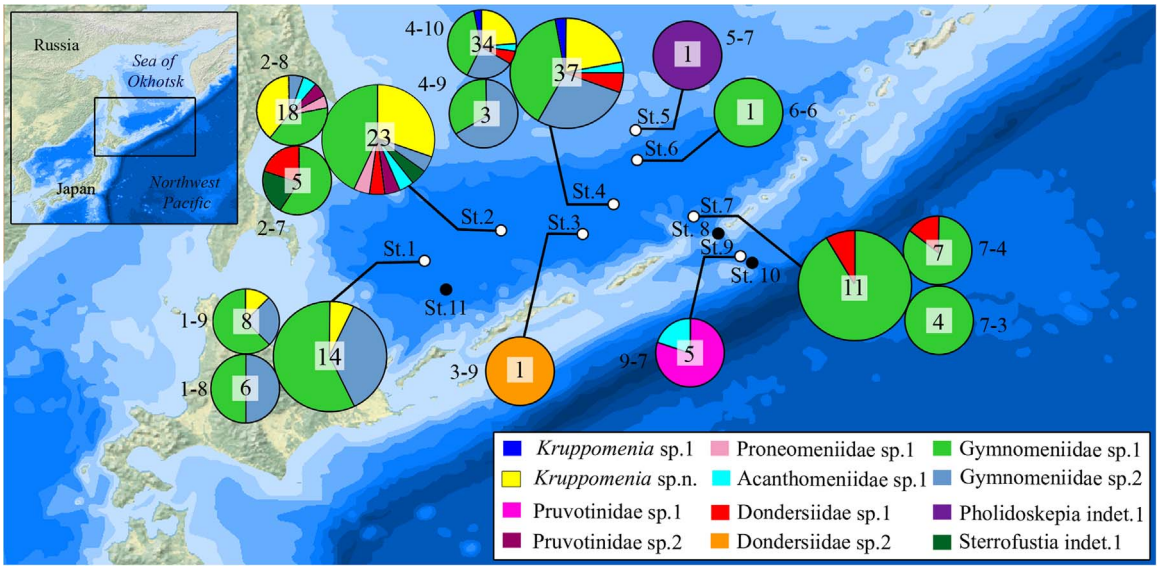

Fig. 1. Occurrence of solenogaster morphospecies collected during the SokhoBio expedition. Small circles depict species composition of single C-EBS hauls (number next to circle); large circles represent total species composition for entire station. Number in circles refers to number of collected individuals. Morphospecies color coded.

The diversity of aplacophoran molluscs (i.e., Solenogastres and Caudofoveata) has been largely neglected in many deep-sea biodiversity surveys, despite their frequent occurrence in deep-sea samples (Scheltema and Schander, 2000). While Caudofoveata are mainly found in soft sediments, Solenogastres show a broader habitat range either leading an epibenthic (partially epizoic lifestyle on their cnidarian prey) or infaunal lifestyle (Todt et al., 2008). There are currently about 300 valid species of Solenogastres (Todt, 2013) with a distribution maximum in the deep sea, and taxonomic experts estimate at least further 60% of the solenogaster diversity still to be discovered (Appeltans et al., 2012), while others estimate the whole number of aplacophoran mollusc species to increase by tenfold (Todt, 2013). Solenogastres are currently grouped within four main orders but cladistics analyses based on morphological characters failed to resolve phylogenetic relationships among the major clades due to a high degree of homoplasy (Salvini-Plawen, 2003) and molecular phylogenetic analyses are still lacking. Taxonomy of Solenogastres is discouraging at first sight due to the variety of taxonomic characters needed for species assignment in these externally rather uniform “worms”. The current classification system demands data on the different features of the scleritome (defined as the entity of all aragonitic elements covering the body surface), radula morphology as well as the histology of the foregut glands, gonopericardial system and sensory structures (García-Álvarez and Salvini-Plawen, 2007); i.e., requiring a number of methodological approaches in a time-consuming identification process (Todt, 2013). Hard-part morphology (i.e., scleritome and radula data) has been discussed as valuable for species discrimination in Solenogastres (Scheltema and Schander, 2000), a concept challenged, however, by the discovery of co-occurring externally cryptic species (Bergmeier et al., 2016). Despite the shortcomings to integrate the discovered diversity into the currently classificatory system, Bergmeier et al. (2017) recently demonstrated the value of combining hard-part morphology with molecular barcoding to reliably assign these challenging aplacophoran molluscs into taxonomic entities – therein offering an efficient approach to tackle their alpha-diversity at local scale for initial biodiversity assessments of the clade. Applying this approach, the authors discovered a wealth of new solenogaster species on the abyssal plain east of the Kuril-Kamchatka Trench, at a depth range from which only few lineages have been previously reported worldwide, thus questioning the rather conservative estimates on the undiscovered diversity of the clade on a global scale (Bergmeier et al., 2017).

Most former taxonomic work on Solenogastres has focused on Antarctic waters (e.g. García-Álvarez and Urgorri, 2003a, 2003b; García-Álvarez et al., 2000a; Salvini-Plawen, 1978) and the East Atlantic (e.g., García-Álvarez et al., 2000b; García-Álvarez and Urgorri, 2001; Gil-Mansilla et al., 2009; Zamarro et al., 2016), with comparably little work in the Pacific (Heath, 1911; Kocot and Todt, 2014; Scheltema, 1990; Scheltema and Schander, 2000). Previous to the recently discovered 19 lineages from the abyssal plain east of the Kuril Island chain (Bergmeier et al., 2017), only eleven species of Solenogastres were recorded from the entire Far Eastern Seas. Most of them are found in the depth ranges of the continental shelf and bathyal (Heath, 1911; Saito and Salvini-Plawen, 2014; Salvini-Plawen, 1997), with so far only one species, the neomeniamorph *Neomenia yamamotoi* Baba, 1975 reported to occur in deeper zones of up to 1500 m in the Sea of Okhotsk (Ivanov, 1996). In general, macrofaunal diversity in bathyal depths at a global scale is considered as an unimodal function of depth with a peak in diversity at intermediate depths (McClain and Rex, 2015). To our knowledge, this depth range is so far entirely unexplored for the diversity of Solenogastres in the Far Eastern Seas, lacking an important step towards a better understanding of the bathymetric and horizontal distribution ranges of known species and hampering beta-diversity approaches between the different regions.

This study aims to increase the current state of knowledge on the species diversity, distribution and overall abundance of Solenogastres in the Far Eastern Seas. We provide an initial characterization of the morphological diversity of all specimens found during the ‘Sea of Okhotsk Biodiversity Studies’ (SokhoBio) cruise via hard-part morphology. Additionally, molecular barcodes for each lineage are included for fast and reliable re-identification of the discovered lineages in future research. One of the most abundant lineages is formally described in full taxonomic detail by combining 3D-microanatomy from histological semithin sections, scanning electron microscopy of the

Table 1
C-EBS station table of the SokhoBio expedition with information on the solenogaster diversity and abundance collected with the C-EBS.

Station	Coordinates	C-EBS haul	Depth [m]	Morphospecies	# of specimens	Total # of specimens
1	46° 08.7' N 145° 59.8' E	1-8	3307	Gymnomeniidae sp.1	3	14
				Gymnomeniidae sp.2	3	
		1-9		Gymnomeniidae sp.1	5	
				Kruppomenia genslerae sp.n.	1	
				Gymnomeniidae sp.2	2	
2	46° 41.1' N 147° 28.0' E	2-7	3351–3353	Dondersiidae sp.1	1	23
				Gymnomeniidae sp.1	3	
				Sterrofustia indet.1	1	
		2-8		Acanthomeniidae sp.1	1	
				Gymnomeniidae sp.1	7	
				Kruppomenia genslerae sp.n.	7	
				Gymnomeniidae sp.2	1	
				Proneomeniidae sp.1	1	
				Pruvotinidae sp.2	1	
3	46° 38.0' N 149° 00.1' E	3-9	3363	Dondersiidae sp.2	1	1
4	47° 12.1' N 149° 37.1' E	4-9	3366	Gymnomeniidae sp.1	1	37
				Gymnomeniidae sp.2	2	
		4-10		Acanthomeniidae sp.1	1	
				Dondersiidae sp.1	2	
				Gymnomeniidae sp.1	13	
				Kruppomenia genslerae sp.n.	9	
				Gymnomeniidae sp.2	8	
				Kruppomenia sp.1	1	
5	48° 37.2' N 150° 00.3' E	5-7	1696–1699	Pholidoskepia indet. sp.1	1	1
6	48° 03.0' N 150° 00.3' E	6-6	3347	Gymnomeniidae sp.1	1	1
7	46° 57.0' N	7-3	3299	Gymnomeniidae sp.1	4	11
	151° 05.0' E	7-4	3300	Dondersiidae sp.1	2	
				Gymnomeniidae sp.1	5	
8	46° 36.5' N 151° 34.2' E	–	2327–2336	–	0	0
9	46° 16.1' N 152° 02.1' E	9-7	3371–3377	Acanthomeniidae sp.1	1	5
				Pruvotinidae sp.1	4	
10	46° 06.8' N 152° 13.3' E	–	4681–4798	–	0	0
11	45° 36.3' N 146° 23.1' E	–	3210	–	0	0

scleritome and molecular barcoding.

2. Material and methods

2.1. Material

The benthic fauna was collected via a camera equipped epibenthic sledge (C-EBS) (Brenke, 2005) at eight stations in the Kuril Basin of the Sea of Okhotsk (Sts. 1–7, 11), one station in the Bussol Strait (St. 8), and two stations close to the Kuril-Kamchatka Trench (Sts. 9, 10) in depths ranging from 1696 to 4798 m in July and August 2015 during the SokhoBio (Sea of Okhotsk Biodiversity Studies) expedition on board of the R/V *Akademik M.A. Lavrentyev* (see Fig. 1 for a cruise map and Table 1 for a station summary). The C-EBS samples were bulk-fixed in 96% ethanol and sorted on ice by the scientists on board. Solenogastres were only retrieved with the C-EBS, all other gear did not recover any solenogaster specimens.

2.2. Hard-part morphology

All collected specimens of Solenogastres were thoroughly investigated externally via light microscopy and photographed under a stereo microscope (Leica Z16 APO; Leica Camera AG; Wetzlar, Germany) at the SNSB-Bavarian State Collection of Zoology (ZSM, Munich, Germany). Based on their habitus and differences in scleritome characters, they were grouped into morphospecies and identified to the most accurate taxonomic level possible based on hard-part morphology. The scleritome was prepared for microscopic investigation by dissolving soft tissue with household bleach in a 1:3 dilution with water. Images were taken under an Olympus CX 41 light microscope with a mounted Olympus DP25 camera using the software Cell^{D} (all Olympus Soft Imaging Solutions GmbH; Tokyo, Japan) at the Biozentrum of the LMU Munich or with a Nikon Camera, 1V1 (Nikon; Tokyo, Japan) attached to a Leica DM RBE microscope (Leica Camera AG; Wetzlar, Germany) at the ZSM. Specimens of the present study are deposited at the Mollusca Section, ZSM. One is deposited at the Museum of National Scientific Center of Marine Biology, Far Eastern Branch of Russian Academy of Sciences (Museum of NSCMB FEB RAS). Radula preparations were done by dissolving the anterior body part in a dilution of household bleach in water (1:3) and by removing surrounding tissue manually to reveal the radula for light microscopic documentation.

Three samples (*Kruppomenia genslerae* sp. nov. ZSM Mol 20170345,

ZSM Mol 20170350; Sterrofustia indet. 1 ZSM Mol 20170368) were prepared for scanning electron microscopy (SEM) via dehydration in an ascending graded acetone series (15 min at each step) and critical-point-dried in a CPD 030 BAL-TEC critical point dryer (Balzers, Liechtenstein). Specimens were mounted on SEM-stubs using self-adhesive carbon stickers and sputter coated with gold (Polaron E5100, Quorum Technologies, United Kingdom) for 150 s in an Argon atmosphere. SEM micrographs were taken with a Leo 1430 VP (Zeiss; Oberkochen, Germany).

2.3. Histology and 3D-microanatomy

The anterior and posterior part of two specimens of *Kruppomenia genslerae* sp. nov. (ZSM Mol 20170344 and ZSM Mol 20170347/Museum of NSCMB FEB RAS MIMB 34435) were used for histology; the mid-sections of each specimen for DNA barcoding (see 2.4). Body parts were rehydrated in a descending graded ethanol series, decalcified overnight in 1% ascorbic acid and post-fixed in 1% osmium tetroxide. After dehydration in an ascending graded acetone series (15 min at each step), specimens were transferred to 100% propylene oxide 3× for 15 min and then to a 1:1 mixture of propylene oxide with Spurr's low viscosity resin (Spurr, 1969) overnight at room temperature. Specimens were then transferred to pure Spurr's resin and hardened overnight at 60 °C.

Resin blocks were trimmed and serial sectioned (1 μm thickness) using a RMC MT 7000 Microtome (Histo Jumbo, Diatome; Biel, Switzerland) with contact cement on the lower cutting edge to form ribbons (Blumer et al., 2002; Ruthensteiner, 2008). The obtained ribbons were stained for 20 s with a solution of 0.25% azure B with 0.75% methylene blue and 0.5% sodium tetraborate decahydrate, ad 100 ml H_2O (dist.) (Richardson et al., 1960).

All sections were digitalized using the software dotSlide in combination with an Olympus BX16VS microscope (both Olympus Soft Imaging Solution GmbH; Tokyo, Japan) saving each section as a single tif image with the software OlyVia (Olympus Soft Imaging Solution GmbH; Tokyo, Japan). Images were converted to grayscale, contrast enhanced and unsharp masked using Photoshop CS4 (Adobe Systems Incorporated; San José, USA). The anterior and the posterior part of one specimen (ZSM Mol 20170344) were used for 3D-reconstruction with the software AMIRA (Version 5.6. TGS Europe, Mercury Computer Systems; Mérignac, France; Visage Imaging GmbH; Berlin, Germany) to reconstruct all major organ systems, following the method outlined in Ruthensteiner (2008).

2.4. Molecular barcodes

DNA was extracted from the middle section of one representative of each morphospecies and 14 individuals of *Kruppomenia genslerae* sp. nov. by combining DNA extraction via CTAB-buffer with DNA recovery via spin-columns (Machery-Nagel Blood and Tissue Set). We amplified mitochondrial 16S rRNA using two sets of primers: 16S-S2 and 16S-a (Simon et al., 1994; Schwenk et al., 1998) and a pair of solenogaster-specific primers, 16Soleno-r and -f (Bergmeier et al., 2017) with 30 s at 98 °C, 35–37 × (5 s at 98 °C, 5 s at 47–50 °C, 20 s at 72 °C) 60 s at 72 °C, and final cooling at 14 °C using the Phire Hotstart II polymerase with the supplied additive 5x reaction buffer (Thermo Fischer). PCR products were cleaned up using the DNA Clean & Concentrator-5 (Zymo Research) and sent off for cycle sequencing on an ABI 3730 capillar sequencer (using Big Dye 3.1) at the Genomics Service Unit of the LMU Munich. Sequences were manually edited using Geneious 7.0.6 (Biomatters Ltd.).

2.5. Phylogenetic analyses and pairwise distances

For phylogenetic analysis, we used all Solenogastres 16S rRNA sequences available from GenBank including 15 sequences of specimens collected from the abyssal plain of the Northwest Pacific. Caudofoveata was used as outgroup (*Chaetoderma nitidulum*, GenBank Nr. AY30451.1). All sequences were aligned using the MUSCLE algorithm (as implemented in Geneious 7.0.6). Ambiguous regions within the alignment were masked with GBlocks with options for less stringent selection (Castresana, 2000). The nucleotide substitution model (GTR + G) was determined with jModelTest (Posada, 2008). We calculated a maximum likelihood analysis using RAxML (Stamatakis, 2014) with 1000 bootstrap iterations for the unmasked (not shown) and masked alignment each. All analyses were done using the CIPRES gateway (Miller et al., 2010). To infer pairwise distances, we aligned all sequences of the respective clade with MUSCLE and trimmed sequences to the same length. 14 successfully amplified *Kruppomenia genslerae* sp. nov. sequences were aligned using MUSCLE (Edgar, 2004) and a parsimonious haplotype network was reconstructed using TCS 1.21 (Clement et al., 2000). All sequences are deposited at GenBank (accession numbers see below).

3. Results

3.1. Solenogaster diversity and abundance

In total, 93 individuals (i.e., entire specimens or only partially damaged ones which still provide the most relevant characters for morphological determination - broken body pieces not included in the present study) were collected during twelve C-EBS deployments at eight stations (out of eleven stations in total; see Fig. 1). 65 individuals (70%) belong to the order Pholidoskepia and 27 (29%) belong to the order Cavibelonia. One individual is provisionally assigned to the order Sterrofustia. No representatives of the solenogaster order Neomeniamorpha were collected.

Overall twelve different morphospecies are delimited based on their general habitus and scleritome characters, with additional information on the radula available for two species. Within Cavibelonia, four families are present in the samples: Simrothiellidae (two morphospecies), Pruvotinidae (two morphospecies), Acanthomeniidae (one morphospecies) and Proneomeniidae (one morphospecies). The most abundant cavibelonian lineage is the simrothiellid *Kruppomenia genslerae* sp. nov. (formal description follows below), with 17 individuals (18% of all specimens) collected at three stations (see Fig. 1). Another simrothiellid morphospecies (*Kruppomenia* sp.1) is collected as a singleton. Pruvotinidae were found at two stations: Pruvotinidae sp.1 was collected outside of the Bussol Strait (5 individuals) and a single specimen of Pruvotinidae sp.2 in the Kuril Basin. Overall three individuals of Acanthomeniidae sp.1 were collected from three stations: two in the Kuril Basin (Station 2 and 4) and one outside of the Bussol Strait (Station 9), close to the Kuril-Kamchatka Trench.

Within Pholidoskepia two morphospecies (5% of all collected Solenogastres, five individuals) are identified as distinct lineages of the family Dondersiidae (Dondersiidae sp.1 and sp.2). Four specimens of the first dondersiid lineage (Dondersiidae sp.1) were collected from three stations, the other lineage (Dondersiidae sp.2) was collected as a singleton. Two other morphospecies have been identified as lineages within the family Gymnomeniidae and provide the highest number of specimens found: 55% (59 specimens) of the Solenogastres in the samples are gymnomeniids. The most common lineage is a gymnomeniid species

(Gymnomeniidae sp.1, Fig. 1) constituting almost 46% (43 individuals) of all collected Solenogastres. This lineage is present at four stations (see Fig. 1) in the Kuril Basin of the Sea of Okhotsk and at one station close to the Bussol Strait – thus exhibiting the most widespread distribution of all collected solenogaster species. Gymnomeniidae sp.2 was an abundant lineage as well: 17% of all individuals (16 specimens) belong to this lineage, which was present at three stations in the bathyal basin. Pholidoskepia indet. sp.1 was collected as singleton on the bathyal northern slope of the Kuril Basin and could not be identified reliably to family level solely based on external features. The identification of one further singleton (Sterrofustia indet.1) remains dubious, but its external features suggest a classification within the order Sterrofustia.

The occurrence of Solenogastres at the sampled stations is highly patchy: at the most diverse station (Station 2), eight species from seven families were sampled during two C-EBS hauls. However, during the first C-EBS haul (2 − 7), only individuals of three morphospecies were collected, while the second haul retrieved overall six morphospecies (see Fig. 1). At Station 4, six morphospecies from four families were found – the first C-EBS haul (4 − 9) collecting only two species and six species sampled during the second one (4 − 10). At three stations, only one individual each was collected: Station 3 and 5 with singletons (Dondersiidae sp.2 respectively Pholidoskepia indet.1) and at Station 6 an individual of widespread Gymnomeniidae sp.1. Overall ten species were sampled at the stations within the Kuril Basin of the Sea of Okhotsk, one at the bathyal slope and two species were collected on the eastern side of the Kuril Islands from Station 9 outside of the Bussol Strait, with Pruvotinidae sp.1 exclusively found there.

3.2. Characterization of the encountered lineages of Solenogastres

The molecular phylogenetic analyses based on the single 16S rRNA marker retrieved all morphospecies (from which more than one sequence was included) as monophyletic entities in the maximum likelihood tree. Deeper phylogenetic relationships are not supported (see Supplement 1) likely due to the inadequacy of the fast evolving mitochondrial barcoding marker to provide resolution on ancient relationships. Supporting information for species delineation, i.e., intra- and interspecific genetic distances of sister clades based on our analyses are reported below.

Systematics and terminology of sclerites follows García-Álvarez and Salvini-Plawen (2007).

PHOLIDOSKEPIA

Dondersiidae sp.1 (Fig. 2A)
Material: ZSM Mol 20170361 (Station 2–7)

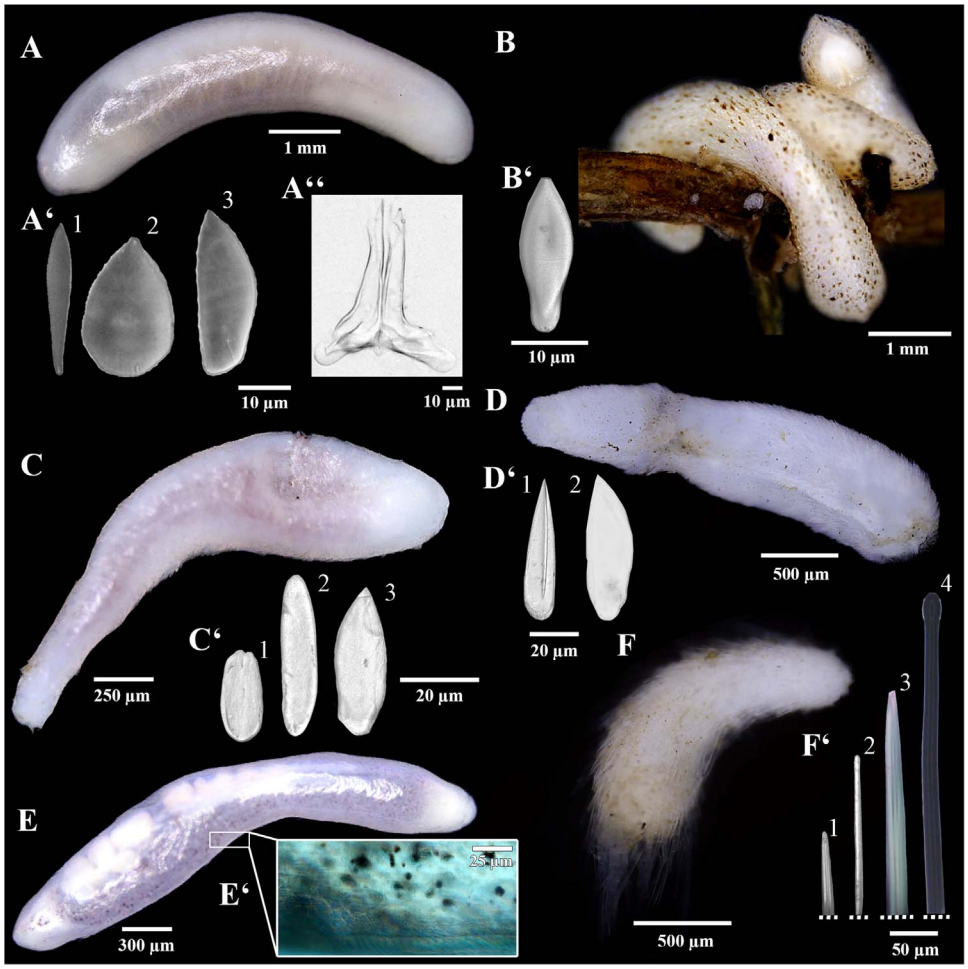

Fig. 2. Morphological variety of hard-part features of Pholidoskepia and Sterrofustia of the SokhoBio cruise. Dotted lines indicate damage on the sclerites. If not specifically indicated; habitus and scleritome images are taken from the same individual. A: Dondersiidae sp.1 (ZSM Mol 20170361); lateral right view. A': Hard parts of A; body scales to the left; foot scale to the right A'': Biserial radula of A. B: Dondersiidae sp.2 (ZSM Mol 20170365); frontal view. B': Body scale of D; no foot scales analysed. C: Gymnomeniidae sp.1 (ZSM Mol 20170363); lateral right view. C': Hard parts of ZSM Mol 20170364; body scales to the left; foot scale to the right. D: Gymnomeniidae sp.2 (ZSM Mol 20170366); lateroventral right view and foot. D': Hard parts of ZSM Mol 20170367; keeled-scale to the left; foot scale to the right. E: Pholidoskepia indet.1 (ZSM Mol 20170362); lateral right view. E': Close up of body surface showing scaly configuration. F: Sterrofustia indet.1 (ZSM Mol 20170368); lateral right view. F': Hard parts of F; soft tissue was not dissolved and sclerites were not removed from the animal's body, therefore there are no whole spicules.

Habitus and size: thin translucent cuticle. Body length 1–6 mm; length width ratio 3:1.

Scleritome: consists of thin, elongated leaf-shaped scales (Fig. 2A′1, approx. 35 µm length), round leaf-shaped scales (Fig. 2A′2; approx. 30 µm length) and blade-shaped foot scales (Fig. 2A′3 approx. 40 µm).

Radula: height 80 µm, width 60 µm; monoserial radula.

Barcode: GenBank Nr. MG603282.

Dondersiidae sp.2 (Fig. 2B)

Material: ZSM Mol 20170365 (Station 3–9)

Habitus and size: epizoic specimen, wrapped around hydrozoan. Body with prominent mid-dorsal keel and covered in scales. Approx. 9 mm in length.

Scleritome: only one type of body scale, approx. 20–30 µm (see Fig. 2B′); excavated with broad stalk at proximal end.

Barcode: GenBank Nr. MG603285.

Taxonomic remarks: Dondersiidae sp.2 does not cluster with Dondersiidae sp.1 (see Supplement 1), however our single marker phylogenetic analyses is likely insufficient to test sister group relationships at family level. We classified the present individual within Dondersiidae due to the high similarity of sclerites with some *Nematomenia* (Salvini-Plawen, 1978; figs. 25, 30). A molecular revision of the family Dondersiidae, whose monophyly has been questioned previously (Scheltema et. al, 2012), is overdue.

Gymnomeniidae sp.1 (Fig. 2C)

Material: ZSM Mol 20170363 (Station 1–8); ZSM Mol 20170364 (Station 1–9; Fig. 2C′).

Habitus and size: length between 1 and 6 mm with thin translucent cuticle. Length width ratio 4:1.

Scleritome: consists of elongated scales with round ends of varying lengths between 25 and 50 µm (Figs. 2C′1, 2) and blade-shaped foot scales (40 µm length, Fig. 2C′3).

Intraspecific genetic distance: 0.1%. Interspecific genetic distance to sister clade Gymnomeniidae sp.2 (see Supplement 1): 10.2–10.5%.

Barcode: GenBank Nr. MG603283, − 83.

Taxonomic remarks: based on scleritome characters Gymnomeniidae sp.1 more closely resembles members of the family Dondersiidae (e.g. *Dondersia namibiensis* (Scheltema et al., 2012; fig. 35–36)). However, in our phylogenetic analysis (see Supplement 1), this lineage clusters together with Gymnomeniidae sp.2 (which was unambiguously identified to family level due to the presence of conspicuous keeled scales) and shows only 10% generic difference. Thus, we assigned this morphospecies to the Gymnomeniidae.

Gymnomeniidae sp.2 (Fig. 2D)

Material: ZSM Mol 20170366 (Station 4–10); ZSM Mol 20170367 (Station 1–9; Fig. 2D′).

Habitus and size: Size from 1 to 6 mm with white body coloration. Length width ratio of about 4:1.

Scleritome: consists only of lanceolate scales with a keel (length approx. 60 µm; Fig. 2D′1) and blade-shaped foot scales (approx. 60 µm length; Fig. 2D′2).

Intraspecific genetic distance: 0.6%.

Barcode: GenBank Nr. MG603286, − 87.

Pholidoskepia indet. 1 (Fig. 2E)

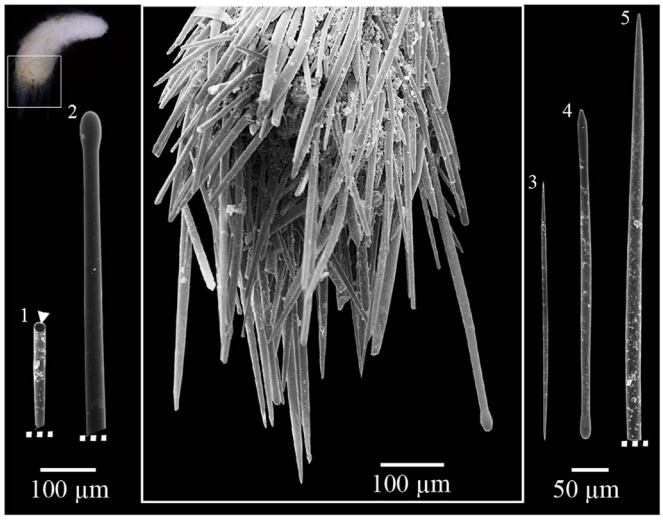

Fig. 3. Scanning electron microscopy (SEM) images of the posterior body of Sterrofustia indet.1 (Fig. 2F; ZSM Mol 20170368). Dotted lines indicate missing part of sclerite. In white box: Overview. Outside the box, from left: 1: Sclerite with breaking point, showing solidity of the sclerites. 2: Pin-shaped sclerite (identical to Fig. 2F′4). 3: Needle-shaped thin sclerite. 4: Acicular sclerite with base. 5: Long pointed sclerite.

Material: ZSM Mol 20170362 (Station 5–7).

Habitus and size: thin cuticle; i.e., gonads visible in the dorsal part of the specimen. Size of 3.1 mm; length width ratio 5:1.

Unfortunately, the specimen was lost after whole mount investigation under the light microscope and we were thus unable to provide details on the scleritome or a molecular barcode. The body surface is densely covered in small iridescent scales (Fig. 2E′).

STERROFUSTIA

Sterrofustia (?) indet. 1 (Fig. 2F)

Material: ZSM Mol 20170368 (Station 2–7).

Habitus and size: remarkably long sclerites relative to its body size (1.5 mm), extending considerably beyond the body. Length width ratio of approx. 3:1.

Scleritome: characterized by three types of solid, acicular spicules. (1) With conspicuous central groove running all the way to the pointed tip (Figs. 2F′1, 3.1,3,4,5). (2) Straight, with round tip (Fig. 2F′2). (3) Only found posterior, with central groove and pin-shaped distal tip (Fig. 2F′4, 3.2), potentially hollow and interpreted as copulatory spicule. All sclerites appear to be solid in SEM (Fig. 3, arrowhead at 3.1).

No molecular data available.

Taxonomic remarks: at present stage of knowledge this individual cannot be reliably assigned to any of the established hierarchical categories within Solenogastres due to its unique scleritome containing pin-shaped sclerites. In SEM examination broken sclerites were all solid and no scales were found, thus we provisionally assigned this lineage to Sterrofustia.

CAVIBELONIA

Pruvotinidae sp.1 (Fig. 4A)

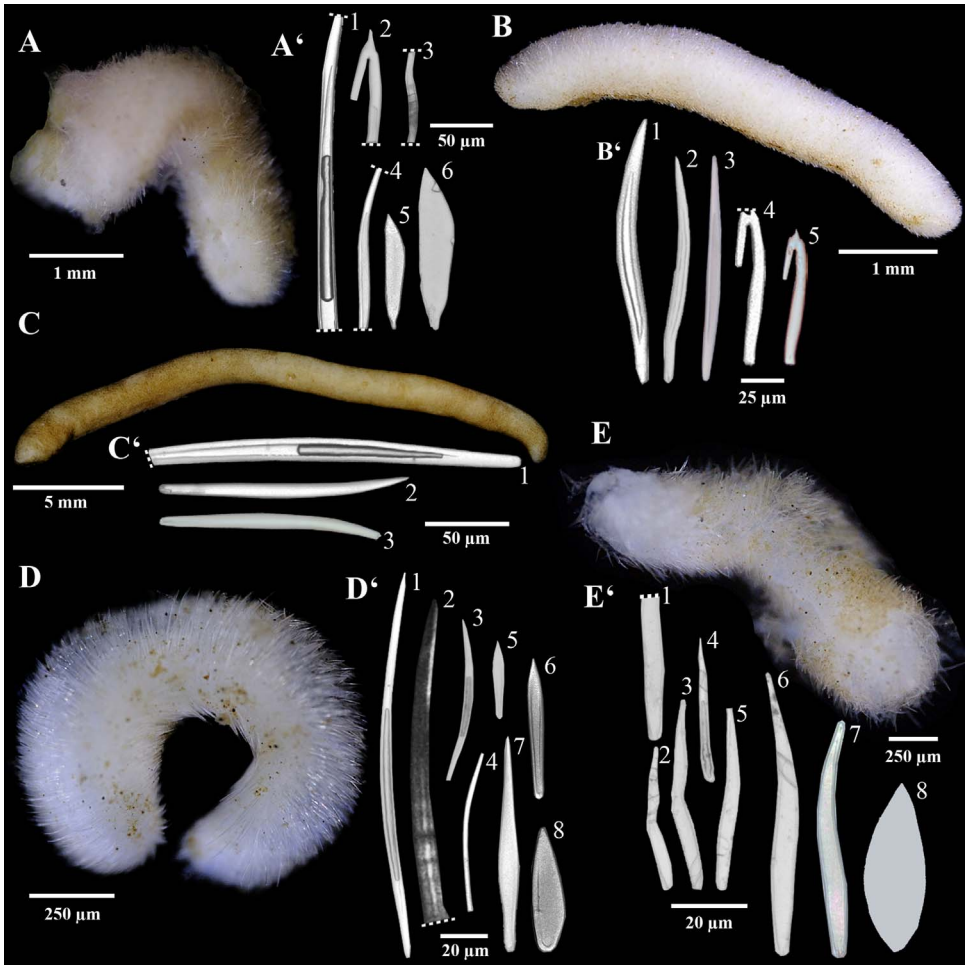

Fig. 4. Morphological variety of hard-part features of Cavibelonia of the SokhoBio cruise. Dotted lines indicate damage on the sclerite. If not specifically indicated; habitus and scleritome images are taken from the same individual. A: Pruvotinidae sp.1 (ZSM Mol 20170369); frontal view, showing ventral side as well. A′: Hard parts of A; to the left: body scleritome, to the bottom far right: foot scales. B: Pruvotinidae sp.2 (ZSM Mol 20170370); lateral left view side. B′: Hard parts of B; no foot scales analysed. C: Proneomeniidae sp.1 (ZSM Mol 20170371); lateral left view. C′: Hard parts of C; no foot scales analysed. D: Acanthomeniidae sp.1 (ZSM Mol 20170372); lateral left view. D′: Hard parts of ZSM Mol 20170390; Scleritome to the left; foot scale to the bottom right corner. E: *Kruppomenia* sp.1 (ZSM Mol 20170391); lateral left view. E′: Hard parts of E; body scleritome to the left; foot scale to the far right.

Material: ZSM Mol 20170369 (Station 9-7)

Habitus and size: fuzzy appearance; size approx. 4 mm; length width ratio approx. 2:1.

Scleritome: in total with four different types of body sclerites: (1) hollow acicular spicules (Fig. 4A′1) with a size of at least 250 µm (only broken sclerites observed); (2) hook-shaped sclerites, pointed at curvature (see Fig. 4A′2; approx. 85 µm). We also retrieved two types of curved, hollow sclerites: (3) One with slight sigmoid shape (Fig. 4A′3 approx. 50–110 µm length); (4) the other with slightly curved distal part (Fig. 4A′4). Elongated foot scales with narrow base of variable size (Fig. 4A′5, 6; approx. 60–100 µm length).

Interspecific genetic distance to sister lineage Pruvotinidae sp.2: 16.4%.

Barcode: GenBank Nr. MG603288.

Pruvotinidae sp.2 (Fig. 4B)

Material: ZSM Mol 20170370 (Station 2–8)

Habitus and size: short sclerites on the dorsal side. Size approx. 5 mm; length width ratio approx. 6.5:1.

Scleritome: consists mainly of three types of hollow acicular spicules (Fig. 4B′1–3): (1) Proximal part bent, then curved in variable sizes (Fig. 4B′1, 2; size of about 110–140 µm); (2) curved sclerite not bent (Fig. 4B′ 3; 125 µm); (3) hook-shaped sclerites with pointed curvature of 65–75 µm size (Fig. 4B′4, 5). No foot scales were retrieved.

Barcode: GenBank Nr. MG603289.

Proneomeniidae sp.1 (Fig. 4C)

Material: ZSM Mol 20170371 (Station 2–8)

Habitus and size: smooth appearance, brownish colouration. Largest specimen within the dataset with a size of 25 mm; elongated body. Length width ratio of 12:1.

Scleritome: two types of acicular spicules: (1) One hollow (Fig. 4C′1, approx. 220 µm; broken); (2) curved acicular type (Fig. 4C′2, 3, approx. 140–150 µm). No foot scales were retrieved.

Barcode: GenBank Nr. MG603290.

Acanthomeniidae sp.1 (Fig. 4D)

Material: ZSM Mol 20170372 (Station 4–10); ZSM Mol 20170390 (Station 2–8; Fig. 4D′)

Habitus and size: size of 1 mm and length width ratio 3:1.

Scleritome: overall five types of body sclerites: (1) Hollow acicular sclerites with a slight central bend (Fig. 4D′1, 3; approx. 80–190 µm); (2) broad hollow spicule observed under the stereo microscope from the posterior end of the animal (Fig. 4D′2, approx. 175 µm for visible part). (3) Additionally solid, thin acicular sclerites with a bend (Fig. 4D′4; approx. 85 µm length, relatively thin). (4) There is also one type of sclerite that tapers towards the tip after broadening close to its proximal part (Fig. 4D′7, approx. 110 µm). (5) Scales with lateral thickened rims (Fig. 4D′5, 6, approx. 30–110 µm) as well as (6) a foot scale (Fig. 4D′7; approx. 60 µm).

Intraspecific genetic distance between the two specimens: 0%. Clusters within *Veromenia* cf. *singula* with 2.7–3.3% pairwise distance (Bergmeier et al., 2017).

Barcode: GenBank Nr. MG603291, −92.

Kruppomeniasp.1 (Fig. 4E)

Material: ZSM Mol 20170391 (Station 4–10)

Habitus and size: fuzzy with sclerites protruding from cuticle in various angles; size approx. 2 mm; length width ratio approx. 3.5:1.

Scleritome: body scleritome consisting of four sclerite types: (1)

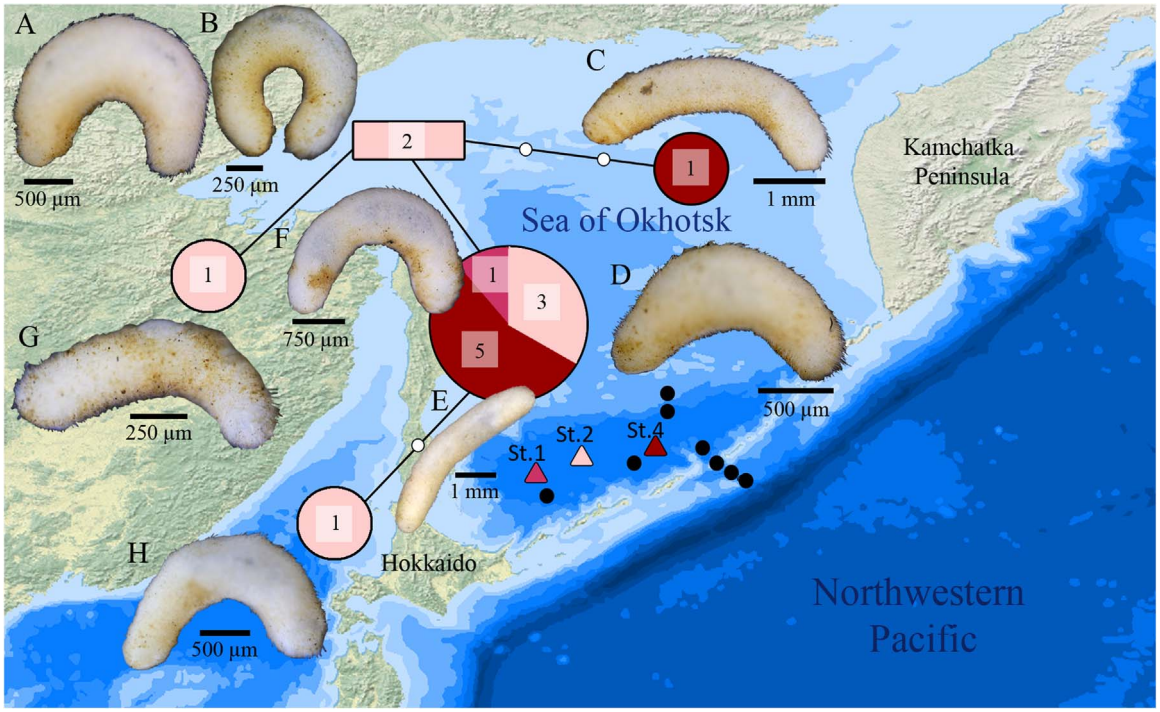

Fig. 5. Haplotype network of *Kruppomenia genslerae* sp. nov. indicating five haplotypes from three stations with different abundances (numbers in small squares), as well as showing variation in external morphology of the species. Number in the circles depicts the number of specimens of this haplotype A: ZSM Mol 20170354. B: ZSM Mol 20170353. C: Paratype 2 (DNA aliquot = ZSM Mol 20170347). D: ZSM Mol 20170355. E: ZSM Mol 20170352. F: ZSM Mol 20170360. G: ZSM Mol 20170356. H: ZSM Mol 20170357.

most common type with a straight base, a curved central part and a thickened rim (see Fig. 4E′5–7; length approx. from 60 to 110 µm). (2) With sharp bend in midsection (see Fig. 4E′2, 3; length approx. 60–80 µm). (3) Straight sclerite, tapering towards the tip (See Fig. 4E′4; length approx. 55 µm). (4) Additionally, broken sclerites were observed with thickened rim, but full description is not possible (see Fig. 4E′1; length of the fragment approx. 70 µm). Foot scale leaf-shaped (Fig. 4E′8 approx. 60 µm length).

Taxonomic remarks: this specimen was collected together with all type specimens of *Kruppomenia genslerae* sp. nov. (see below) at St. 4–10. In our phylogenetic analysis (see Supplement 1), this morphospecies clusters among *K. genslerae* and a hitherto unidentified simrothiellid (Simrothiellidae_sp.1_20170097) from the northwestern abyssal plain. Genetic distance of *Kruppomenia* sp.1 to specimens of *K. genslerae* is 8.0–11.9% and in combination with distinct differences in the scleritome between the specimens (see sclerite with sharp bend (Fig. 4E′) which is lacking in *K. genslerae* sp. nov.) we currently refrain from assigning it to *K. genslerae* sp. nov. until denser sampling and multiple markers are available for northwestern *Kruppomenia*.

Barcode: GenBank Nr. MG603293.

3.3. Species description – *Kruppomenia genslerae sp. nov*

Order CAVIBELONIA Salvini-Plawen, 1978

Diagnosis (modified after Zamarro et al. (2016)): Solenogastres with predominantly hollow, acicular sclerites in one or several layers, or with solid sclerites in combination with a biserial radula together with lateroventral foregut glandular organs of various types, but not of so-called Type A according to Salvini-Plawen (1978) (ducts with subepithelially arranged gland cells) or *Pararrhopalia*-Type according to Handl and Todt (2005). Radula variable or lacking.

Family Simrothiellidae Salvini-Plawen, 1978

Diagnosis (modified after Zamarro et al. (2016)): Cavibelonia with hollow-acicular or solid elongate to scaly sclerites. Radula biserial (rows of paired denticulate radula plates or bars); anterioventral radular sac (when present) paired. Lateroventral foregut glandular organs with various configurations, but not of Type A (according to Salvini-Plawen (1978) or *Pararrhopalia*-Type respectively, according to Handl and Todt (2005).

Genus *Kruppomenia* Nierstrasz, 1902

Diagnosis (modified after Zamarro et al. (2016)): cuticle of varying size, epidermal papillae present or absent. With hollow acicular sclerites in various layers. Mouth within common vestibular-buccal cavity. Radula plates simply serrate, with paired anterioventral radular sac. Lateroventral foregut glandular organs with elongate epithelial gland cells; Type C according to Salvini-Plawen (1978) or *Simrothiella*-Type according to Handl and Todt (2005). Midgut with moderate or no constrictions. Secondary genital opening unpaired. With copulatory stylets, a dorsoterminal sense organ and respiratory organs.

Kruppomenia genslerae Ostermair, Brandt, Haszprunar, Jörger, Bergmeier, sp. nov.

Type material: all material originally fixed in 96% ethanol.

Holotype: 47°12.2′ N 149°36.7′ E, Station 4–10 of the Sea of Okhotsk Biodiversity Studies cruise, in a depth of 3366 m (ZSM Mol 20170344, cross sections, five slides of anterior and six slides of posterior part, DNA aliquot from mid body deposited at the ZSM DNA Bank; 16S rRNA sequence: Genbank Nr. MG603268).

Paratypes 1–7 (ZSM Mol 20170345, ZSM Mol 20170346, Museum of NSCMB FEB RAS MIMB 34435, ZSM Mol 20170348 – 20170350; ZSM 20170352 from the type locality): Paratype 1 (ZSM Mol 20170345) as gold-sputtered SEM sample; Paratype 2 as histological section series of the anterior and posterior part (Museum of NSCMB FEB RAS MIMB 34435) and DNA aliquot of midpart (ZSM 20170347, GenBank Nr. MG603270). Paratypes 3–7 dissolved for hard-part morphology (no material remaining); DNA Barcodes available of ZSM Mol

20170346 (GenBank Nr. MG603269), ZSM Mol 20170348 (GenBank Nr. MG603271), ZSM Mol 20170350 (GenBank Nr. MG603272), ZSM Mol 201703452 (GenBank Nr. MG603273).

Additional material: ten specimens (one lost during radula preparation, nine samples remaining). Anterior and posterior body part from ZSM Mol 20170353 (St. 2–8); ZSM 20170354 (St. 2–8) and ZSM Mol 20170360 (St. 1–9). Anterior body part from ZSM Mol 20170355 – ZSM Mol 20170359 (all St. 2–8). DNA Barcodes from ZSM Mol 20170353 – ZSM Mol 20170360 (GenBank Nr. MG603274 – GenBank Nr. MG603281).

The new species is registered in Zoobank with Life Science Identifiers (LSIs):urn:lsid:zoobank.org:act:FE3565FA-A1E7-4428-AD03-89635C9EA9AF

Etymology: the species is dedicated to Heidemarie Gensler, the long-time technical assistant of the workgroup of GH at the LMU and good fairy to generations of students in the lab. With her technical expertise and support in histological preparations, she has considerably contributed to this – and numerous other – studies and has constantly encouraged junior scientists like LO and FSB with her pep talks in critical phases of their projects.

Distribution: species known from type locality (SokhoBio St. 4–10, 3366 m depth) and the Kuril Basin of the Sea of Okhotsk (SokhoBio Sts. 1–9, 2–8) between 3307 and 3353 m.

Diagnosis: Simrothiellid Solenogastres, with five different types of hollow (one potentially solid) acicular sclerites in several layers. Common vestibular-buccal opening. Biserial radula with small denticles. With paired anteriolateral radular sac. Ventral foregut glands of *Simrothiella*-Type (Handl and Todt, 2005) or Type C respectively (Salvini-Plawen, 1978). Midgut without constrictions. With at least one seminal receptacle at anterioventral part of spawning duct. With two pairs of copulatory stylets and copulatory stylet gland in mature individuals, as well as four pairs of prepallial spicules. With four to six respiratory folds. With an unpaired (potentially fused) dorsoterminal sense organ (DTSO).

Description:

External morphology:

Body vermiform, 1–4.5 mm (Fig. 5A-H). Covered densely by multiple layers of sclerites (Fig. 6A, B). After fixation in ethanol, individuals showed a yellow-brownish colouration with foraminifers and detritus stuck between the sclerites. Cuticle thick (up to 50 µm). Foot originates as a thin ciliated band at the pedal pit, extends midventrally within foot groove and terminates at the pallial cavity. There are no pedal folds visible in the foot groove.

Scleritome:

In total four types of acicular spicules, one type of body scale and one type of foot scales: (1) Straight hollow acicular sclerites present over entire body, up to 100 µm long (Fig. 6D to the left); ultrastructural investigation via SEM revealed longitudinal striae (see Fig. 6C, black arrowheads). (2) Second type with bent tip found in anterior region of specimen, 50–60 µm in length (Fig. 6D; second and third from the left). Type three and four restricted to the posterior part of the animal, unfortunately only damaged sclerites found: (3) probably solid acicular sclerites, slightly curved with a thickened part (Fig. 6D fourth from the left). (4) The fourth type straight, broadened and tapering sclerite with central groove (Fig. 6D fifth from the left). (5) A single type of scales: leaf-shaped, surrounding the dorsoterminal sense organ (Fig. 8F, arrowhead). Foot scales blade-shaped and curved, approx. 40 µm in length (Fig. 6C, D to the right).

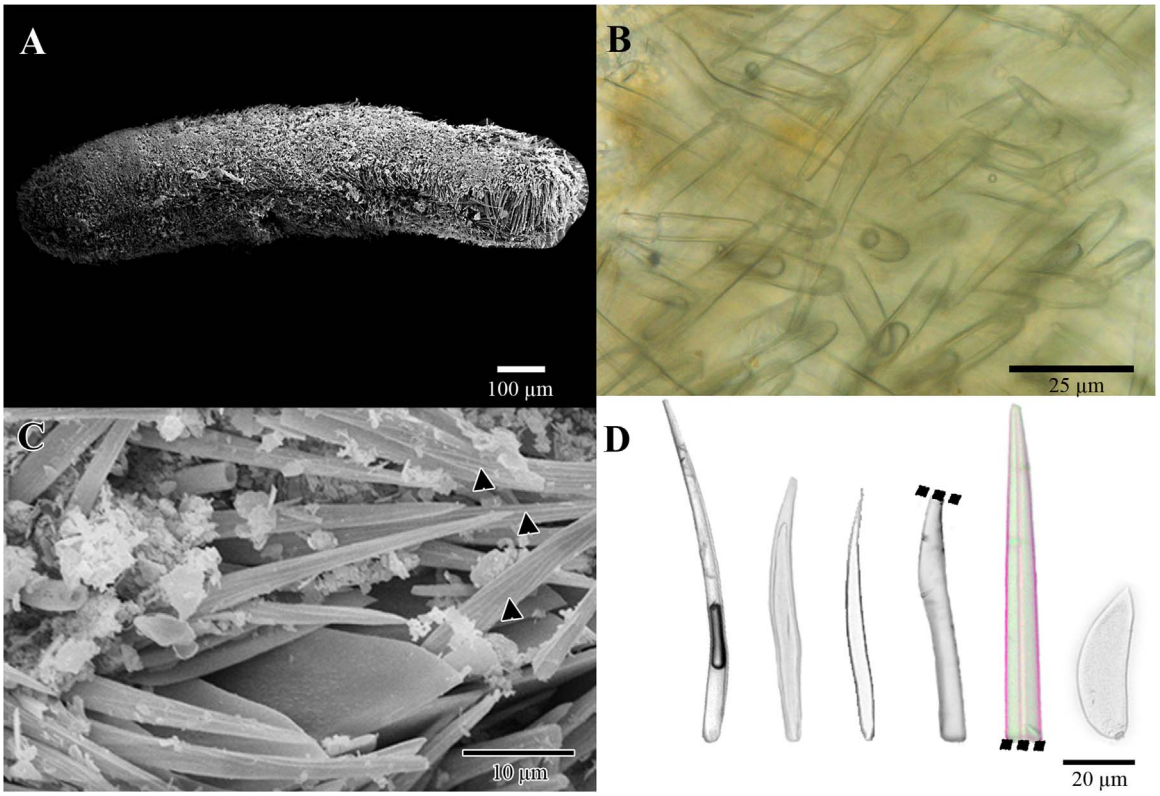

Fig. 6. SEM micrographs and light microscopic images of scleritome features of *Kruppomenia genslerae* sp. nov. A: SEM micrograph of whole specimen (ZSM Mol 20170345). B: Cuticle of ZSM Mol 201703044 showing the multilayered sclerite configuration. C: Close up SEM micrograph of ZSM Mol 20170345, arrowheads indicate the fine lines on the sclerites. D: Different sclerite types of *Kruppomenia genslerae* sp. nov.

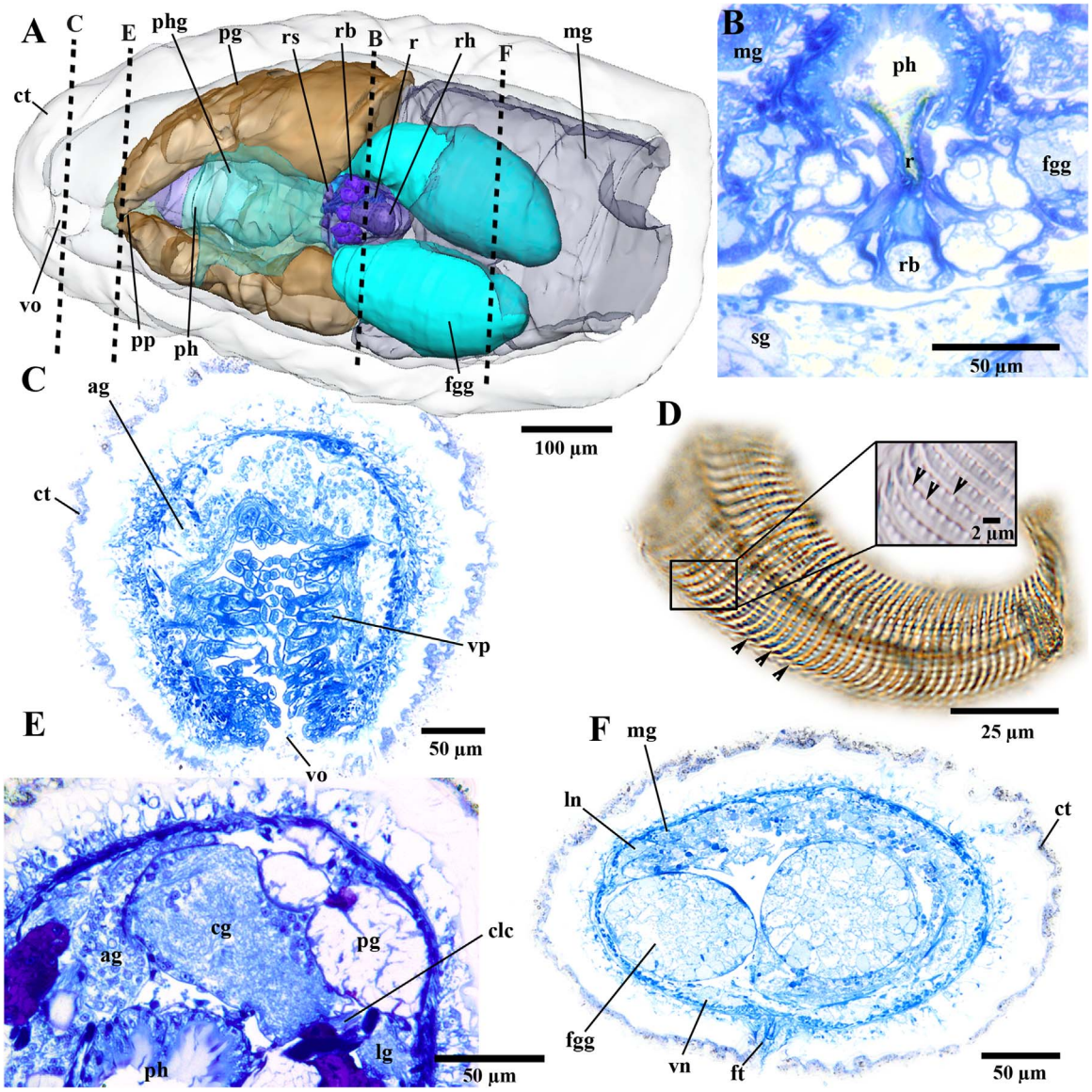

Fig. 7. Descriptive features of the anterior body of *Kruppomenia genslerae* sp. nov. in 3D (A) and histology (B, C, E, F) from ZSM Mol 20170344 A: 3D-reconstruction of the anterior part of the animal (nervous system omitted), ventral view; dotted lines indicate sectional plane of histological images. B: Detail of the radula system. C: Vestibular-buccal cavity with vestibular papillae. D: Light microscopic image of the radula (ZSM Mol 20170348); arrow heads point to single tooth plates. Zoom in on radula teeth shows denticles (indicated by arrowheads). E: Close-up of the cerebral ganglion. F: Foregut glands. **ag** accessory ganglia; **cg** cerebral ganglion; **clc** cerebrolateral connective; **ct** cuticle; **fgg** foregut glands; **ft** foot; **lg** lateral 'ganglion'; **ln** lateral nerve cord; **mg** midgut; **pg** pedal glands; **ph** pharynx; **phg** pharyngeal glands; **pp** pedal pit; **r** radula; **rb** radular bolster; **rh** radular sheath; **rs** radular sac; **sg** sole glands; **vn** ventral nerve cord; **vo** vestibular opening; **vp** vestibular papillae.

Nervous system:

Tetraneurous condition. Fused cerebral ganglia (cg) without central sulcus (approx. 130 µm length × 90 µm width) located dorsal at transition of vestibular-buccal cavity into pharynx (Fig. 7E). Short cerebrolateral connective (12 µm length, Fig. 7E), inconspicuous lateral ganglia (15 µm × 15 µm, Fig. 7E) give rise to lateral nerve cords. Several 'accessory ganglia' (= precerebral ganglia) of roundish or digitiform shape (up to 100 µm × 160 µm) fill the head region anterior to cg surrounding the vestibulum (= atrium) (Fig. 7C, E), medulla and cortex not clearly separated as in true ganglia. Vestibulum with numerous branching digitiform papillae without further subdivision, each up to 40 µm length (Fig. 7C). Paired buccal ganglia (50 µm × 40 µm) located lateral on both sides of pharynx at the level of the radula. Ganglia-like swellings (approx. 50 µm × 20 µm) located ventral, posterior to the pedal pit, but without clear separation in medulla and cortex; cerebropedal connectives exit cg posterioventral to cerebrolateral connectives. Paired lateral nerve cords (approx. 15 µm thick; Figs. 7F, 8B, C) thinner as ventral nerve cords (up to 30 µm thick; Figs. 7F, 8C), the latter lead posterior on both sides of pedal groove. Lateral and ventral nerve cords form suprarectal loop (100 µm × 180 µm), located dorsal to hindgut and pallial cavity. No posterior ganglia found. Two nerves emerge from suprarectal loop in posterioventral direction. They could not be traced for their entire length, but likely they innervate the single, putatively fused dorsoterminal sense organ (DTSO, approx. 45 µm × 70 µm × 70 µm) present on posteriormost part of the animal, extending through cuticle (Fig. 8A, E). DTSO visible externally as small, circular pit surrounded by leaf-shaped scales (Fig. 8F, black arrowheads).

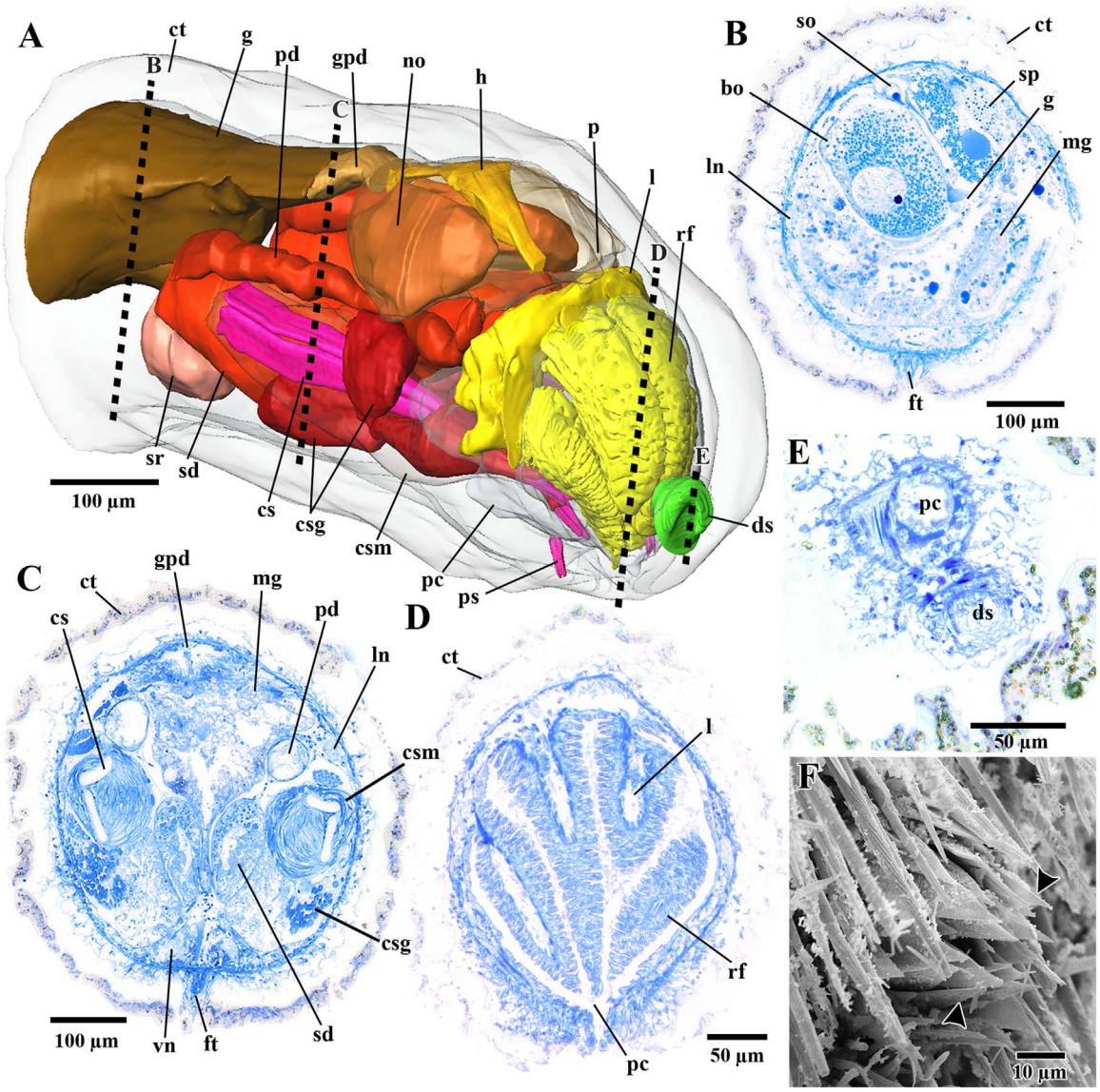

Fig. 8. Descriptive features of the posterior body of *Kruppomenia genslerae* sp. nov. in 3D (A), histology (B-E) from ZSM Mol 20170344 and using SEM (F) from ZSM Mol 20170345. A: 3D-reconstruction of the posterior part (digestive and nervous system omitted), posteriorlateral view on the animal's right side; dotted lines indicate sectional plane of histological images. B: Gonad with oocytes. C: Major features of the genital system. D: Respiratory folds. E: Close-up view of the dorsoterminal sense organ. F: SEM image of scales surrounding the dorsoterminal sense organ; arrowheads pointing on said scales. **bo** big oocytes; **cs** copulatory stylet; **csg** copulatory stylet gland; **csm** copulatory stylet musculature; **ct** cuticle; **ds** dorsoterminal sense organ; **ft** foot; **g** gonad; **gpd** gonopericardioduct; **h** heart; **l** lacuna; **ln** lateral nerve cord; **mg** midgut; **no** nurse 'oocyte'; **p** pericard; **pc** pallial cavity; **pd** pericardioduct; **ps** prepallial spicules; **rf** respiratory folds; **sd** spawning duct; **so** small oocyte; **sp** sperm packages; **sr** seminal receptacle; **vn** ventral nerve cord.

Digestive system:

Oral opening within spacious vestibular-buccal cavity (120 µm × 90 µm; Fig. 7A). Pharynx wide, muscular (180 µm × 300 µm) and ciliated (Fig. 7A, B, E), surrounded by thick mass of unicellular pharyngeal glands (up to 40 µm thickness of layer), each discharging individually into pharynx.

Radula complex located in posterior-most part of pharynx (Fig. 7A, B) consisting of paired radular sac, biserial radula, unpaired radular sheath and numerous radular support cells. Radular sac round (15 µm × 12 µm), located anteriolateral on each side of radula, teeth of radula visible (Fig. 7A). Radula (about 75 µm × 30 µm) with 39–42 rows of thin, slender teeth bearing 15–25 small denticles, all of similar size and shape (Fig. 7D, black arrowheads). Ten radular support cells (approx. 11 × 13 µm) surround radula in a ventral semicircle (Fig. 7A, B). Radular sheath (50 µm × 35 µm) in posterior part of complex, parts of developing radula teeth visible.

Foregut glands (Fig. 7A, B, F) of *Simrothiella*-Type (according to Handl and Todt (2005)) respectively Type C (Salvini-Plawen, 1978) are situated posterior to radula apparatus, ventrolateral to midgut (Fig. 7A). They consist of numerous single extraepithelial glandular cells forming a tube, surrounded by outer muscular layer (Fig. 7F). Connected to pharynx via short paired openings anteriolateral to radula. Pharynx leads directly into midgut without sphincter or histologically distinguishable oesophagus. Midgut extends through almost entire length of the animal. Epithelium consists of large digestive cells. Midgut narrows into thin hindgut lined with regularly arranged cuboidal ciliated epithelium; opens into dorsal part of pallial cavity.

Pedal glands and sole glands:

Paired pedal glands huge, filling most of anterior body between vestibulum and pharynx at the level of the foregut gland ducts (approx. 250 µm × 270 µm × 280 µm) (Fig. 7A, E). Multicellular with large and

Table 2
Species comparison of the genus *Kruppomenia* with distinct characters (modified after Zamarro et al., 2016). P = paired ducts; u = unpaired ducts; p/u = first paired ducts, fusing into unpaired duct. Radula plate width in µm.

Species	Collection site	Water depth [m]	Epidermal papillae	Denticles	Radula plates [width in µm]	Radula sac	Radula sheath	Dorsal caecum	Seminal vesicle	Seminal receptacle	Spawning ducts	Copulatory stylets	Copulatory stylet gland	Respiratory folds
K. minima	Mediterranean	100–1100	X	few	small with few denticles	✓	X	✓	X	✓	p/u	2*2	✓	6 to 12
K. borealis	North Atlantic	110–1191	✓	40–60	50–60*3 µm	✓	X	✓	✓	✓	p/u	2*2	X	12
K. rhynchota	South Pacific	3694	✓	20–25	60*8–10 µm	✓	?	X	?	?	?	?	?	4
K. levis	North Atlantic	4240–4327	✓	numerous	74*2 µm	✓	✓	✓	✓	✓	u	2*4	X	46
K. delta	North Atlantic	4211–4327	?	numerous	78*4 µm	?	?	?	?	?	?	2*2	X	?
K. macrodoryata	Indian Ocean	3716	✓	18–22	60*4 µm	✓	X	✓	X	✓	p/u	2*1	X	11
K. nanodentata	Indian Ocean	520–830	X	numerous	14*2 µm	✓	X	✓	?	?	p/u	2*1	X	5
K. angolensis	South Atlantic	5415	X	26–30	100* 2.5 µm	✓	✓	✓	✓	✓	u	2*2	X	3 to 5 pairs
K. glandulata	South Atlantic	5390–5415	X	15–18	15–20*1.5 µm	X	✓	✓	X	✓	p/u	2*2	✓	up to 5
K. macrodenti culata	South Atlantic	5125–5144	X	8 to 11	35*1 µm	✓	X	X	X	✓	u	2*2	X	2
K. bulla	North Atlantic	788 – 988	X	8–11, hooked	70*2 µm,	✓	✓	✓	✓	✓	p/u	4*2	X	3 to 8
K. vitucoi	North Atlantic	788 – 1004	X	13–15, hooked	20*2 µm	✓	X	✓	X	✓	p/u	2*1	✓	6 to 9
K. genslerae sp. nov.	Northwest Pacific	3366	X	15–25	39–42*?	✓	✓	X	X	✓	p/u	2*2	✓	4 to 6

not clearly delimited cells; some staining faint pinkish, others with dark purple staining secretion. Pedal glands open lateral into pedal pit, pedal pit filled with secretions of pedal glands. Pedal pit located posterior to vestibular-buccal cavity. Dorsal epithelium of pedal pit consists of prismatic cells. Unicellular faint lilac sole glands (Fig. 7B) border foot groove on both sides, discharging secretions into foot groove; occur posterior to pedal pit, extending towards the midregion of the body, not present in the posterior part of the animal.

Gonopericardial system:

Paired tubular gonads located dorsal to midgut (Fig. 8A), containing numerous oocytes of varying sizes (14 µm × 12 µm to 50 µm × 50 µm; Fig. 8B) with large, light-blue staining nucleus and dark blue staining nucleolus surrounded by different amounts of blue staining yolk. Sperm packages (130 µm × 50 µm) found in the posterior third of the gonad lateral to oocytes (Fig. 8B). Gonad connected to pericard through paired short, ciliated gonopericardioducts (45 µm × 75 µm; Fig. 8A, C). Pericard (290 µm × 240 µm) with two big spherical structures interpreted as nurse eggs due to the absence of a nucleus (see Fig. 8A and discussion). Small heart located in dorsal part of pericard (Fig. 8A). Two pericardioducts (380 µm × 35 µm; Fig. 8A, C) emerge lateral from posterior part of pericard. Anterior part of pericardioducts: thin epithelium with poorly distinguishable cells; posterior third of ducts with ciliated cuboidal epithelium. Pericardioducts open into anterior part of paired spawning ducts (40–100 µm in width; Fig. 8A, C). Right spawning duct with spherical pouch at anterioventral part; interpreted as an unpaired seminal receptacle (95 µm × 98 µm in size; Fig. 8A). Paired spawning ducts lined with glandular epithelium, fuse after 220 µm. Fused spawning duct opens into posteriodorsal part of pallial cavity anterior to the anus (Fig. 8A). Overall two pairs of copulatory stylets present: two large stylets on each side of the spawning duct, reaching from center of the body to ventral side (length 350–380 µm; width of 10–40 µm tapering towards the tip; Fig. 8A, C); stylets surrounded by thick layer of musculature (350 µm × 120 µm; Fig. 8A, C) and additional glandular tissue; gland surrounds stylets in their distal last third and comprises two histologically distinct parts: outermost with large, weakly staining cells inner part of glandular cells filled with dark-blue staining secretions (Fig. 8A, C). Anteriolateral to opening of pallial cavity: four pairs of small prepallial spicules (Fig. 8A). Lacunar system posterioventral to pericard, collecting haemolymph from four (holotype) to six (paratype) respiratory folds (Fig. 8A, D), which emerge from dorsal wall of posterior part of pallial cavity (Fig. 8A, D).

Molecular barcoding data:

Based on 16S rRNA sequence data (approx. 450 bp) generated from 14 specimens, *Kruppomenia genslerae* sp. nov. was retrieved as a single haplotype network (Fig. 5) with an intraspecific variability of 0–4.5%. Five different haplotypes are present in the Sea of Okhotsk, with the most common one sampled eight times from three different stations (St.1, 2, 4). At Station 2, four different haplotypes were sampled.

Remarks on taxonomy and biology

The new species can be unambiguously assigned to the genus *Kruppomenia* due to its ventrolateral foregut glandular organs of *Simrothiella*-Type (Handl and Todt, 2005) or Type C, according to Salvini-Plawen (1978), a common vestibular-buccal opening, a simply serrated biserial radula, presence of respiratory folds, dorsoterminal sense organ and copulatory stylets. There are currently twelve valid species within the genus, these species are morphologically delineated by a combination of radula characteristics and features of the reproductive system (see Table 2). Based on morphological and anatomical characters *Kruppomenia genslerae* sp. nov. most closely resembles *K. rhynchota,* the only other lineage of *Kruppomenia* found in the Pacific

(see Table 2). The two species differ in the thickness of the cuticle (50 µm maximum in *Kruppomenia genslerae* sp. nov. vs. 130 µm in *K. rhynchota*) and the presence vs. absence of epidermal papillae (absent in *K. genslerae* sp. nov.). While epidermal papillae are recognized as valuable taxonomic characters, thickness of the cuticle might show some intraspecific variability. Unfortunately, the reproductive system of *K. rhynchota* was not characterized in the original description (Salvini-Plawen, 1978) and thus important comparative data (e.g. on the presence/ absence of the seminal receptacle found in *K. genslerae* sp. nov., see below) is missing. Detailed data on the nervous system of Solenogastres is generally scarce (Scheltema and Schander, 2000; García-Álvarez et al., 2001; Todt and Salvini-Plawen, 2003), and the comparison or evaluation of potential characters between different lineages is currently difficult. Re-investigations and additional data on the already established lineages might reveal further distinguishing characters.

In *Kruppomenia genslerae* sp. nov. we found an unusual, unpaired seminal receptacle attached to the anterioventral part of the spawning duct (Fig. 8A). Seminal receptacles form late during ontogeny and are reported to only be present in certain reproductive phases (Handl and Salvini-Plawen, 2002), thus the presence/ absence of such a structure needs to be evaluated in terms of maturity of the investigated specimen. We found oocyte-like structures in the pericard of *K. genslerae* sp. nov. (see Fig. 8A), which lack a nucleus and were thus interpreted as nurse eggs. If so, this would be the first report on nurse eggs in Solenogastres, but nurse eggs have been described from various gastropods (e.g., Chaparro and Paschke, 1990; Pechenik et al., 1984) and polychaete annelids (Gibson, 1997; Poulin et al., 2001). Additionally there are several reports of brooding species of Solenogastres (Heath, 1911; Todt and Kocot, 2014), but in contrast to *K. genslerae* sp. nov., these species have no or reduced respiratory folds in the pallial cavity to provide space for brooding (Todt and Kocot, 2014). In most cases, respiratory folds are only developed in larger species of Solenogastres to accommodate the higher oxygen demand due to a lower surface area-to-volume ratio. Those are, however, reduced in brooding species in a trade-off towards increased parental care and a higher investment in the offspring (e.g. in *Proneomenia custodiens* (Todt and Kocot, 2014)). Nurse eggs might indicate a special form of adelphophagy, characterized as a certain feeding mode during development with embryos feeding on the yolk of the nurse eggs during growth (Poulin et al., 2001; Rivest, 1983), involving the absence of a planktotrophic larval stage. We thus hypothesize that *K. genslerae* sp. nov. is a low disperser, with a rather restricted distribution range.

4. Discussion

4.1. Solenogaster diversity in the Sea of Okhotsk

By presenting twelve different morphospecies, the present study has considerably augmented the solenogaster diversity of the Sea of Okhotsk with previously only two known species: the gigantic neomeniamorph *Neomenia yamamotoi* reported from the mid bathyal (1500 m) (Ivanov, 1996) and the cavibelonian *Halomenia gravida* Heath, 1911 in the shallow bathyal (420 m) (Heath, 1911). Both species have not been encountered during our survey of the Kuril Basin, suggesting that they are either generally rare or have restricted bathymetric distribution ranges not extending into the depth of the Kuril Basin. However, at least *N. yamamotoi* is unlikely to be sampled via the C-EBS due to its comparably large body size of up to 11 cm, but Agassiz trawl samples from the same station during the cruise have not revealed any specimens of this lineage either.

Concerning the C-EBS data analysed in the present study, a total of eight out of eleven stations harboured specimens of Solenogastres (see Fig. 1), at three of these stations we collected only single specimens. While some morphospecies such as Gymnomeniidae sp.1 (43 specimens, five stations), *Kruppomenia genslerae* sp. nov. (17 specimens, three stations) and Gymnomeniidae sp.2 (16 specimens, three stations) appear to be quite common and widespread throughout the Kuril Basin of the Sea of Okhotsk, the majority of lineages was only encountered at single stations. Five stations revealed morphospecies restricted to single hauls during our sampling effort and repeated hauls at the same stations partially showed strikingly different results in concerns of overall diversity and species composition (see Station 2 and 4). This observed spatial heterogeneity ("patchiness") is a well-known phenomenon in the distribution of deep-sea molluscs (see e.g., Jörger et al., 2014; Linse, 2004; Schrödl et al., 2011; Schwabe et al., 2007) and of macro- and meiofauna in the deep sea in general (Danovaro et al., 2013; Grassle and Maciolek, 1992; Lejzerowicz et al., 2014; McClain et al., 2011). Patchiness and local co-existence of species is usually explained by microhabitat variation and environmental heterogeneity influenced by food availability, bioturbance by megafauna and biogenic structures (McClain et al., 2011; Rex and Etter, 2010; Snelgrove and Smith, 2002). In general, there is a comparably low benthic biomass in the deep sea of the Sea of Okhotsk, potentially related to the low primary production in the surface waters (Tyler, 2002). Most Solenogastres are predators, however, with many species preying on various cnidarians (Todt et al., 2008) and their diversity is linked to morphological variation of the alimentary tracts (i.e., differences in radula morphology, pharyngeal and foregut gland systems) (Scheltema et al., 1994). Different ecological niches related to different prey organisms might explain the presence of closely related co-occurring species of Solenogastres in the deep sea. In contrast to detritus or suspension feeders, the patchiness of potentially selective predators like Solenogastres in the deep sea might be largely influenced by the distribution of their prey organisms.

The patchiness throughout our dataset and the rarity of most of the encountered lineages underline that the present study is just a piece in the puzzle of understanding the diversity of Solenogastres in the Sea of Okhotsk. Only one station (Station 5, see Table 1) sampled the bathyal slope which revealed one distinct morphospecies (Pholidoskepia indet.1) not present at any of the deeper stations in the Kuril Basin, indicating a potential turnover of solenogaster fauna in shallower depth. Based on collections in the Pacific and current species diversity, the major radiation of Solenogastres was hypothesised to have occurred on the continental slope (Scheltema, 1990), thus, these depth ranges should be the main target area for further deep-sea sampling in the Sea of Okhotsk to get a more complete picture of the alpha-diversity of Solenogastres in the region.

4.2. Implications for the solenogaster diversity in the Far Eastern Seas

Unlike the adjacent Sea of Japan, the Sea of Okhotsk is not isolated, but connected with the Pacific via the deep straits between the Kuril Islands and much of the Okhotsk fauna is considered closely linked to the fauna of the adjacent Pacific (Tyler, 2002). So far there are only few records on the solenogaster fauna in the Far Eastern Seas and most lineages described in the region have been collected from the shelf and shallow bathyal (Baba, 1940; Heath, 1911; Saito and Salvini-Plawen, 2014; Salvini-Plawen, 1997). None of these reported twelve shallow deep-sea species were found in our samples of the Kuril Basin in the Sea of Okhotsk. The nearby abyssal plain off the Kuril-Kamchatka Trench revealed an extraordinary high diversity of 19 morphospecies, considerably boosting the known global diversity of abyssal Solenogastres (Bergmeier et al., 2017). Compared to our results of the Kuril Basin in the Sea of Okhotsk, the diversity of the abyssal Northwest Pacific plain is about 1/3 higher, despite similar sampling effort. Since the solenogaster species diversity usually decreases with depth in the abyssal (Scheltema, 1992), this might indicate an impoverished solenogaster fauna in the semi-enclosed Sea of Okhotsk, which might be a general trend also visible in the lower biomass (Tyler, 2002). The formation of anoxic bottom water in the Sea of Okhotsk during interglacial periods (Liu et al., 2006) might deliver one explanation for the poorer solenogaster species diversity in comparison to the open Northwest Pacific

plain.

We find some indication of a faunal overlap between the Kuril Basin in the Sea of Okhotsk and the abyssal plain near the Kuril-Kamchatka Trench shown in the close genetic relationship between *Kruppomenia genserlae* sp. nov. and *Kruppomenia* sp.1 (present study) with a still undescribed simrothiellid from the Northwest Pacific abyssal plain and between Acanthomeniidae sp.1 (present study) with *Veromenia* cf. *singulata* (Bergmeier et al., 2017) (see Supplement 1). Minor differences in the scleritome are present, however, and conspecificity still needs to be tested in molecular species delineation analyses based on multiple molecular markers in the future. Surprisingly, the most common and widespread morphospecies of the present study Gymnomeniidae sp.1 and 2 were not found in the samples of the Northwest Pacific abyssal plain. The only station of the present study sampled outside of the Sea of Okhotsk (Station 9, Fig. 1) retrieved a pruvotinid Solenogastres which has neither been collected inside the Sea of Okhotsk, nor the open abyssal plain south-east of the Kuril-Kamchatka Trench. This indicates that our knowledge on solenogaster diversity is still as patchy as the observed distribution patterns and that the abyss northeast of the Kuril-Kamchatka Trench might harbour a unique fauna. In general little is known on the bathymetric ranges of Solenogastres and the few records of wide bathymetric ranges (e.g. see *Pruovtina* in Salvini-Plawen, 1978) have never been tested with molecular markers.

Concerning species composition, we find a higher percentage of cavibelonian Solenogastres on the abyssal plain (88% cavibelonian) of the Northwest Pacific and more pholidoskepian Solenogastres (70%) in the Kuril Basin of the Sea of Okhotsk. The family composition is similar in both areas but the most dominant family present in the Sea of Okhotsk (i.e., Gymnomeniidae, 55% of all collected specimens) is restricted in its distribution to the Kuril Basin. The other families are present on both sides of the Kuril-Kamchatka Trench and form the major component of the solenogaster diversity in abyssal depths in this area of the Far Eastern Seas. On a global scale, these families are also among the most species rich lineages found in the lower bathyal and beyond (García-Álvarez and Salvini-Plawen, 2007). Our conclusions are still hampered, however, by several discovered morphospecies in our datasets which could not reliably be assigned to family level at present because the investigated (external) characters are either insufficient or because the specimens exhibit features which do not fit within any of the currently existing categories (see e.g., Sterrofustia indet.1). Thus, with further in-depth morphological and molecular work, some additional lineages might broaden the picture.

Source-sink dynamics are suggested to contribute to the diversity in the abyssal, taking the extreme rarity of many lineages into account (Rex et al., 2005). It remains to be explored whether with intensified sampling effort, we discover reproductive populations of the encountered lineages represented by singletons only or whether these rare and scattered individuals settle in the abyssal basins or plains without having conspecifics in reach for reproduction. The horizontal distribution ranges of Solenogastres are also poorly understood and might be highly variable within the clade: typically, Solenogastres develop via planktonic, lecithotrophic larvae (Todt and Wanninger, 2010) and some species are reported with accordingly wide distribution patterns (Scheltema, 2008; Todt and Kocot, 2014). But some species are also brooders and here we report the possible presence of nurse eggs for the first time in Solenogastres – all suggesting limited dispersal abilities and probably high rates of endemism.

5. Conclusions and outlook

The generally poor state of alpha-taxonomic knowledge of the solenogaster diversity in the deep sea of the Northwest Pacific complicates interpretation of our data set in regards to the amount of new vs. established lineages. So far, no solenogaster species is known to span Atlantic as well as Pacific waters in its distribution (Scheltema, 1992) and within most of the encountered lineages our analyses of hard-part characters show differences to related species described from the Pacific. Thus, we conclude based on our preliminary data that likely most of the encountered lineages are new to science. This calls for efficient taxonomic workflows to characterize the discovered diversity. Thorough taxonomic descriptions as exemplified on *Kruppomenia genslerae* sp. nov. are a time-consuming task, but reward not only with reliable morphological data allowing for interpretations on adaptations to certain ecological niches, but also with new biological data (e.g. the presence of putative nurse eggs) adding to the knowledge of this poorly understood clade of aplacophoran-molluscs. The well-known shortage of trained taxonomist and funding sources (“taxonomic impediment”) to deal with the vast amount of discovered, but yet undescribed diversity in marine molluscs (Bouchet et al., 2016), however, hinders “deep descriptions” on all lineages and molecular-based “turbo-taxonomic” approaches can be suitable to provide formal descriptions of closely related lineages. Thus, it should be the primary target to establish multi-marker molecular datasets of the new encountered deep-sea lineages as well as the already described diversity of Solenogastres for reliable molecular species delineation in future research within a phylogenetic framework. Based on this, key lineages to our understanding of solenogaster evolution and diversity can be identified and reliable and fast tools for species identification are provided to support future beta-diversity studies.

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Author contributions

LO conducted morphological analyses, 3D-microanatomy and drafted the first version of the manuscript.

AB organised the SokhoBio expedition.

GH supported histological examination of *Kruppomenia genslerae*.

KMJ designed and supervised the study.

FSB conducted the molecular and phylogenetic work and supervised the morphological determination.

All authors contributed to the final version of the manuscript.

Appendix A. Supplementary material

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Of basins, plains, and trenches: Systematics and distribution of Solenogastres (Mollusca, Aplacophora) in the Northwest Pacific

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ABSTRACT

Solenogastres (Mollusca, Aplacophora) form a common part of the benthic malacofauna in the deep sea, but their diversity in the Northwest Pacific is poorly understood. In this study, we analyze the systematics and distribution of Solenogastres sampled during the Kuril-Kamchatka Biodiversity Studies II expedition to the hadal Kuril-Kamchatka Trench, in the framework of previous expeditions to the shallow bathyal areas of the Japanese coast, the abyssal Kuril Basin in the Sea of Okhotsk, and the open abyssal plain of the Northwest Pacific. This unique dataset from adjacent regions extending from bathyal to hadal zones, enables us to study bathymetric and geographic distribution ranges in this neglected deep-sea clade for the first time. Our applied species delineation in these taxonomically challenging aplacophoran molluscs relies on an integrative approach, combining sclerite characters with molecular analyses. A first molecular phylogenetic study based on two mitochondrial markers reveals conflict with traditional systematics and suggests the need to revise at least two polyphyletic families and the polyphyletic order 'Cavibelonia'. In total, 192 specimens could be grouped within 60 candidate species, which present a surprisingly rich abyssal fauna and includes the first representatives of Solenogastres at hadal depths. We found a high proportion of singletons and little faunal overlap between the investigated regions and depths, suggesting that the diversity of the abyssal and hadal Solenogastres is not entirely determined by source-sink dynamics from the bathyal slope. Rather, there is evidence for abyssal source populations, supporting the presence of endemic abyssal and potentially even hadal species. Our molecular data reject the Kuril-Kamchatka Trench as an insurmountable barrier of dispersal for Solenogastres, with one species present at both sides of the trench. Remarkably, one species from the Kuril-Kamchatka Trench shows a vertical distribution extending over more than 6,000 m. Overall, our study more than doubles the global number of solenogaster species from abyssal depths, provides first records of a unique hadal solenogaster fauna and delivers valuable insights into distribution ranges of these deep-sea molluscs.

1. Introduction

The deep oceans are the largest marine habitat and although less than 0.01% of the deep-sea floor has been explored, its biodiversity is estimated to be among the highest on Earth (Etter and Mullineaux, 2001; Snelgrove and Smith, 2002; Ramirez-Llodra et al., 2010). Even though large parts of the deep-sea benthos are still unexplored, hypotheses on bathymetric gradients of benthic biodiversity suggest that species diversity decreases with increasing depth, after a peak at the transition between the lower bathyal and abyssal zones (Stuart et al., 2003). Source-sink dynamics have been suggested to play a major role in sustaining abyssal diversity, which is formed by a range extension of a subset of the bathyal diversity according to this hypothesis (Rex et al., 2005). Modelling larval source-sink dynamics in the abyss, Hardy et al. (2015) concluded that abyssal diversity is unlikely entirely sustained by larval influx from slope sources with higher food availability, among

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others due to too long distances between the bathyal "source" and abyssal "sink". Abyssal diversity might also be sustained by abyssal regions with higher productivity (Hardy et al., 2015), arguing towards a self-sustaining and truly endemic abyssal fauna. To contribute to a better understanding of biodiversity patterns and endemism in the deep sea, comparative data on alpha-diversity of deep-sea clades from adjacent areas is needed in conjunction with data on food availability and larval biology.

The Pacific Ocean harbors the largest abyssal region on Earth. At its continental margins, its deep-sea floor is considerably structured with a system of oceanic trenches and marginal seas.

In the Northwest Pacific, from the western part of the Aleutian Trench, the Kuril-Kamchatka Trench extends southwards along the Kamchatka Peninsula and the Kuril Islands until it intersects with the Japan Trench off the coast of Hokkaido which continues until the Mariana Trench in the south. The Kuril-Kamchatka Trench forms one of the largest hadal habitats on Earth, as 90% of the trench floor lie within the hadal zone, estimated to reach depths around 9,6000 m (Dreutter et al., in press). Oceanic trenches potentially act as barriers for the distribution of shallower abyssal and bathyal benthic species (Jamieson, 2015) and thus form important areas for comparative biodiversity surveys. The East China Sea, southeast of Japan, is a marginal sea of the warm temperate Northwest Pacific and influenced by the warm, northbound Kuroshio current (Spalding et al., 2007; Wang and Oey, 2016). The cold Oyashio current originates in the Bering Sea and flows towards the south, where it strongly influences the open Northwest Pacific plain, as well as the semi-isolated Sea of Okhotsk (Qiu, 2001). The Sea of Okhotsk is a marginal sea of the cold temperate Northwest Pacific, between the far eastern parts of Russia, the Kamchatka Peninsula, Hokkaido, and Sakhalin Island. While the mean depth of the Sea of Okhotsk is rather shallow (812 m), the Kuril Basin of the Sea of Okhotsk averages at abyssal depths of 3,300 m. The basin is connected to the open Northwest Pacific and the open abyssal plain (mean depth of 5,000 m (Zenkevitch, 1963)) via two deep straits (Bussol Strait at 2,318 m and Krusenstern Strait at 1920 m), both proposed to allow faunal exchange between the two regions (Tyler, 2002). This geographically diverse deep-sea region has been intensively studied by the R/V *Vityaz* during ten expeditions in the 1950ies and 1960ies, which have contributed majorly to recent knowledge on abyssal fauna (Ebbe et al., 2010; Brandt and Malyutina, 2015), and more recently by a series of German-Russian expeditions (Malyutina and Brandt, 2013; Brandt and Malyutina, 2015; Malyutina et al., 2018) as well as Japanese cruises.

Molluscs constitute an important component of benthic deep-sea macrofauna (Gage and Tyler, 1991) and the diversity of the two dominating and most speciose classes (i.e., Gastropoda and Bivalvia) has been relatively well-studied in the Northwest Pacific (e.g., Fukumori et al., 2018, 2019, Kamenev, 2015, 2018a). In contrast, the two classes of aplacophoran molluscs have been comparably neglected in deep-sea research and in the Northwest Pacific in specific. Aplacophora comprises the worm-shaped Caudofoveata (=Chaetodermomorpha) and Solenogastres (=Neomeniomorpha), which lack a shell but are characterized by a covering of calcareous sclerites. According to recent phylogenomic analyses, they are sister to Polyplacophora, and together form the clade Aculifera as sister to all remaining molluscs termed Conchifera (Kocot et al., 2011; Smith et al., 2011). Solenogastres exclusively inhabit the marine environment and currently about 300 species are described (García-Álvarez and Salvini-Plawen, 2007; Todt, 2013). In extrapolations based on recent sampling efforts from the intertidal to the deep sea, their diversity is estimated to be at least ten-times higher, with a diversity peak likely in deeper waters (Todt, 2013). With the exception of a few striking taxa (e.g., large-sized *Epimenia* Nierstrasz, 1908 or colorful *Anamenia amabilis* Saito & Salvini-Plawen, 2010), the majority of species are inconspicuous "worms" with body sizes of only a few millimeters, posing a challenge for taxonomist due to their external, macroscopic uniformity. Solenogastres are classified into four orders: the majority of species are contained within Cavibelonia (184 spp.) and Pholidoskepia (62 spp.), whereas Neomeniamorpha (26 spp.) and Sterrofustia (13 spp.) are far less speciose. 14% of solenogaster genera are monotypic (Scheltema et al., 2012) and species descriptions are often based on single animals collected from remote locations (Salvini-Plawen, 1978a, 1978b). So far classification within Solenogastres lacks a phylogenetic perspective and has been established based on morphological similarities. The first phylogenomic analyses on higher classification of Solenogastres have revealed major conflicts with the traditional classification, rendering e.g. "Cavibelonia" paraphyletic (Kocot et al., 2019). Currently, solenogaster identification requires detailed investigations of the "scleritome", i.e., the calcareous sclerites covering their entire body surface. They occur in a variety of shapes, as flatly arranged scales and/or solid or (partially) hollow spicules with occasional intricate microstructures (e.g., serrations or hooks on the distal ends of the sclerites). Of further taxonomic importances are microanatomical features such as the radula and the histology of glands associated with the foregut (Salvini-Plawen, 1978a; Salvini-Plawen, 1978b; Handl and Todt, 2005). The highly diversified types of pharyngeal and foregut glands likely reflect adaptations in feeding, but observations are rare (but see Scheltema and Jebb, 1994; Sasaki and Saito, 2005) and they have been mostly suggested to feed on cnidarians, based on histological investigations showing cnidocysts in the midgut (Salvini-Plawen, 1972; Bergmeier et al., 2016), or remains of polychaetes (Todt and Salvini-Plawen, 2005). In general, the reproductive output of Solenogastres correlates with their size, and small species lay batches with a small number of eggs (Todt and Wanninger, 2010). Few Solenogastres retain their offspring within their mantle cavity (e.g., Heath, 1911; Todt and Kocot, 2014). Experiments showed that lecithotrophic larvae (of *Wirenia argentea* Odhner, 1920) can remain up to ten days in the water column before metamorphosis, but may continue feeding on their yolk reserves for up to one further month (Todt and Wanninger, 2010). Larval planktotrophy has never been observed in any solenogaster species.

Solenogastres occur circum-globally in tropical to cold waters. Our knowledge on their diversity is strongly biased towards the Antarctic (e.g., monographs by Salvini-Plawen, 1978a; Salvini-Plawen, 1978b) as well as the Atlantic (Pedrouzo et al., 2014; Zamarro et al., 2015) and the Mediterranean (e.g., Salvini-Plawen, 2003a), while the Pacific has been less extensively surveyed (but see e.g., Heath, 1911; Scheltema, 1990; Kocot and Todt, 2014) with a prominent lack of knowledge in the Northwest Pacific. Until a few years ago, only eleven species of Solenogastres were known as described from the continental slope and upper bathyal of the Northwest Pacific (Heath, 1911; Baba, 1940, 1975; Salvini-Plawen, 1997; Saito and Salvini-Plawen, 2010, 2014), and two additional records of undescribed Solenogastres were available from abyssal depths (i.e., *Helicoradomenia* Scheltema & Kuzirian, 1991 and another yet unidentified specimen (Ramirez-Llodra, 2005; JAMSTEC, 2016)). This has changed considerably due to two recent studies on the diversity of Solenogastres, which explored the Sea of Okhotsk (Ostermair et al., 2018) and the abyssal plain east of the Kuril-Kamchatka Trench (Bergmeier et al., 2017) and discovered a high abyssal diversity of 30 species belonging to six different families. Both studies applied an integrative taxonomic approach, characterizing the encountered specimens based on their scleritome and providing molecular barcodes for more rapid species identification in future research (Bergmeier et al., 2017; Ostermair et al., 2018).

The present study describes the diversity of Solenogastres from the KuramBio II expedition to the Kuril-Kamchatka Trench, exploring for the first time the hadal environment of a deep-sea trench for this clade. Together with novel data from additional Japanese cruises, we discuss these findings within a molecular phylogenetic framework of all recently collected Northwest Pacific Solenogastres.

This allows us for the first time to test the validity of the current, morphology-based taxonomy at family level, which is entirely based on morphological features. We compare the solenogaster diversity

between the investigated bathyal zone, the abyssal Kuril Basin and the open abyssal plain as well as the hadal Kuril-Kamchatka Trench. We explore bathymetric and geographic distribution ranges of encountered species to find answers for the following questions: (1) does the Kuril-Kamchatka Trench form an insurmountable barrier for Solenogastres, which are considered to have limited dispersal abilities? (2) Is there evidence for endemic abyssal (and even hadal) solenogaster diversity, or does it represent a non-self-sustaining "sink" of bathyal "source" populations?

2. Material and methods

2.1. Sampling, sorting, and fixation

Benthic deep-sea fauna was collected at overall 11 stations around the Kuril-Kamchatka Trench area during the KuramBio II cruise (KB II) in 2016 on board of R/V *Sonne*. The first sampling transect forms an extension of the Bussol Strait and crosses the Kuril-Kamchatka Trench (KB II stations 3, 4, 5 and 6), while stations 8, 2, 9, and 10 form a parallel transect further to the southwest. The third transect (KB II stations 1, 4, 7, 9, and 11) samples along the deepest parts in the middle of the Kuril-Kamchatka Trench and thus intersects with both other transects at stations 4 and 9 (see Fig. 1). CTD, Multi-Plankton Sampler, Box-Corer, Multi-Corer, Agassiz Trawl (AGT, 3.5 m wide × 0.7 m height, 8.7 m net length, 10 mm mesh size), and a camera-equipped Epibenthic Sledge (C-EBS, 1.2 m w × 1.8 m h × 3.6 m l; Brandt et al., 2013) were deployed at the different stations, resulting in overall 93 gear deployments during the cruise (for details on depth and co-ordinates of the deployments that yielded Solenogastres, see Table 1). Solenogasters were retrieved only from AGT and EBS samples. The sieved AGT fractions were sorted on ice and all encountered Solenogastres were directly fixed in 96% ethanol. After sieving, EBS samples were bulk fixed in ice-cold 96% ethanol and sorted into phyla after at least 48 h of storage in a −20 °C freezer.

During Japanese research expeditions to adjacent regions of the Northwest Pacific (Fig. 1, Table 1), including the R/V *Hakuho-Maru* cruises KH-01-02, KH-14-02, R/V *Tansei-Maru* cruises KT-08-27 and KT-11-12, and R/V *Nagasaki-Maru* cruise N342, benthic sampling was conducted using 3m or 4m beam trawls. Sediment was sieved through different mesh sizes and obtained fractions were searched for animals. After fixation in ethanol, samples were stored at the Atmosphere and Ocean Research Institute, The University of Tokyo, Japan (AORI) or the National Museum of Nature and Science, Tsukuba, Japan (NSMT). A subset of the Solenogastres collected during these cruises is included in this study. Museum vouchers and/or DNA aliquots of the material analyzed herein are deposited at the Bavarian State Collection of Zoology (ZSM Munich, Germany) or at the National Museum of Nature and Science (NSMT, Tsukuba, Japan) (see Supplementary data 1).

In the present study, we analyze the new material collected during the KB II and above mentioned five Japanese expeditions, within the framework of previous studies on the diversity of the Northwest Pacific Solenogastres from the KuramBio I and SokhoBio expeditions (see Fig. 1 and Table 1 for stations of the previous cruises; more details available in Brandt et al., 2015, 2018a). Due to different sampling strategies and gear (i.e., AGT, EBS, and beam trawls) the generated data are not compared quantitatively between stations and cruises.

2.2. Morphological investigations

All specimens were photographed with a Leica camera on a Leica Z16 APO dissecting microscope to document the general habitus. Pictures were taken after fixing the animals in ethanol, except those of Amphimeniidae sp.2 and Neomeniidae sp.1 which were photographed prior to fixation. In large-sized specimens, a piece of mediodorsal tissue was used for DNA extraction. Smaller animals were trisectioned, with the mid part used for DNA extraction and the anterior and posterior

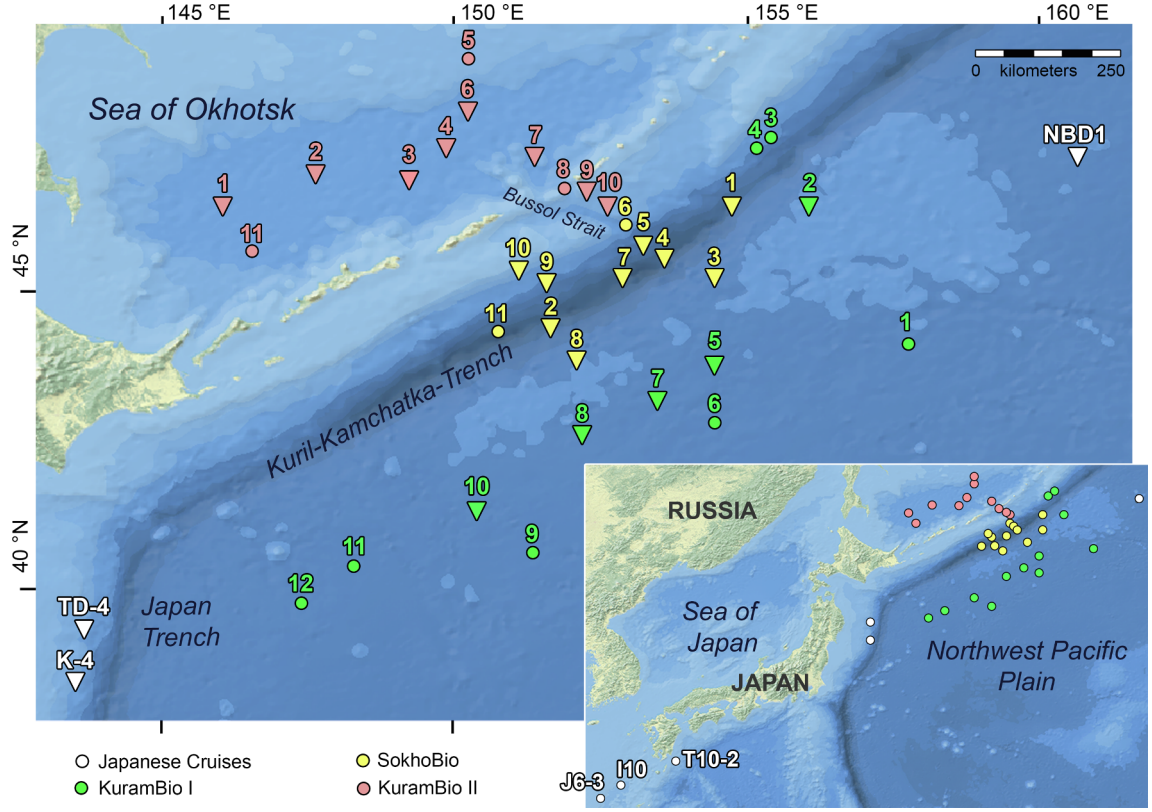

Fig. 1. Sampling sites in the Northwest Pacific, with a close up of the area of the Kuril-Kamchatka Trench. Numbers correspond to the stations of the respective cruises. Triangles mark stations from which Solenogastres were collected.

Table 1
Expedition overview with information on stations and deployments which yielded Solenogastres.

Cruise	Station	# Haul, Gear	Latitude [N°]	Longitude [E°]	max. Depth [m]	Date
KB II	8	8–1, EBS	43° 49.55′–43° 48.59′	151°46.25′–151°46.47′	5,136	19.08.2016
KB II	8	10, EBS	43° 49.43′–43° 48.45′	151°46.96′–151°47.17′	5,120	20.08.2016
KB II	1	17, EBS	45° 52.04′–45° 51.40′	153° 51.39′–153°50.41′	8,191	22.08.2016
KB II	1	18, AGT	45° 50.86′–45° 51.95′	153° 49.56′ −153°51.25′	8,200	23.08.2016
KB II	5	40, EBS	45° 38.00′–45° 40.83′	152° 55.95′–152°57.68′	7,081	29.08.2016
KB II	5	41, AGT	45° 39.23′–45° 40.11′	152° 56.68′–152°58.36′	7,154	29.08.2016
KB II	5	42, EBS	45° 39.62′–45° 40.26′	152° 56.39′–152° 57.63′	7,123	30.08.2016
KB II	4	52, EBS	45° 29.77′–45° 29.18′	153° 12.16′–153° 11.13′	8,737	06.09.2016
KB II	3	65, EBS	45° 09.85′–45° 10.16′	153° 43.34′–153° 44.05′	5,755	09.09.2016
KB II	7	77, EBS	45° 13.71′–45° 14.21′	152° 51.21′–152° 49.95′	9,584	13.09.2016
KB II	10	85, EBS	45° 02.26′- 45° 01.64′	151° 02.14′–151° 03.68′	5,265	15.09.2016
KB II	9	89, EBS	44° 40.12′–44° 39.05′	151° 27.35′–151° 27.34′	8,221	16.09.2016
KB II	2	97, EBS	44° 05.68′–44° 06.94′	151° 24.88′–151° 24.88′	8,575	18.09.2016
KH-01-02	TD-4	BT-4m	39°24.55′–39°29.16′	143°36.85′–143°38.52′	3,200	26.09.2001
KT-08-27	K-4	BT-3m	38°28.13′–38°30.83′	143°37.71′–143°33.20′	3,045	23.10.2008
KT-11-12	T10-2	BT-3m	31°07.82′–31°06.69′	131°39.03′–131°39.03′	1,082	23.06.2011
N342	J6-3	BT-3m	28°33.57′–28°32.57′	127°02.55′–127°02.07′	630	17.11.2011
N342	I10	BT-3m	29°20.46′–29°19.81′	127°42.30′–127°41.61	1,018	19.11.2011
KH-14-02	NBD1	BT-4m	47°0.22′–47°00.91′	160°02.62′–160°01.29′	5,223	27.05.2014

parts partially used for scleritome investigations. The scleritome was accessed by either dissolving pieces of the mantle in a 3:1 solution of distilled water and household bleach or by scratching sclerites off of the cuticle with fine insect needles; sclerites were subsequently photo-documented. Additional pictures of all investigated specimens and their respective scleritomes are deposited on figshare, accessible under DOI:10.6084/m9.figshare.9943577.

Specimens were grouped into morphospecies based on their general habitus and scleritome characters, and then identified to the most accurate taxonomic level possible.

2.3. Sequence amplification and phylogenetic analyses

Genomic DNA was extracted using a combination of the standard CTAB extraction protocol and spin-columns (Macherey-Nagel NucleoSpin Tissue Blood and Tissue Set). We aimed to amplify two mitochondrial markers, i.e., partial sequences of the cytochrome *c* oxidase subunit I (COI) and 16S rRNA genes, using the Phire II Hotstart polymerase (Thermo Fischer). A modified version of the Folmer et al.'s (1994) universal COI primers (LCO_Apl TTTCTACTAAYCATAARGATATTGG and HCO_Apl TGATTTTTTGGTCACCCTGAAGTTTA) courtesy of Kevin M. Kocot (University of Alabama), and solenogaster-specific 16S primers (16Soleno-r YYTAATCCAACATCGAGGTC and 16Soleno-f RRGAGTWAGRCCTGCCCAGT) (Bergmeier et al., 2017) were used. PCR reaction was conducted with the following conditions for COI: initial denaturation for 30 s at 98 °C, 35–37 cycles of [5 s at 98 °C, 5 s at 47–53 °C, 20 s at 72 °C] and final elongation for 60 s at 72 °C. Annealing temperatures were 47–50 °C for 16S. Amplification resulted in products of approx. 670 bp for COI and 360 bp for 16S rRNA. For the present study, we augmented the previous datasets (Bergmeier et al., 2017; Ostermair et al., 2018) by amplifying and sequencing 238 additional 16S rRNA and COI sequences (see Supplementary data 1). PCR products were purified using the DNA Clean & Concentrator (Zymo Research) and cycle-sequenced on an ABI 3730 capillar sequencer at the Genomics Service Unit of the LMU Munich.

Specimens collected during the Japanese cruises were processed at the AORI. DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen); amplification was performed with the same procedure as described above. Amplicons were purified with ExoSAP-IT (Affymetrix) and cycle-sequencing was conducted on an ABI PRISM 3130xl.

All sequences were edited using Geneious v.11 (Biomatters Ltd). To check for contamination, each sequence was blasted against the GenBank database via the BLASTN program with the BlastN algorithm (Altschul et al., 1990). All verified sequences are deposited in GenBank (see Supplementary data 1 for accession numbers). Phylogenetic trees were rooted by using the caudofoveate *Chaetoderma nitidulum* as the outgroup (GenBank accession numbers MG264122.1 and AY340451). The COI and 16S rRNA datasets were aligned separately using the MUSCLE algorithm (Edgar, 2004) as implemented in Geneious v.11. The COI alignment was converted into amino acids to check for potential errors. Ambiguous blocks within the 16S rRNA alignment were masked with Gblocks using all options for less stringent selection (i.e., allowing for smaller final blocks, gap positions within the final block, and less strict flanking positions) (Castresana, 2000). The nucleotide substitution model GTR + I + G was determined by jModelTest2 under the Akaike Information Criterion (Darriba et al., 2012). We performed maximum likelihood analyses using RAxML 8.2.10 for each individual gene and the concatenated two-gene dataset partitioned by gene. For the single-gene analysis of COI we also included all solenogaster COI sequences available from GenBank (see Supplementary figure 1 (16S rRNA) and Supplementary figure 2 (COI) for the single gene trees). Bootstrap values were obtained by rapid bootstrapping with 1000 replicates. All phylogenetic analyses were conducted via the CIPRES gateway (Miller et al., 2010). The concatenated alignment is listed in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S25146).

2.4. Species delineation

Species are delineated herein based on unique morphological characters (i.e. habitus and scleritome, see 2.2). Recognized morphospecies are cross-validated via the phylogenetic analyses as reciprocally monophyletic clades in both gene trees, showing smaller intraspecific than interspecific genetic distances. To account for putative cryptic species, we reanalyzed monophyletic clades of externally similar Solenogatsres with low genetic distances by generating haplotype networks with popART (Leigh and Bryant, 2015) and TCSv1.21 (Clement et al., 2000) for the COI and 16S rRNA alignments of these subsets. We take separate haplotype networks as an indication for independently evolving lineages, i.e., species.

3. Results

3.1. Distribution and abundance of Northwest Pacific Solenogastres

During the KuramBio II expedition, Solenogastres were collected from 12 out of 18 EBS hauls (55 individuals) and two out of 16 Agassiz Trawls (three individuals) at nine stations, resulting in a total of 58 individuals (Fig. 2B; Table 2). More than half of all Solenogastres (31

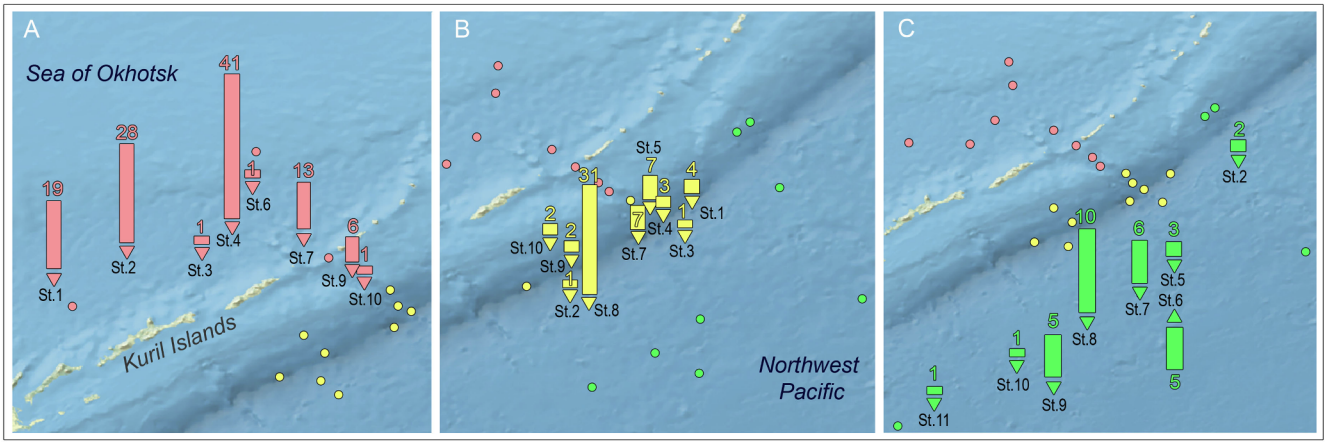

Fig. 2. SokhoBio (A), KuramBio II (B) and KuramBio I (C) stations with plotted numbers of collected specimens of Solenogastres. Note: in total, the generation of molecular data failed in 34 specimens, which are therefore not included in the present analyses.

individuals, 53.3%) were collected at KB II station 8 on the open abyssal plain from a maximum depth of 5,352 m (Table 2). A remarkable proportion of specimens (27.6%, 16 individuals) were retrieved from four stations at the bottom of the KKT in hadal depths between 8,200 m and 9,584 m (see KBII-stations 1, 4, 7, 9 in Tables 1 and 2). To our knowledge, the seven individuals collected from station 7 at 9,580 m present the current depth record of Solenogastres. The stations of the western slope (i.e., continental side) of the Kuril-Kamchatka Trench yielded nine specimens (15.5%): seven individuals were found at station 5 (7,081–7,122 m) in extension of the Bussol Strait (Fig. 1), and two solenogaster at the southern station 10 (5,265 m). Only two individuals were collected from the eastern slope stations of the Kuril-Kamchatka Trench (st. 2 and 3).

In combination with the data collected during eight other cruises to the Northwest Pacific and its marginal seas (Fig. 1, Table 1), morphological and molecular data from 192 specimens of Solenogastres are now available. In summary, specimens were collected at overall 29 sites, which cover the upper bathyal of the East China Sea and extend to the northern part of the Japan Trench, as well as the Kuril-Kamchatka Trench. The majority of sampling events took place in the vicinity of the Kuril-Kamchatka Trench and the adjacent abyssal plain of the open Northwest Pacific (Fig. 1). We provide a qualitative analysis of the available material; results between cruises and deployments are not standardized and cannot be directly compared quantitatively. Nevertheless, our data still show some general trends on the diversity and the abundance of Northwest Pacific Solenogastres: overall the sampling success among stations and the different geographic areas varies, with numbers of collected individuals varying considerably even at small local scale or direct vicinity (e.g., SokhoBio st. 3 and 4, KuramBio II st. 2 and 8 (Fig. 2A, B, Table 2)).

In summary, the abyssal basin of the Sea of Okhotsk and the Bussol Strait (ranging from about 3,300–4,800 m depth) yielded the highest number of specimens (57.3%, 110 individuals) (Fig. 2A). Close to a quarter (24.5%, 47 individuals) of all specimens were collected from the open abyssal plain of the Northwest Pacific from stations between 4,860 and 5,380 m – a relatively low number of individuals, taking into account the number of sampling events and the size of the covered area (Figs. 1 and 2B, C). The stations at hadal depths at the bottom of the trench below 8,000 m were sparsely populated (8.3% of all collected individuals, 16 individuals) and the lowest numbers of specimens were sampled from the abyssal slope stations of the trench (6.25%, 12 individuals) (see Fig. 2B). The low number of individuals from bathyal depths (seven specimens, 600–1,100 m) from two different Japanese cruises (see Fig. 1, Table 1) represents only a subset of more collected specimens (not available for the present study).

In all samples from the eight cruises analyzed herein (i.e. SokhoBio, KuramBio I and II, KH-01-02, KH-14-02, KT-08-27, KT-11-12, and N342), the two solenogaster orders Cavibelonia (= 97 specimens) and Pholidoskepia (= 93 specimens) are equally common in terms of numbers of specimens. We found only a single individual for each of the two remaining orders Sterrofustia and Neomeniamorpha. Our dataset contains at least nine different families of Solenogastres and 15 genera according to the currently recognized classificatory system (García-Álvarez and Salvini-Plawen, 2007).

The families Acanthomeniidae, Proneomeniidae, Pruvotinidae, and Simrothiellidae are widespread according to our dataset and were found on both sides of the Kuril Islands, and except for Acanthomeniidae also off of the Pacific coast of southern Japan (st. TD-4, T10-2) and the East China Sea (Proneomeniidae and Simrothiellidae (st. I10)) (Table 2). Remarkably, the widespread Proneomeniidae and Simrothiellidae were absent in the samples collected from the bottom of the trench below 8,000 m.

Dondersiidae (as traditionally defined) was the most abundant family in the Kuril Basin of the Sea of Okhotsk (55 specimens, equivalent to 53.4% of all Solenogastres collected there), and was also discovered in samples of the slope and hadal bottom of the Kuril-Kamchatka Trench and the Northwest Pacific plain. While Gymnomeniidae were present in the Sea of Okhotsk and also on the abyssal plain of the Northwest Pacific, they were not found on the slopes or the bottom of the trench. Singletons representing Neomeniidae, Macellomeniidae, and Phyllomeniidae occurred only in the eastern part of the Bussol Strait close to the trench (Neomeniidae) and on the Northwest Pacific plain (Macellomeniidae, Phyllomeniidae). We found Amphimeniidae only at the bottom of the Kuril-Kamchatka Trench below 7,000 m.

3.2. Systematics and species diversity of Northwest Pacific Solenogastres

The phylogenetic maximum-likelihood analysis based on two mitochondrial markers reveals three major clades within Northwest Pacific Solenogastres (Fig. 3), albeit all of the deeper nodes lack bootstrap support: Amphimeniidae (order Cavibelonia) forms the sister group to all remaining Solenogastres which are split into two clades. One clade unites various cavibelonian families such as (polyphyletic) “Pruvotinidae” (Pruvotinidae *sensu stricto* contains *Pruvotina* Cockerell, 1903 sp., identified based on anatomical traits (Jäger et al., 2017); formerly as Pruvotinidae sp.1 in Ostermair et al. (2018)), Simrothiellidae, Proneomeniidae and one representative of Neomeniidae (order Neomeniamorpha). The second clade contains representatives of the order Pholidoskepia (families “Dondersiidae”, Gymnomeniidae, Macellomeniidae), Cavibelonia (family Acanthomeniidae), and a single Sterrofustia (*Plicaherpia* sp., Phyllomeniidae). Acanthomeniidae are recovered as monophyletic with moderate support on some nodes

Table 2
Overview over the number of species and specimens collected at all investigated stations of the KuramBio I (KBI), SokhoBio (SB), KuramBio II and Japanese Expeditions (KH-01-02, KH-14-02, KT-08-27, KT-11-12, N342).

Cruise	KuramBio I						SokhoBio								KuramBio II										Japanese Expeditions					
Species/Station	2	5	6	7	8	10	1	2	3	4	6	7	9	10	1	2	3	4	5	7	8	9	10		NB D1	TD −4	K −4	T10 −2	I 10	J6 −3
Cavibelonia sp.1																														1
Cavibelonia sp. KBI-1						1																								
Cavibelonia sp. KBI-2		1																												
Acanthomeniidae sp.1																				1										
Acanthomeniidae sp.2																					3									
Acanthomeniidae sp.3																					1									
Acanthomeniidae sp.4																						1								
Acanthomeniidae sp.5																						1								
Acanthomeniidae sp.6								1												6										
Acanthomeniidae sp.7																					1									
Acanthomeniidae sp.8																					1									
Acanthomeniidae sp. SB-1								1		1			1																	
Veromenia cf *singula*			2	2																										
Amphimeniidae sp.1																			1											
Amphimeniidae sp.2															2				1											
Dorymenia sp.1																												1		
Dorymenia sp.2																										2				
Dorymenia sp.3																												1		
Dorymenia sp.4																											1			
Proneomeniidae sp.1																												1		
Proneomeniidae sp. SB-1								1																						
Pruvotinidae sp.1																												1		
Pruvotinidae sp.2																							1							
Pruvotinidae sp.3																							1							
Pruvotinidae sp.4																					1									
Halomeniinae sp. KBI		2																												
Pruvotinidae sp. KBI-1	1																													
Pruvotinidae sp. KBI-2		1		2	1																									
Pruvotina sp. SB													4																	
Pruvotinidae sp. SB-2								1																						

Cruise	KuramBio I						SokhoBio								KuramBio II															
Species/Station	2	5	6	7	8	10	1	2	3	4	6	7	9	10	1	2	3	4	5	7	8	9	10		NB D1	TD −4	K −4	T10 −2	I 10	J6 −3
Simrothiellidae sp.1																												1		
Simrothiellidae sp.2																			1											
Simrothiellidae sp.3																1														
Simrothiellidae sp.4																					1									
Simrothiellidae sp.5																					1									
Simrothiellidae sp.6					1																2									
Simrothiellidae sp.7																					7									
Simrothiellidae sp.8																									1					
Simrothiellidae sp.9																					1									
Simrothiellidae sp.10																					2									
Spiomenia sp.1																					2									
Spiomenia sp.2																					1									
Kruppomenia genslerae					1		1	7		7	1	3																		
Dondersiidae sp.1																					1									
Dondersiidae sp.2																					1									
Dondersiidae sp.3																	1													
Dondersiidae sp.4																			1											
Dondersiidae sp.5															2			3												
Lyratoherpia sp.																					1									
Nematomenia sp.																			3											
Dondersiidae sp. SB-1								1		2		1																		
Dondersiidae sp. SB-2									1																					
Dondersiidae sp. SB-3										1		3	1																	
Dondersiidae sp. SB-4							8	14		18		5																		
Gymnomeniidae sp.1																					2									
Gymnomeniidae sp.2																					1									
Gymnomeniidae sp. SB-2							10	2		12		1																		
Macellomenia sp.																					1									

Cruise	KuramBio I						SokhoBio								KuramBio II															
Species/Station	2	5	6	7	8	10	1	2	3	4	6	7	9	10	1	2	3	4	5	7	8	9	10		NB D1	TD −4	K −4	T10 −2	I 10	J6 −3
Neomeniidae sp.														1																

(*continued on next page*)

Table 2 (*continued*)

Cruise	KuramBio I						SokhoBio								KuramBio II										Japanese Expeditions					
Species/Station	2	5	6	7	8	10	1	2	3	4	6	7	9	10	1	2	3	4	5	7	8	9	10	NB D1	TD −4	K −4	T10 −2	I 10	J6 −3
Plicaherpia sp.																					1									
Total number of specimens	**1**	**4**	**2**	**4**	**3**	**1**	**19**	**28**	**1**	**41**	**1**	**13**	**6**	**1**	**4**	**1**	**1**	**3**	**7**	**7**	**31**	**2**	**2**	**1**	**1**	**1**	**4**	**1**	**1**
Total number of species	**1**	**3**	**1**	**2**	**3**	**1**	**3**	**8**	**1**	**5**	**1**	**5**	**3**	**1**	**2**	**1**	**1**	**1**	**5**	**2**	**20**	**2**	**2**	**1**	**1**	**1**	**4**	**1**	**1**

within the family, as are Gymnomeniidae (Fig. 3). "Dondersiidae" is polyphyletic: parts form the sister clade to Gymnomeniidae with moderate support and another part forms the sister clade to Acanthomeniidae + Cavibelonia sp.2 + *Plicaherpia* sp.. *Macellomenia* sp. (Macellomeniidae) is placed within the latter subgroup of "Dondersiidae", but the majority of the recovered relationships lack bootstrap support.

Overall 60 putative species of Solenogastres are present in the analyzed material. 19 of those species have been investigated in previously published studies (Bergmeier et al., 2017; Ostermair et al., 2018), and 41 species are novel discoveries and herein shown for the first time (Figs. 4 and 5). The majority of these species can be delineated by comparative diagnostics based on their habitus (such as shape (e.g. elongated/ stout/ with "tail"), appearance (smooth/ rough/ spiny), and to some extent also size) and unique scleritome characters (see below, and further discussion under 4.1).

The discovered morphospecies of the order Cavibelonia (Fig. 4A–E and 5A) are all characterized by mainly hollow, acicular (i.e. needle-like) or solid spicules, occasionally in combination with scales. Representatives of the order Pholidoskepia (Fig. 5B–D) are mostly covered in scale-like elements. We assigned one morphospecies (Fig. 4F) to the order Neomeniamorpha based on the presence of solid elements characteristic for this order, and another one (Fig. 4G) to the order Sterrofustia, based on different types of acicular sclerites.

The general habitus and sclerites of the largest-sized species within our dataset (Fig. 4A) best fit the family Amphimeniidae. Pruvotinidae were identified based on the characteristic combination of hook-shaped sclerites and sclerites with serrated ends (Fig. 4B). While Pruvotinidae sp.1, sp.2, and sp.3 all share the same three types of sclerites (1. straight, 2. with serrated distal end, 3. hook-shaped with pointed distal curvature and bent at the proximal base, see Fig. 4B), sp.2 and sp.3 bear each an additional shorter type of sclerites (sigmoid shape in sp.2, short scale and sharply bent sclerite in sp.3). The hook-shaped sclerite of Pruvotinidae sp.4 considerably differs from the other pruvotinids due to its overall length and the compact shape of the distal hook.

We assigned specimens in our dataset to the family Simrothiellidae (Fig. 4C) based on the unique combination of hollow acicular spicules to more solid-elongated elements, and a biserial radula formed by paired, denticulate plates (see Fig. 4C). We delimited the simrothiellid species based on differences in the scleritome such as length and shapes of the spicules (e.g. only straight in sp.3, straight to curved in sp.7, sharply bent in sp.10; see Fig. 4C), but see below for sp.4, sp.5, sp.6. *Spiomenia* (Fig. 4C) can be identified because of the 'captate' distal ends of some of its spicules which are unique for this genus. Both *Spiomenia* species bear this diagnostic character (see Fig. 4C, black arrowhead), but differ e.g. in the presence of sigmoid-shaped spicules (only present in sp.1) and scales (only present in sp.2) (Fig. 4C).

The Proneomeniids in our dataset are in general of long and slender body shape and differ considerably in size (9 mm up to 5.3 cm) and also form (i.e. *Dorymenia* species with a narrow, pointed "tail-like" posterior end see Fig. 4D).

Neomeniidae sp. is characterized by a stout body shape and sclerites as solid needles and groove-shaped elements (compare Fig. 4F with Fig. 1B in Salvini-Plawen and Paar-Gausch, 2004).

We identified the phyllomeniid as *Plicaherpia* (Fig. 4G) due to the overall high similarity to the type species *P. papillata* (compare to Fig. 5A in García-Álvarez et al., 2010), and the congruence of three out of four sclerite types (1. slightly curved acicular, 2. long and narrow groove-shaped sclerites with elongated pointed distal end, 3. long and wide groove-shaped scale; compare Fig. 4G (this publication) and Fig. 5B in García-Álvarez et al., 2010). However, we did not observe any hook-shaped elements as mentioned for the original *P. papillata,* either because they are truly absent or not present in the part of the cuticle used for the sclerite preparation.

All Acanthomeniidae (Fig. 5A) bear hollow, acicular spicules in combination with scaly elements. Spicules are curved (sp.3, sp.5, sp.6, sp.7) or straight with a sharply bent distal part (sp.1, sp.2; also sp.6 and sp.7) with serrations in Acanthomeniidae sp.2 and sp.6 (Fig. 5A, black arrowheads). Scales are of various shapes, often elongated or drawn out into pointed distal ends (sp.2), and with thickened rims (sp.1-sp.7).

Pholidoskepian Dondersiidae (Fig. 5B) usually appear smooth and somewhat "shiny", due to the flatly arranged scales, occasionally interspersed with solid, short needles. The general habitus and coloration vary between the encountered species (e.g. conspicuously elongated and slender body of Dondersiidae sp.3, whitish appearance (e.g. *Lyratoherpia* sp., Dondersiidae sp.4), or translucent integument (Dondersiidae sp.1)), and the scleritome details deliver further characters for species delineation: Dondersiidae sp.1, sp.2, sp.4, and sp.5 all have one type of leaf-shaped scale which varies in size (compare sp.1 and sp.4 in Fig. 5B) or form (compare symmetrically shaped scales of sp.1 and sp.4 with asymmetrical proximal base of sp.2), as do the short and solid spicules. Sclerites of *Lyratoherpia* sp. and *Nematomenia* sp. are highly similar to new species of the two genera from the South Atlantic abyssal basin (M. Carmen Cobo, University of Santiago di Compostela (Spain), pers. comm.).

The scleritomes of Gymnomeniidae sp.1 and sp.2 (Fig. 5C) consist mostly of keeled sclerites (except foot scales, see Fig. 5C asterisks), yet are distinctively different: Gymnomeniidae sp.1 has at least three different types of keeled sclerites (short and scale-like to acicular, thus the "spiny" habitus), while the scleritome of sp.2 is dominated by keeled scales with interspersed knife-like elements (black arrowhead in Fig. 5C). Keeled sclerites have so far been only reported for the family Gymnomeniidae.

Macellomeniidae is a monotypic family with unique sclerites, characterized by a basal plate from which the sclerites rise (often referred to as "nail-shaped") (see Fig. 5D).

However, within the families Simrothiellidae and "Dondersiidae" scleritome-based species delineation is problematic for some species. While Simrothiellidae form a monophylum in the concatenated dataset (see Fig. 3), the family is polyphyletic in single gene analyses (Supplementary figure 1 and 2). Simrothiellidae sp.4, sp.5, and sp.6 all have an inconspicuous habitus and similar size (3–5 mm), and share hollow, acicular sclerites of the same type which vary only in terms of their length and degree of curvature (from straight to sigmoid) (Fig. 4C).

The respective specimens with these highly similar scleritomes form a monophylum in the phylogenetic analysis of the concatenated two genetic markers (Fig. 3). Parsimonious haplotype networks of the three simrothiellid species sp.4, sp.5, sp.6 reveal three independent networks (corresponding to 6.6–12.9% interspecific and 0–0.3% intraspecific distances in 16S rRNA; COI missing for sp.5 and partially sp.6),

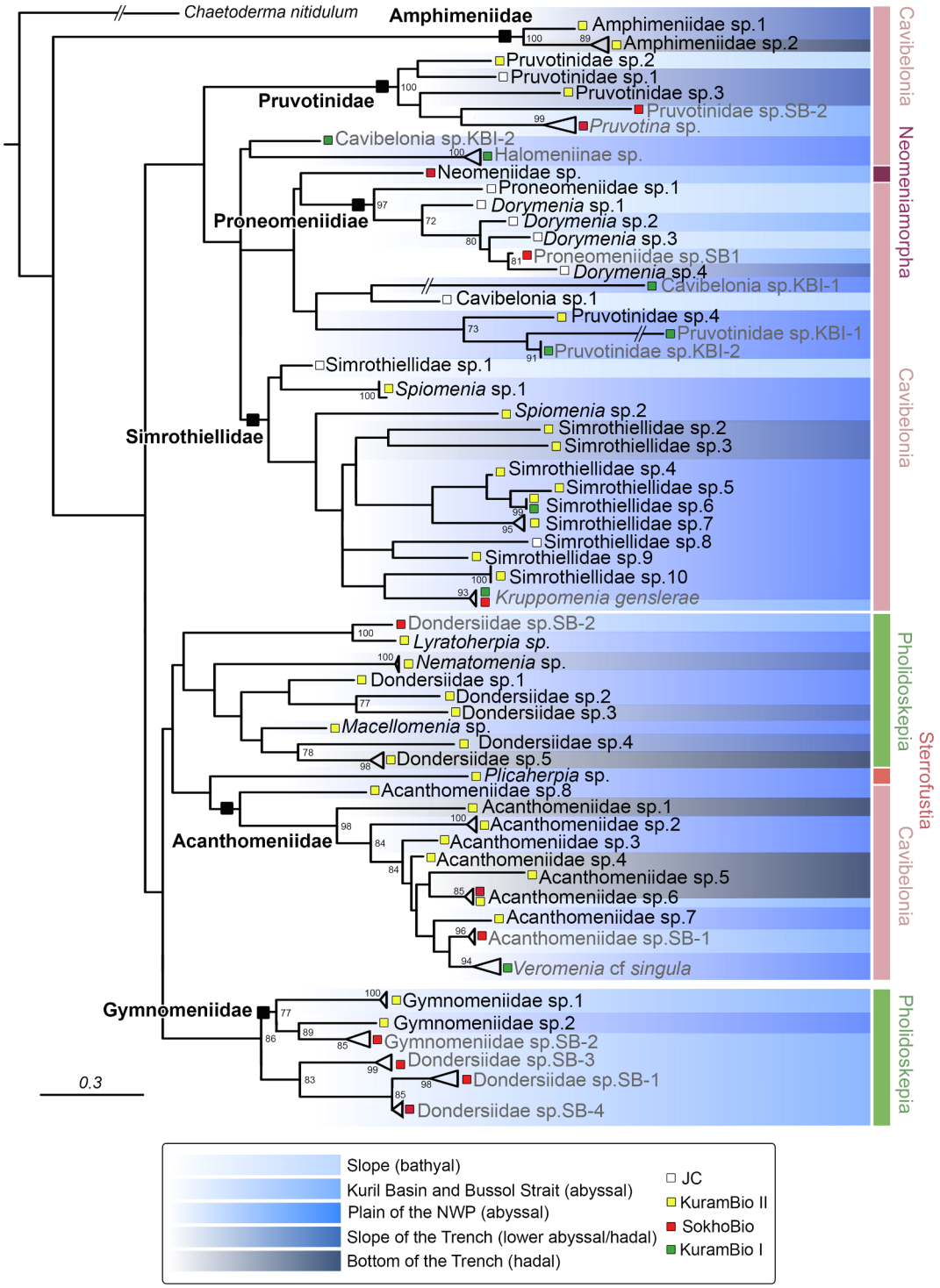

Fig. 3. Maximum-likelihood tree of Northwest Pacific Solenogastres based on the concatenated alignment of 16S rRNA and COI sequences. Bootstrap values below 70 not shown. Taxa already included in previous studies (Bergmeier et al., 2017; Ostermair et al., 2018) in light grey; abbreviation in taxa name: SB – SokhoBio expedition, KBI – KuramBio I expedition. Underlying shades of blue indicate depth profile. Colored squares refer to respective cruises (compare with Fig. 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

suggesting the presence of potentially three separate species.

Simrothiellidae sp.4 clusters among Simrothiellidae sp.7 on the 16S rRNA single gene tree (Supplementary figure 1), but outside of sp.7 and as sister to sp. 6 on the COI tree (Supplementary figure 2), suggesting a case of incomplete lineage sorting and recent diversification within the representatives of this family included in the present study.

Dondersiidae sp.SB-3 and Dondersiidae sp.SB-4 (Fig. 3) were previously identified by Ostermair et al (2018) as a single gymnomeniid species, based on external morphology (Fig. 2 C in Ostermair et al. (2018)). In our analyses, this morphospecies, previously misclassified due to a lack of microanatomical characters and ambiguous scleritome (see discussion), is not recovered as monophyletic (Fig. 3), suggesting two morphologically cryptic lineages.

Species boundaries between Dondersiidae sp.SB-1 and Dondersiidae sp.SB-4 remain problematic: While the single gene analysis based on 16S rRNA (Supplementary figure 1) retrieves them not even in a sister

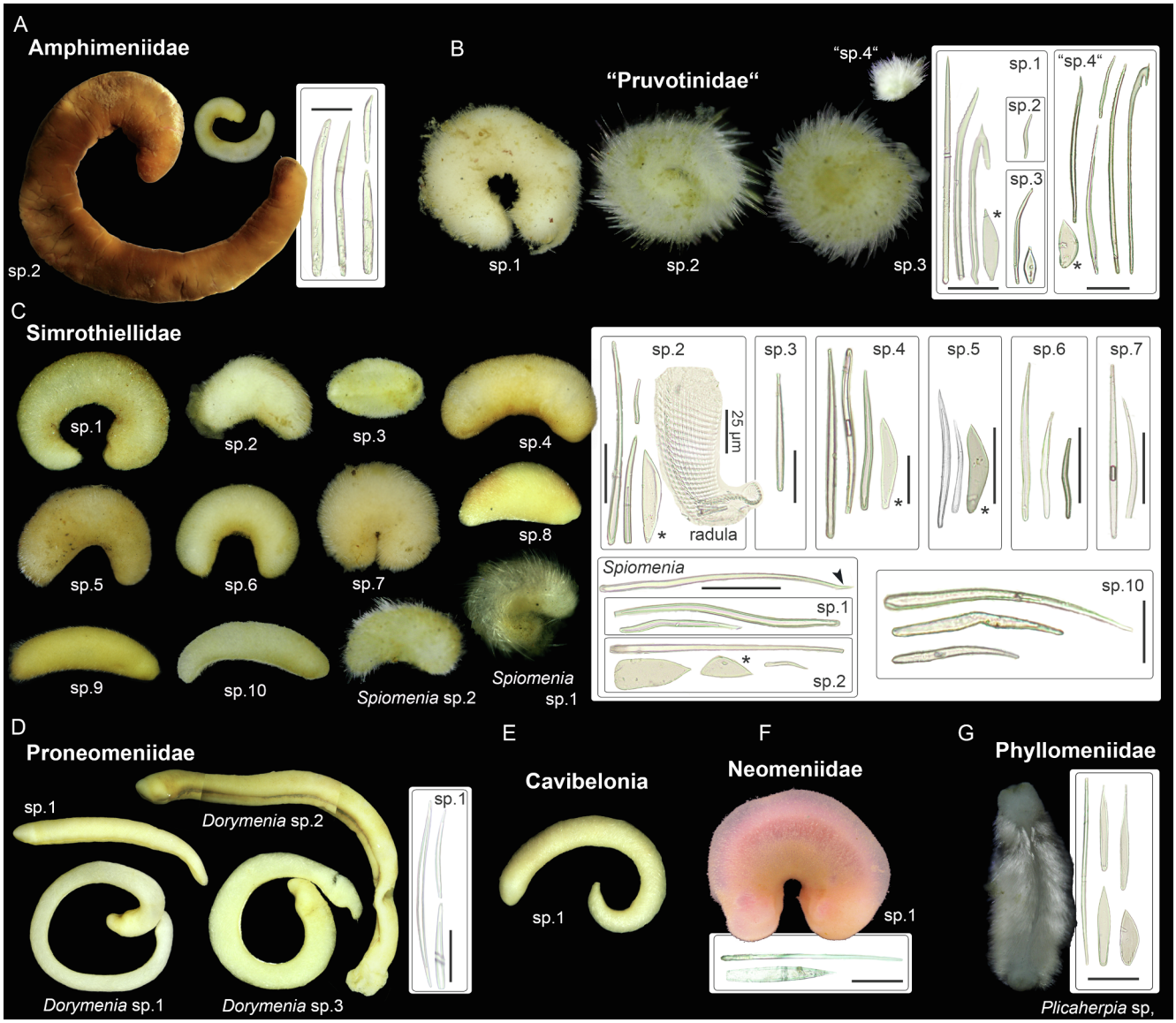

Fig. 4. Diversity of Cavibelonia (A–E), Neomeniamorpha (F), and Sterrofustia (G) from the Northwest Pacific. External morphology and characteristic sclerites are shown if available. Foot scales are marked by an asterisk. If not stated otherwise, all scale bars are of 50 µm length. A. Amphimeniidae sp.2 (ZSM Mol 20,190,583 & 20190582, juvenile), 70 mm and 15 mm. B. "Pruvotinidae": sp.1 (ZSM Mol 20190589), 5 mm; sp.2 (ZSM Mol 20190590), 2 mm; sp.3 (ZSM Mol 20190591), 2 mm; sp.4 (partial specimen, ZSM Mol 20190592), 2 mm; C. Simrothiellidae: sp.1 (ZSM Mol 20190602), 5.3 mm; sp.2 (ZSM Mol 20190603), 1.1 mm, sp.3 (ZSM Mol 20190604), 1 mm; sp.4 (ZSM Mol 20190605), 3.5 mm; sp.5 (ZSM Mol 20190606), 3 mm; sp.6 (ZSM Mol 20190608; sclerites: ZSM Mol 20190607), 3–5 mm; sp.7 (ZSM Mol 20190610; sclerites: 20190612), 1.5–3 mm; sp.8 (ZSM Mol 20190615), 2.5 mm; sp.9 (ZSM Mol 20190616), 2 mm; sp.10 (ZSM Mol 20190618), 1.5–2 mm; *Spiomenia* sp.1 (ZSM Mol 20190619; sclerites: ZSM Mol 20190620), 1.5 mm; *Spiomenia* sp.2 (ZSM Mol 20190621), 1–1.5 mm, black arrowhead points to 'captate' end; D. Proneomeniidae: sp.1 (ZSM Mol 20190621), 9 mm; *Dorymenia* sp.1 (ZSM Mol 20190585), 22 mm; *Dorymenia* sp.2 (NMST 73197, damaged posterior end), 53 mm; *Dorymenia* sp. 3 (ZSM Mol 20190586), 18 mm; E. Cavibelonia sp.1 (ZSM Mol 20190563), 14 mm; F. Neomeniidae sp.1 (ZSM Mol 20190844), 32 mm; G. Phyllomeniidae: *Plicaherpia* sp. (ZSM Mol 20190845), 2 mm.

group relationship with large pairwise distances (17.5–25.1% distance), sp.SB-1 clusters within sp.SB-4 in the COI tree with only 0.4–6.1% pairwise distance (Supplementary figure 2). Anatomical characters and additional genetic information are needed to further address and reliable delineate these two dondersiids.

3.3. Bathymetric and geographic ranges of Northwest Pacific solenogaster species

We collected six species from bathyal depths (sampling locations off southern Japan, st. T10-2, I10, J63), 42 species from abyssal depths (sampling locations near the Japan Trench and Kuril-Kamchatka Trench, Kuril Basin, Northwest Pacific Plain), nine species from the lower abyssal and hadal slopes of the Kuril-Kamchatka Trench, and six species from the hadal bottom stations of the Kuril-Kamchatka Trench.

40 out of 60 species investigated in this study, were collected only as singletons. In most of the investigated families, the majority of species are represented by single findings only. Pholidoskepian Gymnomeniidae and "Dondersiidae" are comparably common and bear fewer singletons. Only 10% of all singletons (4 spp.) were collected from the Kuril Basin of the Sea of Okhotsk and the Bussol Strait between 3,000 m and 4,500 m (Fig. 6A); at those stations, which yielded the highest solenogaster abundance (Fig. 2A, Table 2), the majority of species were represented by multiple individuals. The rate of singletons was highest at depths of around 5,000–5,500 m on the Northwest Pacific plain (Fig. 6A), where 67.9% of the encountered species (19 out of

Fig. 5. Diversity of Cavibelonia (A) and Pholidoskepia (B–D) from the Northwest Pacific. External morphology and characteristic sclerites are shown if available. Foot scales are marked by an asterisk; cs: copulatory spicules. All scale bars are of 50 µm length. A. Acanthomeniidae: sp.1 (ZSM Mol 20190564, damaged), 2 mm; sp.2 (ZSM Mol 20190566), 1.5–2 mm; sp.3 (ZSM Mol 20190568; damaged), 4 mm; sp.4 (ZSM Mol 20190569), 1.2 mm; sp.5 (ZSM Mol, 20190570), 3 mm; sp.6 (ZSM Mol 20190575; sclerites: ZSM Mol 20190572) 1.5–2 mm; sp.7 (ZSM Mol, 20190578) damaged), 4.5 mm; sp.8 (ZSM Mol 20190579), 1.5 mm; black arrowheads mark serrations. B. "Dondersiidae": *Lyratoherpia* sp. (ZSM Mol 20190682), 13 mm; *Nematomenia* sp. (ZSM Mol 20190684), 1.5–4 mm; "Dondersiidae" sp.1 (ZSM Mol 20190622; damaged), 2 mm; sp.2 (ZSM Mol 20190623), 6 mm; sp.3 (ZSM Mol 20190624), 9 mm; sp.4 (ZSM Mol 20190625; damaged), 1 mm; sp.5 (ZSM Mol 20190629; sclerites: ZSM Mol KB2 20190630), 1–2.3 mm; C. Gymnomeniidae: sp.1 (ZSM Mol 20190687; sclerites: ZSM Mol 20190686), 1.5–2 mm; sp.2 (ZSM Mol 20190688, damaged), 2.7 mm; black arrowhead marks knife-shaped sclerite; D. Macellomeniidae sp. (ZSM Mol 20190712), 2 mm.

28 spp.) are represented by single individuals only, constituting 47.5% of all singletons in the present study. The numbers of Solenogastres collected from the slopes and bottom of the trench were overall low (Fig. 2B, Table 2) and 75% of slope species (8 out of 12 spp.) and 50% of trench species (3 out of 6 spp.) are represented as singletons only (Fig. 6A). Among the species with multiple collected individuals, eight species were found only at single locations, with overall low abundances between two and six individuals. Of the remaining 12 species which were collected at more than one station, the majority (8 spp.) exhibited restricted depth ranges of only a few hundred meters (Fig. 6B). Remarkably, three species were distributed over a depth range of more than 1000 m: Amphimeniidae sp.2 (three specimens), with a body length of up to 7 cm the largest-sized Solenogastres within our dataset (Fig. 4A), was found only at the bottom of the Kuril-Kamchatka Trench between 7,100 and 8,200 m (Fig. 6B). Simrothiellid *Kruppomenia genslerae* Ostermair, Brandt, Haszprunar, Jörger & Bergmeier, 2017, which was among the most abundant and widely distributed species in the Sea of Okhotsk, was found at around 3,300 m in the Kuril Basin (Ostermair et al., 2018). A single specimen of *K. genslerae* was also collected from the Northwest Pacific plain at 5,100 m during the KuramBio I expedition (KB I st. 8, Fig. 7A), extending the depth range to almost 2,000 m (Fig. 6B). *Kruppomenia genslerae* is also the only species in our dataset with confirmed occurrence on both sides of the Kuril-Kamchatka Trench (genetic distances in 16S rRNA: 0–0.3%). A parsimonious haplotype network revealed overall five different haplotypes, and the ancestral haplotype was shared between the

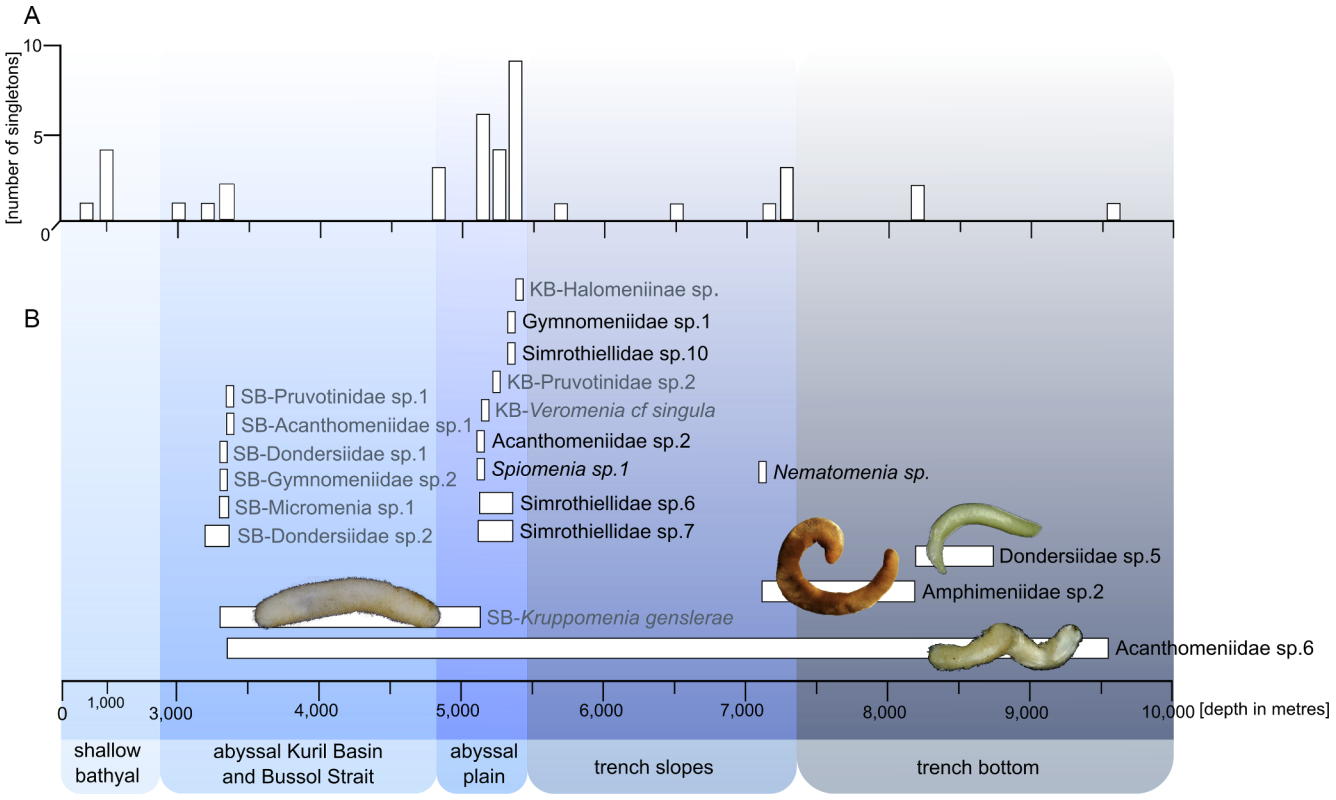

Fig. 6. Bathymetric distribution of Northwest Pacific Solenogastres analyzed in the present study. A. Occurrence of species collected as single individuals only in relation to depth. B. Bathymetric distribution of species (sampled with multiple individuals).

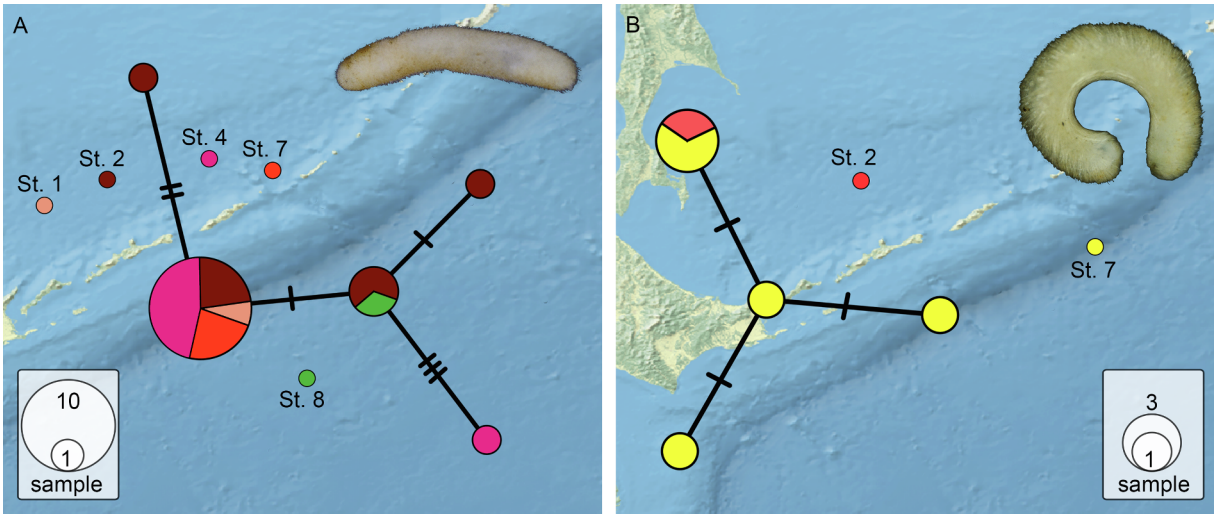

Fig. 7. Parsimonious haplotype networks based on 16S rRNA for *Kruppomenia genslerae* (A) and Acanthomeniidae sp.6 (B). Colors of haplotypes correspond to station colors on the underlying map, where haplotypes were sampled. KuramBio I station – green, KuramBio II stations – yellow, SokhoBio stations – shades of red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

individuals from SokhoBio st. 2 and the open Northwest Pacific plain (Fig. 7A).

Acanthomeniidae sp.6 was collected over an astonishing depth range (Fig. 6B). We sampled six individuals at the bottom of the Kuril-Kamchatka Trench at 9,580 m (KB II st. 7, in vicinity of the Bussol Strait (Fig. 1)). A single individual of the same species (genetic distance in 16S rRNA: 0–0.6%) was collected from SokhoBio st. 2 at ~3,300 m. All representatives of Acanthomeniidae sp. 6 are retrieved in a single parsimonious haplotype network (Fig. 7B). In total, four different haplotypes are present, with the ancestral haplotype collected at the bottom of the trench (Fig. 7B). Overall, Acanthomeniidae sp.6 covers a depth range of 6,200 m from the Kuril Basin down to the bottom of the Kuril-Kamchatka Trench.

4. Discussion

4.1. Systematics of Northwest Pacific Solenogastres

Studies on solenogaster systematics are still limited and current classification is largely based on scleritome and microanatomical data (dominantly on characters of radula, foregut glands and the reproductive system). A first morphocladistic approach on solenogaster

families by Salvini-Plawen (2003b) did not resolve internal relationships of Solenogastres due to a putative high degree of homoplasy in the included characters. The first phylogenomic study, based on a dataset of more than 500 genes including representatives of all traditional Solenogastres orders, has already revealed considerable conflict with the traditional classificatory system, rendering one of these orders (resp. Cavibelonia) polyphyletic (Kocot et al., 2019) and retrieving the former cavibelonian family Amphimeniidae as sister to all remaining Solenogastres. This morphologically surprising result indicates either multiple independent origins of hollow sclerites in "cavibelonian" clades or multiple losses, weakening this character for higher classification (Kocot et al., 2019).

Our phylogenetic study of Northwest Pacific Solenogastres is based on two fast-evolving mitochondrial markers, which are not ideal to resolve deep solenogaster relationships, whose origin has been estimated in the early Paleozoic (Vinther et al., 2012). Thus, unsurprisingly, the majority of deeper nodes within our analysis are only poorly supported (see Fig. 3). Nevertheless we also recover Amphimeniidae, a family with unique foregut glands (Type D, García-Álvarez and Salvini-Plawen, 2007), as sister group to all remaining Solenogastres. This result, based on different representatives of Amphimeniidae and other genetic markers than in Kocot et al. (2019), supports the early split-off of the family and consequently rejects previous hypotheses on the evolution of Solenogastres which placed Pholidoskepia in a "basal" position (Salvini-Plawen, 1985).

Pholidoskepia form a monophylum in the present study, but also include "cavibelonian" Acanthomeniidae (taxa missing in Kocot et al., 2019) and sterrofustian *Plicaherpia* sp. (see Fig. 3). Interestingly, genus-level morphocladistics by Salvini-Plawen (2003b) have recovered partial pholidoskepian Dondersiidae and Acanthomeniidae in a polytomy. Therefore, Scheltema et al. (2012) already suggested revision of the placement of at least *Dondersia* Hubrecht, 1888 and *Acanthomenia* Thiele, 1913, due to similarities in taxonomically important traits (i.e., scleritome, radula, and foregut glands). Our study delivers further evidence that Acanthomeniidae has been previously misplaced within "Cavibelonia" and should rather be classified among Pholidoskepia; the latter order is currently diagnosed by almost exclusively scaly scleritomes (scale-like sclerites in various forms are present in Acanthomeniidae, albeit do not dominate the scleritome (García-Álvarez and Salvini-Plawen, 2007)) and two types of foregut glands (one of which can also be found in Acanthomeniidae) (Salvini-Plawen, 1978a; García-Álvarez and Salvini-Plawen, 2007).

In phylogenomic analyses, sterrofustian *Phyllomenia* Thiele, 1913 is sister to "cavibelonian" Pruvotinidae (Kocot et al., 2019), while our study suggests a sister group relationship of sterrofustian *Plicaherpia* sp. with Acanthomeniidae. *Plicaherpia* (Phyllomeniidae) and Acanthomeniidae share the same type of foregut glands (García-Álvarez et al., 2010) and there are similarities in scale-like elements (compare Fig. 4G with Fig. 5 A, sp.1, sp.2, sp.3 and sp.7), which both support this unexpected relationship. The type of radula, however, differs considerably (monoserial or lacking in Acanthomeniidae (e.g., Scheltema, 1999; Handl and Salvini-Plawen, 2002; Gil-Mansilla et al., 2008) and distichous in the genus *Plicaherpia* (García-Álvarez et al., 2010)) and while needle-like elements of Acanthomeniidae are hollow, needles of *Plicaherpia* are solid – one of the main diagnostic characters of Sterrofustia. It should also be noted that the affiliation of the Northwest Pacific specimen to this genus is based on a highly similar habitus and three out of four identical sclerite types, but we did not find the fourth type of sclerites (i.e. hook-shaped sclerites) as described for the type specie *P. papillata*. However, the status of Sterrofustia as an independent order of Solenogastres has been questioned previously (Kocot et al., 2019) and its monophyly requires re-investigation.

The non-monophyly of "Cavibelonia" as traditionally defined (Kocot et al., 2019) is supported by our study. The position of Neomeniidae (representative of the order Neomeniamorpha) is, however, in direct conflict with larger phylogenomic analyses. Kocot et al. (2019) recover Neomeniamorpha sister to a clade of *Phyllomenia* + Pruvotinidae (albeit with weak support), while our study results in a common clade of neomeniamorph Neomeniidae sp. + cavibelonian Proneomeniidae (also with weak support). In contrast to Proneomeniidae, Neomeniidae (respectively all Neomeniamorpha) lack ventral foregut glands, and sclerites are hollow in Proneomeniidae but solid in Neomeniidae. Proneomeniid radulae are polyserial, which also occur in one out of three genera constituting Neomeniamorpha (i.e. *Archaeomenia* Thiele, 1906 (Hemimeniidae)). The other two neomeniamorph genera (*Neomenia* Tullberg, 1875, Neomeniidae) and *Hemimenia* Nierstrasz, 1902 (Hemimeniidae) both lack a radula (Salvini-Plawen and Paar-Gausch, 2004; Salvini-Plawen, 1978a,b). Nevertheless the relationship between Proneomeniidae and Neomeniidae as recovered in our phylogenetic analyses remains dubious from a morphological perspective based on current knowledge.

Here we present for the first time a molecular study of multiple species per family, partially also from different genera, testing the validity of established taxonomic entities. Amphimeniidae, Proneomeniidae, Simrothiellidae, and Acanthomeniidae all result in monophyla based on our concatenated two-marker analyses. "Pruvotinidae" and "Dondersiidae" however, are both recovered as polyphyletic (Fig. 3). Pruvotinidae form a species rich family (33 spp.) and are currently classified in five subfamilies (MolluscaBase, 2019). The diagnosis of Pruvotinidae is fairly general (high degree of variation in scleritome and radula types, also all except one type of foregut glands present (García-Álvarez and Salvini-Plawen, 2007)) and our study indicates – albeit with low bootstrap support – that "Pruvotinidae" as traditionally defined might represent an artificial assemblage. Microanatomical data is needed from the different clades of "Pruvotinidae" recovered herein (Fig. 3) to evaluate whether these can be assigned to existing (sub-) families, which in this case should be erected to family level in future reclassification of Solenogastres.

"Dondersiidae" have traditionally served as a "taxonomic catchbasin" for hard-to-place lineages (e.g., Klink et al., 2015), as the family diagnosis is based on a loose mosaic of character combinations, several of which are not unique (e.g., thin cuticle, varying combinations of scale-like and solid needle-like elements) (Scheltema et al., 2012). In our phylogeny, parts of "Dondersiidae" + macellomeniid *Macellomenia* sp. form a poorly supported monophylum together with Acanthomeniidae (+phylomeniid *Plicaherpia* sp.) (Fig. 3). Monogeneric Macellomeniidae can be well identified based on their unique sclerites with a basal plate (Fig. 5D; Scheltema, 1999: fig.12; Kocot and Todt, 2014: Fig. 3). The scleritome of dondersiid *Lyratoherpia* sp. is highly similar to a new *Lyratoherpia* Salvini-Plawen, 1978 species from the South Atlantic abyssal basin off Brazil (M. Carmen Cobo, University of Santiago di Compostela (Spain), pers. comm.). *Nematomenia* sp. shares scleritome elements with other *Nematomenia* species (e.g., *N. glacialis* Thiele, 1913; Salvini-Plawen, 1978a: figs. 28–30) and a newly discovered *Nematomenia* from the abyssal Angola Basin (Carmen M. Cobo, University of Santiago di Compostela (Spain), pers. comm.). The other "dondersiid" lineages investigated herein are hard to place among the different genera of the family, as they all have variously shaped scales in common, combined with some kind of paddle-shaped elements (see Dondersiidae sp.1, sp.2, sp.4 in Fig. 5B). For generic identification of these species, it is thus necessary to refer to microanatomical characters (e.g., radula, foregut glands, reproductive system) in future research.

Our study also reveals a problematic case of externally cryptic species and own misidentification: the phylogenetic analyses splits representatives of a species formerly classified as "SB-Gymnomeniid sp.1" (see Fig. 2C in Ostermair et al. (2018)) into two externally cryptic lineages (see Fig. 3, Dondersiidae sp. SB-3 and sp.SB-4) as sister to Gymnomeniidae. Initial classification among Gymnomeniidae was based on scleritome characters only (see Fig. 2D, D′ in Ostermair et al. (2018)), which was not unambiguous as highly similar scleritomes are also known for Dondersiidae. Re-allocation within "Dondersiidae" in the present study is based on subsequent histological investigations of

the two cryptic dondersiid lineages (formerly classified as "SB-Gymnomeniidae sp.1"), which (1) supports them as independent species and (2) revealed a *Micromenia*-type monostichous radula (own unpublished data). At present, reliable assignment to an existing dondersiid genus is hampered by conflicting characters between the radula type (suggesting affinities with *Micromenia* Leloup 1948) and sclerites (as found e.g., in *Nematomenia*). This underlines the need of molecular and morphological revision of the family Dondersiidae, as our study indicates that some established genera might be more closely related to Gymnomeniidae than to other dondersiid genera. Gymnomeniidae sp.1, sp.SB-1, and sp.3 all bear the typically keeled scales found only in the gymnomeniid *Wirenia* Odhner, 1920 (Fig. 5C in the current study and Fig. 2D, D′ in Ostermair et al. (2018)), but with additional characters (i.e., almost needle-like keeled elements (sp.1) and short knife-shaped elements (sp.2), Fig. 5C)).

Our study highlights that the family "Pruvotinidae" also requires a thorough re-investigation, and additional families might arise as problematic with more taxa included in molecular analyses. Combining scleritome characters with multiple molecular markers allows for integration into the currently existing classificatory system (based on hard parts) and robust species delineation (molecular markers). The molecular barcodes generated during the course of this project provide a first step in facilitating (re)identification for fast alpha- and beta-diversity assessments, but cannot replace traditional taxonomic investigations. For formal description of the discovered diversity, investigations of traditional anatomical characters (such as radula, foregut glands and configurations of the reproductive system) are still indispensable.

4.2. Boosting knowledge on the diversity of Solenogastres on regional and global scale

Our knowledge on solenogaster diversity in the Northwest Pacific was based on very few records scattered over a large geographic area. Prior to the series of expeditions studied herein, only eleven species of Solenogastres have been reported from the Northwest Pacific region between 1911 and 2014 (Heath, 1911; Baba, 1940, 1975; Salvini-Plawen, 1997; Saito and Salvini-Plawen, 2010, 2014; Sirenko, 2013). All of those species have been described from the continental shelf and bathyal depths between 40 and 1,500 m and the majority occurs in coastal waters surrounding Japan. Published datasets (Ramirez-Llodra, 2005; JAMSTEC, 2016) report two Solenogastres from abyssal depths, i.e. an unidentified specimen from the slopes of the northern Japan Trench at 5,345 m and simrothiellid *Helicoradomenia* sp. from the Mariana Ridge in 3,600 m. Unfortunately, no further data exist which would allow for identification in comparison to the present dataset. All of the 19 species, which were characterized or formally described based on morphological and molecular data from the KuramBio I and SokhoBio expedition (as well as 12 additional morphospecies lacking molecular barcodes), present first records for the Northwest Pacific (Bergmeier et al., 2017; Ostermair et al., 2018). Overall, we have raised the number of species known from the continental shelf and bathyal zone in the region from 11 to 17. With overall 45 newly recorded species and 160 records, the investigated abyssal zone (approx. 3,000–6,000 m) harbors a rich solenogaster fauna: 10 species were found in the abyssal Kuril Basin of the Sea of Okhotsk, three species in the Bussol Strait, two each on the western abyssal slope of the Kuril-Kamchatka Trench and close to the Japan Trench, and 31 species on the open abyssal plain of the Northwest Pacific. In contrast to the investigated abyssal plain of the Northwest Pacific, the solenogaster fauna of the Sea of Okhotsk is considerably less diverse, with a single widespread dondersiid species (Dondersiidae sp.SB-4) accounting for 40% of the solenogaster records in the Sea of Okhotsk. This lower diversity might be a result of low primary production in the surface layer above the Kuril Basin and low levels of oxygen at the bottom (Tyler, 2002). The formation of oxygen-poor bottom waters in the basin during interglacial periods (Liu et al., 2006) might also explain the potentially impoverished fauna.

Our data currently indicate an overall considerably higher diversity of Solenogastres at abyssal depths than on the continental shelf (0–200 m) and in the bathyal zone (200–3,000 m) of the Northwest Pacific. This would contradict the general trend of decreasing benthic deep-sea diversity with increasing depth (e.g., Rex et al., 1990), which has also been hypothesized for Solenogastres (Scheltema, 1992). Current distribution records of Solenogastres show that the clade is most species-rich on the continental shelf and in the upper bathyal down to 1,000 m, which harbor more than 50% of the described species diversity (Todt, 2013). In these depths they can also occur in locally high abundance, e.g., on the continental slope of the northern European Atlantic coast and the Northeast Pacific (e.g., Scheltema, 1990; Todt, 2013). Currently, the comparably high abyssal diversity in the Northwest Pacific should be considered as a result of sampling bias, as the adjacent bathyal zone has not yet been systematically explored for Solenogastres. Extrapolating from the high degree of collected singletons and overall rarity in the abyss favors a higher diversity on shelf and bathyal areas as previously proposed.

Globally, only 31 Solenogastres species have been reported between 3,000 and 6,000 m. Our study more than doubles the number of solenogaster species from the abyssal zone to 75 species in total. Prior to the discovery of Neomeniidae sp. (Fig. 4F, from SokhoBio St.10, at 4,803 m), only a single species of the order Neomeniamorpha (i.e. *Neomenia trapeziformis* Salvini-Plawen, 1978) has been found below 2,000 m (at roughly 2,100 m at the margin of the Bounty Plateau off Southeast New Zealand in the South Pacific) while all other neomeniamorph species are recorded from the shallower bathyal. The potential sterrofustian *Plicaherpia* sp. (Fig. 4G) is only the second species of this order recorded outside of Antarctic waters (only *Imeroherpia laubieri* Handl, 2002 was collected in the Northern Hemisphere as well, but in the North Atlantic at 2,246 m).

Recent investigations of the Angola Basin (DIVA-1 expedition (Arbizu and Schminke, 2005)) reported additional undescribed candidate species (Gil-Mansilla, 2008; Gil-Mansilla et al., 2008; Gil-Mansilla et al., 2009; Gil-Mansilla et al., 2012) from the lower abyss, showing that - on a global scale - the abyssal zone is catching up with shallower depths in terms of species-level diversity. The current increase in reports on Solenogastres from the abyss correlates with extensive sampling efforts (e.g., Arbizu and Schminke (2005); Brandt et al. (2018b)) and new gear specifically designed to collect benthic macrofauna at great depths (Brandt et al., 2013).

Characterized by a total absence of light, enormous hydrostatic pressure, and dependence on nutrient influx from upper layers resulting in very limited food-supply (Jamieson, 2015), the deepest regions of the oceans have long been considered void of life, until the first hadal animals were collected during the *Galathea* expedition in the middle of the 20th century (Belyaev and Brueggeman, 1989). Up to the present study, all classes of molluscs have been reported as part of the hadal malacofauna (Schwabe, 2008; Jamieson, 2015; Linse and Schwabe, 2018) – except for Solenogastres, which were not sampled even in most recent exploration of trenches (Linse and Schwabe, 2018). This gap in knowledge is filled by the herein recorded 11 species (24 individuals) from the slopes and bottom of the Kuril-Kamchatka Trench. The two acanthomeniid species (Fig. 5A, sp.1 and sp.6) collected from the bottom of the Kuril-Kamchatka Trench (KB II st. 7, Fig. 1) at 9,584 m present the current depth record of Solenogastres, exceeding the previous record by almost 4,000 m (*Plawenia schizoradulata* Salvini-Plawen, 1987, collected at 5,931 m near the South Shetland Islands (Salvini-Plawen, 1978b).

Our study gives first insights into a surprisingly diverse hadal solenogaster fauna, even though all discovered hadal species are rare: they were collected in low individual numbers (one to six specimens) and restricted to either the slopes or the bottom of the trench, except for large-sized Amphimeniidae sp.2, which was collected at two stations

over a "horizontal" distance of about 60 km on both the slope and bottom of the trench (KB II st. 1 and st. 5), and Acanthomeniidae sp.6 with an astonishing distribution range (see discussion below). Apart from Acanthomeniidae sp.6, there is no faunal overlap on species level between the hadal slopes and bottom of the trench with the shallower investigated regions, i.e. on the nearby abyssal plain or towards the Sea of Okhotsk. Even hadal trenches in close proximity (e.g., Kuril-Kamchatka Trench and Japan Trench) probably present disjunct habitats, as they are separated through shallower (i.e., abyssal and bathyal) regions which might lead to high rates of endemism (Belyaev and Brueggeman, 1989; Blankenship-Williams and Levin, 2009; Jamieson et al., 2010).

4.3. Distribution over depth and distance – And a trench as a barrier to dispersal?

Within our dataset, the majority of species exhibit a restricted distribution as they were either collected as singletons (40 spp.) or only found at single stations (8 spp.). We discovered two species, *Macellomenia* sp. and *Veromenia* cf *singula* which exhibit high morphological (and in the latter case also anatomical (see Bergmeier et al., 2017 for details)) similarity to described deep-sea species from the Atlantic, i.e. *Macellomenia aciculata* Scheltema, 1999 from the west European Basin and *Veromenia singula* Gil-Mansilla, García-Álvarez and Urgorri, 2008 from the Angola Basin. Molecular data is needed from the Atlantic specimens to evaluate their relationships to the Pacific species and test if amphi-oceanic species do exist or whether both findings present cases of morphological crypsis.

We found overall seven individuals of Acanthomeniidae sp.6 which were collected from the bottom of the Kuril-Kamchatka Trench (six individuals), as well as the Kuril Basin of the Sea of Okhotsk (one individual). The maximum vertical distribution range for this species thus covers 6,226 m, and constitutes a link between the upper abyssal and hadal zones. Vertical ranges of around 2,000 m have been described for several solenogaster species, usually from the continental shelf down to the upper bathyal (e.g. *Dondersia namibiensis* Scheltema, Schander & Kocot, 2012 in Scheltema et al. (2012)). *Pruvotina longispinosa* Salvini-Plawen, 1978 has been described over a vertical range of more than 3000 m depth (64 m to 3,890 m), and horizontal distribution from the Adelaide Archipelago off Chile to the Antarctic South Shetland Islands. However, this study presents the first wide bathymetric range confirmed via molecular data. Several benthic amphipods of the Kuril-Kamchatka Trench region have been described with similar distribution ranges, and even though the morphological basis of identification of these widely distributed species has been questioned, a bathymetric range over 3,200 m was confirmed via molecular barcoding for a single amphipod species, which suggests that dispersal of this species might not be impeded by the Kuril-Kamchatka Trench (Lörz et al., 2018).

The currently known longest time spent by lecithotrophic Solenogastres larvae in the water column is a maximum of 10 days before final settlement (Todt and Wanninger, 2010) rendering wide distribution ranges rather unlikely at present state of knowledge. However, this period might be significantly longer in very cold water. Dispersal between the investigated regions might be strongly influenced by the Kuril-Kamchatka Trench, as hadal trenches potentially act as dispersal barriers for benthic species of shallower regions. Remarkably, we found evidence of one species crossing this potential distribution barrier, as specimens of *Kruppomenia genslerae* were found on both sides of the Kuril-Kamchatka Trench (Fig. 7A), i.e., in the Kuril Basin of the Sea of Okhotsk and on the Northwest Pacific plain. Potential connectivity between the Kuril Basin of the Sea of Okhotsk and the Northwest Pacific plain has been explored for other benthic invertebrate species, but molecular data currently rejects a cross-trench colonization in sternaspid annelids (Kobayashi et al., 2018). Morphological data indicates that the bivalve fauna in the Sea of Okhotsk contains several bathyal-abyssal Pacific species (Kamenev, 2018b), but for gastropods, Fukumori et al. (2018) suggest that problematic taxonomic assignment in gastropods resulted in the assumption that species from the Kuril Basin are widely distributed in the Northwest Pacific. Thus, based on current knowledge the present study presents the first case of cross-trench distribution confirmed by molecular data.

4.4. Sunken loners or true endemics in the abyssal and hadal zone?

Based on distribution patterns and bathymetric ranges of other molluscs, source-sink dynamics have been proposed to explain the rarity of abyssal species in a food-limited environment (Rex et al., 2005). The source-sink hypothesis considers the encountered abyssal diversity as a subset of bathyal source populations sunken to the seafloor, but not representing self-sustaining, endemic abyssal populations (i.e., with reproduction rate not compensating mortality, but instead relying on a constant influx of dispersing larvae from bathyal assemblages) (Rex et al., 2005). This scenario likely applies to marine deep-sea organisms dispersing via planktonic larvae and the influence of source-sink dynamics on their abyssal diversity clearly depends on the survival times of the larvae drifting in the water column and the distance of the abyssal plains from their putative bathyal sources (Hardy et al., 2015). Modelling larval source-sink dynamics in the abyss shows that the investigated area of the Northwest Pacific provided an intermediate "larval slope source index" due to is relative proximity to the nearest shore (see Fig. 1a in Hardy et al., 2015). Several species of deep-sea Solenogastres are brooding (Heath, 1911; Todt and Kocot, 2014), which disqualifies them for source-sink dynamics. Others have lecitotrophic larvae which remain in the water column only for a minimum of eight to ten days (Okusu, 2002; Todt and Wanninger, 2010), showing considerably less dispersal potential than other molluscs, e.g., gastropods with planktotrophic larvae (e.g., Yahagi et al., 2017). However, our data provides one striking distribution example supporting source-sink dynamics: the above-mentioned *Kruppomenia genslerae* (Fig. 7A) formed one wide-spread population in the abyssal Kuril Basin of the Sea of Okhotsk (Table 2) and only a single individual was found in the lower abyssal zone of the Northwest Pacific plain – interestingly sharing the ancestral haplotype with one of its conspecifics from the Sea of Okhotsk (Fig. 7A). *K. genslerae* presumably develops via lecitotrophic larvae (Ostermair et al., 2018), which would suffice to disperse between the Sea of Okhotsk and the abyssal plain across the Kuril-Kamchatka Trench, e.g. via currents in both directions through the bathyal Bussol and Krusenstern Straits. In this case, larvae colonizing the abyssal plain are "sourced" from populations in the abyssal basin, not the slope.

Our study, however, also provides a potential example of a "reversed source-sink" scenario: Acanthomeniidae sp.6 exhibits an astonishing depth range of more than 6,000 m, with its ancestral haplotype sampled from the bottom of the Kuril-Kamchatka Trench in more than 9,500 m depth (six collected specimens), and only a single individual found in shallower depths during the investigation of the abyssal Kuril Basin (Fig. 7B). Strong upwelling zones in the trench which might propel individuals of hadal "source" species into a shallower abyssal "sink" seem unlikely, and the presence of the ancestral Acanthomeniidae sp.6 at the bottom of the trench probably results from undersampling.

In general, the solenogaster fauna of the investigated abyssal plain and hadal zone (i.e., slopes and bottom of the trench) is characterized by extreme rarity of the collected lineages. Overall the comparably high rate of abyssal and hadal solenogaster singletons (67.9% of abyssal species and 61% of hadal species investigated herein) potentially indicates that they might not represent individuals of self-sustaining abyssal and hadal populations, which were accidentally not sampled. However, patchiness is common among deep-sea molluscs across all depth ranges (Schwabe et al., 2007; Jörger et al., 2014) and deep-sea fauna in general (McClain et al., 2011; Danovaro et al., 2013), and is often linked to heterogeneous habitats and food-source availability (Rex and Etter, 2010; McClain et al., 2011); the occurrence of

individually sampled species (i.e., singletons) thus does not necessarily imply rarity of the respective species and populations. The lack of faunal overlap and encounter of species uniquely collected from abyssal and hadal depth potentially indicates rather self-sustaining and endemic abyssal and hadal species. The potential existence of a unique solenogaster trench fauna is further supported by specializations in their prey preferences, as revealed by molecular analyses of gut contents (own unpublished data), suggesting the hadal species are well adapted to these depths. However, the lack of data from the shelf region might obscure a putative faunal overlap with the deeper zones and further sampling is needed to evaluate whether these singletons are potential offspring of bathyal fauna (and hereby corroborating possible slope source – abyssal sink mechanics at work) or actually true endemic abyssal and hadal lineages. Especially data on non-isolated regions like the Japanese continental shelf or east of the Kuril Islands chain would be of interest, as populations of these regions might potentially contribute to sustaining abyssal populations in a source-sink scenario. The unique abyssal and hadal fauna in our study provides some evidence that the abyss harbors self-sustaining populations, which present the source for other abyssal regions, suggesting that local high-productivity zones in the abyss significantly contribute to the diversity of Solenogastres. This can also explain high diversity of Solenogastres found in the northern Angola Basin (Gil-Mansilla, 2008), which is subject to high nutrient input from the African continental slope and the Congo River (Kröncke and Türkay, 2003; Brandt et al., 2005). Similar diversity trends have been observed in well-studied Antarctic abyssal gastropods, where species richness is likely influenced by habitat heterogeneity and elevated seasonal primary production resulting in high nutrient availability and diversity not entirely depends on slope-abyss source-sink dynamics (Schrödl et al., 2011). In general, modelling of larval source-sink dynamics revealed that the diversity of abyssal species is unlikely only a subset of bathyal populations but that adaptive evolution in high-productivity sink habitats function as source to other low-productivity areas ("oligotrophic sink hypothesis") (Hardy et al. 2015).

4.5. Conclusion

Our study discovers a hitherto unknown and still largely undescribed species diversity of Solenogastres in the Northwest Pacific. Molecular barcoding is a quick and efficient way to assess species diversity in these molluscs. Our molecular phylogenetic analyses reveal the need for revision of the current classificatory system (rendering the order "Cavibelonia" as well as the two families "Pruvotinidae" and "Dondersiidae" polyphyletic), which currently hampers reliable placement within the Linnaean System. Extrapolating from discoveries of the present study and additional still unpublished data from extensive sampling in the region (YK and HS, own unpublished data), the currently known species numbers and records present only a fraction of the actual Northwest Pacific solenogaster fauna. In summary, Solenogastres inhabit the marine environment across all depth ranges and habitats, as part of the shallow-water meiofauna of the intertidal to hadal trench communities, with the most likely peak in species diversity along the bathyal slope. Comparisons between abyssal and hadal depths zone and the different investigated regions of the Northwest Pacific suggest the presence of endemic (self-sustaining) abyssal and maybe even hadal solenogaster species.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pocean.2019.102187.

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CHAPTER 7

SOLENOGASTRES

DIVERSITY AND DISTRIBUTION OF SOLNOGASTRES (MOLLUSCA) ALONG THE NW PACIFIC

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1. Introduction

Solenogastres (= Neomeniomorpha) are exclusively marine, vermiform molluscs. Together with Caudofoveata (= Chaetodermomorpha), they form the clade Aplacophora, a name referring to their body, which lacks a shell. Solenogastres is a comparatively species poor and understudied class of Mollusca with currently 293 described species organized in 24 families and four orders. Most Solenogastres are minute and reach only a few millimeters in body length and are thus usually collected with sampling gear designed for benthic meiofauna (only few giant species reach exceptional body lengths of up to 30 cm, and can be retrieved through macrobenthic sampling).

In Solenogastres, the molluscan foot is reduced to a narrow ciliary gliding sole, usually visible as a fine median line running along the ventral side of the animal and they lack a head shield (both characters help to distinguish them from equally worm-shaped Caudofoveates). Aragonitic sclerites protrude from the chitinous cuticle surrounding the entire body. These sclerites (comprising the so-called scleritome) are highly diverse, ranging from solid or hollow needles to solid scale-like elements. Depending on the composition of the scleritome, Solenogastres often appear smooth and shiny, shaggy, or very spiny. Together with the organization and histology of the digestive and reproductive system, the scleritome serves as one of the main taxonomic characters required to differentiate and identify solenogaster species. Most scientific work conducted on this group focuses on traditional taxonomy, but recent phylogenomic studies have begun investigating internal evolutionary relationships and rendered several parts of the current classificatory system (i.e. the order Cavibelonia Salvini-Plawen, 1978) paraphyletic (Kocot et al. 2019). Solenogastres systematics will thus likely receive major revisions in the near future.

1.1. Biology and Ecology

Solenogastres are commonly found among benthic fauna, even though they are seldom encountered in high individual numbers. They prey on marine invertebrates, mainly cnidarians (preferably hydrozoans) and polychaetes.

Little is known about the biology and ecology of Solenogastres, and observations are restricted to a few well-studied taxa. They are hermaphrodites and after copulation most species are assumed to deposit small batches of fertilized eggs from which lecitotrophic swimming larvae hatch (Todt and Wanninger 2013). A few species of Solenogastres brood and retain the encapsulated larvae within their pallial cavity until juveniles emerge, altogether suggesting limited dispersal abilities (Todt and Kocot 2014).

1.2. Habitat

Solenogastres inhabit a wide range of sediments from coarse shell gravel and volcanic sands to fine, silty sediments. Several species have been found living epizoically on cnidarians (Figure 1) or in association with sponges (Kocot et al. 2019).

While a few species can be collected in knee-deep waters of the shallow intertidal zone, the lower continental shelf is currently assumed to harbor the highest species diversity (Todt 2013).

The solenogaster fauna of the world's vast abyssal plains and the hadal zone of oceanic trenches still remain largely unexplored.

Figure 1. Dondersiidae sp. SB-2 (Pholidoskepia), from the Kuril Basin of the Sea of Okhotsk. Found wrapped around a cnidarian. Head to the right. Scale bar: 1 mm.

1.3. Geographical Distribution

Solenogastres are known from all oceans, sampled from the Arctic to the Antarctic. Most taxonomic work has focused on historical samples from Antarctica (see monographs by Salvini-Plawen 1978a; 1978b) and the North Atlantic along the western European coast, thus the majority of species has been described from these regions. To date, out of 293 recognized solenogaster species on a global-scale, 56 species are known from the entire Pacific Ocean and only 17 have been recorded from the NW Pacific. They occur mostly in the shallow bathyal around the Japanese coast (11 species, 27-600 m), the Sea of Japan (one species, 200-600 m), the Sea of Okhotsk (2 species, 200-400 m), and the Bering Sea (1 species, 880 m) (García-Álvarez and Salvini-Plawen 2007; Sirenko 2013).

2. Objectives

The present chapter aims to compile the current knowledge on the diversity and distribution of Solenogastres in the investigated area of the NW Pacific, recorded from the deep sea below 2,000 m. Based on this data, we explore putative patterns of species richness and distribution comparing the open NW Pacific and the semi-isolated adjacent Sea of Okhotsk.

3. Material and Methods

3.1. Coverage Area:

The KuramBio I and II (Kuril-Kamchatka Biodiversity Studies I and II, see Brandt et al. 2015, 2020) and SokhoBio (Sea of Okhotsk Biodiversity Studies, Brandt et al. 2018) Expeditions between 2012 and 2016 explored the benthic deep-sea fauna of the open NW Pacific and its adjacent regions. Solenogastres were collected during these expeditions using Agassiz trawls and epibenthic sledges. Overall, the investigated area ranges from 120-180°E and 40-60°N. It partially covers the open NW Pacific abyssal plain and the semi-isolated Kuril Basin of the Sea of Okhotsk, which is connected to the open NW Pacific via two deep straits. East of the Kuril Islands, the Kuril-Kamchatka Trench extends southwards reaching hadal depths of almost 9,600 m.

3.2. Depth Gradient

We have compiled data on Solenogastres occurring in the coverage area from bathyal (2,000-3,000 m), upper (3,000-4,000 m) and lower abyssal (4,000-6,000 m), and hadal depths (6,000 m and below).

3.3. Latitudinal Gradient

This chapter covers the deep-sea Solenogastres found in the temperate open NW Pacific and the Sea of Okhotsk with a latitudinal gradient of 40-60°N. Sampling sites correspond to the stations investigated during the recent KuramBio I (2012) and II (2016) (Brandt et al. 2015, 2020) and the SokhoBio (2015) (Brandt et al. 2018) expeditions.

4. Results

4.1. Richness Patterns

Prior to this recent expedition series to the deep NW Pacific no Solenogastres were described from the investigated area of the NW Pacific below 2,000 m. However, these expeditions revealed a unique solenogaster diversity: 66 candidate species were collected between the Kuril Basin of the Sea of Okhotsk, the open NW Pacific Plain, and the Japanese and Kuril-Kamchatka Trench, spanning a depth range from 3,000 to more than 9,500 m (see Bergmeier et al. 2017, 2020; Ostermair et al. 2018).

Following the currently recognized classificatory system of Solenogastres (García-Álvarez and Salvini-Plawen 2007), these 66 species cover all four traditional solenogaster orders and represent at least 10 families (see the Species Check-List in Chapter 1, Table 1). The two orders Cavibelonia Salvini-Plawen, 1978 and Pholidoskepia Salvini-Plawen, 1978 constitute in mostly equal parts for 98% of all collected Solenogastres in the area, and are both distributed from the upper abyssal down to the hadal zone. These two most common groups can be usually differentiated directly in the field under a stereomicroscope, as Cavibelonia are in general characterized by a spinier and rough appearance due to a scleritome largely composed of needle shaped elements, whereas Pholidoskepia are rather smooth and shiny, predominantly covered in scale-like elements).

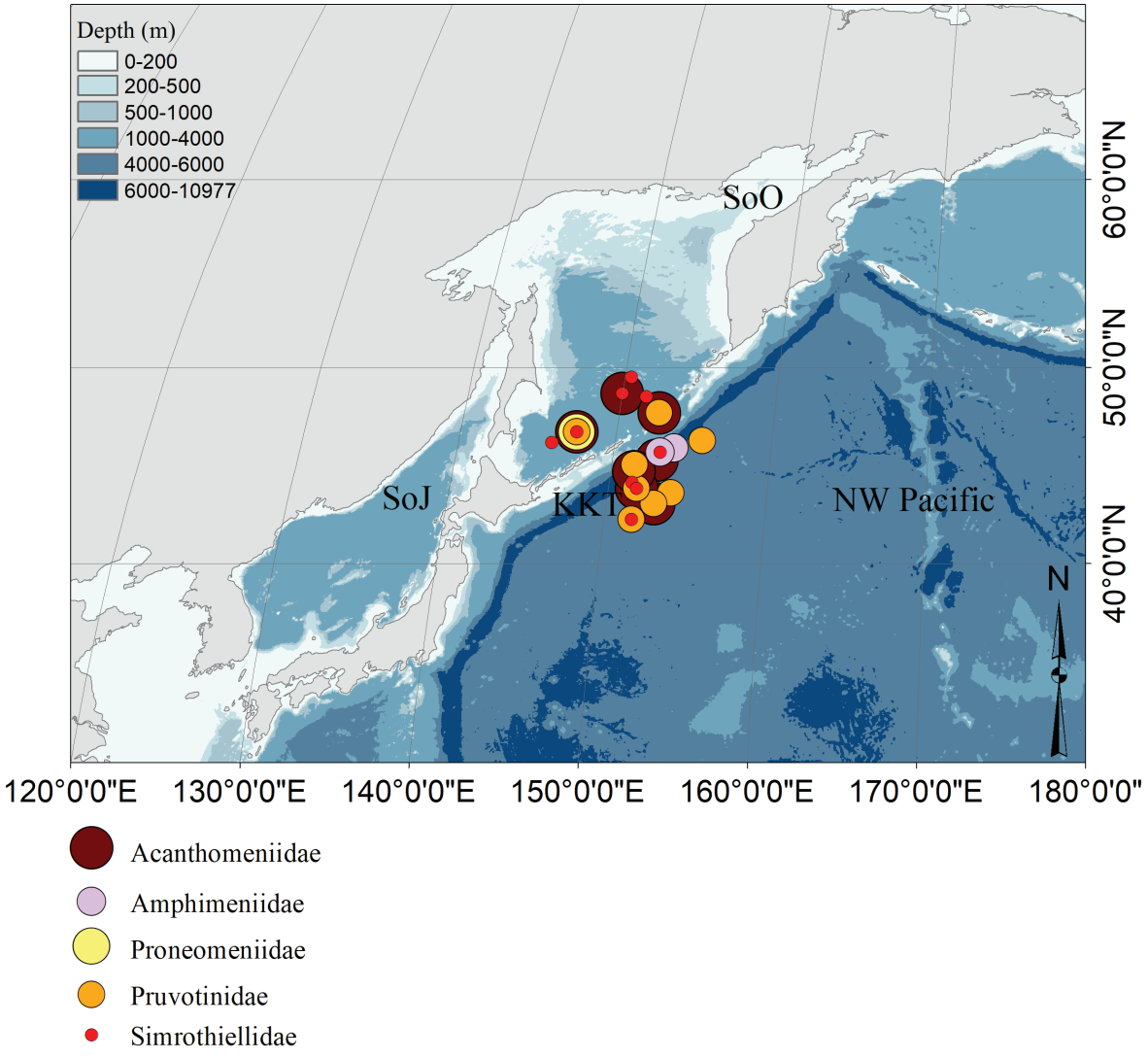
The five cavibelonian families are represented by 44 species, and while species of Acanthomeniidae, Amphimeniidae, Pruvotinidae, and Simrothiellidae all have been reported from the abyssal zone before (in the Atlantic, Indian, South Pacific and Southern Ocean), abyssal Proneomeniidae are currently only known from the NW Pacific (Map 1). Three families of Pholidoskepia (20 species) are present in the investigated regions and are among the first records of this order below 2,500 m (Map 2), apart for a single dondersiid species from the abyssal Atlantic (Cobo et al. 2020).

The remaining orders Neomeniamorpha and Sterrofustia are both rare and only account for one (Neomeniamorpha) and two (Sterrofustia) species, and while neomeniamorph Solenogastres are known from the bathyal NW Pacific, Sterrofustia have only been found once outside of the Southern Ocean before.

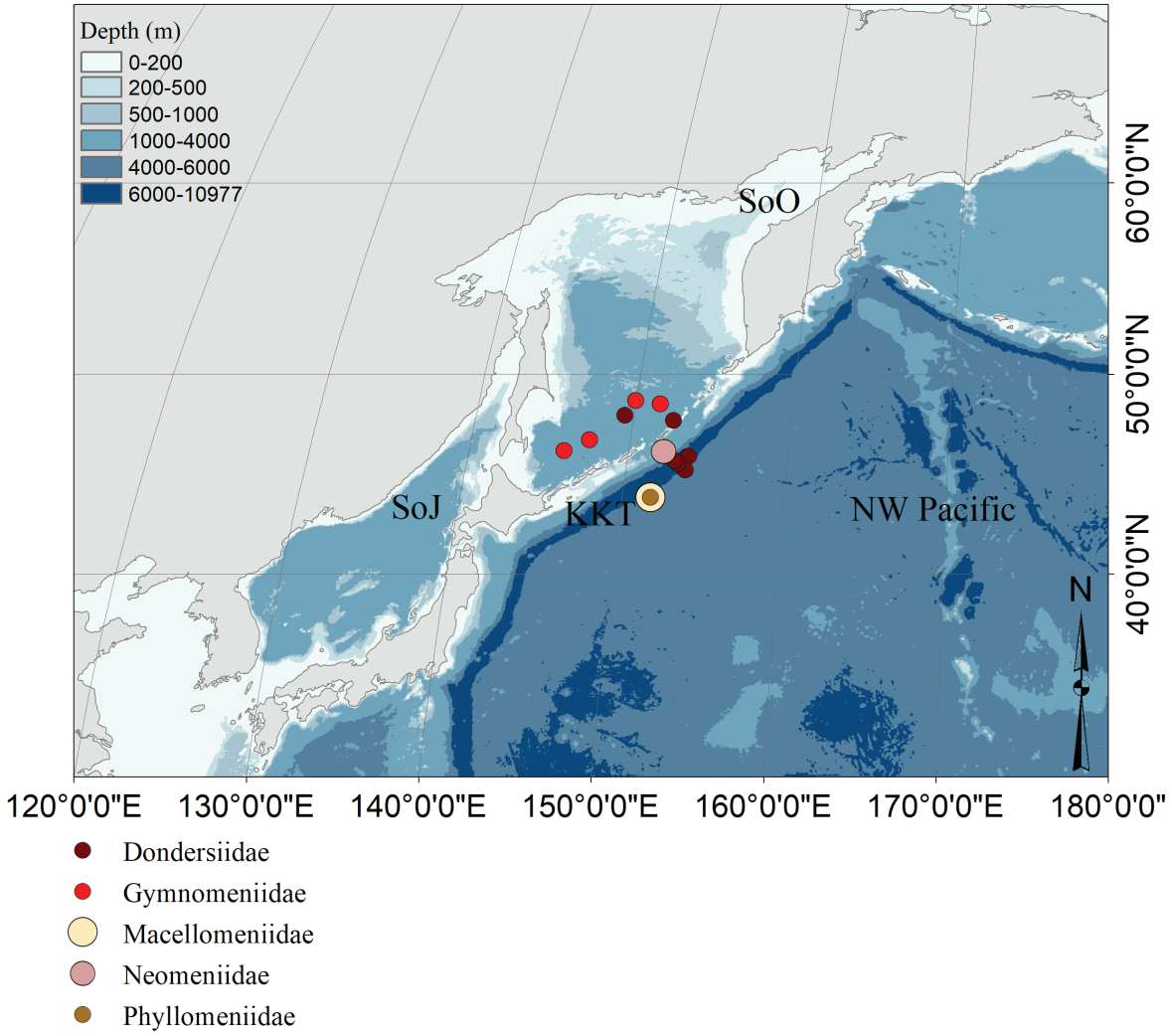
Species diversity varies along a depth gradient: 15 species are present in the upper abyss (3,000-4,000 m), 43 species in the lower abyssal (4,000-6,000 m), and 11 species in the hadal zone (6,000-9,577 m). Most of the upper abyssal species are recorded at around 3,300 m throughout the Kuril Basin in the Sea of Okhotsk (12 species) and the Bussol Strait (3 species) between the Sea of Okhotsk and the open sea (Table 1, Map 3 and 4). Nevertheless, the lower diversity of Solenogastres in the semi-isolated Kuril Basin when compared to the open NW Pacific plain might be a result of

Table 1. Species numbers (on familial level) in the investigated Northwest Pacific regions.

Family	Kuril Basin, Sea of Okhotsk (ca. 3,300 m)	Slopes and bottom of the Kuril-Kamchatka-Trench (ca. 5,200-9,577 m)	Open Northwest Pacific (abyssal plain) (ca. 4,800-5,400 m)
Acanthomeniidae	2	4	8
Amphimeniidae	-	2	-
Proneomeniidae	1	-	2
Pruvotinidae	1	2	6
Simrothiellidae	1	2	12
Dondersiidae	4	4	6
Gymnomeniidae	1	-	3
Macellomeniidae	-	-	1
Neomeniidae	-	-	1
Phyllomeniidae	-	-	1



Map 1. Records of cavibelonian solenogaster families (Acanthomeniidae, Amphimeniidae, Pruvotinidae, and Simrothiellidae) in the Northwest Pacific.



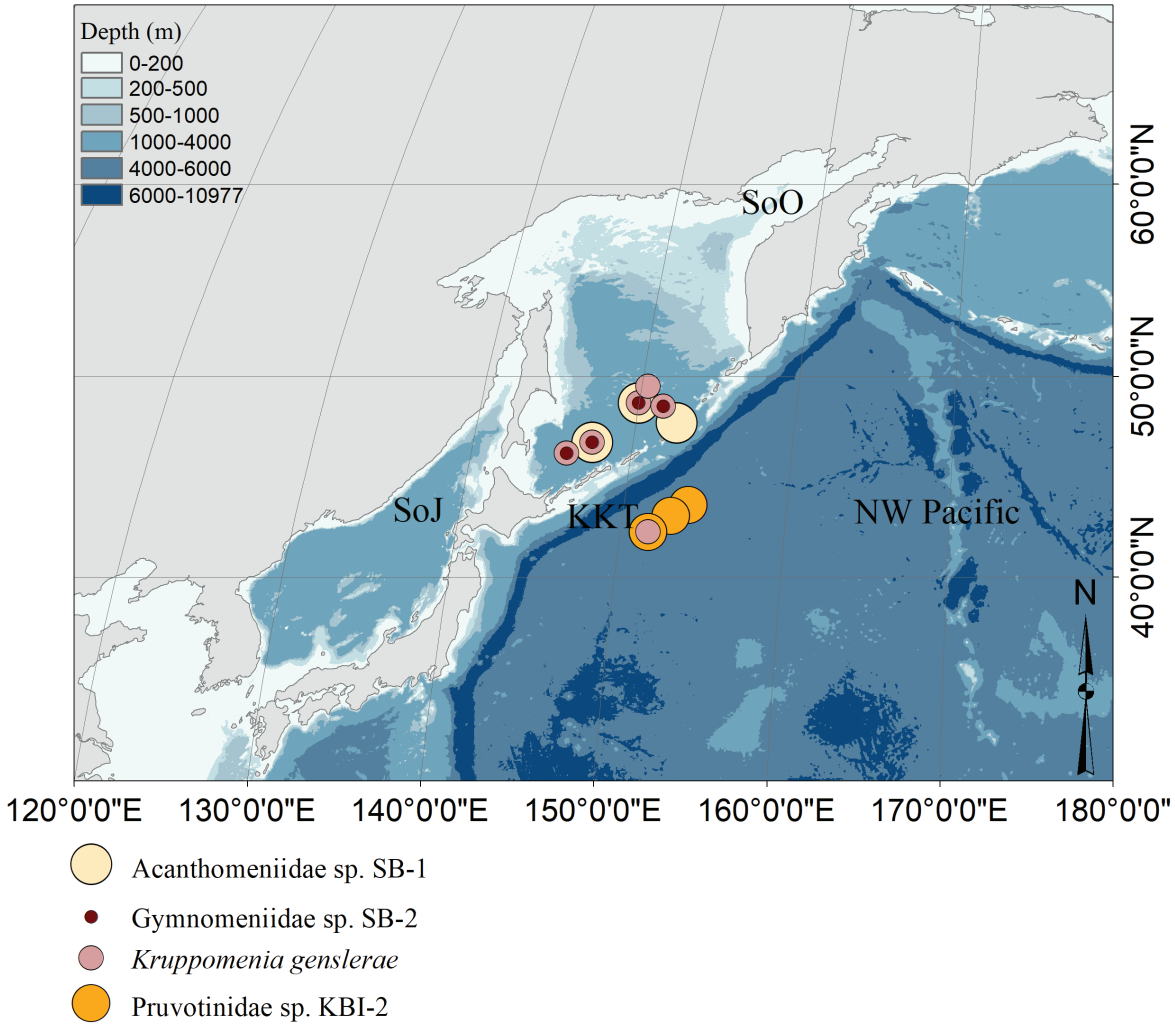
Map 2. Records of pholidoskepian (Dondersiidae, Gymnomeniidae, Macellomeniidae), neomeniomorph (Neomeniidae), and sterrofustian (Phyllomeniidae) solenogaster families.

oxygen-depleted bottom waters, formed during interglacial periods (Liu et al. 2006). 41 species are currently known from the open NW Pacific and its abyssal plain, while the slopes of the Kuril-Kamchatka Trench harbor nine species (5,200-7,200 m). Overall six species were sampled at four localities along the bottom of the trench, for the first time demonstrating the presence of Solenogastres in the hadal zone of oceanic trenches.

4.2. Biogeographic Patterns

Overall, the known solenogaster fauna of the abyssal and hadal zone of the NW Pacific is characterized by a high rate of singletons (i.e. species collected as single individuals only). Currently, within the investigated region, 45 out 66 species are collected only as singletons, and eight additional species were found only at a single location. This suggests that they might generally occur at low densities and/or with patchy distribution and consequently render potential hypotheses on their biogeographic and bathymetric distributions difficult based on the current state of knowledge.

Out of 10 families, four (Acanthomeniidae, Pruvotinidae, Simrothiellidae, Dondersiidae) are



Map 3. Distribution of dondersiid species recorded at three or more localities in the Sea of Okhotsk.

widely distributed across the Sea of Okhotsk, the Kuril-Kamchatka Trench and the open NW Pacific. Two families (Proneomeniidae, Gymnomeniidae) are present on both sides of the Kuril-Kamchatka Trench (albeit not recorded from the slopes or bottom), and four have only been recorded with restricted distribution, e.g. the large-sized Amphimeniidae (Figure 2) are currently only known from the lower slope and bottom of the Kuril-Kamchatka Trench (Table 1, Map 1 and 2).

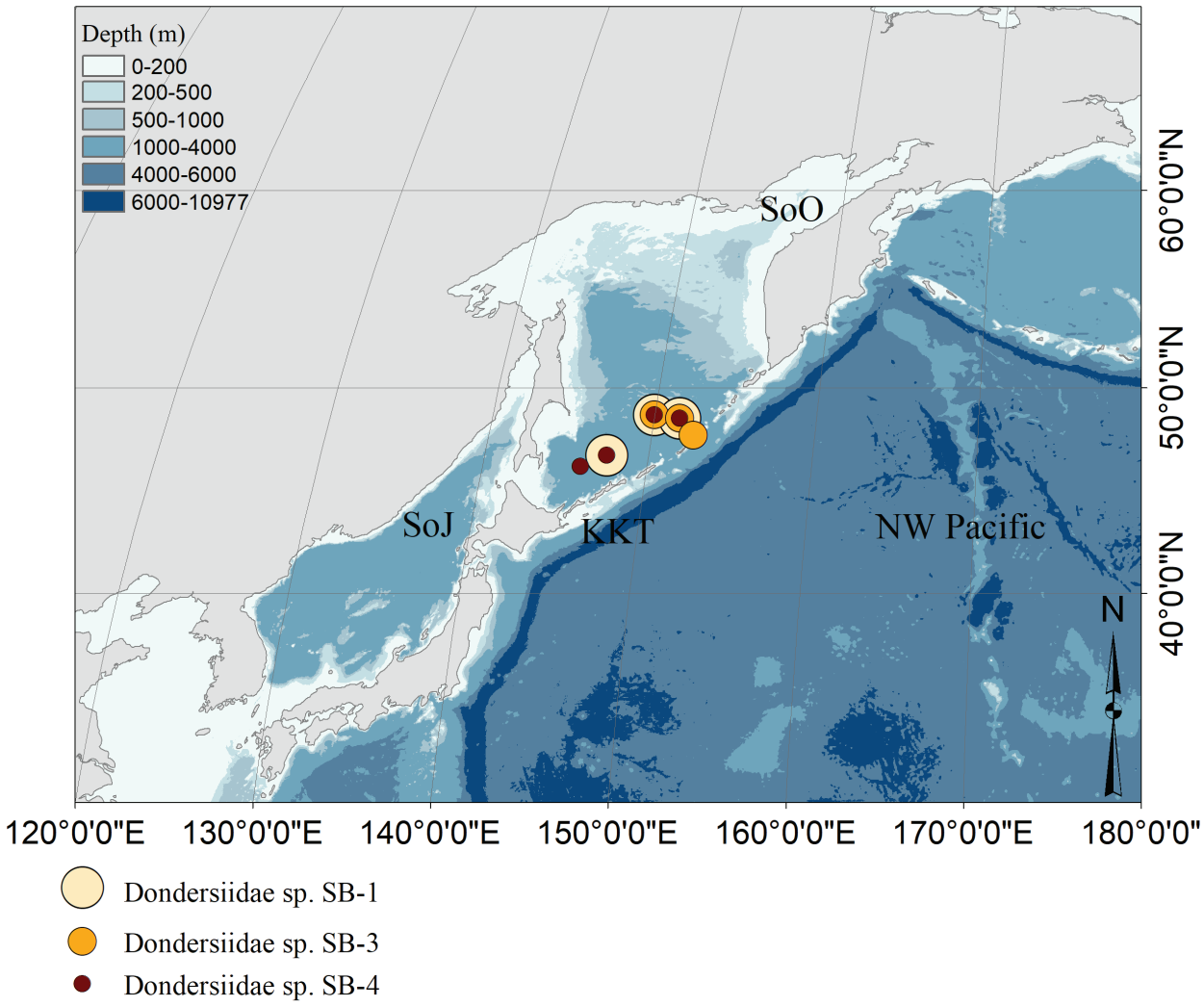
Figure 2. A large-sized Amphimeniidae sp.2 (Cavibelonia), found between 7,100 and 8,200 m at the bottom of the Kuril-Kamchatka Trench. Head to the left. Scale bar: 1 cm.

In the Sea of Okhotsk, 55% of the species are comparatively common, i.e. present at three or more localities (Map 3 and 4). Dondersiidae sp. SB-4 (Figure 3) is one of three common dondersiid

species in the Kuril Basin (Map 3), and accounts for 40% of the local solenogaster fauna. The families Acanthomeniidae, Gymnomeniidae, and Simrothiellidae are each represented by single species, albeit collected at several locations within the Kuril Basin (Map 4). 98% of species from the NW Pacific abyssal plain are highly restricted in their occurrence, and only a single species (Pruvotinidae sp.KBI-2) was found at three different localities, all in close vicinity (Map 4).

Overall there is only little faunal overlap on species level between the Sea of Okhotsk and the open NW Pacific: *Kruppomenia genslerae* Ostermair, Brandt, Haszprunar, Jörger & Bergmeier, 2018 (Figure 4) is so far the only solenogaster species reported from both sides of the Kuril-Kamchatka Trench (Map 4), as confirmed via molecular barcoding, suggesting a connection between the abyssal NW Pacific Plain and the semi-isolated Kuril Basin of the Sea of Okhotsk.

Most deep-sea Solenogastres known from the NW Pacific all show restricted depth ranges of max. 1,800 m. However, Acanthomeniidae sp. 6 exhibits an astonishing vertical distribution of more than 6,000 m, as conspecifity between five individuals recorded from the bottom of the



Map 4. Distribution of pholidoskepian (Gymnomeniidae sp.SB-2) and cavibelonian solenogaster species recorded at three or more localities in the Northwest Pacific.

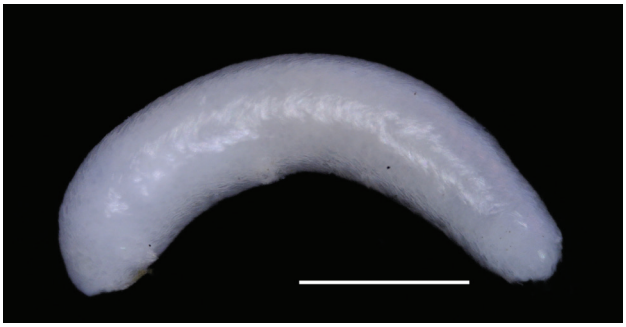


Figure 3. Dondersiidae sp.SB-4 (Pholidoskepia), a common species found in the Sea of Okhotsk. Note the shiny, smooth appearance due to the flatly arranged scales. Head to the left. Scale bar: 1 mm.

Figure 4. Holotype of *Kruppomenia genslerae* Ostermair, Brandt, Haszprunar, Jörger & Bergmeier, 2018 (Cavibelonia). Note the spiny outer appearance (needle-like, püreojectinv spicules). Head to the left. Scale bar: 1 mm.

Kuril-Kamchatka Trench and a single individual from the Sea of Okhotsk was confirmed via molecular barcoding (Bergmeier et al., in press).

5. Discussion

Within the last couple of years, the number of deep-sea species of Solenogastres (below 2,000 m) recorded from the NW Pacific has risen from zero to 66 candidate species, with the majority new to science and still pending formal descriptions.

It is generally assumed that solenogaster diversity is the highest on the continental shelf (Todt 2013) and decreases with increasing depth, which is a general trend in benthic deep-sea diversity (Rex et al. 1990). The comparably high number of abyssal species in the NW Pacific is most likely result from sampling bias, as the solenogaster fauna of the adjacent bathyal zone currently remains largely unexplored.

The currently known species recorded in the NW Pacific and summarized in this chapter present only a fraction of the actual diversity of deep-sea Solenogastres in the region, and we expect them to continuously rise with increasing sampling efforts.

Acknowledgements

We wish to express our gratitude to the editors, Hanieh Saeedi and Angelika Brandt, for the opportunity to join this compilation on benthic NW Pacific deep-sea fauna. Thanks to Hanieh Saeedi for creating the maps of the present chapter and the proofread of the manuscript, Peter C. Kohnert (ZSM Munich) for providing pictures of the amphimeniid Solenogastres, and Bastian Brenzinger (ZSM Munich) for suggestions on the manuscript. We also wish to thank all crew members and scientists participating in the SokhoBio, KuramBio I and II expeditions for their support during the cruises and their sampling efforts. This chapter was part of the “Biogeography of the NW Pacific deep-sea fauna and their possible future invasions into the Arctic Ocean project (Beneficial project)”. The Beneficial project (grant number 03F0780A) was funded by Federal Ministry for Education and Research (BMBF – Bundesministerium für Bildung und Forschung) in Germany.

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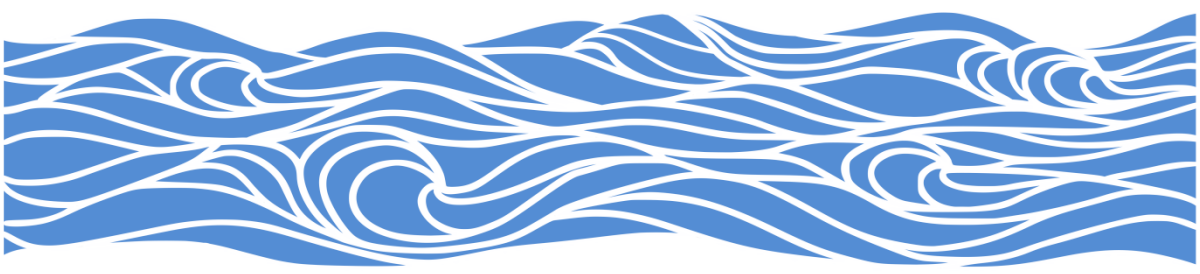
Part III. Insights into deep-sea food webs



Chapter 10. Bergmeier FS, Ostermair L & Jörger KM. **Specialized predation by deep-sea Solenogastres revealed by gut-content sequencing.** *Current Biology* (accepted).

This article will be publicly available online at: https://www.cell.com/current-biology/home 12 months after its initial publication.

Specialized predation by deep-sea Solenogastres revealed by sequencing of gut contents

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eTOC Blurb: Trophic ecology in the deep sea is still poorly understood. Bergmeier et al. indirectly sequenced gut contents of abyssal and hadal Solenogastres. These enigmatic molluscs are revealed as highly specialized predators on diverse deep-sea invertebrates and scavengers of jelly-falls with food preferences linked to adaptations in their digestive tract.

"Who eats whom?" is a fundamental question useful for furthering knowledge on the ecological roles and interactions of deep-sea organisms. However, the tools needed to analyse trophic relationships remain limited, especially with regard to studying meiofauna and small macrofauna in abyssal and hadal depths. We present results from indirect molecular analyses of the gut contents of abyssal and hadal Solenogastres (Mollusca, Aplacophora) of the Northwest Pacific. Our data revealed a high food specialization and a surprising diversity of food sources among these inconspicuous worm-shaped predators. We hypothesize that Hydrozoa forms the ancestral food source of Solenogastres, and that specialization on non-cnidarian prey (such as annelids, nemerteans, and bivalves) evolved independently along with modifications in the digestive tract. Despite being intuitively advantageous in the nutrient-limited deep sea, we found only one widespread generalist feeder (potentially associated with scavenging).

Trophic ecology in the deep sea is still poorly understood, with research focusing on food web analyses via stable isotopes[1] or on megafauna, as the latter allows for ROV-observation or direct analyses of gut contents[2], while the most abundant and diverse meiofauna remains inaccessible. Little is known about Solenogastres, mostly meiofaunal (1–4 mm) predators in the deep sea. Anatomical diversity relates mostly to the digestive system, with different radula morphologies and complex glands associated with the foregut (see Supplemental Information). This implies diversification was largely triggered by trophic niche specialization. Knowledge on their food sources is yet limited and mainly derived from (1) direct observations of large-bodied species living and actively feeding on anthozoans and (2) indigestible remains in their gut suggesting that cnidarians are their prevailing food source[3].

From 2012 to 2016, three deep-sea expeditions explored the benthic fauna of the Kuril-Kamchatka Trench, Sea of Okhotsk, and the abyssal plain of the Northwest Pacific from 3,300 to 9,750 m. Integrating molecular barcoding with morphological data from almost 200 individuals revealed a diverse solenogaster fauna with 60 candidate species, representing the currently known deep-sea diversity of these molluscs in the region[4]. Amplification of solenogaster nuclear 18S ribosomal RNA is hampered by its secondary structure[5] which generally leads to amplification of exogenous DNA[5,6]. Taking advantage of similar problems in 28S rRNA, we used universal primers to indirectly sequence gut contents from genomic DNA extracts of these Solenogastres. BLAST searches of gut contents revealed extraordinary diversity, with over 26 food sources among four different phyla. While one third of studied

species feed exclusively on cnidarians, others were for the first time reported specialized predators on various benthic invertebrates. Surprisingly, generalist feeding was only documented in one species.

Plotting available food sources on the phylogeny of Northwest Pacific Solenogastres revealed that hydrozoans represent the most common and widespread food source, found in ten families (Figure 1A, Supplementary Table 1). We hypothesize that preying on hydrozoan colonies is plesiomorphic in Solenogastres and, accordingly, the phylogenetically widespread tweezer-like distichous radula and foregut glands, formed as an invagination of the foregut epithelium, secreting into simple ducts (Figure 1B). Contrarily, the digestive system of Amphimeniidae – sister group to all remaining Solenogastres – with monostichous or no radula and a system of ramified, compound foregut glands[7] likely presents a unique adaptation in this family related to specialisation on alcyonaceans (see Supplemental Information). Apparently, independent origins of secondary 'gigantism' are associated with anthozoan food sources (e.g., Proneomeniidae feeding on Alcyonacea (Figure 1A) or Neomeniidae on Actiniaria[8]).
Surprisingly, most deep-sea Solenogastres prey on Siphonophorans: in parts on pelagic medusae, indicating scavenging on jelly fall, and in parts on organisms related to benthic, Rhodallidae – (see Supplement) providing indirect data on their occurrence at the respective sampling sites. We discovered several clades of Solenogastres specialized on non-cnidarian prey. Simrothiellidae prey exclusively on sessile, terebellid polychaetes which were uncommon at respective stations compared to other polychaetes[9]. We interpret their biserial radula (Figure 1D) to be a modified multi-hooked tool for grasping polychaete tentacles and ripping out soft tissue. Our approach does not artificially restrict results to food sources, which are histologically traceable in the digestive tract, surprisingly revealing bivalves and nemerteans as solenogaster prey. Especially monostiliferan hoplonemerteans are expected to be strongly self-protective, thus we consider egg or juvenile predation plausible scenarios. Solenogastres feeding on equally sized, brooding bivalves are likely scavengers. We hypothesize that the strong muscular pharynx is used for sucking, with or without the aid of a piercing monoserial radula (Figure 1C).

Most deep-sea Solenogastres are specialized feeders (a single food source was found in 90% of conspecific specimens from different stations, see Figure 1A). Only one widespread species is a putative generalist feeder, feeding on siphonophorans, trachymedusae, and annelids (Figure 1, Table S1). This species (Dondersiidae sp.SB-4, provisionally placed within a family requiring taxonomic revision[4]) appears at first to have a simple digestive system (distichous radula, no distinct foregut glandular organs, numerous unicellular pharyngeal glands), showing less complexity than specialized feeders preying on similar organisms. Despite this basal setting, our phylogenetic hypothesis suggests that generalist feeding is derived from specialized cnidarian predation. Thus, the seemingly simple digestive system is secondary, and as in the case of the closely related Gymnomeniidae[4, 10] might reveal cellular diversity on an ultrastructural level. A generalist feeding strategy potentially linked with scavenging is advantageous in the nutrient-limited deep sea and likely contributes to the success of the species, reflected in its relative commonness and broader distribution range.

Our study shows that the usefulness of setbacks in the lab, such as contamination in molecular analyses, is often underestimated when it comes to scientific discoveries. This unconventional approach to gut content analysis is a powerful method, both for advancing our knowledge of

food sources in meiofaunal predators and for contributing to the diversity and distribution records of food organisms (such as delicate Siphonophorae, often destroyed during regular sampling). The high food specificity in deep-sea Solenogastres revealed in the present study correlates with narrow distribution ranges, highlighting trophic dependencies and the vulnerable ecology in the deep sea. This in turn calls for efficient, close-knit conservation strategies.

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Author Contributions
Conceptualization, F.S.B. and K.M.J; Investigation, F.S.B and L.O.; Writing - Original Draft, F.S.B. and K.M.J.; Writing – Review & Editing, F.S.B., K.M.J., and L.O.; Funding Acquisition, K.M.J. and L.O.; Supervision, K.M.J.

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Declaration of Interests

The authors declare no competing interests.

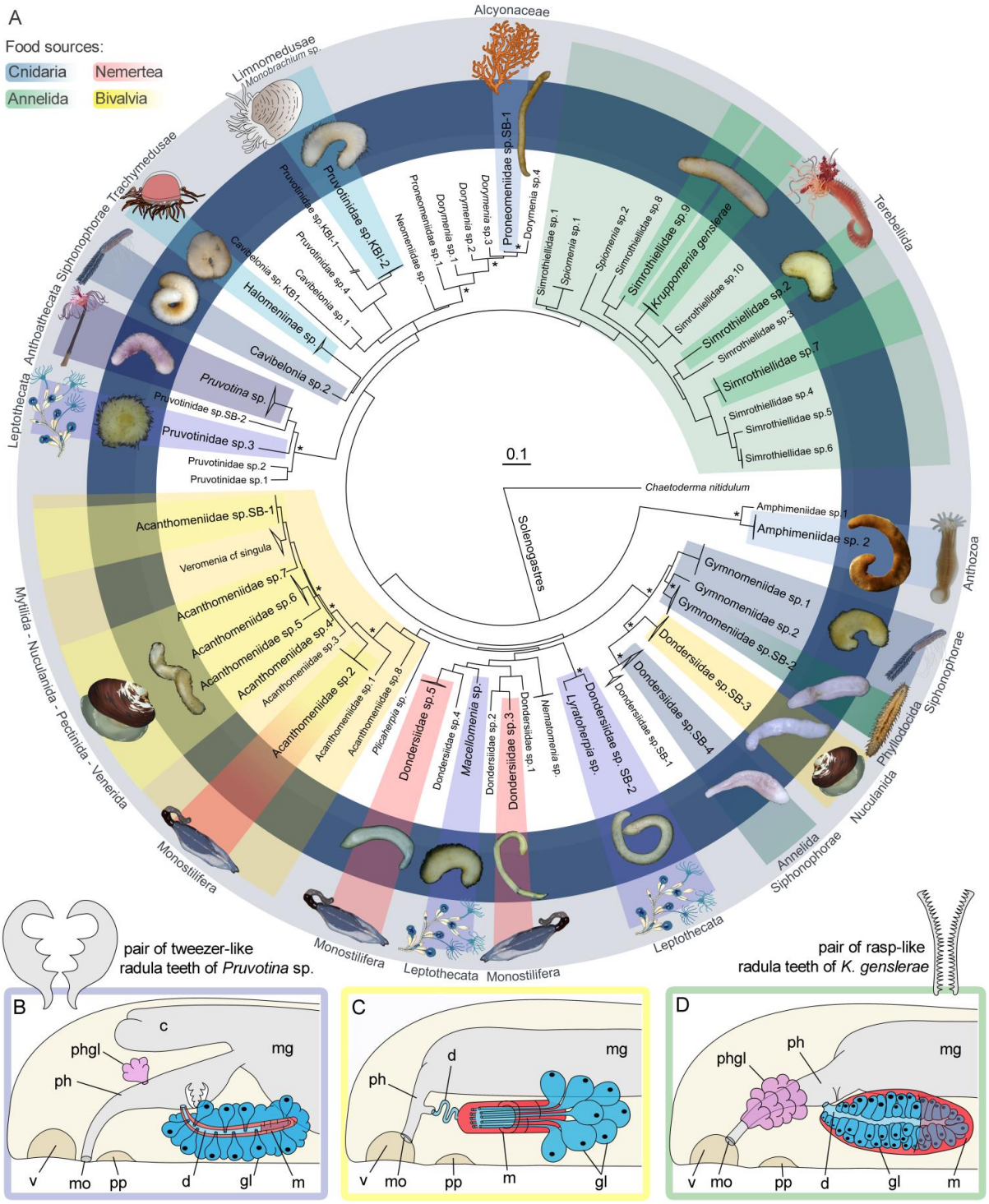

Figure 1. Food source specializations in deep-sea Solenogastres from the Northwest Pacific, as revealed by indirect sequencing of gut contents. A. Maximum likelihood tree of 193 Solenogastres based on mitochondrial 16S rRNA and COI markers. Asterisks indicate bootstrap values of 90 or above. Taxa with successful amplification of putative gut contents are highlighted in large font. The dark inner circle depicts Solenogastres (body sizes mostly between 2–8 mm, largest specimens are Amphimeniidae sp.2 (up to 6 cm) and Proneomeniidae sp.SB-1 (up to 3 cm)). Food organisms of the respective solenogaster species are plotted on the outer circle. Each clade's prey preference is indicated by coloured sections. Shades of blue: Cnidaria; green: Annelida; red: Nemertea; yellow: Bivalvia. B–D: Schematic

drawings of the head region of three differently specialized solenogaster species, showing their glandular digestive organs. The anterior digestive system of: B. cnidarivorous *Pruvotina* with a distichous radula, pharyngeal glands, and "exoepithelial" (multicellular, invagination of foregut epithelium) glandular organs formed by a muscular duct surrounded by multiple gland cells (*Pararrhopalia*-Type). C. A bivalve-feeding acanthomeniid solenogaster (Acanthomeniidae sp.SB-1) lacking radula but with a muscular sucking pharynx and large, multicellular foregut glands (*Acanthomenia*-Type). D. Simrothiellid *Kruppomenia genslerae* which preys on terebellid annelids, with denticulated radula teeth, numerous unicellular pharyngeal glands and a complex of glandular cells surrounded by a muscular sheath (*Simrothiella*-Type).
c = midgut caecum, d = duct of glandular organ, gl = glandular cells, m = muscle layer, mg = midgut, mo = mouth opening, ph = pharynx, phgl = pharyngeal gland, pp = pedal pit, v = vestibulum.

Supplemental information: **Specialized predation by deep-sea Solenogastres revealed by sequencing of gut contents**

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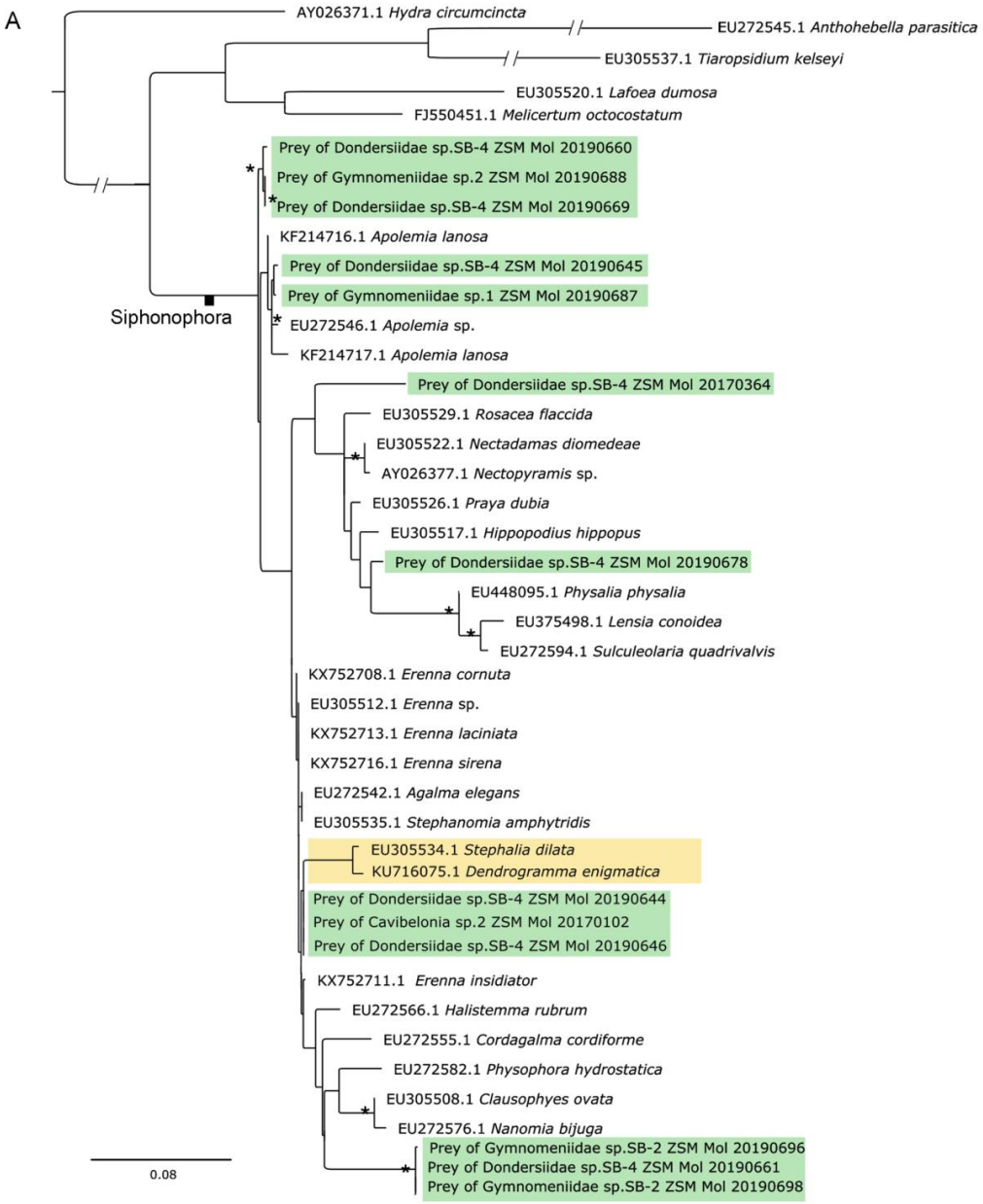

Figure S1. Analyses of 28S rRNA sequences amplified from potential gut contents. A, Maximum likelihood tree of Siphonophorae based on partial 28S rRNA sequences retrieved from GenBank. Asterisks indicate bootstrap values above 85. Solenogaster prey sequences highlighted in green. Benthic Rhodaliidae highlighted in yellow. **B,** Positions and secondary structures of bubble and helical regions of 28S rRNA sequenced from Dondersiidae sp.SB-4 (ZSM Mol 20190670) corresponding to putative foreign RNA amplified during PCR (highlighted blue). Helix 1 (120 bp length): highest Blast match with *Rhyssoplax olivaceus* (Spengler, 1797) (QC: 57 %, PW-ID: 78.38 %); Bubble (176 bp length): highest Blast match with Onychophora sp. (QC: 90 %, PW-ID: 87.04 %); Helix 2: Blast matches with green algae (QC: 6 – 25 %, PW-ID: 72 – 93.75 %. Green: 90 bp of true solenogaster 28S rRNA (Blast match with *Neomenia carinata* Tullberg, 1875 MG878441.1 (QC: 100 %, PW-ID: 90%). Yellow: 425 bp of its annelid prey (Blast match with *Glyphidrilus* sp. (QC: 96%, PW-ID: 74.47%). Red: 105 bp of potential laboratory cross-contamination (blast match with *Oreochromis* (QC: 48%, PW-ID: 86.14%).

Supplemental Experimental Procedures

Solenogaster diversity in the Northwest Pacific

The present study analysed gut contents of nearly 200 solenogasters collected during several deep-sea cruises to the Northwest Pacific (KuramBio I, II, and SokhoBio Expedition) between 2012 and 2016. In a previous study[S1], 193 specimens of Solenogastres were collected and assigned via a combined molecular (phylogenetic analyses based on barcoding markers COI and 16S rRNA) and morphological (external scleritome data) approach to 60 candidate species. All successfully amplified COI and 16S rRNA sequences were separately aligned using MUSCLE algorithm[S2], concatenated, and maximum likelihood analyses performed using RAxML 8.2.10[S3] under the GTR+I+G nucleotide substitution model. The tree was rooted using the caudofoveate *Chaetoderma nitidulum* Lovén, 1844 (GenBank Accession MG264122.1 and AY340451) as outgroup. The resulting phylogenetic hypothesis is concordant with latest phylogenomic analyses of Solenogastres[S4] and serves as guide tree to plot the encountered food sources.

Sequencing and analyses of gut contents

DNA was extracted from the mid region of animals or entire specimens due to their meiofaunal body size, after manual cleaning and rinsing of the specimens under the dissecting microscope. This approach includes the risk of contamination of the samples through food organisms, as the digestive tract occupies most part of the body cavity. Here, we took advantage of a contamination problem in the amplification of nuclear markers in Solenogastres[S5,S6] and intentionally analysed the DNA extracts of all 193 specimens listed in Bergmeier *et al.*[S1] with the universal 28S rRNA primers 28S-C1'[S7] and 28S-D3[S8] with the following protocol using Phire Hot Start II polymerase (Thermo Fisher): 98 °C for 90 s, 31-39x (98 °C for 15 s, 55-63 °C for 5 s, 72 °C for 30 s), 72 °C for 60 s. Successful PCR products were purified using the DNA Clean & Concentrator Kit (Zymo Research). Multiple bands visualized in gel electrophoresis, indicating the presence of multiple amplified fragments, were extracted individually with x-tracta Gel Extractor (Promega) and purified with the QIAquick Gel Extraction Kit (QIAGEN). All samples were sequenced using the 28S-C1 and 28S-D3 PCR primers with Big Dye 3.1. on an ABI 3730 capillary sequencer by the Sequencing Service of the Genetics Department, LMU Munich. The obtained sequences ranged in size between 863 and 1211 bp.

We edited the sequences using Geneious (version 11.0.5, Biomatters Ltd) and conducted a BLASTn search[S9] against the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi, last accessed 10th of March, 2021). Table S1 lists the GenBank numbers of the food source sequences retrieved in the present study, including the voucher numbers of the source specimens (i.e., Solenogastres documented in Bergmeier *et al.*[S1], deposited at the Bavarian State Collection of Zoology Munich, Germany). Additionally, it lists the closest match in GenBank including query coverage (QC) and pairwise identity (PW-ID). 140 28S rRNA sequences from overall 193 solenogaster DNA extracts were successfully amplified and sequenced. Our results restrict the presented sequences to those evaluated as putative food sources (i.e., we excluded sequences likely associated to contamination due to storage conditions or potential laboratory cross-contamination of fungal, plant or terrestrial origin). Further 1) single data with no informative matches in GenBank were excluded (for an exception see below "Testing for chimeric sequences"), and 2) data with poor sequencing quality due to an overlap of signal were excluded, introducing a potential bias towards single food sources in single specimens. After filtering all amplified potential food source sequences, we plotted our final dataset of 61 food sources onto the maximum-likelihood phylogeny of all 193 solenogaster individuals. We cannot exclude that an external contamination of individual Solenogastres herein is misleadingly interpreted as putative food source. However, we consider this unlikely based on identical sequence data from conspecifics collected from different stations and cruises, while sequences of co-occurring Solenogastres collected from the same epibenthic sledge hauls differ considerably.

Ancestral state reconstruction of solenogaster food sources

We conducted an ancestral state reconstruction in Mesquite 3.61 using parsimony and standard categorical characters for the identified food sources (Hydrozoa, Anthozoa, Annelida, Bivalvia and Nemertea) (tree not shown). Our dataset is still patchy and includes nearly 70% missing data, but at present Hydrozoa is the plesiomorphic food source for all main clades of Solenogastres, specialisation on non-cnidarian prey evolved several times independently. The basal sister group relationship of Amphimeniidae, which feed on Anthozoa, to all remaining Solenogastres influences also the hypothesis of the ancestral food source, revealing feeding on Anthozoa might be plesiomorphic for all Solenogastres. However, Amphimeniidae present a highly derived clade based on their complex foregut gland configuration (clusters of glandular cells secreting into a branching system of ducts) and also on their monostichous (= unipartite) or absent radula (but see Kocot *et al.*[S4] for alternative hypotheses) and we consider anthozoan feeding as unique adaptation in this clade.

Anatomy of Solenogaster digestive tracts

We exemplarily studied the anatomy of the digestive systems of three different species (*Kruppomenia genslerae* Ostermair, Brandt, Haszprunar, Jörger & Bergmeier, 2018 ZSM Mol 20170344, *Pruvotina* sp. ZSM Mol 20190594, Acanthomeniidae sp.SB-1 ZSM Mol 20190580) based on semi-thin (1.2 µm thickness) histological serial sections. Different types of foregut glandular organs related to the anterior digestive system can be classified according to their location, histology of the glandular cells, and associated musculature and morphology of ducts[S10, S11]. Despite some general patterns and hypothesis on plesiomorphic conditions and the evolution of radula configurations and glandular settings[S12], there is high anatomical variability also within clades and among species feeding on a common food source such as Hydrozoa, indicating a high species-specific specialization.

Siphonophorae as food sources

We conducted a phylogenetic analysis of prey sequences blasting among Siphonophorae and all publically available 28S rRNA sequences of siphonophores on GenBank (last accessed 14.04.21 – 44 sequences in total, 29 after excluding duplicates from the same species and sequences which lacked overlap with our sequence data). Four species of Lepthotecata were chosen to root the tree (see Cartwright *et al.*[S13] for sister group relationships) and *Hydra circumcincta* Schulze, 1914 as outgroup. 43 sequences were aligned using the MUSCLE algorithm[S2] as implemented in Geneious Prime 2021.1.1 with the standard settings and the alignment was trimmed to focus on the 28S rRNA region sequences in the present study (1051 base pairs). We removed ambiguously aligned parts of the alignment via Gblocks[S14] using options for a less stringent selection, resulting in a total alignment length of 958 base pairs. GTR+G+I was determined as best fitting nucleotide substitution model under the Akaike Information Criterion via jModelTest[S15]. We performed maximum likelihood analysis using RAxML 8.2.11[S3] as implemented in Geneious Prime 2021.1.1 with 1000 rapid-bootstrapping iterations, presented in Figure S1A showing that Solenogastres prey on a diverse range of siphonphores, suggesting scavenging on pelagic forms as well as predation on benthic rhodaliid-like animals.

Testing for chimeric sequences

Seven amplified 28S rRNA sequences blasted among different trochozoan taxa (Annelida, Mollusca), with a lumbricid annelid (*Glyphidrilus* sp., GenBank accession code HQ728961.1) retrieving highest query coverage (QC 45%) and pairwise identity (PW-ID 74.47%) (e.g. 28S rRNA sequence amplified from Dondersiidae sp.SB-4 (ZSM Mol 20190670): ACCGCGATTCCCTCGGTAGCGGCGAGCGAAGAGGGAAAAGGCCAGCGCTGAATC CCCGAGACCCTTGTCCCGTGGGAGGTGTAGCGTTACGGGCGGGATCAACCGTGCC GCCGGCGCGCCCGAGTTCCCACGAACGGGACCCCGGAGCGGGTGTTAGGCCTCT ACGGAGCGCCGCGCGGTGCGGACGGTGTCGACCCATCGAGTACGGTTGCCTGAG ATCGCAACCCGAAGAGCGAGGTAAACTCCTCGTAAGCCTAAATATCCGTGCGTGT CCGATAGCGAACAAGTACCGCGAGGGAAAGTTGAAAAGGACTTTGAAGAGAGAG TTCAACAGTACGTGAAACCGTTCGGAGCGAAACGGACGGAGCCGTCAAGTCGGC CCGCGAGATTCGTCGGCTTTGTCGGGGTTCTGTCGCGTCGTGCGGCACTTCGCCCG TCGTCGTTCCTTCGGGTTCGTCGTCGGCGTGGTCGTCGTTCGGCGTGCTCCACTTC GAGGAGTCGCTCGCGTCTCGCGCGCGCCGTGCGTCGCGACCGATTCATGGGCGAT CCGCCACCCGGCGGCGTGGTGATCGGGCGGCGATAGCCGGTGTTTCGACGGAGG TTCGTTCGGGTCGCCGCCGACGTACGGCACCCGGCGTTAGTCCACGTCGAGCCGC CGATGTCCCGCCACCACACTAGAGGCCGGTAAGACGCGCCCCGTCGGATCGAGC AAATAGTAACTGTGCATGTGCCGCCCTGACTCACCCTCTAGGCAAAGTGACCGCC GGTAGGGGTTTGGCCGGGCGCGATCTAGGGAATGGTCCGTCGCTACCCGCCCCTC CGACGACTACTCGTTTTACCCTTGCGCCAAGGTGTTAAAATCGAAGGTACACGGC CAGTCAGCGACGATGTCCGGTAACCCGTCCGACCCGTCTTGAAACACGGACCAAG GAGCGCGACGGTCGCGCGAGTCGAAGGGTCTCACGAAACCCCCAGGCGAACGCG AGAACGAGAGACCCTTGCCCGCCTAATGCCCTTCCGGAGTCCCTCAGGGGACCGA CGAGACGGTGGCGGGTTTAATAGCAAGCGTGGCGCGATCTGAGTACCTACCTCAG CGTAGCGCCGCCCGTTCCGACGTCACCGGCCCGCGCGGGTCGGGAGCGGATCGA GGCNANAGCGCGACCGTCGCTACCCGAAAGATGGTGATCTATTCCCGAGCAGGG CGAAGTCGGGG). To check for potential chimeric sequences resulting from PCR-amplification, we followed the workflow outlined in Meyer *et al.*[S5]. We generated stitch

profiles of *Glyphidrilus* sp. 28S rRNA (GenBank accession code HQ728961) using standard parameters[S16]. The resulting stitch profile was aligned to the potential chimeric sequences and bubble and helical regions identified (Figure S1B). Secondary structures were determined with RNAfold[S17] as implemented in Geneious (using standard settings and Turner energy Model) (Figure S1B). We separately blasted corresponding helical and bubble regions and stretches of sequences in between. Based on these results, our obtained sequences might present a chimera composed of stretches from 1) the solenogaster, 2) its annelid prey and potentially 3) cross-contamination from the lab (Figure S1B). Thus, we chose – at present stage of knowledge – not to submit these sequences to GenBank. Remarkably, we obtained highly similar 28S rRNA sequences (99.07% - 99.91% pairwise identity) from all collected individuals of Dondersiidae sp.SB-4 and Gymnomeniidae sp.SB-2 (from different sampling localities[S1]). This is a strange coincidence for chimeric sequences, indicating that they (mainly) originate from the combination of the true solenogaster sequences and its common prey.

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Table S1: List of solenogaster food sources as revealed by BLAST analyses of sequenced gut contents. Taxon ID and voucher number refer to solenogaster source specimen (deposited at the Bavarian State Collection of Zoology, Munich Germany). Pairwise identity (PW-ID) and query coverage (QC) values refer to query sequence and closest BLAST search match on GenBank (accessed on 13. March 2021).
*- marks sequences which have ambiguous BLAST hits with poor QC and PW-ID (see section "Testing for chimeric sequences").

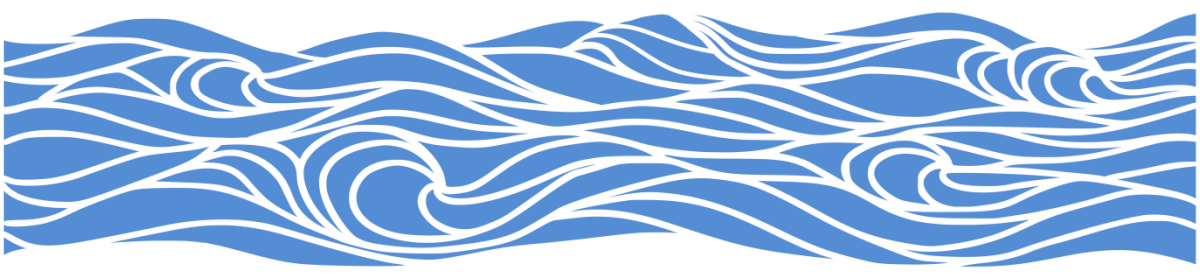
Solenogastres		**Sequenced Gut Content**				
Taxon ID	**Voucher Number ZSM Mol**	**Higher taxonomic rank**	**Closest match BLAST search and GenBank Accession**	**PW-ID [%]**	**QC [%]**	**GenBank Accession**
Acanthomeniidae sp.2	20190565	Mollusca, Bivalvia, Mytilida	*Dacrydium* sp. KX713372	98.8	100	MZ027160
Acanthomeniidae sp.2	20190566	Nemertea, Hoplonemertea, Monostilifera	*Galathenemertes giribeti* Chernyshev & Polyakova, 2019; MN211452	94.3	100	MZ027161
Acanthomeniidae sp.4	20190569	Mollusca, Bivalvia, Venerida	*Turneroconcha magnifica* (Boss & R. D. Turner, 1980) KC429487	96.8	100	MZ027162
Acanthomeniidae sp.5	20190570	Mollusca, Bivalvia, Venerida	*Turneroconcha magnifica* KC429487	96.5	100	MZ027163
Acanthomeniidae sp.6	20190576	Mollusca, Bivalvia, Nuculanida	*Megayoldia japonica* (A. Adams & Reeve, 1850) LC144747	96.67	100	MZ027168
Acanthomeniidae	20190572	Mollusca, Bivalvia,	*Megayoldia japonica*	96.65	100	MZ027164

sp.6		Nuculanida	LC144747			
Acanthomeniidae sp.6	20190573	Mollusca, Bivalvia, Nuculanida	*Malletia humilior* Prashad, 1932 LC144748	96.99	100	MZ027165
Acanthomeniidae sp.6	20190574	Mollusca, Bivalvia, Nuculanida	*Megayoldia japonica* LC144747	96.65	100	MZ027166
Acanthomeniidae sp.6	20190575	Mollusca, Bivalvia, Nuculanida	*Megayoldia japonica* LC144747	96.59	100	MZ027167
Acanthomeniidae sp.6	20190577	Mollusca, Bivalvia, Nuculanida	*Megayoldia japonica* LC144747	96.59	100	MZ027169
Acanthomeniidae sp.7	20190578	Mollusca, Bivalvia, Pectinida	*Parvamussium carbaseum* Dijkstra, 1991 AB102751	85.2	100	MZ027170
Acanthomeniidae sp.SB-1	20170390	Mollusca, Bivalvia, Nuculanida	*Katadesmia cuneata* (Jeffreys, 1876) KC984810	100	97.85	MZ027172
Acanthomeniidae sp.SB-1	20170372	Mollusca, Bivalvia, Nuculanida	*Yoldiella inconspicua* Verrill & Bush, 1898 KC984807	98.71	94	MZ027171
Acanthomeniidae sp.SB-1	20190580	Mollusca, Bivalvia, Nuculanida	*Yoldiella inconspicua* KC984807	98.71	94	MZ027173
Amphimeniidae sp.2	20190584	Cnidaria, Anthozoa, Actiniaria	*Edwardsia timida* Quatrefages, 1842 KJ483088	93.2	100	MZ027175
Amphimeniidae sp.2	20190582	Cnidaria, Anthozoa, Actiniaria	*Edwardsia timida* KJ483088	92.1	99.11	MZ027174
Cavibelonia sp.2	20170102	Cnidaria, Hydrozoa, Siphonophorae	*Erenna laciniata* Pugh, 2001 KX752715	99.7	98.72	MZ027176
Dondersiidae sp.3	20190624	Nemertea, Hoplonemertea, Monostilifera	*Proamphiporus kaimeiae* Hookabe, Tsuchida, Fujiwara & Kajihara, 2020	92.8	100	MZ027177
Dondersiidae sp.5	20190626	Nemertea, Hoplonemertea, Monostilifera	*Galathenemertes giribeti* MN211452	94.3	100	MZ027178
Dondersiidae sp.5	20190628	Nemertea, Hoplonemertea, Monostilifera	*Galathenemertes giribeti* MN211452	94.3	100	MZ027179
Dondersiidae sp.5	20190629	Nemertea, Hoplonemertea, Monostilifera	*Galathenemertes giribeti* MN211452	94.3	100	MZ027180
Dondersiidae sp.SB-3	20190638	Mollusca, Bivalvia, Nuculanida	*Megayoldia japonica* LC144747	92.2	100	MZ027181
Dondersiidae sp.SB-4	20170363	Cnidaria, Hydrozoa, Narcomedusae	*Aeginura grimaldii* Maas, 1904 MG979307	99.5	100	MZ027182
Dondersiidae sp.SB-4	20190662	?		< 80	< 40	*
Dondersiidae sp.SB-4	20190659	?		< 80	< 40	*
Dondersiidae sp.SB-4	20190672	?		< 80	< 40	*
Dondersiidae sp.SB-4	20190655	?		< 80	< 40	*
Dondersiidae sp.SB-4	20190670	?		< 80	< 40	*
Dondersiidae sp.SB-4	20190642	?		< 80	< 40	*
Dondersiidae sp.SB-4	20190645	Cnidaria, Hydrozoa, Siphonophorae	*Apolemia lanosa* Siebert, Pugh, Haddock & Dunn, 2013 KF214716	99.7	100	MZ027190

Dondersiidae sp.SB-4	20190660	Cnidaria, Hydrozoa, Siphonophorae	*Apolemia lanosa* KF214716	99	100	MZ027186
Dondersiidae sp.SB-4	20190669	Cnidaria, Hydrozoa, Siphonophorae	*Apolemia lanosa* KF214716	99.2	99.48	MZ027188
Dondersiidae sp.SB-4	20190644	Cnidaria, Hydrozoa, Siphonophorae	*Erenna laciniata* KX752715	99.8	98.24	MZ027184
Dondersiidae sp.SB-4	20190646	Cnidaria, Hydrozoa, Siphonophorae	*Erenna laciniata* KX752715	99.2	98.75	MZ027185
Dondersiidae sp.SB-4	20170364	Cnidaria, Hydrozoa, Siphonophorae	*Erenna laciniata* KX752715	90.2	100	MZ027183
Dondersiidae sp.SB-4	20190661	Cnidaria, Hydrozoa, Siphonophorae	*Erenna laciniata* KX752715	94.8	98.76	MZ027184
Dondersiidae sp.SB-4	20190678	Cnidaria, Hydrozoa, Siphonophorae	*Hippopodius hippopus* (Forsskål, 1776) EU305517	97.9	95.63	MZ027189
Gymnomeniidae sp.SB-2	20190708	?		< 80	< 40	*
Gymnomeniidae sp.SB-2	20190698	Cnidaria, Hydrozoa, Siphonophorae	*Erenna laciniata* KX752715	94.4	100	MZ027194
Gymnomeniidae sp.SB-2	20190696	Cnidaria, Hydrozoa, Siphonophorae	*Erenna laciniata* KX752715	94.5	100	MZ027193
Gymnomeniidae sp.1	20190687	Cnidaria, Hydrozoa, Siphonophorae	*Apolemia lanosa* KF214716	99.4	100	MZ027191
Gymnomeniidae sp.2	20190688	Cnidaria, Hydrozoa, Siphonophorae	*Apolemia lanosa* KF214716	99.1	100	MZ027192
Gymnomeniidae sp.SB-2	20170366	Annelida, Errantia, Phyllodocida	*Dysponetus caecus* (Langerhans, 1880) EU555028	98	30.17	MZ027195
Halomeniinae sp.	20170077	Cnidaria, Hydrozoa, Trachymedusae	*Arctapodema* sp.	94.4	98.76	MZ027196
Kruppomenia genslerae	20170353	Annelida, Sedentaria, Terebellida	*Terebellides californica* Williams, 1984 JN936478	97.6	72.62	MZ027200
Kruppomenia genslerae	20170354	Annelida, Sedentaria, Terebellida	*Terebellides californica* JN936478	97.7	71.6	MZ027201
Kruppomenia genslerae	20170357	Annelida, Sedentaria, Terebellida	*Terebellides californica* JN936478	97.6	71.6	MZ027202
Kruppomenia genslerae	20170358	Annelida, Sedentaria, Terebellida	*Terebellides californica* JN936478	97	71.52	MZ027203
Kruppomenia genslerae	20170344	Annelida, Sedentaria, Terebellida	*Terebellides californica* JN936478	97.6	70.3	MZ027197
Kruppomenia genslerae	20170347	Annelida, Sedentaria, Terebellida	*Terebellides californica* JN936478	97.3	71.75	MZ027198
Kruppomenia genslerae	20170348	Annelida, Sedentaria, Terebellida	*Terebellides californica* JN936478	97.7	71.23	MZ027199
Lyratoherpia sp.	20190682	Cnidaria, Hydrozoa, Leptothecata	*Lafoea dumosa* (Fleming, 1820)	92.7	96.91	MZ027204
Macellomenia sp.	20190712	Cnidaria, Hydrozoa, Leptothecata	*Earleria panicula* (G.O. Sars, 1874) FJ550452	94.9	92.84	MZ027205
Proneomeniidae sp.SB-1	20170371	Cnidaria, Anthozoa, Alcyonacea	*Placogorgia* sp.	94.3	100	MZ027206
Pruvotina sp.SB	20190593	Cnidaria, Hydrozoa, Anthoathecata	*Corymorpha groenlandica* (Allman, 1876) JN594037	99.3	100	MZ027207
Pruvotina sp.SB	20190594	Cnidaria, Hydrozoa, Anthoathecata	*Corymorpha groenlandica* JN594037	99.2	100	MZ027208
Pruvotinidae sp.3	20190591	Cnidaria, Hydrozoa, Leptothecata	*Earleria panicula* FJ550452	95	91.68	MZ027209
Pruvotinidae sp.KBI-2	20170079	Cnidaria, Hydrozoa, Limnomedusae	*Monobrachium parasitum* Mereschkowsky, 1877 MG979299	99.4	99.9	MZ027210

Simrothiellidae sp.2	20190603	Annelida, Sedentaria, Terebellida	*Polycirrus* sp. EU418866	84.8	99.89	MZ027211
Simrothiellidae sp.KBII-7	20190612	Annelida, Sedentaria, Terebellida	*Polycirrus* sp. EU418866	91	100	MZ027212
Simrothiellidae sp.KBII-9	20190616	Annelida, Sedentaria, Terebellida	*Amphitrite ornata* (Leidy, 1855) DQ790022	92.9	100	MZ027213

Part IV. Science communication and public outreach



Chapter 11. AplacBase – an online database for Mollusca's most neglected classes

Both groups of aplacophoran molluscs (Solenogastres + Caudofoveata) are virtually unknown to the general public and are relegated to a niche existence even among invertebrate taxonomists. As a first step in an international community effort towards a better understanding of aplacophorans, Dr. Carmen M. Cobo, Meghan Yap-Chiongco (both University of Alabama, USA) and I have brought together aplacophoran researchers from Brazil, Japan, Norway, Russia, Spain, and the USA to initiate AplacBase (https://aplacbase.weebly.com/), an online database dedicated to Aplacophora (Fig. 2).

AplacBase offers a variety of resources surrounding Solenogastres and Caudofoveata, and our main aims to provide are:

1.) Searchable database of recognized species and their distribution:
This searchable database is based on an up-to-date species list of both groups and includes query forms for classification, type localities (geo coordinates, depth ranges, maximum depth, marine province and ecoregion), taxonomic literature and institutes hosting holotypes (Fig. 2D). We additionally provide an interactive map depicting all type localities (Fig. 2E).

2.) Resources for the identification of aplacophorans:
AplacBase provides quick ID guides to aplacophoran families, focusing only on easily accessible scleritome and radula. In cases where unambiguous identification to family level is not possible, the quick ID guides offer advice which alternative families should also be considered. The guides are illustrated with photos of "living" animals and the taxonomically relevant characters (Fig. 2F). As further support for identification, AplacBase contains a continuously growing photo database of recognized as well as undescribed species (Fig. 2B).

3.) Compilation of 150 years' worth of aplacophoran literature (Fig. 2G).
AplacBase is intended as an integrative supplement to other databases and repositories. In contrast to the World Register of Marine Species (WoRMS) and the Ocean Biodiversity System (OBIS), AplacBase focuses on providing practical support in the identification of aplacophoran molluscs. In contrast to WoRMS, AplacBase additionally contains information on yet undescribed species (e.g. distribution and morphology) and makes distribution and morphological information quickly available by side-stepping formal species descriptions. Most of the offered resources are based on our own experience and difficulties when starting out with aplacophoran taxonomy, and we thus hope to support and facilitate future aplacophoran biodiversity research.

An introduction into solenogaster diversity and systematics as well as the regularly updated Blog on aplacophoran research are written for an audience beyond the scientific community (Fig. 2C). We aim to ensure that all resources are also accessible to interested non-experts, by explaining scientific jargon and providing a glossary of relevant (aplacophoran) terminology. Together with our international contributors we are continuously expanding AplacBase since its launch in January 2021 and planning its integration into the World Register of Marine Species (WoRMS).

One of the main future aims for AplacBase is to develop it from an online repository and database to an additional web-based bioinformatics pipeline for molecular-based initial species identification and delineation (see Conclusion and Outlook).

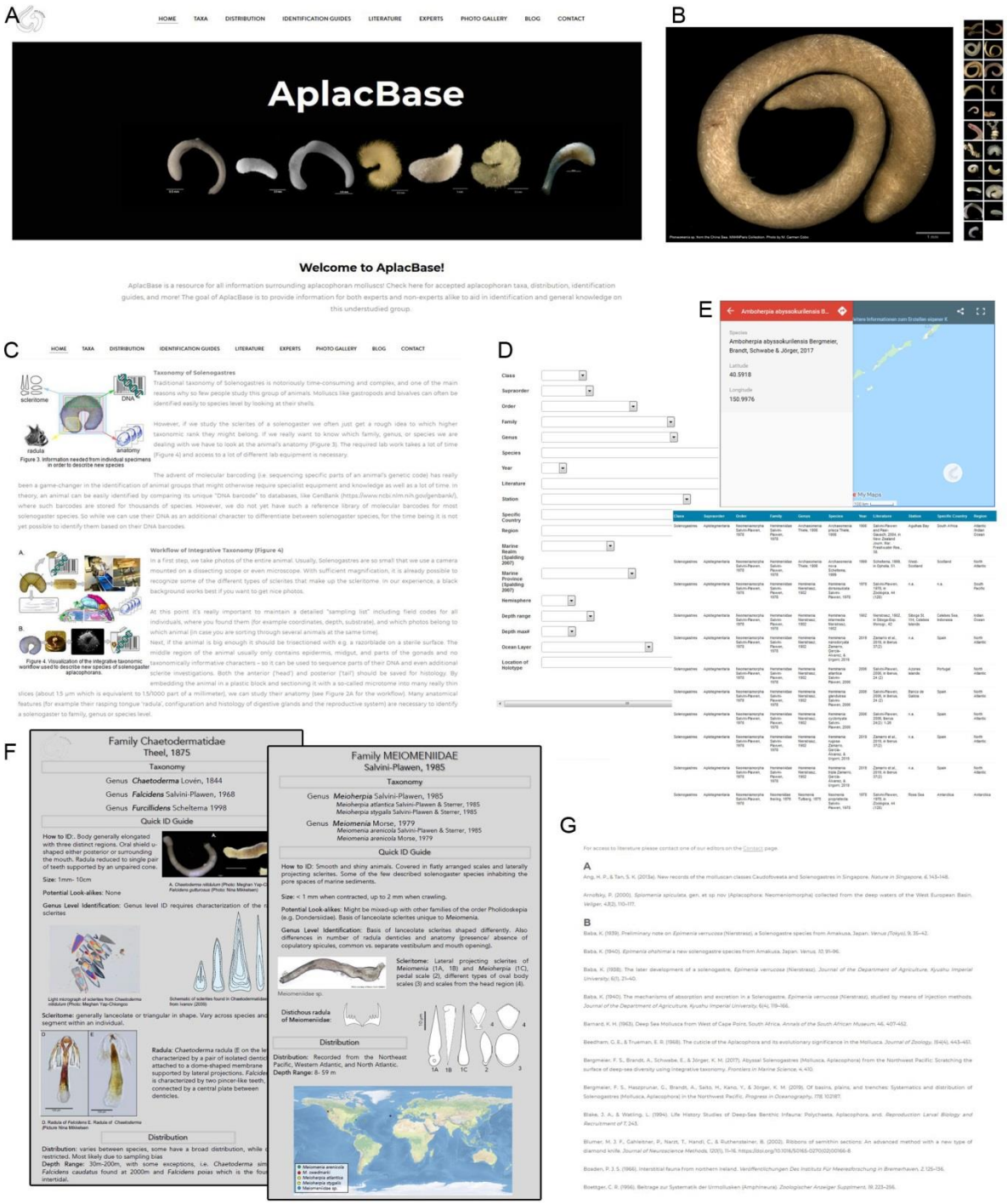


Figure 2. Screenshots of main features of AplacBase (31.01.2021). **A.** Header and Welcome. **B.** Gallery of Aplacophoran species. **C.** Introduction to Aplacophoran taxonomy and systematics. **D.** Searchable species database. **E.** Interactive map of Aplacophoran type localities. **F.** Quick-ID-Guides to aplacophoran families. **G.** Database of aplacophoran literature.

Chapter 12. Pardo JCF, Araújo TQ, Capucho AT, Yap-Chiongco MK, Buckenmeyer A, Jondelius Y, Aramayo V, **Bergmeier FS**, Andrade LF, Cherneva I, Petrunina A, Peixoto AJM, Mikhlina A, Davidson AM, Engelhardt J, Frade F, Ellison C, Roberts NG, Costa AC & Jörger KM. **Tiny animals do live in the sand: a report of meiofaunal focused active-learning activities to increase ocean literacy in primary-school children.** *Açoreana* (in press): 1-11.

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TINY ANIMALS DO LIVE IN THE SAND: A REPORT OF MEIOFAUNAL FOCUSED ACTIVE-LEARNING ACTIVITIES TO INCREASE OCEAN LITERACY IN PRIMARY-SCHOOL CHILDREN

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ABSTRACT

Ocean literacy is essential to increase awareness and educate people about the importance of our ocean. Students are among the main actors to build an ocean-literate society. Outreach initiatives, for instance, have an educational potential to transfer knowledge in a practical way. Here, we report an outreach activity with students about meiofaunal communities, an ecological important and underexplored group in marine environments. Through interactive talks and hands-on activities, we showed primary school children the main characteristics of the group and their importance to marine habitats. Using information obtained during the "Exploring the Diversity of Marine Meiofauna of the

Azores" summer school, the activity was regionally focused providing a broad perspective of the topic. Taxa and methods to deal with meiofauna were explained by dividing the topics into stations and knowledge transferred via playful and interactive activities. Students were involved in the activities interacting with mentors and raising questions about meiofauna and the marine environment. Direct contact with animals endemic to the Azores provided students with a new understanding of these understudied groups within the local marine environment. This interaction can increase the knowledge and awareness about marine life achieving the principles of ocean literacy.

RESUMO

A Literacia do Oceano é essencial para sensibilizar e educar pessoas sobre a importância do oceano. Os estudantes estão entre os principais actores na construção de uma sociedade consciente sobre o ambiente marinho. Actividades de divulgação, por exemplo, possuem um potencial educativo na transferência de conhecimento de uma forma prática. O presente relatório descreve uma actividade de divulgação realizada com estudantes sobre as comunidades de meiofauna, um grupo ecologicamente importante e subexplorado em ambientes marinhos. Utilizando informações obtidas durante a escola de verão "Explorando a Diversidade de Meiofauna Marinha dos Açores", a actividade foi realizada com um enfoque regional, porém promovendo uma visão mais ampla sobre o assunto. Táxons e métodos para trabalhar com meiofauna foram explicados através de estações divididas por tópicos, e o conhecimento transferido através de actividades interactivas e lúdicas. Os estudantes mostraram-se interessados nas actividades, interagindo com os monitores e levantando questões sobre a meiofauna e o ambiente marinho. O contacto directo com animais endémicos dos Açores proporcionou aos estudantes um novo conhecimento sobre o ambiente marinho local. Esta interação aumenta o conhecimento e consciencialização sobre a vida marinha e, por consequência, promove os princípios da Literacia do Oceano.

INTRODUCTION

Understanding the ocean is essential to comprehending and protecting our planet and ultimately ensuring our own health and well-being (Santoro *et al*., 2017, Ghilardi-Lopes *et al*., 2019). Ocean literate citizens know the relationship between man and ocean using the knowledge and awareness about the environment in their daily-life (Cava *et al*., 2005). Promotion of ocean literacy becomes important as traditionally, policymakers have scarce knowledge about the ocean, and therefore do not incorporate it when making decisions (Cava *et al*., 2005; Uyarra & Borja, 2016). Given that our ocean is under several threats (e.g., environmental pollution, climate change), mostly due to anthropogenic actions in the last decades, ocean literacy is a matter of urgent necessity, especially when it comes to younger generations (Santoro *et al*., 2017, Ghilardi-Lopes *et al*., 2019).

Considering that students are future critical-thinking citizens, ocean literacy activities are essential tools to ensure the sustainable use of the ocean and its natural and social resources (Santos *et al*., 2018). Understanding complex systems like the ocean can be made less daunting, through strategies such as experiential learning

which has proved to be an effective way to improve knowledge in this area (Santoro *et al.*, 2017). In primary level schooling, the academic curricula over the world lack ocean-literacy related subjects (Visbeck, 2018). Young students are also an important bridge transferring the environmental knowledge, attitudes and behaviors to their peers and family (Hartley *et al*., 2015). Engaging learners in first-hand experiences and playful teaching practices help them build personal connections, making it easier to learn and ultimately motivating them to act on behalf of the ocean (NOAA 2013, Guest, 2015, Stefanelli-Silva *et al.*, 2019). Even small scientific dissemination activities aimed at a wide audience can greatly impact our perception of caring for the marine environment and its diversity. These efforts are aligned with the objectives of the UN - Decade of Ocean Science for Sustainable Development (2021-2030), which stimulate and promote public awareness activities about our aquatic ecosystems (Intergovernmental Oceanographic Commission [IOC], 2019). Both in person and remote dissemination activities can be key to socializing scientific knowledge to students. This can contribute positively to collective ideas about the conservation and study of natural habitats, and eventually have implications even in schoolchildren's educational curriculum (Mogias *et al*., 2019). For teachers and education professionals, it is always important to develop strategies that may increase the student's interest in the given activity, including the use of original materials, hands-on activities, and interaction between the students (Dohn, 2011). Potential positive experiences retain students' interest and may also influence their career and life aspirations in marine science (Wang & Staver, 2001).

Marine life is wondered by most children where underwater fauna and flora are very appealing due to their high diversity of body shapes, conspicuous behaviours and life-strategies (Ballantyne, 2004; Guest *et al*., 2015). The use of this diversity to teach and justify protection actions arise as a good strategy to promote ocean literacy. Marine life has been suffering from anthropogenic actions resulting in e.g. coral bleaching and ocean acidification, and several species have become extinct without being accessed, discovered and described (Dulvy *et al*., 2013; Zeppelli *et al*., 2015). According to Cerca *et al*. (2018), 27 out of the 34 phyla of Metazoa have at least some microinvertebrate representatives living among the sand grains, collectively called meiofauna. Meiofauna is a diverse aquatic community of organisms grouped by the small size between micro- and macrofauna (Giere, 2009; Schmidt-Rhaesa, 2020). Despite their importance as a major component of benthic habitats and having a key ecological role (Nascimento *et al*., 2012; Schratzberger & Ingels, 2018), it has never been a popular topic in marine science, let alone in ocean literacy. Because of their hidden habitat and small size, meiofaunal organisms tend to go unnoticed by most people. It is difficult for scientists to convey examples which show that tiny organisms are of utmost importance in maintaining the functionality of the whole ecosystem (Uyarra & Borja, 2016). The absence of charismatic species, with some exceptions i.e., tardigrades, has also always hindered the massive diffusion of this group of small organisms among the non-scientific public.

In the present study, we report the outreach activity with primary school-age children conducted during the summer school "Exploring the Diversity of Marine Meiofauna of the Azores: from discovery to scientific publication" in 2019. Our aim was to raise awareness about meiofaunal organisms, introduce students to the concept of the group, their relationship and importance to the marine environment, and explain the work we performed during the summer school.

'VW-SUMMER SCHOOL – EXPLORING THE MEIOFAUNA OF THE AZORES' ACTIVITY

Our outreach activity was performed in partnership with EXPOLAB - "Centro de Ciência Viva", an educational center in São Miguel Island, Azores, promoting scientific literacy on Natural Sciences and Technology via laboratory and experimental activities. Two groups of 10 primary-school children were introduced to the topic of meiofauna in an auditorium lab via an interactive talk and playful activities (one hour per group). We shortly introduced ourselves (~5 minutes) and asked about their prior knowledge of the group. Students were then divided into pairs and encouraged to take part in hands-on activities subdivided into sections to get more familiar with the different groups and methodologies used in the meiofaunal research.

Extraction station

Before hands-on activities with meiofaunal animals, students were given an explanation on these "tiny animals living in the sand" are and how to find them (Figure 1A, 1B). We carried out a real-time extraction of animals from sand collected the day before and described every step of the process. First, sand was put into a 1L Erlenmeyer flask and filled with half sea water and half 7% magnesium chloride ($MgCl_2$) solution. It was explained to the children that the solution was meant to relax animals in order to loosen them from the sand grain as many meiofaunal organisms have adhesive glands. After swirling the flask, the water was decanted into a 60µm sieve. The sieve was then washed with sea water into a petri dish in order to visualize the meiofauna from the extraction. These petri dishes were then projected to show the children the results of the extraction that was just demonstrated in order to highlight the life that can be found between the sand grains. To see the animals better, the children studied the organisms in the dishes under a stereomicroscope. They could see numerous animals living in a small amount of sand and appreciate the diversity of forms, as there were representatives of different types: polychaetes, crustaceans, flatworms etc. After a first round of demonstrating the technique, some of the children tried the extraction themselves. Since neither toxic chemicals nor sharp objects are involved in this form of animal extraction, it can be safely conducted by supervised children. At subsequent stations, the children were able to gain a deeper understanding of each major group found within the meiofaunal community.

Crustacean station

Crustaceans are one of the most popular and known invertebrate groups by the general public. The activity started by

asking the students about examples of animals within this group. As expected, all of them were able to cite at least one organism, being all of them belonging to the order Decapoda, whose most popular representatives are crabs, shrimps and lobsters. They were then shown a picture with many silhouette drawings of the silhouettes of invertebrates with varying body shapes and were asked to point out which one they thought could be a crustacean (Figure 1C). Brief explanations of the importance of the various structures to the success of the organism in a meiofaunal habitat along with the importance of each organism to its environment were given as the students participated in this activity. Crustaceans collected during the Summer School were sorted into different taxa such as amphipods, isopods and tanaids. Some living individuals of each group were placed in petri dishes under a stereomicroscope and shown to the students, highlighting the differences among each group in an accessible explanation. We also mounted some zoological slides with glycerin containing appendices of tiny crustaceans, so they could observe details of their structures under a stereomicroscope. They were also asked to identify well-known crustaceans in an image to highlight that various shrimps, crabs and lobsters were actually crustaceans.

Polychaete station

Among invertebrates, polychaetes are largely unknown by the general public and, save for a few exceptions, are inconspicuous, hold no commercial value, and are not used for human consumption (Read, 2019). The station was conducted similarly to the Crustacean station. Upon arriving, students were asked about the group, revealing no prior knowledge on the existence of polychaetes. We defined and explained the importance and general characteristics of the group and showed under stereomicroscope living specimens collected during the summer school (Figure 1D). Students could interact with the specimens using a fine paintbrush, while we offered simple explanations on examined specimens' differences, morphology, and mobility.

Worms station

"Worms" is a rather broad term, but we used it to keep things simple, referring to the phyla Platyhelminthes, Xenacoelomorpha and Nematoda. They may not be the most appealing critters to kids, but we tried to make them so by comparing them to animated characters. The station consisted of matching pictures of different worms (a flatworm, an acoel and a nematode) to their literal translated Portuguese name and each animal's similarity to a Disney character. In this way, students were able to remember the most distinguishable feature of each group and they started to get more excited about these animals. Then, after seeing a triclad flatworm at the microscope, students guessed to which of the three groups it belonged, making comparisons with the characters (Figure 1D). Finally, students colored a drawing of a flatworm to take home (Figure 1E).

Baby station

In addition to mature meiofaunal animals, which children got to know about at the previous stations, the larval and

juvenile stages of many benthic invertebrates can be also meiofaunal. Two sets of pictures were then presented to the students: larval and juvenile pictures taken by us during the meiofauna course, and pictures of adult invertebrates taken from the Internet (Figure 1F). The children were asked to find an appropriate pair for the larva/juvenile, often surprised by how vastly different the larval stage of each animal could be from the adult. Explanations about the organisms were then made and questions raised by the students answered during the activity.

Metabarcoding station

As the summer school included the collection of samples for a small metabarcoding study, we included a station meant to introduce the students to the basics of metabarcoding - the concept of identifying the species present in a sample without actually seeing them. This is done based on genomic data which was not available for the Expolab environment. In the sand used for the extraction station, we placed 10 items of various kinds, such as eppendorf-tubes, pine-cones and rocks and gave the students 60 seconds to find them all (Figure 1G). The miscellany of items was to illustrate the applicability of metabarcoding to all animal groups. After 60 seconds the sand was sieved, revealing more than what the students had originally spotted or managed to dig up in the limited time frame. Students were able to gain a deeper understanding of the concept of metabarcoding (i.e. what you find in a sample is not always only what is there), while getting hands-on experience with the sand.

Build your animal station

After passing through all stations, students arrived at this station to "build" their own meiofaunal organism (Figure 1H). We divided the organism's body parts into three different aspects of meiofaunal life: "eating", "sensation" and "attachment to substrate". We explained using non-academic language the function of different types of feeding strategies (e.g., pharynx pump, scalids, oral stylets, jaws); the function of sensorial structures, which help organisms find food, move among the sand grains, and avoid potential predators (e.g., tentacles, sensorial bristles, ocelli); and different organs that these tiny animals use to attach to the substrate (e.g., adhesive organs, adhesive tubes, suction pads). Following this introduction, the students were able to build their own animals from the available organs. Some of them chose jaws, spines and adhesive tubes to allow their animals to be accurate predators or some chose smooth structures to hide between the sand grains more efficiently. This experience provided an approachable and immersive opportunity to understand the function of important morphological features of meiofaunal animals.

Discussion and conclusion remarks

Our activity consisted of showing primary school students part of the Azorean biodiversity of tiny organisms living between sand grains. By introducing the group, we discussed their role in the environment and importance to preserving marine habitats. In this short dissemination activity, students were encouraged to

FIGURE 1. Activities conducted during the outreach. **A**, general schematization of the sieving method; **B**, students and mentors during the activity in the different stations; **C**, Crustacean station, **D**, Polychaetes and Worms stations; **E**, Worms station; **F**, Baby station; **G**, Metabarcoding station; **H**, Build your animal station.

connect with the understudied group of meiofaunal species through direct observation and hands-on activities.

Environmental literacy is considered a priority on a global scale and has been seen as such in the Autonomous Region of the Azores. The region has indeed an important bond with the coastal and marine environment, relying upon economically from marine-related activities such as fishing and tourism attractions (e.g., whale watching, scuba diving) (Calado *et al*., 2011, Carvalho *et al*., 2011). The Azorean biodiversity is also an open door to initiatives focused on the understanding and conservation of its natural legacy. For example, the program "Parque Escola[1]", developed by the Environmental Regional Directorate, provides students with a multidisciplinary educational offer, with activities designed to promote environmental awareness both inside and outside the school. Other similar activities are provided by the "Observatório do Mar dos Açores"[2] (Azores Sea's Observatory), which is a non-profit technical, scientific, and cultural association linked to science communication and promotion of environmental literacy in the scope of marine science.

Museums and educational centers, for instance, are ideal settings for active and contemplative learning, having a significant role in raising the curiosity of young students in life and earth sciences (Ramey-Gassert *et al*., 1994; Bozdoğan & Yalçın, 2009; Mifsud, 2015). The structure and activities are of great support to explore one of the essential principles of Ocean Literacy: 'The ocean supports a great diversity of life and ecosystems' (Santoro et al., 2019). Biodiversity is indeed a topic of interest for children, supporting the retention of related environmental concepts (Ballantyne, 2004; Baram-Tsabari & Yarden, 2007, Guest *et al*., 2015; Stefanelli-Silva *et al*., 2019). During our activities, students were impressed by the variety of forms of organisms, thus being engaged in the activity and creating a smooth knowledge transfer. Student engagement and attention is more important to the retention of a specific information rather than the time spent in the activity, highlighting the importance of active and hands-on learning (Wittrock, 1986). The use of local sensitivity approaches is also known to be efficient in environmental education activities (Jenkins *et al*., 2003). Meiofaunal organisms seem to entice a student's natural curiosity, therefore creating a natural path towards open discussion and introduction of more broad environmental questions and ocean literacy-related topics.

Although we have not obtained quantitative or qualitative data, our report may be useful for similar activities on the topic benefiting from our outreach description. Stations appeared to be a fruitful strategy when dealing with short-time educational activities for large groups. Activities conducted in all stations were straightforward, budget-friendly and easy to implement. During each activity, students raised several questions about the organisms and the environment in which

[1] http://educarparaoambiente.azores.gov.pt/epas/138/parque-escola

[2] https://www.oma.pt/

they live, which is an important indicator of motivation in the learning process (Chin & Osborne, 2008 and literature within). Previously, the concept of sediment composition was shown to properly address Ocean Literacy standards in children through the use of an engaging and playful activity, reminiscent of the activities designed for the ExploLab (Parrish et al., 2015). To our knowledge, this is the first report of a meiofauna-focused environmental outreach activity. Meiofauna is a crucial component of the marine environment, therefore activities that can positively spark the interest of young students to ocean-related topics are crucial to potentially inspiring the next generation of marine scientists. Improving and increasing understanding of biodiversity and the human/ocean relationship will change how students see the marine environment, hopefully instilling a desire to advocate for a healthier and sustainable ocean (Uyarra & Borja, 2016), supporting the achievement of UNESCO's Sustainable Development Goal 14, "Life below Water"[3].

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[3] https://en.unesco.org/sustainabledevelopmentgoals

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3. Discussion

3.1 From shallow sands to deep-sea trenches: evolutionary history and novel insights into solenogsater diversity and distribution

Prior to my dissertation, two-thirds of all known solenogaster species were known from along the lower continental shelf between 200 and 1,000 meters (type localities of 189 species) (AplacBase, 2021). Only a handful of species had been recorded from the intertidal and shallow subtidal, and no Solenogastres were known from the hadal zone below 6,000 m (Fig. 3). Of almost 350 solenogaster specimens investigated during the course of my study, about 20% were collected during several field trips and meiofauna workshops in shallow waters around the world (see Part I of this thesis and further unpublished data) and 80% during deep-sea expeditions to the Northwest Pacific (see Part II and III of this thesis).

The majority of investigated specimens constitute new candidate species and the pending formal descriptions will require a strong taxonomic effort (see discussion below). Overall, the compiled dataset has allowed catching a first glimpse into putative patterns of solenogaster diversity, distribution and feeding ecology.

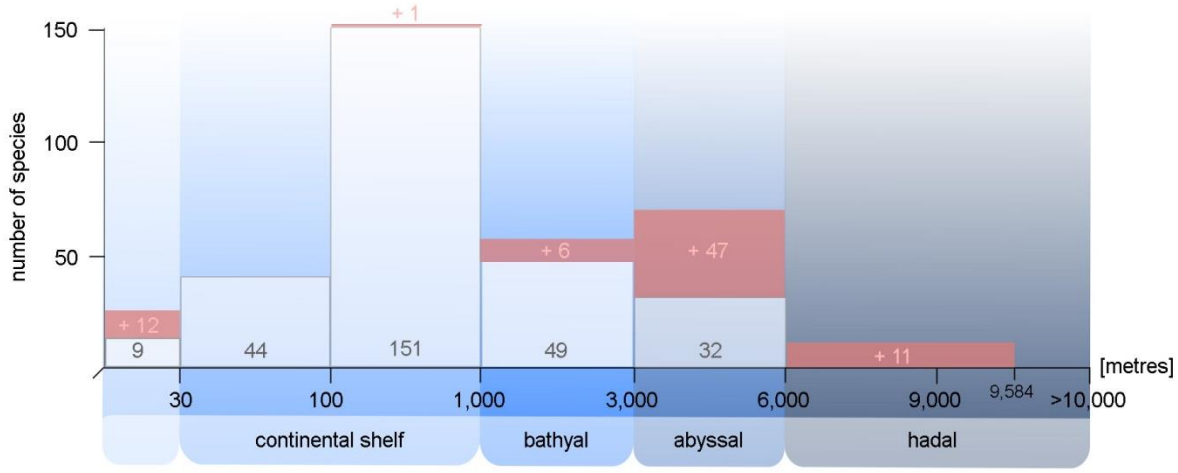

Figure 3. Depth distribution of solenogaster species before (white boxes) and after this thesis (red boxes). White boxes refer to depth of type localities only.

Interstitial Solenogastres from shallow sands

Overall 23 described solenogaster species fall within meiofaunal dimensions (herein restricted to a body length of up to 3 mm). Ten species are assumed to live permanently within the interstitial habitat and only four of those species have been recorded from the intertidal and shallow subtidal above 30 m (Bergmeier & Jörger, 2020).

Sampling for shallow-water mesopsammic Solenogastres in different areas of the Atlantic and the Pacific has revealed at least 12 additional (morphology-based) candidate species, all preliminary assigned to families with known meiofaunal representatives from the intertidal and also below 30 m (i.e. Dondersiidae Simroth, 1893, Lepidomeniidae Pruvot, 1902, Meiomeniidae Salvini-Plawen, 1985, Phyllomeniidae Salvini-Plawen, 1978, Simrothiellidae Salvini-Plawen, 1978) (García-Álvarez et al., 2000; Klink et al., 2015; Bergmeier et al., 2016a; Bergmeier & Jörger, 2020; Neusser et al., 2021).

Based on our own observations from sampling sites in southern Japan (Okinawa and Honshu), New Caledonia, Bermuda, South America and northern Europe, a dondersiid-like morphotype presents the most common and widespread taxon in the mesopsammon (see e.g. Klink et al., 2015; Bergmeier et al., 2016a; Neusser et al., 2021; further own unpublished data). During this targeted sampling for interstitial Solenogastres, findings of entirely new taxa have been surprisingly rare (but see for example Hawaiian Phyllomeniidae sp. in Bergmeier and Jörger (2020) and Azorean Pholidoskepia sp. in Neusser et al. (2021)). Molecular data of these tiny animals is urgently needed to assess whether some of these clades are actually wide-spreading species or if their seemingly large distribution ranges can be attributed to cryptic species complexes as in other meiofaunal taxa (see e.g. Jörger et al., 2012; Cerca et al., 2018 and references therein).

Taking into account the few and scattered occurrence records as well as the lack of genetic data and phylogenetic background, hypotheses on conspecificity and distribution patterns among interstitial Solenogastres remain highly speculative for now. The presence of interstitial Solenogastres in the sands of volcanic, mid-oceanic islands (i.e. Azores and Hawaiian archipelago) (Bergmeier & Jörger, 2020; Neusser et al., 2021 own unpublished data) suggests colonization routes either from continental shelfs requiring respective dispersal abilities or alternatively from source populations harbored in the deep sea (Neusser et al., 2021). Based on the aplacophoran phylogeny reconstructed by Kocot et al. (2019) and own unpublished data, these habitat shifts into the interstitial have occurred independently and multiple times during the evolutionary history of Solenogastres. In contrast to interstitial gastropods, where these shifts required major morphological and anatomical adaptations (e.g. loss of shell, vermification, general reduction of head, foot and appendages – see 'meiofauna syndrome' in Brenzinger et al. (2013)), the only major change required in the bauplan of worm-shaped and generally small Solenogastres to switch from a presumably epibenthic to infaunal lifestyle is further miniaturization. The surprising results of recent solenogaster phylogenetic studies retrieving large-bodied Solenogastres as the earliest branching clade and in several other positions across the tree (Bergmeier et al., 2019; Kocot et al., 2019), do not necessarily imply large-sized animals as the most parsimonious ancestral condition of Solenogastres (but see Kocot et al., 2019 for a different interpretation). According to earlier versions of the Aculifera hypotheses, both groups of aplacophoran molluscs have originated through progenesis and their last common ancestor was most likely a small-sized (i.e. several millimeters), worm-shaped animal (Scheltema, 1993). Taking into account the overall small body size (in the range of several millimeters) of aplacophoran Caudofoveates (Scheltema & Ivanov, 2009) and following the evolutionary scenario that recent larger animals (several centimeters) present derived forms, the hypothetical ancestral solenogaster was probably a few millimeters in size and lived epibenthically in depths of the continental shelfs.

With the colonization of shallower waters, novel adaptations were required to deal with increasing predator pressure. Recent shallow-water species are mostly known living wrapped around anthozoan and hydrozoan colonies (see e.g. Salvini-Plawen, 1981a; Sasaki & Saito, 2005) that simultaneously serve as food source while their stinging cells offer protection from potential predators. Contrary to previous hypotheses (Salvini-Plawen, 1981a), an epizoic lifestyle presents a derived rather than plesiomorphic condition. Extreme miniaturization and a permanent shift into the sediment is likely an alternative strategy to avoid predation. So far, the only known novel anatomical adaptation linked to the interstitial habitat is a posterior adhesive glandular organ. Overall, their ancestral bauplan can be considered as the ideal prerequisite to colonize the mesopsammic habitat.

Solenogastres of the deep Northwest Pacific: diversity and distribution

Prior to my dissertation, eleven species from Japanese coastal waters and the adjacent continental shelf constituted the entire known solenogaster fauna of the Northwest Pacific (NWP) (see e.g. Baba, 1940; Baba, 1975; Saito & Salvini-Plawen, 2010; Sirenko, 2013; Saito & Salvini-Plawen, 2014).
73 putative candidate species of Solenogastres (see discussion 3.2 for details) were discovered in the studied area of the NWP (Fig. 1), amounting to more than 200 occurrence records and representing at least nine families and 15 genera (Bergmeier et al., 2017; Ostermair et al., 2018; Bergmeier et al., 2019) among the four traditional solenogaster orders (Salvini-Plawen, 1978a; Salvini-Plawen, 1978b). Comparison between the investigated regions and depth zones revealed regional differences in solenogaster species diversity and allowed a first glimpse into putative distribution patterns. Solenogaster diversity in the semi-isolated abyssal Kuril Basin of the Sea of Okhotsk was low (12 spp.), despite two-and-a-half times as many comparable sampling events as on the NWP plain. A single wide-spread species accounted for 40% of all solenogaster records in the Kuril Basin (Ostermair et al., 2018; Bergmeier et al., 2019). Similar observations of comparative low diversity have been made on prosobranch gastropods (Fukumori et al., 2018). The overall low diversity might be attributed to low levels of primary production in the surface layers and oxygen-poor bottom waters (Zhang et al., 2001; Tyler, 2003), both factors influencing diversity and abundance in the deep-sea benthos (Levin et al., 2001; Thistle, 2003). Especially levels of dissolved oxygen have been identified as the main factor predicting deep-sea alpha diversity in the NWP (Saeedi et al., 2020).

The investigated abyssal and hadal zones of the NWP plain and the Kuril-Kamchatka Trench (KKT) harbored a rich diversity (48 spp. in total). 11 species sampled from the slopes and bottom of the KKT are the first Solenogastres recorded from the hadal zone (max. depth: 9,584 m at Station 77, Fig. 3) and exceed the hitherto known deepest occurrence of a solenogaster by almost 4,000 m (Salvini-Plawen, 1978b). The prior lack of solenogaster records from the hadal zone cannot entirely be attributed to the global undersampling of these depths and the resulting gaps in knowledge: in investigations of the mollusc assemblage of the Atlantic Puerto Rico Trench, Solenogastres were reported as absent (Linse & Schwabe, 2018) despite applying the same benthic sampling strategy as in the NWP (as outlined in Brandt et al., 2018b). In contrast to the Puerto Rico Trench which is located in an oligotrophic area, the NWP is characterized by general higher primary production which increases the KKT's potential to sustain species richness (Tyler, 2003).

Our dataset currently suggests an overall higher abyssal diversity in the NWP than along its continental shelf and bathyal zone. However, Solenogastres are by no means rare in shallower depths in the NWP, as indicated by locally low numbers of described species. They rather constitute a frequent component (albeit in low abundance) of the benthic fauna of the nearby Japanese continental shelf (pers. comm. by Dr. Yasunori Kano (Atmosphere and Ocean Research Institute, University of Tokyo) and Dr. Hiroshi Saito (National Museum of Science and Technology, Tsukuba)).
The rising numbers of solenogaster species discovered in further abyssal deep-sea basins in the Atlantic (see e.g Gil-Mansilla et al., 2008; Cobo, 2021; Cobo & Kocot, 2021) illustrate that their current global distribution curve across depth zones (Fig. 3) is biased through undersampling, especially of the abyssal and hadal zone.

There is little overlap between the solenogaster fauna of the different NWP regions. However, findings of the simrothiellid *Kruppomenia genslerae* Ostermair, Brandt, Haszprunar, Jörger & Bergmeier, 2018 provide a first link between the semi-isolated Kuril Basin and the open NWP abyssal plain (Bergmeier et al., 2019). This indicates that neither the hadal depths of the trench nor the

shallower bathyal passageways between the Kuril Islands and the Kuril Basin (see Fig. 1B) are strict depth-induced biogeographic barriers to solenogaster distribution. The vertical range of 6,000 m of a species present in the Kuril Basin and at the bottom of the trench by far exceeds any previously recorded depth range of Solenogastres (second next largest range are approx. 3,500 m for *Pruvotina longispinosa* Salvini-Plawen 1978) and is further evidence for true eurybathy (Bergmeier et al., 2019).

The remarkable diversity of abyssal and hadal Solenogastres is characterized by a high proportion of singletons: two-thirds of all species collected on the plain and from the trench stations are represented by single individuals. While the presence of singletons can be an indicator for "true" rarity, species distribution in the deep sea is notoriously patchy (Danovaro et al., 2013). This patchiness has been linked to heterogeneous habitats and the availability of food-sources (Rex & Etter, 2010; McClain et al., 2011). In terms of their habitat structure, abyssal plains have been commonly regarded as vast and homogeneous sediment covered stretches of ocean-floor, interspersed with hard substrates. Riehl et al. (2020) showed that these areas are in general far more heterogeneous and complex with respective implications for harboring higher species diversity (Smith, 2020). Indirect sequencing of gut contents has revealed astonishing taxon-specific prey preferences among NWP Solenogastres on a variety of invertebrates (Bergmeier et al., accepted). As highly specialized micropredators of the deep-sea benthos, their occurrence might be strongly linked to the presence of their food-sources, explaining their patchiness during sampling. The relative commonness and wide distribution of the single presumably scavenging and generalist species lends further support to this hypothesis (Bergmeier et al., accepted). Thus, attributing the high proportion of singletons to patchiness rather than rarity would implicate that the specimens found in the deep NWP are part of actual self-sustaining populations. Alternatively, rarity of abyssal mollusc species has been explained through source-sink mechanisms (Rex et al., 2005b). In this scenario, abyssal species are a subset of populations from shallower water ("source") which have coincidentally drifted to the sea floor ("sink"). To sustain these abyssal "populations", a constant influx of drifting larvae from shallower depths is required. While the current lack of overlap between the investigated depth zones points towards endemic abyssal and hadal fauna, sampling along the NWP continental shelf and bathyal zone is required to evaluate the presence of potential source populations in shallower depths. Despite the immense sampling effort during the three deep-sea campaigns (KuramBio I, SokhoBio, KuramBio II) that provided most of the studied samples, only 0.1 km² of ocean floor was investigated with epibenthic sledge hauls, highlighting the magnitude of undersampling of the deep sea floor, even in this well studied area.

3.2 Taxonomy of Solenogastres: rapid approaches and current impediments

While working up the deep-sea samples from the Northwest Pacific, I discovered a total of 226 Solenogastres which were assigned to 60 candidate species (see chapters 6-9 of this thesis) following the unified species concept of De Queiroz (2005) (Amplification of genetic data for additional 12 putative candidate species and *Amboherpia abyssokurilensis* Bergmeier, Brandt, Schwabe & Jörger, 2017 failed). Species are herein defined as independently evolving lineages of metapopulations, which are supported through different lines of evidence (De Queiroz, 2005; De Queiroz, 2007). In my thesis, respective candidate species were delineated based on an integrative taxonomic approach, combining scleritome characters with reciprocal monophyly in phylogenetic analyses based on two mitochondrial genetic markers.

Of the 60 proposed candidate species, two species new to science have been formally described and anatomical data is provided for three additional species (Bergmeier et al., 2017; Ostermair et al., 2018). From the moment a novel solenogaster species is collected, its photo documentation, DNA extraction, complete histological workflow (embedding, sectioning, 3D-reconstruction of anatomy) to a formal species description in the form of a publishable manuscript takes about an estimated 4-5 months (based on discussions with fellow aplacophoran taxonomist Dr. M. Carmen Cobo (University of Alabama) and own experience). Parallel descriptions of congeners can streamline the taxonomic process, but likewise the required time might easily increase if the novel lineages warrant establishing a new entity on higher taxonomic rank, i.e. on genus or family level. Non-invasive imaging techniques like µ-computed tomography (µCT) have been hailed to speed up anatomical investigations (Faulwetter et al., 2013), but key taxonomic features of Solenogastres like radula configuration and cytology of foregut glands cannot be discerned with current staining methods and resolution (Candás et al., 2016; Candás et al., 2018; own trial with nanoCT at LRZ TU München). If assuming an average description time of 4.5 month for each of the not yet described 59 candidate species characterized in Bergmeier et al. (2017); Ostermair et al. (2018); Bergmeier et al. (2019), total time designated to full taxonomic descriptions will be 21 years. Considering molecular work, morphological and histological investigations are already partially completed and several animals can be processed simultaneously, time spent on species descriptions could possibly be reduced to a third of the time. This would still add up to a total of more than 7 years of non-stop taxonomic work. Spending seven consecutive years on the description of 59 solenogaster species might be considered an honorable venture among dedicated taxonomists, but is hardly feasible.

If left “as is”, the 59 candidate species will not be diagnosed or named as required by the International Code of Zoological Nomenclature (“The Code”, ICZN) to be recognized as formal species. Instead they remain under their provisional IDs, characterized based on their respective scleritomes and a maximum of two genetic markers. Not every research questions requires its study object to have a formally recognized name. Publishing provisional candidate species without formal names is by now common practice to deal with the current high rates of species discovery and the increasing backlog of pending formal species descriptions (Pante et al., 2015; DeSalle & Goldstein, 2019). Most of the time these discovered entities are efficiently circumscribed as molecular operational taxonomic units (MOTUs) based on their respective genetic distance (Floyd et al., 2002). MOTUs are broadly used in large-scale environmental assessments and biodiversity studies (see e.g. Logares et al., 2014; De Vargas et al., 2015; Leray & Knowlton, 2015; de Araujo et al., 2018; Morinière et al., 2019) and serve as definable units in the continuous discovery of cryptic species complexes (e.g. Jörger & Schrödl, 2013; Brasier et al., 2016; Nygren et al., 2018). As a result, the amount of MOTUs and “dark taxa”, i.e. “species […] that lack formal names” (Page, 2016) deposited in online repositories like GenBank, GBIF and BOLD (for sequence data) or OBIS (for distribution records) has also been increasing rapidly. Relying on MOTUs can deliver fast and efficient results in confirming (or rejecting) conspecificity, and are especially useful to assess baseline biodiversity or study patterns of diversity and distribution.

However, left in transitional state between discovery and description, MOTUs are at risk of turning volatile: lacking a formally recognized name or even a consistent naming system, it becomes difficult to trace MOTUs across different data bases, platforms, and follow-up publications (Schindel et al., 2010; Minelli, 2017; Minelli, 2019; Minelli, 2020). For purposeful communication about provisional candidate species and to enable future research based on unnamed taxa, best practice agreements on standardized, consistent nomenclature and the deposition of associated raw data (i.e. voucher

specimens) are urgently needed but currently still lacking (Schindel et al., 2010; Guralnick et al., 2015; Morard et al., 2016; Minelli, 2017). Without the context of a formal naming system such as Linnaean nomenclature, these entities are isolated from potentially hundreds of years of taxonomic literature (Minelli, 2020). Additionally, MOTUs cannot be integrated or transcribed into ranks or species under the Linnaean system, and the risk persists of establishing a parallel system of taxonomy and classification (Minelli, 2020). Through the use of MOTUs, the practical challenges currently impeding taxonomic research (i.e. the long process of classifying specimens and providing diagnoses for candidate species) are ultimately just shifted from one system to another.

The necessary connection between molecular-based species discovery and subsequent description can be efficiently provided by DNA taxonomy. Over the last decade, the number of species descriptions based on diagnostic genetic characters (e.g. nucleotides or proteins) has steadily increased (Renner, 2016). DNA taxonomy has been used across a variety of plant and animal species (Renner, 2016 and citations therein), but despite the broad and common application of genetic data to identify and delimit species, it is overall still sparsely used to actually diagnose them (Renner, 2016; Fedosov et al., 2019).

Among gastropod molluscs, DNA taxonomy has been used to diagnose cryptic species (Ornelas-Gatdula et al., 2012; Jörger & Schrödl, 2013; Zielske & Haase, 2015) and also morphologically highly similar congeners (Egger et al., 2020). By identifying diagnostic nucleotides in conjunction with selected external morphological characters, authors ensured that novel species are integrated within the existing classificatory context, and can allocate them into respective families and genera (Jörger & Schrödl, 2013; Zielske & Haase, 2015; Egger et al., 2020). This shows that DNA taxonomy delivers an efficient and rapid approach to species diagnoses and thus formal descriptions in mollusc groups which can be placed within existing genera based on external, easily accessible traits (for example like gastropod shells). However, the majority of Solenogastres unfortunately cannot be assigned reliably to genus level based on external morphology only. In a taxonomic “best-case scenario”, scleritome characters suffice to place the animals within a family. As an example, in the current dataset of novel Northwest Pacific Solenogastres, only 10 out of 60 candidate species (accounting for 17 out of almost 200 individuals) could be identified to genus level based on external scleritome data. Additionally, they mostly represent evolutionary distant entities and are therefore not suitable for straight forward DNA taxonomy (see discussion below).
As a consequence, a differentiated approach combining DNA taxonomy with selected anatomical traits is needed in Solenogastres. The necessary connection to the Linnaean System and therewith the taxonomic history of Solenogastres will - for now - still require time consuming anatomical investigations of numerous candidate species to lay the required groundwork to enhance rapid DNA-based taxonomic research in the future. The following workflow is thus proposed (Figure 4).

Step 1: Documenting external morphology.
Each individual is photo-documented under a dissecting scope or light microscope (when dealing with minute animals) focusing on its scleritome. In some instances, unique scleritome characters or combinations of them (e.g. needle-like sclerites or scales, hollow or solid spicules) might already allow to place morphological entities within existing classifications. Considering that the scleritome is still the most easily accessed of all traditionally used solenogaster traits, sclerite types should be thoroughly documented even if it will not be enough to identify specimens to family level or beyond.
Step 2: Getting the most out of each specimen for integrative taxonomy.

Subsequently, specimens need to be trisectioned to guarantee that all relevant taxonomic characters are extracted from the same individuals (see e.g., Ostermair et al., 2018; Bergmeier et al., 2019; Kocot et al., 2019) as is considered the current taxonomic standard in light of externally cryptic, co-occurring species. The taxonomically uninformative middle region serves for DNA extractions and its cuticle for detailed light microscopic scleritome analyses. Anterior and posterior parts contain important anatomical characters (i.e. radula type, foregut glands, reproductive system) required for traditional taxonomy and species descriptions and should thus be stored for potential "deep taxonomy" (i.e. microanatomical investigations through histological serial sectioning, supplemented by scleritome and genetics) in the future. If individuals are too small to be confidently trisectioned (often the case when dealing with tiny interstitial Solenogastres), the posterior half should be used for DNA extractions and the anterior half saved for anatomical investigations. Based on sclerites, radula and foregut glands identification of specimens to genus level should be possible, potentially even on species level.

Alternatively, entire minute animals can be investigated via scanning electron microscopy and subsequently used for DNA extractions (Bergmeier et al., 2016b). When using whole mounted individuals, some scleritome details will be inevitably missed during investigations. Sclerite info derived from SEM micrographs thus usually suffices to identify specimens to order level only, but in few cases with unique scleritomes down to family level (e.g. nail-shaped sclerites as only present in Macellomeniidae Salvini-Plawen, 1978).

Step 3: Inferring the backbone phylogeny from genetic markers.
A phylogenetic guide tree is needed to 1) identify taxonomic units for subsequent approaches of molecular species delineation and extraction of molecular diagnostic characters and 2) to identify potential candidate species for deep taxonomic workflow.

Solenogaster-specific primer pairs for partial mitochondrial 16S rRNA (Bergmeier et al., 2017) and COI (courtesy of Kevin M. Kocot, University of Alabama) have considerably enhanced PCR amplification success rates when compared to generic universal primer pairs. After amplification, alignments of each single marker and a concatenated dataset should be tested for their respective best fitting nucleotide substitution model (e.g. using JModelTest2 (Darriba et al., 2012)). Based on Maximum Likelihood or Bayesian Analyses, phylogenetic relationships are inferred from alignments and compared for inconsistencies between single and paired gene trees to check for deviations through incomplete lineage sorting. Solenogaster classification currently does not reflect the evolutionary history of the clade and the inferred phylogenetic hypothesis is expected to lack statistical support on many deeper nodes. However, with regard to the central aim of identifying recent speciation events resulting in cluster of closely related lineages, the lack of support is in this case negligible.

Step 4: Combining morphological and molecular data into a primary species hypotheses to differentiate between candidate species for rapid vs. deep taxonomical approaches
In plotting morphological data onto the molecular phylogeny, two lines of evidence are combined: reciprocal monophyly in conjunction with scleritome traits leads to the establishment of a primary species hypotheses. Candidate clades are now selected for rapid taxonomy (continuing with Step 5) if 1) available character sets permit assignment to genus level and 2) comparative molecular data of closely related lineages is available, i.e. relatively broad taxon sampling for the respective genus.

Consequently, deep taxonomy is indispensable in cases where candidate species 1) cannot be identified to genus level based on generated morphological and molecular data and/ or 2) represent evolutionary distant lineages within the dataset. Clades and singletons thus resulting in long branches in the inferred phylogeny are not suitable for subsequent molecular-based species delineation and formal species diagnosis based on nucleotides. Mining these sequences for diagnostic nucleotides carries the risk of falsely claiming homoplasies for diagnostic DNA characters. To include these specimens in the subsequent workflow (Step 5 to 6), either broader taxon sampling covering closer related lineages is required, or additional molecular markers with more conserved mutation rates need to be amplified (see Outlook). Until then, these candidate species should only be diagnosed and formally named via traditional taxonomy, as morphological and anatomical characters form an established set of characters to evaluate putative apomorphies.

Step 5: Rapid taxonomy I - molecular species delineation of target clades and formulation of secondary species hypotheses.

Monophyletic clusters which have been previously identified as suitable for rapid taxonomy are analyzed using several independent methods of molecular-based species delineation. Distance/ similarity-based approaches (e.g. ABGD (Puillandre et al., 2012)) should be combined with model-based tree and coalescence approaches (Bayesian inference, GMYC (Monaghan et al., 2009)) as further support for independently evolving lineages, i.e. molecular candidate species. These approaches can be further supplemented by haplotype networks based on statistical parsimony (Clement et al., 2000) to estimate species boundaries. Resulting separate networks are taken as further support for the presence of independently evolving lineages. Candidate species established under this secondary species hypothesis can now be diagnosed and named.

Step 6: Rapid taxonomy II - species diagnosis through molecular characters and subsequent formal description.

Like traditional morphological characters, molecular diagnostic characters are discrete apomorphic traits, differentiating the target species from closely related lineages (Jörger & Schrödl, 2013; Merckelbach & Borges, 2020). Diagnostic nucleotides can be extracted from both mitochondrial markers using the currently available softwares and packages e.g. FASTACHAR (Merckelbach & Borges, 2020), DeSignate (Hütter et al., 2020) or QUIDDICH (Kühn & Haase, 2020). Formal species names receive a unique life science identifier through registration in ZooBank (http://zoobank.org/) and adequate type material is designated (e.g. DNA type) and deposited in a natural history collection.

Step 7: Deep taxonomy and species descriptions.

Time-consuming microanatomical investigations are proposed to primarily focus on lineages which cannot be unambiguously assigned on genus-level based on available morphological characters, or form long branches in the phylogenetic guide tree and thereby constitute evolutionary distant lineages. Obviously, additional candidate species might be selected for deep taxonomy to mine for special anatomical adaptations even among genetically closely related lineages due to inherent interest (i.e. because of a specific habitat, biological association or presumed ecological role).

At least one (ideally mature) individual of each monophyletic cluster needs to undergo “deep taxonomy”, i.e. needs to be embedded, serial sectioned and investigated for the relevant taxonomic characters (radula type, type of digestive glands, and configuration of the reproductive system). Subsequent diagnoses should focus on traits distinguishing the candidate species from its congeners.

Following the detailed investigations of one selected candidate species of a target cluster and its generic placement, the remaining candidate species can be diagnosed based on genetic data and placed within the same nominal genus, thereby satisfying the notion of genus rank foremost reflecting close relations of its subsumed species. Formally described species are registered in ZooBank, type material is designated and deposited accordingly.

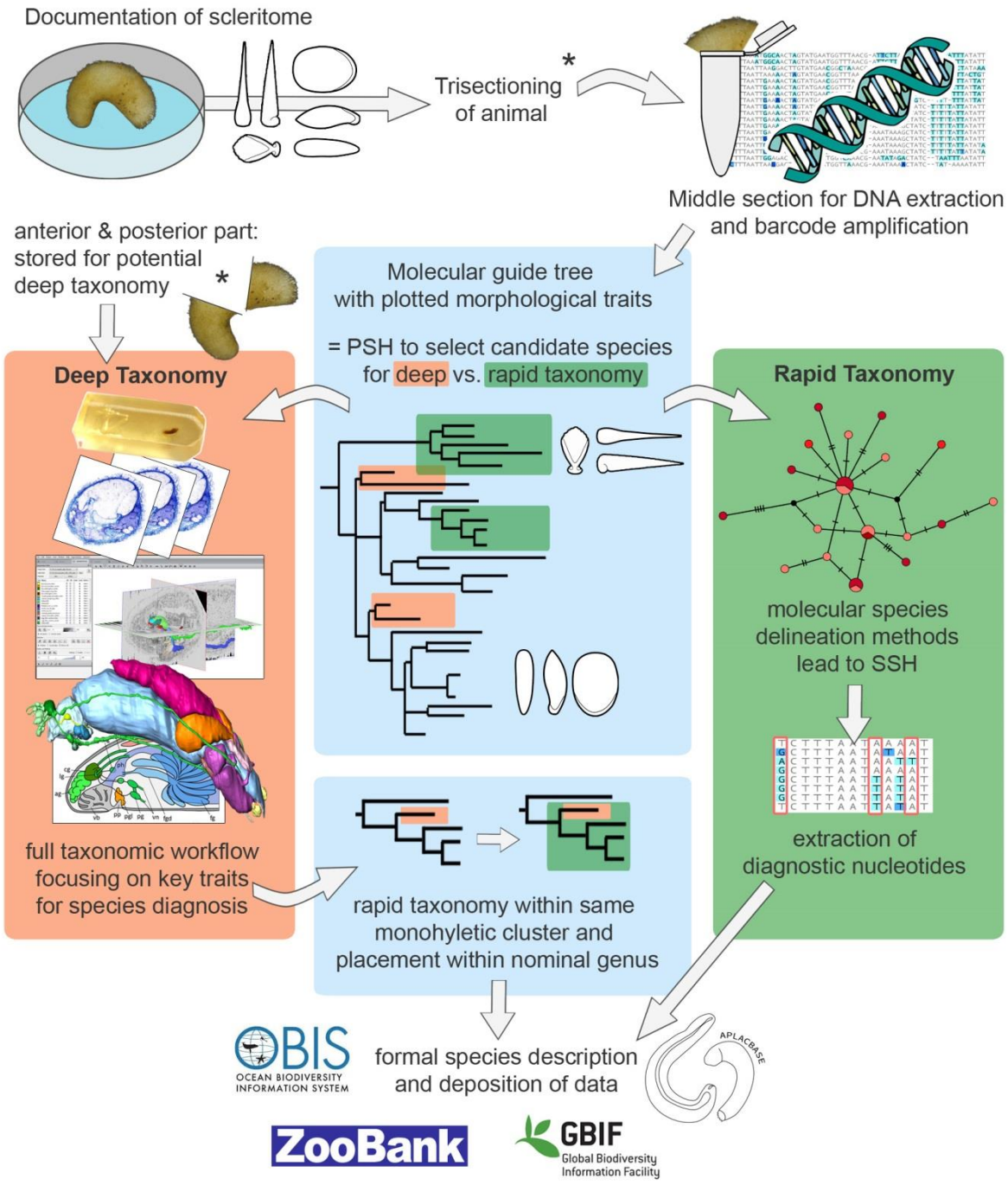

Figure 4. Visualization of the proposed workflow of deep and rapid DNA taxonomy in Solenogastres. Abbreviations: PS, Primary Species Hypotheses; SSH, Secondary Species Hypotheses.

4. Conclusion and Outlook

The proposed combinatory workflow of deep and rapid taxonomy is currently residing on the side of time-consuming deep taxonomy and will do so for some time to come. A broad application of the proposed rapid taxonomic workflow is hampered by the gaps in baseline data resulting from the sheer magnitude of still undiscovered diversity as well as the lack of genetic data from "historical" species (i.e. species described prior to 2014). For now, molecular phylogenies of Solenogastres based on few fast-evolving mitochondrial gene segments will reflect these gaps through long branches and uncertain phylogenetic placement of evolutionary distant lineages, impeding rapid DNA taxonomy based on these unstable guide trees.

Targeted sampling initiatives and large-scale biodiversity studies across geographical regions and depth zones as in the deep Northwest Pacific will contribute to gradually shedding light on still largely undiscovered diversity of Solenogastres. The connection to the already established "historical" diversity of Solenogastres can be formed via so called 'Museomics' approaches (i.e. genetic data generated from original type material in Natural History Collections). Modern high throughput sequencing technology promises to deal with the usually highly degraded DNA of old museums material (Tin et al., 2014; Derkarabetian et al., 2019; Colella et al., 2020). Most Solenogastres type material is currently long-time stored in Ethanol generally suitable for the preservation of DNA (but the entire fixation history is in many cases unknown). The success of extracting DNA from old mollusc museum material highly depends on 1) the amount of input tissue for DNA extraction and 2) the quality of the storage medium, which is often unknown (own unpublished data). Current trials using the universal probe set designed by Moles and Giribet (2021) to target ultraconserved elements from "fresh" solenogaster DNA will hopefully pave the way to generate a specific probe set based on solenogaster mitochondrial genomes. Further test series and development of highly sensitive target capture approaches are needed in the future.

In conjunction with broader taxon sampling to stabilize the guide tree, an increase in molecular markers will improve resolution and reliability of the phylogenetic tree (and thus primary species hypotheses), subsequent molecular species delineation approaches and the resulting secondary species hypotheses. Large-scale phylogenomic analyses by Kocot et al. (2019) suggest higher taxonomic classification of Solenogastres (i.e. order level) requires revisions, an implication reflected also on lower taxonomic level through phylogenetic analysis with broader taxon coverage but with low resolution (Bergmeier et al., 2019). As standard Sanger-sequencing of conservative nuclear markers is hampered in Solenogastres (see Okusu & Giribet, 2003; Meyer et al., 2010) high throughput sequencing of mitochondrial genomes is an efficient alternative to obtain additional markers suitable to reliably reflect at least recent splits within their phylogeny, the prerequisite for rapid taxonomy and to link novel species data based on barcoding markers to large scale transcriptomic datasets as in Kocot et al. (2019). Based on genome-scale data of one Solenogaster, we extracted the first complete mitochondrial genome of this class of molluscs (see Fig. 5). Comparative mitogenomics across the phylum Mollusca have been unsuitable to resolve deep molluscan relationships due to effects of long branch attraction and supposed convergent evolution of gene arrangements (Stöger & Schrödl, 2013; Stöger et al., 2016). However, within denser taxon samplings they are highly promising to infer interclass relationships (see e.g. Uribe et al., 2017; Mikkelsen et al., 2018; Kong et al., 2020; Guo et al., 2021; and own unpublished data on (partial) mitochondrial genomes of three solenogaster species).

Once a stable backbone phylogeny combining available datasets is available, fewer markers will hopefully suffice to reliably place new taxa within the existing framework. To speed up the shift towards a future of rapid and partially automated identification and description of Solenogastres, a joint effort of the community of aplacophoran researchers is required.

The online database AplacBase was established by the international community of aplacophoran researchers and is currently built as an online repository and initial identification tool surrounding aplacophoran research. In the future, AplacBase is intended to additionally serve as a bioinformatics platform for rapid, automated taxonomy based on input sequence data. AplacBase Taxonomy Tool shall provide an initial identification based on query sequences (if possible) and support decision-making between rapid species descriptions and deep taxonomy. Facilitating identification and description will open Aplacophorans to a wider range of researchers. Hopefully, AplacBase will also contribute towards communicating about the wonders of these hardly known molluscs outside of the scientific community.

Figure 5. Unpublished annotated mitochondrial genome (15,347 bp) of an Antarctic Pruvotinidae (3.5 mm body length) showing coding sequences and rRNA (created with Geneious 2021.0).

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7. Appendices

7.1 Congress Contributions

The following section contains abstracts of talks and copies of first-author posters that directly resulted from my thesis (ordered chronologically). A full list of all congress contributions in which I was involved is available in my Curriculum Vitae (see 7.4).

Talks

2015 – 14th Deep-Sea Biology Symposium, Aveiro (Portugal):

Disparate curiosities: an integrative approach to the diversity of abyssal Solenogastres in the Kuril-Kamchatka region

Bergmeier FS, Schwabe E, Brandt A, Jörger KM

Solenogastres (Neomeniomorpha) are a clade of marine, aplacophoran worm-like molluscs. They are globally distributed and the majority of the close to 300 described species occurs below the shelf area. Due to their difficult taxonomy requiring amongst others time-consuming histological analyses, they present a group largely neglected in biodiversity and biogeography studies of deep-sea regions. During the KuramBio (Kuril-Kamchatka Biodiversity Study) cruise on board of the R/V Sonne in 2012, the Kuril-Kamchatka trench and the adjacent abyssal plain were explored using standardized sampling gear. At all twelve stations, a camera-equipped epibenthic sledge retrieved aplacophoran molluscs from abyssal soft clay sediments (4830-5780 m). The aplacophoran diversity within these samples proved to be surprisingly species rich, albeit with relatively low numbers of individuals including several lineages only represented by singletons. Following the preliminary identification of fourteen different Solenogastres morphospecies based on light microscopy, we used an integrative approach to thoroughly delineate the collected specimens. Firstly, details of the scleritome were reinvestigated via rotational scanning electron microscopy. Additionally, we provide microanatomical information based on semithin histological serial-sections and comparatively visualize all major organ systems of the main lineages via computer-based 3D-reconstructions (e.g., nervous system, digestive tract and gonopericardial system). To enhance the accessibility of aplacophoran molluscs for biodiversity and biogeographical studies in the future, we amplified standard DNA barcodes linked to our in-depth morphological analyses. Finally, the neomeniomorph diversity of the Kuril-Kamchatka region is comparatively discussed with findings from the 2010 SoJaBio expedition to the more isolated Sea of Japan and first preliminary results from a recent expedition to the Sea of Okhotsk in 2015. Prior to this study, only three neomeniomorph species have been reported from the Far Eastern seas. Our first records of abyssal Solenogastres from the Kuril-Kamchatka region do not only by far exceed this locally known diversity, but also nearly double the global number of neomeniomorph species described from abyssal depths.

2016 – World Congress of Malacology (Penang, Malaysia):

All quiet on the eastern front? Diversity and Biogeography of deep-sea Solenogastres from the Sea of Okhotsk

Bergmeier FS, Ostermair L, Schwabe E, Brandt A, Haszprunar G, Jörger KM

Solenogastres are a small and understudied clade of worm-shaped molluscs occurring mainly in deep waters. Many species are described from single localities only and their bathymetric and geographic distribution ranges are poorly explored. To our knowledge, there are currently only nine records of Solenogastres from the Far Eastern Seas, with eight of them dating back to the early 20th century. As part of a large scale project aimed at understanding biodiversity and biogeographic patterns in the region of the Northwest Pacific, the 'Sea of Okhotsk Biodiversity Studies' expedition set out in 2015 to explore the deep-sea benthos of the Sea of Okhotsk (Russia). During this cruise, we collected around 100 entire specimens (and additionally several partial and damaged individuals) of Solenogastres from the bathyal Kuril basin at eight stations from 1696 m to 3377 m depth. Preliminary investigations revealed 16 morphospecies, including one lineage with a relatively common occurrence at six stations. Using a combination of morphological methods (i.e., light and scanning electron microscopy as well as microanatomical 3D-reconstructions) and molecular barcoding, we present a first analysis of the hidden molecular and morphological diversity of Solenogastres found in the Sea of Okhotsk. We compare our results with the solenogaster diversity of the Kuril-Kamchatka trench and its adjacent abyssal plains (sampled during the 'Kuril-Kamchatka Biodiversity Studies' KuramBio-Cruise), which are connected with the semi-enclosed Kuril basin via two bathyal straits, the Bussol and the Krusenstern Strait. During the KuramBio-Cruise 35 solenogaster specimens were collected from 4830 m to 5780 m, which represent diverse lineages. Our integrative taxonomic approach not just leads to an immense boost in the hitherto very restricted diversity of Solenogastres in the Far Eastern Seas, but it also significantly raises the global number of Solenogastres from these depths of the world's oceans.

2016 – Annual Meeting of the Network of Biological Systematics Austria (Linz, Austria): Invited Talk after winning the 2016 NOBIS DNA Sequencing Grant

Barcoding the unknown: deep-sea Solenogastres (Mollusca) from the Sea of Okhotsk (Russia)

Bergmeier FS, Ostermair L, Brandt A, Haszprunar G, Jörger KM

The 'SokhoBio Expedition' set out in 2015 to explore the deep-sea benthos of the Sea of Okhotsk (Russia, Northwest Pacific). During the cruise, the bathyal Kuril Basin of the Sea of Okhotsk was sampled at twelve different stations using a variety of gear. Among the samples were more than 100 specimens of Solenogastres. This small clade of aplacophoran worm-molluscs forms an important component of the deep-sea fauna, and could contribute to a better understanding of the biological interactions and distributional patterns in deep-sea organisms. However, Solenogastres often remain unidentified due to their time-consuming and challenging taxonomy, requiring a complex set of morphological and anatomical characters. In an effort to elucidate solenogaster diversity in the Kuril Basin, we pursued an integrative taxonomic approach combining molecular barcoding with imaging methods (e.g., light and scanning electron microscopy, anatomical 3D-reconstructions derived from histological serial sections). Due to the low amplification success with standard barcoding primers, we designed and established a set of solenogaster specific 16S rRNA primers. The obtained combination of characters allows for robust species delineation and a characterization of the species diversity found in bathyal depths of the Sea of Okhotsk. We compare our results with the solenogaster diversity of the Kuril-Kamchatka trench and its adjacent abyssal plain (sampled during the 'Kuril-Kamchatka Biodiversity Studies - KuramBio Expedition'), which are connected with the semi-enclosed Kuril Basin via two bathyal straits. The Solenogastres collected during this cruise represent diverse lineages, however with little faunal overlap in comparison to the Sea of Okhotsk. Our integrative taxonomic approach leads to an immense boost in the hitherto little explored diversity of Solenogastres in the Far Eastern Seas. Additionally, the obtained barcodes can facilitate future identification of Solenogastres and hopefully help transform this neglected taxon into a clade more accessible for biogeographical and ecological studies.

2018 – 15th Deep-Sea Biology Symposium (Monterey Bay, California, USA)

Bathyal slope to hadal trench: diversity and biogeography of Solenogastres (Mollusca) in the Northwest Pacific

Bergmeier FS, Kohnert P, Brandt A, Jörger KM

Several recent joint German-Russian research cruises investigated the deep-sea benthos in the Northwest Pacific with the aim to identify biogeographic links or isolating factors between the semi-enclosed Sea of Okhotsk and the open Pacific. Solenogastres are a clade of shell-less, worm shaped molluscs and a frequent albeit not overly abundant component of the collected deep-sea fauna. Due to their inaccessibility and complex taxonomy, Solenogastres are generally understudied and underestimated – the latter at least in regard to their diversity, which is estimated to be at least ten-fold above the currently known 300 species. Many of these species are based on single findings and as a consequence vertical and horizontal distribution ranges of the group are poorly explored.

We studied approximately 150 specimens of Solenogastres, collected from the bathyal slope and basin of the Sea of Okhotsk, the abyssal plain of the Northwest Pacific, and hadal depths of the Kuril-Kamchatka Trench. We used an integrative taxonomic approach combining multiple mitochondrial markers, 3D-microanatomy based on histology, and scanning electron microscopy to delimitate different lineages and investigate their bathymetric and biogeographic distribution ranges.

In total, we discovered more than 40 species in the region, largely new to science and by far exceeding the previously known solenogaster diversity in the area. Solenogastres have rarely been reported from hadal depths, but we discovered several lineages at 9,500 m. Overall there is a unique solenogaster fauna in each of the sampled regions with few lineages present on both sides of the Kuril-Kamchatka Trench, which we think might act as a barrier to their dispersal. This is the first comprehensive study evaluating phylogenetic relationships, population genetic patterns and distribution ranges in Solenogastres on a regional scale. It provides a reliable barcoding library for easier identification in future faunistic surveys and therein a first baseline for beta-biodiversity comparisons.

Posters

Bergmeier FS, Haszprunar G, Todt C & Jörger KM (2014) **Comparative 3D-microanatomy of mesopsammic Meiomeniidae (Pholidoskepia, Solenogastres)**. 3rd International Congress on Invertebrate Morphology – ICIM 3 (Berlin, Germany).

Comparative 3D-microanatomy of mesopsammic Meiomeniidae (Pholidoskepia, Solenogastres)

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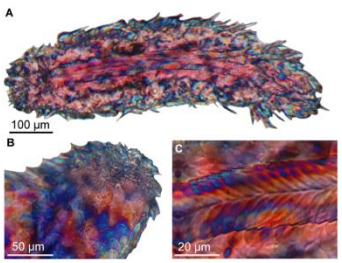


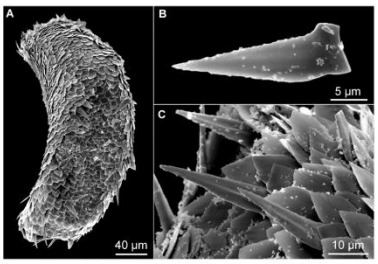
Light microscopic images using bipolarized filters of a specimen identified as *Meioherpia atlantica*. A. Ventral view. B. Head region. C. Scales surrounding the ventral pedal groove.

Background

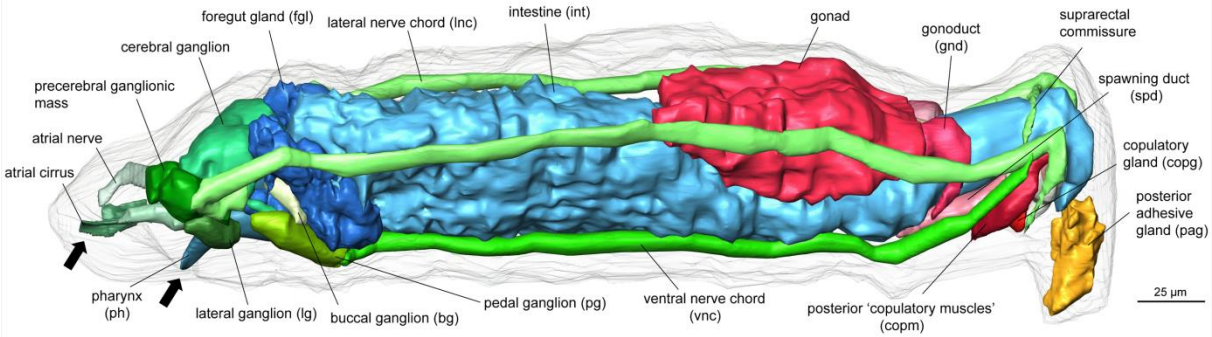
Most Solenogastres (Neomeniomorpha) - a globally distributed clade of vermiform mollusks - inhabit bathyal and abyssal zones and probably lead an epibenthic lifestyle. Meiomeniidae Salvini-Plawen, 1985 is a small clade of pholidoskepid Solenogastres which inhabits the shallow subtidal mesopsammon in temperate and tropical zones. Three of its four valid species (*Meioherpia stygalis*, *Meioherpia atlantica* and *Meiomenia arenicola*) are described from Bermuda, where they form a locally common part of the interstitial meiofauna. Original descriptions of these tiny mollusks are largely limited to hard structures (i.e., scleritome and radula), whereas the anatomical diversity is poorly explored.

Objective

We aim to provide a microanatomical characterisation of all known Bermudan meiomeniid lineages for a better understanding of the diversity of this unique clade.



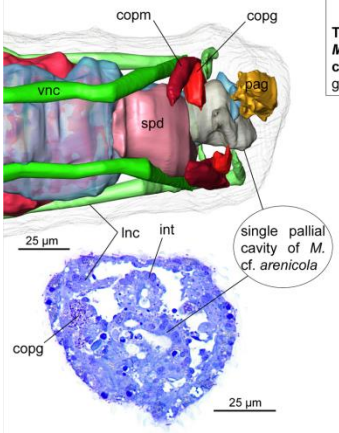
Scanning electron microscopic image of *Meioherpia* sp. A. Overview of the scleritome. B. Dorsal body scale. C. Lateral projecting lanceolate scale and leaf shaped body scales.

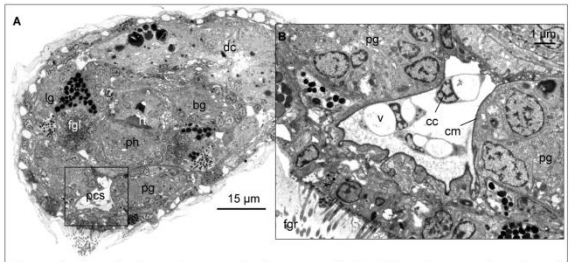


3D reconstruction using Amira® 5.3.3 based on a series of semi-thin sections of a meiomeniid identified as *Meiomenia* cf. *arenicola*. All investigated specimens were characterized by the presence of separated mouth and atrial openings (see arrows). **Green** nervous system, **blue** digestive system, **red** reproductive system.

The typical **tetraneural nervous system** comprises the fused cerebral ganglion and the paired pedal, buccal and lateral ganglia. Remarkable is the unpaired 'pedal commissural sac'.

The **digestive system** is formed by paired foregut glands opening into the pharynx on both sides of the distichous radula. The intestine is a straight tube terminating in the pallial cavity.

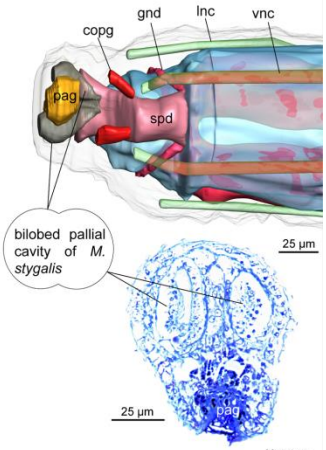




Transmission electron micrographs (cross-section) of the pharyngeal region of *Meioherpia atlantica*. A. Overview. B. Close-up of the pedal commissural sac (pcs). **cc** central cell, **cm** central membrane, **dc** dorsal caecum of the intestine, **fgr** foot groove, **rt** radula tooth, **v** vacuole.

The **reproductive system** comprises tubular, paired gonads each connected by a gonoduct to the single spawning duct. Neither heart nor pericard could be detected on histological sections.

The only clear microanatomical distinction between the examined meiomeniid species relates to a single versus bilobed **pallial cavity**.



Conclusions and Outlook

- Comparison of the original descriptions revealed little interspecific variation across meiomeniid scleritomes which makes reliable identification of newly collected material and species delineation of Meiomeniidae solely based on external morphology highly problematic.
- Comparative 3D microanatomy of putative species herein showed morphological uniformity across all major organ systems. A valuable diagnostic character could be the organisation of the pallial cavity.
- The co-occurrence of externally uniform meiomeniids whose type material is inappropriately fixed for microanatomy make taxonomic assignment of recollected material impossible - at present.
- Hopefully additional character sets (e.g., molecular barcoding markers) will allow to link new material to the respective type material in the future.

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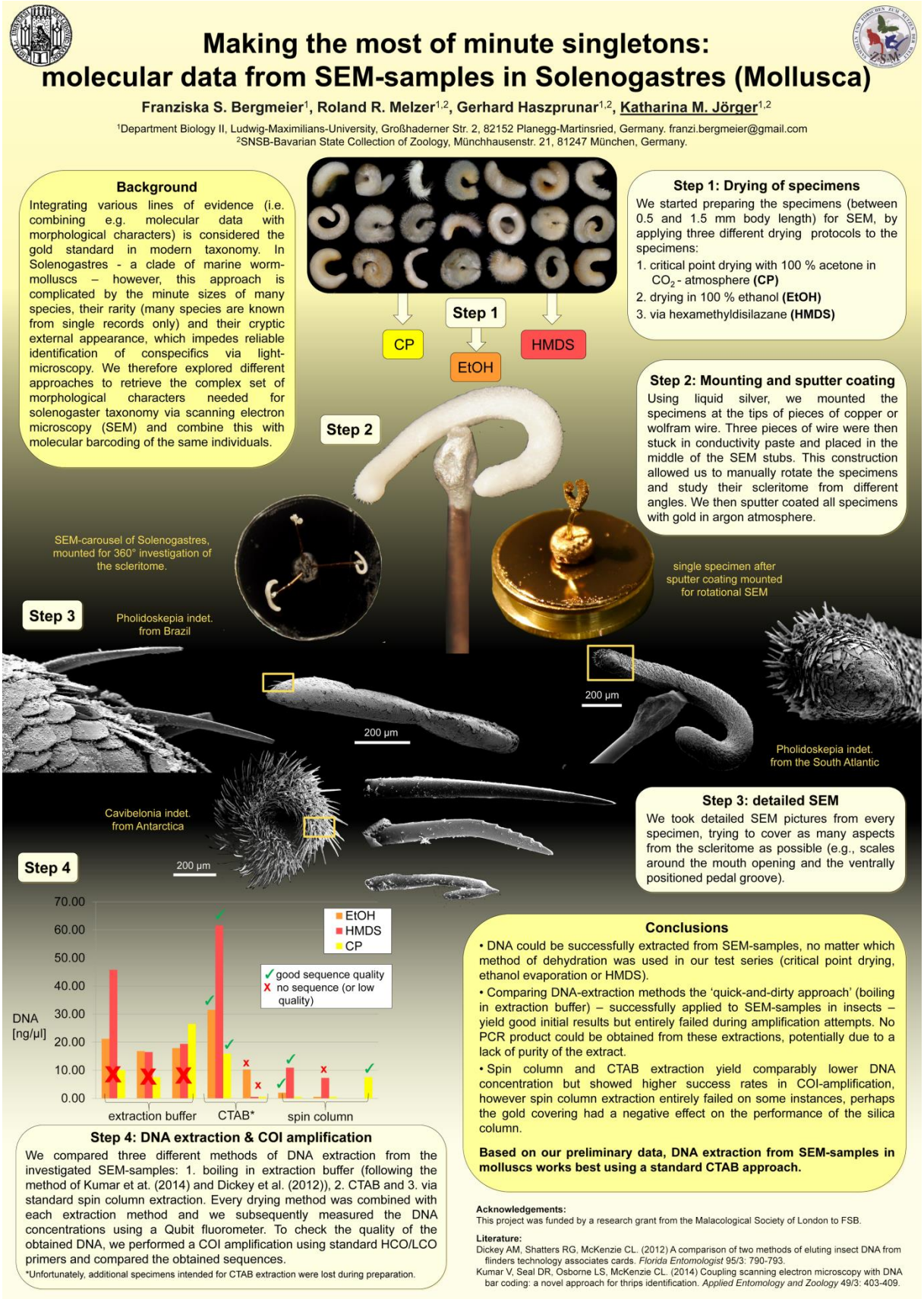
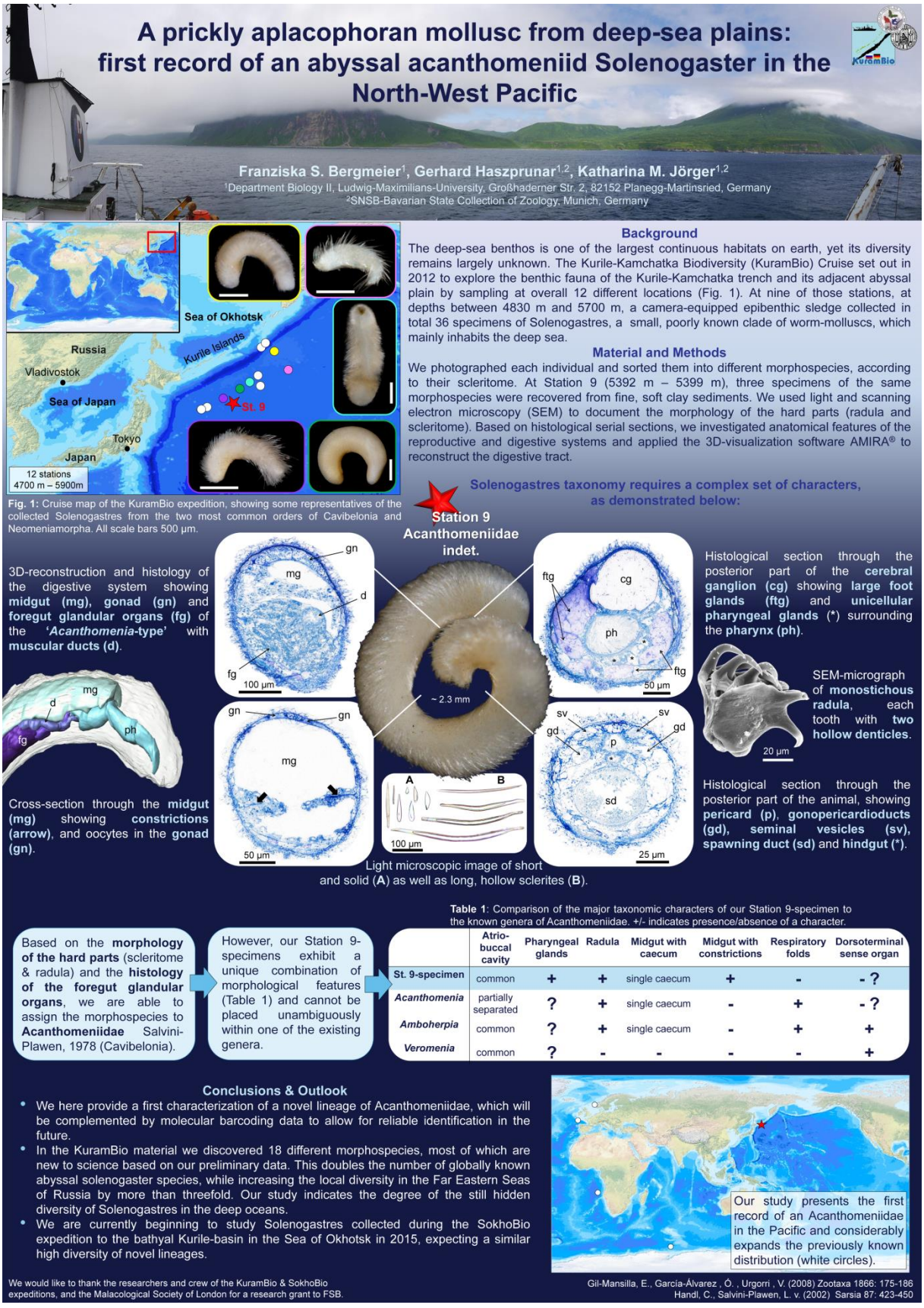
Dr. Joachim Haug (LMU) is thanked for an introduction to birefringence microscopy and Alenka Kerin (LMU) for support with the TEM images.

Literature
Salvini-Plawen, Luitfried. "New interstitial solenogastres (Mollusca)." *Stygologia* 1.1 (1985). 101-108.

Bergmeier FS, Melzer R, Haszprunar G & Jörger KM (2015) **Making the most out of minute singletons: molecular data from SEM-samples in Solenogastres (Mollusca)**. 108th Jahrestagung der Deutschen Zoologischen Gesellschaft – DZG (Graz, Austria).

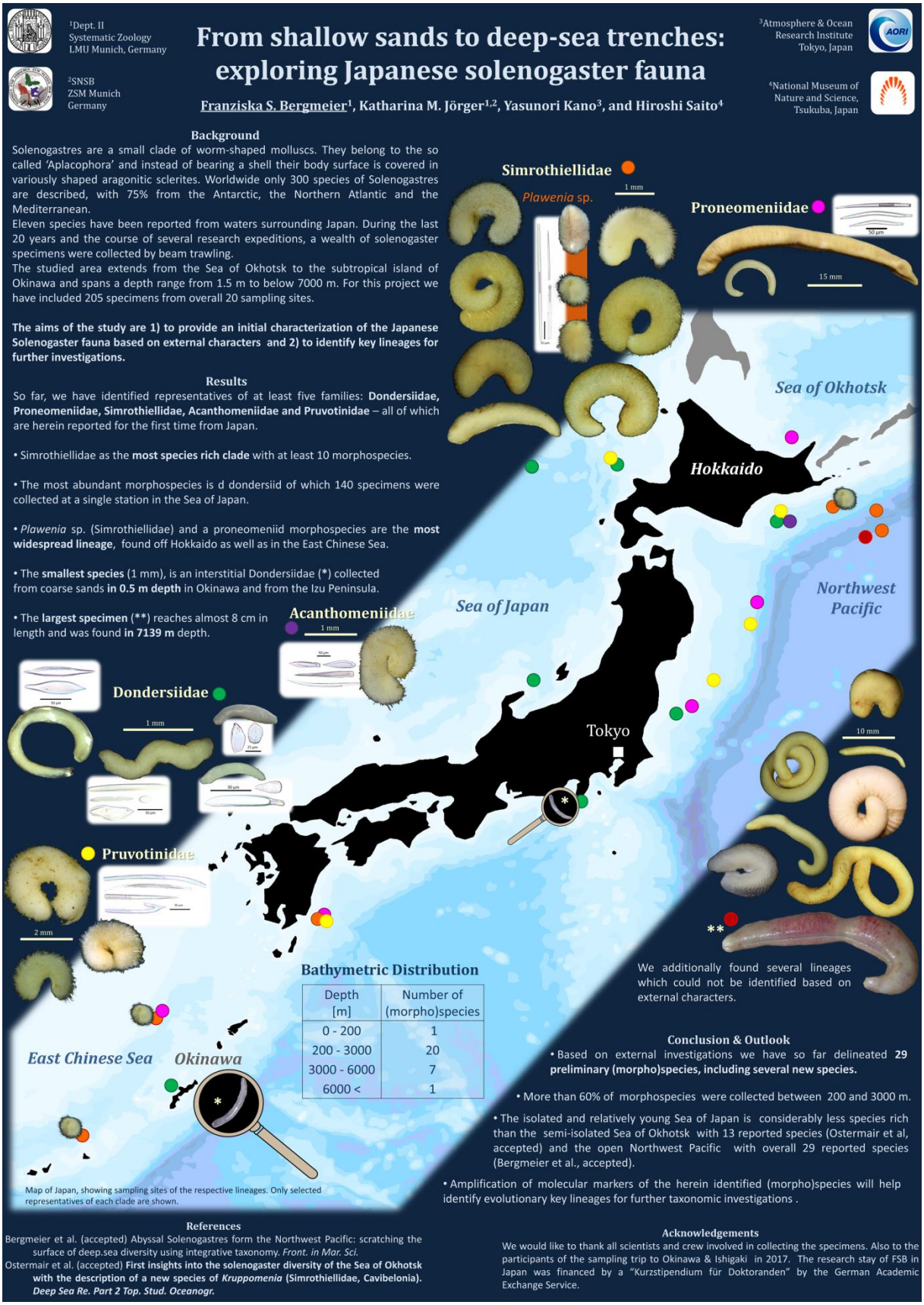


Bergmeier FS, Haszprunar G & Jörger KM (2016) **A prickly aplacophoran mollusc from deep-sea plains: first record of an abyssal acanthomeniid Solenogaster in the North-West Pacific**. 17th Jahrestagung der Gesellschaft für Biologische Systematik – GfBS. (Munich, Germany).

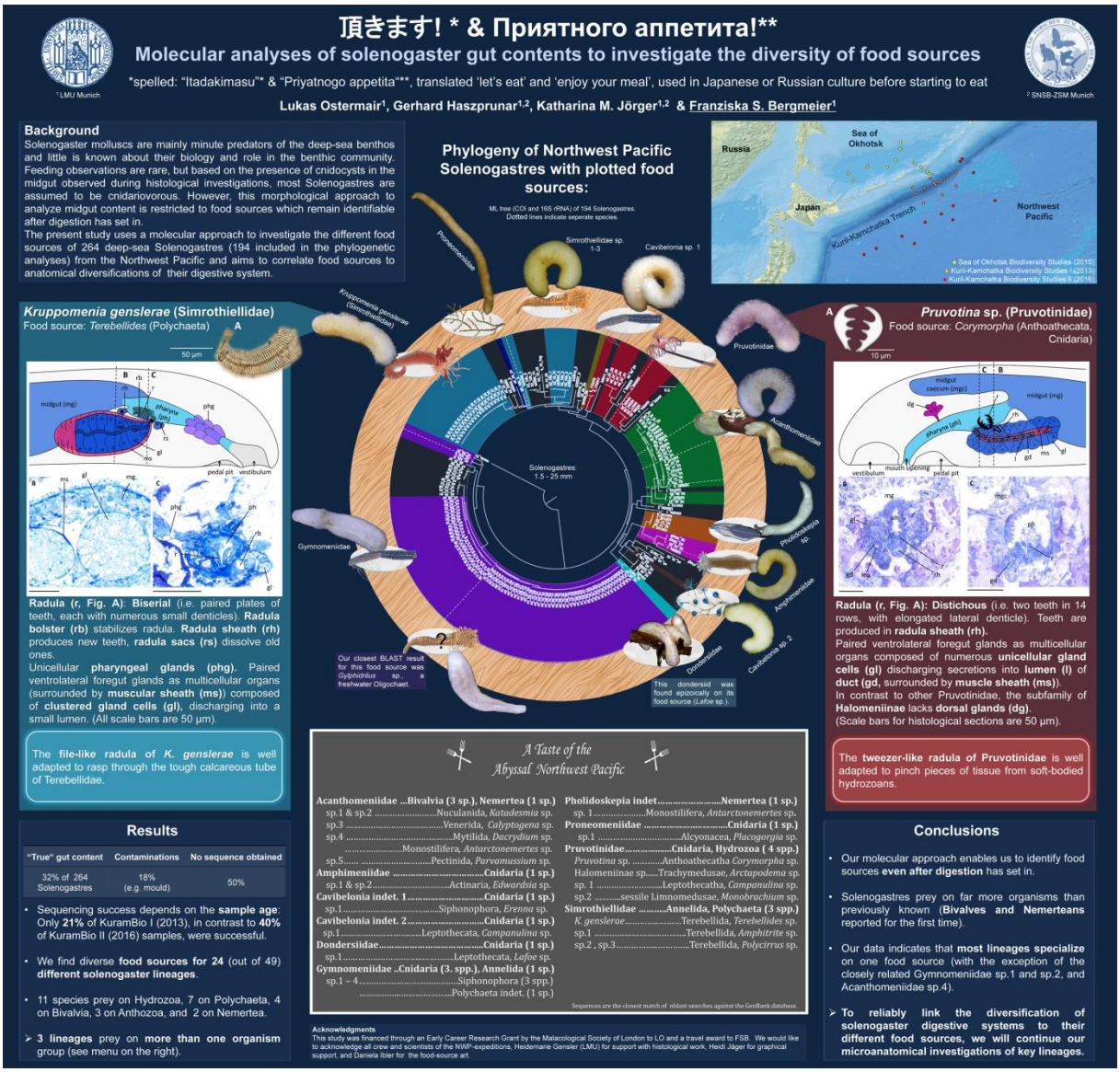


	Atrio-buccal cavity	Pharyngeal glands	Radula	Midgut with caecum	Midgut with constrictions	Respiratory folds	Dorsoterminal sense organ
St. 9-specimen	common	+	+	single caecum	+	-	- ?
Acanthomenia	partially separated	?	+	single caecum	-	+	- ?
Amboherpia	common	?	+	single caecum	-	+	+
Veromenia	common	?	-	-	-	-	+

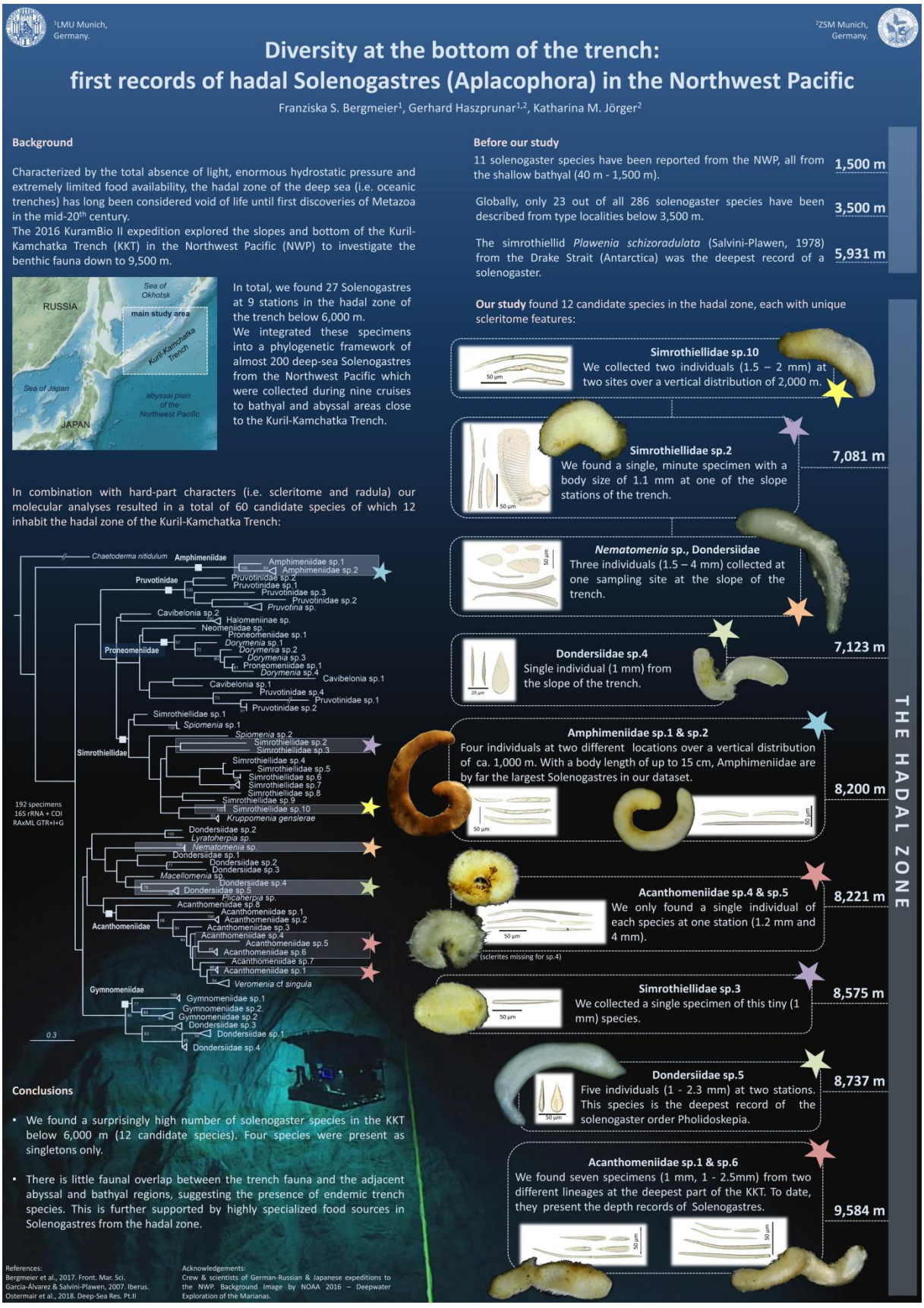
Bergmeier FS, Jörger KM, Kano Y, Saito H (2017) **From shallow sands to deep-sea trenches: exploring Japanese solenogaster fauna**. Molluscan Forum (London, Great Britain).



Ostermair L, Haszprunar G, Jörger KM & **Bergmeier FS** (2018) **頂きます! * & Приятного аппетита!** Molecular analyses of solenogaster gut contents to investigate the diversity of food sources.** 16th Deep Sea Biology Symposium – DSBS (Monterey bay, California, USA).



Bergmeier FS, Haszprunar G & Jörger KM (2019) **Diversity at the bottom of the trench: first records of hadal Solenogastres (Aplacophora) in the Northwest Pacific.** World Congress of Malacology – WCM (Asilomar, California, USA).



7.2 Statement of own contributions to each peer-reviewed publication

Chapter 1. Bergmeier FS, Haszprunar G, Todt C & Jörger KM (2016) Lost in a taxonomic Bermuda Triangle: comparative 3D-microanatomy of cryptic mesopsammic Solenogastres (Mollusca). *Organisms Diversity & Evolution*, 16: 613-639.

I performed morphological (SEM) anatomical investigations (embedding, sectioning, 3D- reconstruction), designed and created figures and tables, drafted and wrote the manuscript.

Chapter 2. Klink, P, **Bergmeier FS**, Neusser TP & Jörger KM (2015) Stranded on a lonely island: description of *Dondersia* (?) *todtae* sp. nov., the first shelf solenogaster (Mollusca, Aplacophora) from the Azores. *Açoreana,* 10(4): 603-618.

I assisted during morphological (SEM) and anatomical investigations (embedding, sectioning, 3D-reconstruction) and contributed to the draft of the manuscript as well as its final version.

Chapter 3. Neusser TP, **Bergmeier FS**, Brenzinger B, Kohnert P, Egger C, Yap-Chiongco MK, Kocot K, Schrödl M & Jörger KM. Shallow-water interstitial Malacofauna of the Azores. *Açoreana* (in press): 1-23.

I was involved in collecting the studied animals, have written the parts of the manuscript on solenogaster fauna and designed and created the respective figures (Figures 1, 2, 3). I have contributed to the overall draft of the manuscript and its final version.

Chapter 4. Bergmeier FS & Jörger KM (2020). Aplacophoran molluscs: Solenogastres and Caudofoveata. In A Schmidt-Rhaesa (Ed.), *Guide to the Identification of Marine Meiofauna* (pp. 308-320). München: Verlag Dr. Friedrich Pfeil (non-peer reviewed)

I contributed to the designed and creation of the figures, wrote the draft and final version of this book chapter.

Chapter 5. Bergmeier FS, Melzer R, Haszprunar G & Jörger KM (2016) Getting the most out of minute singletons: molecular data from SEM-samples in Solenogastres (Mollusca). *The Malacologist*, 66: 23-25 (non peer-reviewed).

I designed the experimental set-up, conducted the investigations, designed and created the figures and wrote the draft and final version of this methodological research report

Chapter 6. Bergmeier FS, Brandt A., Schwabe E & Jörger KM (2017) Abyssal Solenogastres (Mollusca, Aplacophora) from the Northwest Pacific: Scratching the Surface of Deep-Sea Diversity Using Integrative Taxonomy. *Frontiers in Marine Science*, 4, 410: 1-22.

I performed morphological and anatomical investigations, conducted molecular lab work (DNA extraction, amplification) and phylogenetic analyses, designed and created figures and tables, drafted and wrote the manuscript.

Chapter 7. Ostermair L, Brandt A, Haszprunar G, Jörger KM & **Bergmeier FS** (2018) First insights into the solenogaster diversity of the Sea of Okhotsk with the description of a new species of *Kruppomenia* (Simrothiellidae, Cavibelonia). *Deep sea Research Part II*: *Topical Studies in Oceanography*, 154: 214-229.

I participated in the SokhoBio expedition to collect deep-Sea Solenogastres from the Sea of Okhotsk, supervised and instructed morphological and anatomical investigations, conducted molecular lab work and phylogenetic analyses and contributed to the draft and final version of the manuscript.

Chapter 8. Bergmeier FS, Haszprunar G, Brandt A, Saito H, Kano Y & Jörger KM (2019) Of basins, plains, and trenches: Systematics and distribution of Solenogastres (Mollusca, Aplacophora) in the Northwest Pacific. *Progress in Oceanography*, 178, 102187: 1-17.

I performed morphological and anatomical investigations, conducted molecular lab work (DNA extraction, amplification) and phylogenetic analyses, designed and created figures and tables, drafted and wrote the manuscript.

Chapter 9. Bergmeier FS & Jörger KM (2020) Solenogastres: Diversity and distribution of Solenogastres (Mollusca) along the NW Pacific. In H Saeedi & A Brandt (Eds.), Biogeographic Atlas of the Deep NW Pacific Fauna (pp. 115-123) Sofia: Pensoft Publishers.

I provided solenogaster distribution data and wrote the draft and final version of this book chapter.

Chapter 10. Bergmeier FS, Ostermair L & Jörger KM. Specialized predation by deep-sea Solenogastres revealed by gut-content sequencing. *Current Biology*, accepted.

I co-supervised anatomical investigations and lab work, performed parts of the formal analysis, designed and created figures and wrote draft and final version of the manuscript.

Chapter 12. Pardo JCF, Araújo TQ, Capucho AT, Yap-Chiongco MK, Buckenmeyer A, Jondelius Y, Aramayo V, **Bergmeier FS**, Andrade LF, Cherneva I, Petrunina A, Peixoto AJM, Mikhlina A, Davidson AM, Engelhardt J, Frade F, Ellison C, Roberts NG, Costa AC & Jörger KM. Tiny animals do live in the sand: a report of meiofaunal focused active-learning activities to increase ocean literacy in primary-school children. *Açoreana* (in press): 1-11.

I contributed to the conceptualization and realization of the described activities, provided photographs for Figure 1, helped draft and write parts of the manuscript (“Extraction Station”) and discussed the final version of the manuscript.

I hereby confirm the above statement. Munich, 26.05.2021

M. Sc. Franziska S. Bergmeier Prof. Dr. Gerhard Haszprunar

7.3 List of Publications

Peer-Reviewed

1. **Bergmeier FS**, Ostermair L & Jörger KM. Specialized predation by deep-sea Solenogastres revealed by gut-content sequencing. *Current Biology* (accepted).
2. Neusser TP, **Bergmeier FS**, Brenzinger B, Kohnert P, Egger C, Yap-Chiongco MK, Kocot KM, Schrödl M & Jörger KM. Shallow-water interstitial malacofauna of the Azores. *Açoreana* (accepted): 1-23.
3. Pardo JCF, Araújo TQ, Capucho AT, Yap-Chiongco MK, Buckenmeyer A, Jondelius Y, Aramayo V, **Bergmeier FS**, Andrade LF, Cherneva I, Petrunina A, Peixoto AJM, Mikhlina A, Davidson AM, Engelhardt J, Frade F, Ellison C, Roberts NG, Costa AC & Jörger KM. Tiny animals do live in the sand: a report of meiofaunal focused active-learning activities to increase ocean literacy in primary-school children. *Açoreana* (in press): 1-11.
4. **Bergmeier FS**, Haszprunar G, Brandt A, Saito H, Kano Y & Jörger KM (2019) Of basins, plains, and trenches: Systematics and distribution of Solenogastres (Mollusca, Aplacophora) in the Northwest Pacific. *Progress in Oceanography*, 178, 102187: 1-17.
5. Ostermair L, Brandt A, Haszprunar G, Jörger KM & **Bergmeier, F.S**. (2018) First insights into thee Solenogaster diversity of the Sea of Okhotsk with the description of a new species of *Kruppomenia* (Simrothiellidae, Cavibelonia). *Deep Sea Research Part II* 154: 214-229.
6. **Bergmeier FS**, Brandt A, Schwabe E & Jörger KM (2017) Scratching the surface of deep-sea diversity: abyssal Solenogastres (Mollusca, Aplacophora) from the Northwest Pacific. *Frontiers in Marine Sciences* 4: 1-22.
7. **Bergmeier FS**, Haszprunar G, Todt C & Jörger KM. (2016) Lost in a taxonomic Bermuda Triangle: Comparative 3D microanatomy of cryptic mesopsammic Solenogastres. *Organisms Diversity and Evolution* 16, 3: 613-639.
8. Klink SP, **Bergmeier FS**, Neusser TP & Jörger KM (2015) Stranded on a lonely island: description of *Dondersia* (?) *todtae* sp. nov., the first shelf Solenogaster (Mollusca, Aplacophora) from the Azores. *Açoreana* 10, 4: 604-618.

Further Publications (non peer-reviewed)

9. **Bergmeier FS**, Melzer R, Haszprunar G & Jörger KM (2016) Getting the most out of minute singletons: molecular data from SEM-samples in Solenogastres (Mollusca). Research report in *The Malacologist*, 66: 23-25.
10. **Bergmeier FS** & Jörger KM (2020) “Solenogastres (Mollusca, Aplacophora)” in Biogeographic Atlas of the deep Northwest Pacific fauna (Eds: H Saeedi, A Brandt). PenSoft Advanced Books. https://doi.org/10.3897/ab.e51315.
11. **Bergmeier FS** & Jörger KM (2020) “Aplacophoran Molluscs: Solenogastres and Caudofoveata” in Guide to the determination of marine Meiofauna (Ed: A Schmidt-Rhaesa). Verlag Dr. Friedrich Pfeil, München.