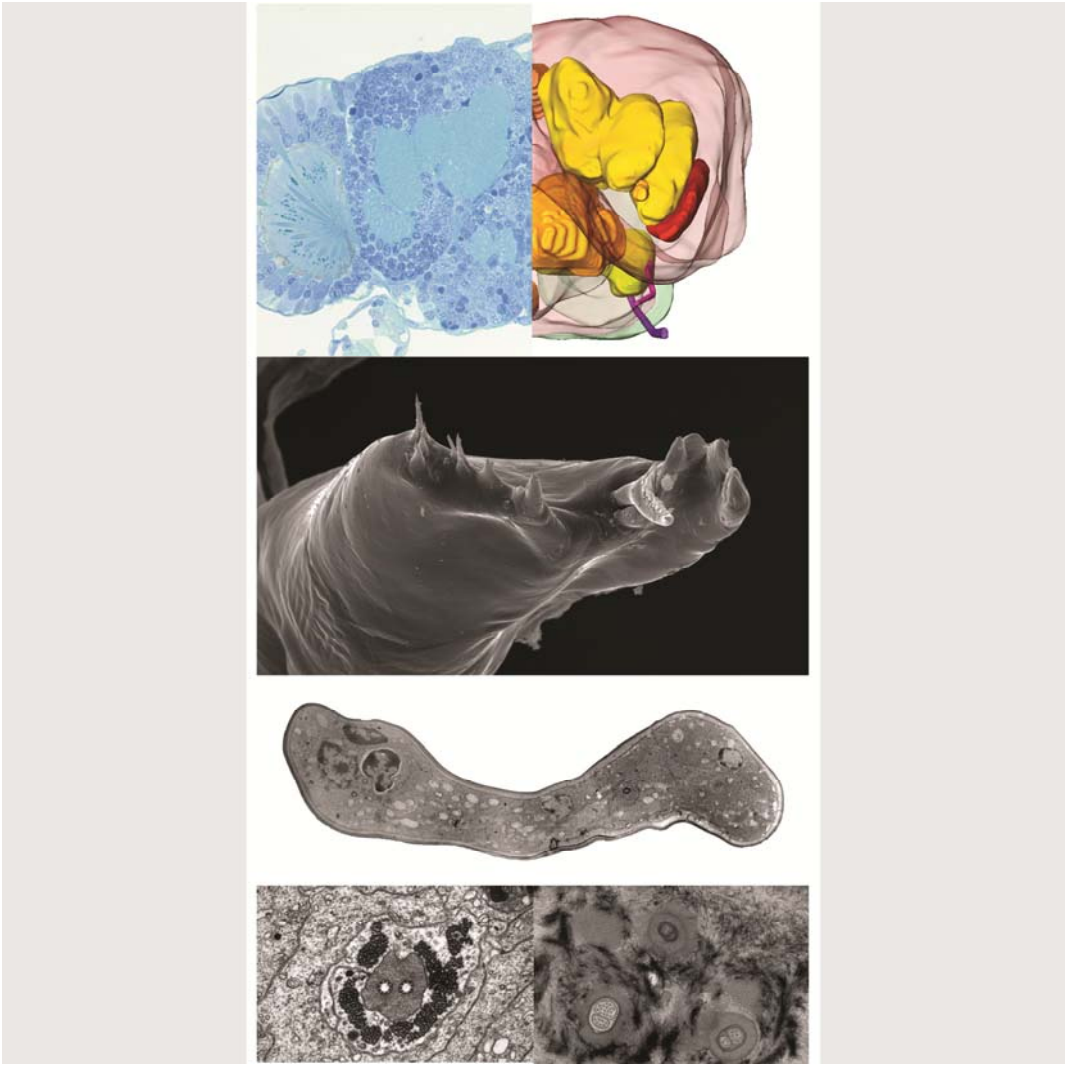
Morphology and Evolution of Malacostraca:
Structure of Central Nervous Systems, Mandibles and Sensilla



DISSERTATION

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)
an der Fakultät für Biologie
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Vorgelegt von

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Coverpicture: Semi-thin section of a zoea I larva of *Hippolyte inermis* (Leach,1815) showing the protocerebrum and optic neuropils (upper left). 3D reconstruction of a zoea I of *Hippolyte inermis* showing structure of the brain (upper right). SEM-picture of the right mandible of zoea I in *Palaemon elegans* Rathke, 1837 (middle above). TEM-picture showing overview of the ultrastructure of the right mandible of zoea I in *Palaemon elegans* (middle below). TEM-pictures of sensilla on mandible of zoea I in *Palaemon elegans* (below).

Erstgutachter: Prof. Dr. Roland R. Melzer

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Tag der mündlichen Prüfung: 30.07.2014

Erklärung:

Diese Dissertation wurde im Sinne von § 12 der Promotionsordnung von Prof. Dr. Roland R. Melzer betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

_____ _____

Ort, Datum Dipl.-Biol. Hannes Geiselbrecht

Eidesstattliche Erklärung:

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

_____ _____

Ort, Datum Dipl.-Biol. Hannes Geiselbrecht

List of publications

Paper I

Geiselbrecht, H., Melzer, R.R., 2013a. Nervous systems in 3D: A comparison of caridean, anomuran, and brachyuran zoea-I (DECAPODA). Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 320, 511-524.

Paper II

Batel., A., Melzer, R.R., Anger, K., Geiselbrecht, H., 2014. Heterochrony in mandible development of larval shrimp (Decapoda: Caridea) - a comparative morphological SEM study of two carideans. Journal of Morphology (under review)

Paper III

Geiselbrecht, H., Melzer, R.R., 2013b. How do mandibles sense? The sensory apparatus of larval mandibles in *Palaemon elegans* Rathke, 1837 (DECAPODA, PALAEMONIDAE). Arthropod Structure & Development 42, 1-16.

Paper IV

Geiselbrecht, H., Melzer, R.R., 2014. Fine structure and ecdysis of mandibular sensilla associated with the lacinia mobilis in *Neomysis integer* (Leach, 1814) (CRUSTACEA, MALACOSTRACA, PERACARIDA). Arthropod Structure & Development (published online 7 February 2014).

Declaration of author's contribution

Author's contribution to Paper I (Geiselbrecht and Melzer, 2013):
Hannes Geiselbrecht collected and prepared the specimens, accomplished the 3D-Reconstruction and the morphological analyses and drafted and wrote the manuscript. Roland R. Melzer supervised the methodical part and the mansucript writing.

Author's contribution to Paper II (Batel, Melzer, Anger, Geiselbrecht, 2014) :
Hannes Geiselbrecht collected the egg bearing females of *Palaemon elegans*, conducted the rearing experiments and specimen fixation at the Bavarian State Collection of Zoology and the Sea Life Center in Munich. *Macrobrachium amazonicum* larvae were reared and fixed at the Helgoland Marine Biological Laboratory (BAH) by Klaus Anger. Annika Batel accomplished the microscopy and the morphological and statistical analyses and drafted the manuscript under supervision of Roland R. Melzer and Hannes Geiselbrecht. Hannes Geiselbrecht revised and completed the manuscript. Klaus Anger revised the manuscript.

Author's contribution to Paper III (Geiselbrecht and Melzer, 2013b):
Hannes Geiselbrecht collected and prepared the specimens, accomplished the histology and microscopy and the morphological analyses and drafted and wrote the manuscript. Roland R. Melzer supervised the methodical part and the mansucript writing.

Author's contribution to Paper IV (Geiselbrecht and Melzer, 2014):
Hannes Geiselbrecht collected and prepared the specimens, accomplished the histology and microscopy and the morphological analyses and drafted and wrote the manuscript. Roland R. Melzer supervised the methodical part and the mansucript writing.

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1. Zusammenfassung

In diesem Dissertationsprojekt werden vergleichend-morphologische Studien am Nervensystem sowie der Mandibelstruktur und der sensorischen Ausstattung der Mandibeln bei Vertretern der Decapoda und Peracarida vorgestellt und im Hinblick auf die Evolution der Taxa interpretiert. Es handelt sich um eine kumulative Dissertation und die Ergebnisse wurden in mehreren getrennten Veröffentlichungen erarbeitet. Es wurden sowohl larvale als auch adulte Merkmale, sowie die Ontogenese bestimmter Merkmale analysiert. Dabei wurden vielseitige Bildgebungstechniken angewendet, von einfacher Lichtmikroskopie bis zur detaillierten Ultrastrukturuntersuchung mittels Transmissionselektronenmikroskopie. Das Hauptaugenmerk lag auf der Beschreibung und Etablierung bisher unbekannter Merkmalskomplexe, welche in eine Rekonstruktion der Phylogenie der Crustacea einbezogen werden können.

Das adulte Nervensystem der Decapoda ist seit langem ausführlich untersucht und zeigt sehr spezifische taxon-typische Anpassungen. In der vorliegenden Arbeit wurde nun erstmals auch das larvale Nervensystem in seiner Gesamtheit vergleichend untersucht. Mittels computergestützter 3D-Rekonstruktionen wurden allgemeine und artspezifische Merkmale analysiert und die Grundelemente beschrieben, dazu gehören die segmentalen Ganglien und ihre Neuropile sowie die segmentalen Hauptnerven. Das larvale Nervensystem zeigt sich in einem Übergangszustand zur adulten Organisation wobei die Grundelemente bereits weitgehend ausdifferenziert sind. Ebenso spiegelt ein phasenspezifischer Aufbau Anpassungen des larvalen Stadiums wieder. Die untersuchten Arten repräsentieren jeweils eine der drei Hauptlinien der Decapoda, d.h. der Caridea, Anomura und Brachyura. Unterschiede in der Ausdifferenzierung bestimmter Ganglien können vor diesem Hintergrund am besten mit Verschiebungen im zeitlichen Ablauf von morphogenetischen Ereignissen, also Heterochronie, erklärt werden.

Die Untersuchung von Heterochronie als zugrundeliegender Motor von evolutiven Anpassungen ist ein weiteres Kernthema der Arbeit. Neben der zuvor beschriebenen Studie wurde dazu auch die Morphologie und Feinstruktur der decapoden Mandibel während der Larvalentwicklung untersucht. Dies wurde anhand von zwei nahe verwandten Arten, die zudem unterschiedliche Ernährungsweisen im ersten Zoea-Stadium zeigen, untersucht. So konnte getestet werden, ob die Mandibelmorphologie in den frühen Larvalstadien lediglich von der Ernährungsweise abhängt oder ob ein evolutives Grundmuster in der Mandibelmorphologie auch bei Arten erkennbar ist, die im ersten Zoea-Stadium nicht fressen. Im Falle eines Vergleichs der Mandibeln, der nur auf

die Merkmale des ersten Zoea-Stadiums beschränkt ist, können durch Anpassungen an die Ernährungsweise möglicherweise ontogenetische Verschleierungen der taxon-spezifischen Merkmale auftreten. Durch detaillierte Betrachtung konnte aber nachgewiesen werden, dass sogar im ersten Zoea-Stadium von nicht fressenden Arten die gleichen apomorphen Grundmerkmale des zugehörigen Taxons erkannt werden können wie bei „fressenden“ Arten. Dies bekräftigt die Hypothese, dass sich in der larvalen Mandibelmorphologie phylogenetisch relevante Merkmalsätze finden lassen.

Die Monophylie der Mandibulata basiert hauptsächlich auf der Hypothese einer Homologie der Mandibeln der Myriapoda, Hexapoda und Crustacea, jedoch ist bisher erstaunlich wenig über die Sinnesstrukturen des gnathalen Lobus der Mandibeln und noch weniger über ihre Ultrastruktur bekannt. Die Erarbeitung dieser Merkmalskomplexe stellt ein weiteres bedeutendes Ziel dieses Projekts dar. Dazu wurde die Ultrastruktur des gnathalen Lobus der Mandibeln des ersten Zoea-Stadiums einer Felsengarnele untersucht. Besonderes Augenmerk wurde dabei, neben der äußeren Struktur und Verteilung und der Analyse der modalitätsspezifischen Strukturen sämtlicher sensorischer Elemente, im speziellen auf die Merkmale der „Lacinia mobilis“ gelegt. Insgesamt konnten sieben verschiedene Typen von Sensillen die durch vier spezielle Dendritentypen innerviert werden, beschrieben und verglichen werden. Darunter befinden sich (1) mechanorezeptive Haar-Sensillen und (2) mutmaßliche Kontakt-Chemo-Rezeptoren, sowie (3) Sensillen ohne äußere Strukturen und (4) Sensillen die mit starren Dornen assoziiert sind. Die Ergebnisse liefern neue Einblicke in die funktionelle Morphologie larvaler decapoder Mandibeln und stellen einen signifikanten Merkmalskomplex aus Fein- und Ultrastrukturmerkmalen dar. Dieser Merkmalskomplex wurde weiterführend mit der Analyse entsprechender Merkmale bei einem Vertreter der Peracarida ergänzt. Auch hier konnte ein Überblick der sensorischen Elemente der Mandibel dargestellt werden sowie die Ergebnisse einer detaillierteren Analyse der Lacinia mobilis, gestützt sowohl auf ultrastrukturelle Merkmale als auch auf Merkmale im Zusammenhang mit der Häutung. Im Vergleich kann eindeutig gezeigt werden, dass es sich bei der Lacinia mobilis auf der rechten Mandibel der Peracarida, und ebenso bei der entsprechenden Struktur der Decapoda, um ein mechanosensitives Sensillum handelt. Die Schlussfolgerungen bekräftigen die Hypothese einer möglichen Homologie dieser Strukturen. Für die Strukturen auf der linken Mandibel ist eine differenzierte Betrachtung notwendig. Die Ergebnisse lassen hier keine eindeutige Interpretation zu und es bleibt zu klären ob die Lacinia mobilis der linken

Mandibel ein abgewandeltes Sensillum darstellt oder ob es sich um eine zusammengesetzte Struktur mit mehreren Sensillen handelt.
Durch die Anwendung verschiedenster, teils modernster Techniken und die umfassende Diskussion der Ergebnisse konnte ein bedeutender Beitrag zur phylogenetischen Betrachtung der Eumalacostraca, wenn nicht Crustacea, geleistet werden. Es konnten Merkmalssätze etabliert werden, die verschiedene Organisationsstufen des Arthropodenkörpers umfassen. So konnten zunächst phylogenetisch relevante Signale im Grundbauplan des larvalen Nervensystems und der Feinstruktur der Mandibeln der Decapoda dargestellt werden. Des Weiteren wurden äußerst komplexe und detaillierte Merkmalssätze der Mandibelultrastruktur erarbeitet, die ein umfassendes Bild der sensorischen Fähigkeiten einer eumalacostraken Mandibel liefern und im Vergleich bereits Rückschlüsse auf die Homologie der Lacinia mobilis zuliessen. Dadurch lassen sich auch die phylogenetischen Einordnungen der jeweiligen Taxa bekräftigen.

Summary

In this dissertation project comparative morphological studies on the nervous system, mandible structure and sensory equipment of Decapoda and Peracarida are presented and interpreted with regard to the evolution of the taxa. This is a cumulative dissertation and the results were obtained in several separate publications. Both larval and adult characters as well as the ontogeny of certain features were included and analysed using various sets of imaging techniques ranging from conventional light microscopy to ultrastructure research with transmission electron microscopy. Attention was focused on the description and development of previously unexplored sets of characters which can be included in a reconstruction of crustacean phylogeny.
The adult nervous system in Decapoda has been extensively studied for a long time and shows very specific taxon generic adaptations. In the present thesis now also the larval nervous system was comparatively investigated in its entirety for the first time. By use of computer assisted 3D reconstruction general and species specific features were analysed and basic elements were described, including the segmental ganglia and their neuropils as well as the segmental nerves. The larval nervous system is in a transitory stage to the adult organization, already showing well differentiated basic elements. Likewise the phase-specific structure reflects adaptations to larval life. The studied species respectively represent one of the three decapod main lineages, i.e. Caridea, Anomura and Brachyura. Against this background variations in the differentiation of

certain ganglia can best be explained with shifts in the timing of morphogenetic events, i.e. heterochrony. The studies on heterochrony as motors of evolution are another core topic of this project. Along with the latter study also the morphology and finestructure of decapod mandibles during larval development was investigated, based on two closely related species, showing different feeding modes in the zoea I. Thus, it could be tested whether the mandible structure in early larval stages only depends on feeding modes or an evolutionary ground pattern is recognizable even in species with non-feeding zoea I. In case of a comparison of mandibles, restricted only to the features of the zoea I, adaptations to food preferences may obscure taxon-specific features. In detailed inspection, however, it could be shown that even in species with non- feeding zoea I apomorph basic features of the related taxon can be recognized. This supports the hypothesis of the presence of phylogenetic relevant character sets in larval mandible morphology.

The monophyly of the Mandibulata is manly based on hypotheses defending the homology of the mandibles in Myriapoda, Hexapoda and Crustacea, nevertheless, knowledge on sensory structures located on the gnathal lobe is astonishingly limited, even less is known about their ultrastructure. The development of this complex of characteristics represents a further aim of this project. For this purpose the ultrastructure of the mandibular gnathal lobe of the zoea I of a rockpool prawn was analysed. Besides external structure and location and an analysis of the modality specific structures, special attention was paid to the features of the lacinia mobilis. In total a number of seven different types of sensilla, innervated by four different types of dendrites, could be described and compared, including (1) mechanoreceptive hair-sensilla and (2) putative contact-chemo-receptors, as well as (3) sensilla without external structures and (4) sensilla associated with inflexible spines. The results reveal new insights into the functional morphology of larval decapod mandibles and constitute a significant character complex including fine- and ultrastructural features. Following-up the character complex was completed by investigations of respective features of a peracarid representative. The results also present an overview of the sensory elements of the mandible as well as a detailed analysis of the lacinia mobilis based on their ultrastructure and features related to ecdysis. By comparison it can be shown, that the lacinia mobilis on the right mandible in Peracarida and also the respective structure in Decapoda are mechanosensitive sensilla. In conclusion the hypothesis of a possible homology of the latter structures gains further support. Concerning the structures on the left mandible a differentiated consideration is necessary. No unambiguous conclusions can be made and it remains to be

resolved if the lacinia mobilis on the left mandible is a derived sensillum or a compound structure equipped with multiple sensilla.

With the application of many different state-of-the-art technics and the overall discussion of the results an important contribution to eumalacostracan phylogeny, maybe even crustacean phylogeny, could be made. Character sets comprising different levels of organization of the arthropod body could be established. Primarily phylogenetic relevant signal in the basic elements of the larval nervous system and the mandibles in Decapoda could be presented. Furthermore, highly complex and detailed character sets of the mandible ultrastructure were developed, revealing a comprehensive presentation of the sensory capacities of eumalacostracan mandibles and by comparison already allowed conclusions about the homology of the lacinia mobilis. Thus, also the phylogenetic position of the respective taxa can be confirmed.

2. General introduction

2.1. *Introduction to Decapoda and Peracarida*

The core topics of the present thesis are comparative morphological studies on the decapod larval nervous system and on decapod and peracarid mandible structure and sensory equipment. To provide previously unexplored sets of characters for reconstructing crustacean phylogeny a various set of imaging techniques including conventional light microscopy and computer assisted 3D reconstruction, as well as scanning and transmission electron microscopy was applied. The present chapter therefore introduces the two taxa dealt with, Decapoda and Peracarida, their ontogenetic stages and general *bauplan* and ideas on phylogeny. Subsequently the main aims of the study are presented.

One of the most diverse and common animal groups living on earth are the Crustacea. Belonging to the phylum Arthropoda, Crustacea show an impressive diversity and can be found in nearly every kind of habitat. Unlike insects, that are the most abundant animals on land, crustaceans mainly live in marine ecosystems. The estimated number of described species in Crustacea is approximately 52.000 (Martin and Davis, 2001), the largest groups amongst others are eumalacostracan taxa, including Decapoda with 15.000 species (De Grave et al., 2009) and Peracarida with 12.000 species (Barnes and Ruppert, 2004). The decapods are probably most familiar to laymen, because they are edible yet mostly considered as delicacies. There are the commonly known lobsters (Astacidae), spiny lobsters (Palinura), shrimps (Caridea) and crabs (Brachyura), and furthermore the hermit crabs (Anomura). Rather inconspicuous or with a hidden lifestyle are the mud lobsters and ghost shrimps (Thalassinidae) or the coral shrimps (Stenopodidae). Among the Dendrobranchiata are the Penaeidae, with the tiger prawn (*Penaeus monodon*) being one of the most important subjects of commercial sea food farming. Updated taxonomic surveys are given e.g. in Števčić (2005), Ng et al. (2008), Chan (2010) and De Grave and Fransen (2011).

Although they are also very common in most marine habitats, the peracarids are rather unknown. They are mostly small animals of less than 2 cm in length and besides the seas also inhabit freshwater environments and sometimes even can get on land, e.g. terrestrial isopods like the common woodlouse. Peracarida includes the shrimp-like Mysidacea, the partly superabundant and hyperdiverse Amphipoda, Cumacea and Tanaidacea, the already mentioned Isopoda and the

freshwater or deep-sea inhabiting Thermosbaenacea, Spelaeogriphacea and Mictacea (Poore, 2005).

The similarity of the general habitus can be very high between particular members in Decapoda and Peracarida, e.g. the shrimp-like body in Caridea and Mysida, however, in both groups also highly derived forms can be found. Shared characters are the malacostracan autapomorphies like standardized tagmosis with a constant number of head, thorax and abdominal segments, location of the female gonopores on the sixth thoracic segment and male gonopores on the eighth and an always biramous antenna I. Eumalacostracan autapomorphies present in both groups are the tail fan, formed by the uropods and a flattened telson, and the scaphocerit, a flattened exopod of the antenna II (Schminke, 2007). Due to the huge ecological license of the malacostracan body plan the animals are very well adapted to their specific life style. Consequently, the biodiversity in this group of animals is outstanding and in the different orders or infraorders extremely variously shaped body forms are present.

Decapods are predominantly bottom dwelling and most abundant in marine intertidal zones but they can also be found from the sublittoral down to the shelf edge and some species in even higher depths of the deep sea. However, the primary features of the decapod body are still noticeable in all groups. Basically, the body is divided into two tagmata: the cephalothorax and the pleon. The cephalothorax is composed by a fusion of the primary cephalon and the following eight thoracomeres. It bears several partly strongly modified pairs of limbs or appendages, primitively showing a biramous structure with a basal protopod and two attached rami, termed endopod and exopod. The biramous antenna I (= antennule) and antenna II (= antenna) on the 1st and 2nd somite are followed by the three mouthpart bearing somites with the mandibles and the maxilla I (= maxillule) and maxilla II (= maxilla). Posteriorly the appendages of the next three somites are maxillipeds, still showing a biramous structure and also with a function in feeding. The remaining five pairs of thoracic appendages are the 10 pereopods from which the name Decapoda is derived. The pereopods are uniramous and the first one is frequently enlarged and distally bears a strong euchela, which is formed by a dactylus that is medially attached on a distally elongated propodus and thus both becoming opposable. The following pereopods may be chelate as well, then of subchelate type, but usually are stenopodous walking legs. The carapace forms a robust shell, dorsally attached to the cephalothorax and laterally enclosing the gills, which are modified epipodits of the appendages. The pleon extends posteriorly from the

cephalothorax, it is well segmented, the pleopods are biramous swimming legs and a tail fan is formed by the appendages of the last pleon segment, the uropods, and the telson. In general there are two extreme body forms: the shrimplike (caridoid) form and the crablike (cancroid) form. A slightly aberrant habitus or intermediate forms are found in the Anomura (Barnes and Ruppert, 2004; Schminke, 2007).

Showing many primitive malacostracan characters, the most distinctive and autapomorph character of the peracarids is a female ventral brood pouch, the marsupium. It is formed by enlarged, plate-like oostegites extending medially from the posterior thoracic coxae. They also mostly have fused anterior thoracic segments to form a cephalothorax and the appendages of these segments are maxillipeds. A more delicate but also characteristic feature that can be found between the incisor and molar process of the mandible is the lacinia mobilis. A carapace may be present or not (Barnes and Ruppert, 2004).

2.2. *Phylogeny of Malacostraca*

The primary classification of the Malacostraca was introduced by Calman (1904). The system placed the Leptostraca at the basis as sistergroup to all other Malacostraca (the Eumalacostraca). Eumalacostraca again are divided in the Hoplocarida (Stomatopoda) and the Caridoida, including Syncarida, Pancarida (Thermosbaenacea), Peracarida and Eucarida (Euphausiacea + Decapoda), but over a long period many controversial classifications were published (i.a. Giesbrecht, 1913; Grobben, 1919; Schram, 1969; Schram, 1981; Bowman and Abele, 1982; Dahl, 1983; Hessler, 1983; Richter and Scholtz, 2001).

From the late 19th century on decapod species have frequently served as laboratory model organisms in physiological, morphological and behavioral studies (e.g. Huxley, 1884; Bethe, 1895). Early taxonomic classification divided the decapods into the swimming lineages (Natantia) and the crawling lineages (Reptantia) (Boas, 1880). Later Burkenroad (1963; 1981) broke with this concept and introduced the suborders Dendrobranchiata and Pleocyemata based on gill structure. Since then still many changes and arrangements have been suggested. The classification by Bowman and Abele (1982) accepted Burkenroad´s suborders and also several modern handbooks abandoned the Natantia concept by elevating the Penaeoidea (prawns) to the rank of a separate suborder (the Dendrobranchiata) and placing the rest of the Natantia (shrimps) plus the Macrura Reptantia (marine lobsters, freshwater crayfishes, spiny lobsters, slipper lobsters and mud lobsters), plus the Anomura (hermit crabs), plus the Brachyura (crabs) in the

Table 1: Eumalacostracan classification after Martin & Davis (2001)

Class **Malacostraca** Latreille, 1802
Subclass **Eumalacostraca** Grobben, 1892
Superorder **Syncarida** Packard, 1885
Order Bathynellacea Chappuis, 1915
Order Anaspidacea Calman, 1904
Superorder **Peracarida** Calman, 1904
Order Spaeleogriphacea Gordon, 1957
Order Thermosbaenacea Monod, 1927
Order Lophogastrida Sars, 1870
Order Mysida, Haworth, 1825
Order Mictacea, Bowman et al., 1985
Order Amphipoda Latreille, 1816
Order Isopoda Latreille, 1817
Order Tanaidacea Dana, 1849
Order Cumacea Kroyer, 1846
Superorder **Eucarida** Calman, 1904
Order Euphausiacea Dana, 1852
Order Amphionidacea Williamson, 1973
Order Decapoda Latreille, 1802

single suborder Pleocyemata (Holthuis, 1993). Holthuis (1993), however, had a critical opinion about this classification and rather preferred the primary one. A consensus of the decapod evolution is not established even to date. With an estimated divergence time of 437 million years the origin of the Decapoda is placed in the early Silurian (Porter et al., 2005). This broad timescale and the morphological diversity and complexity results in a difficult interpretation of the phylogeny and many conflicting hypothesis (see Bracken et al., 2009b). The most recent higher classification of living and fossil Decapoda lists 233 families containing 2,725 genera and 17,635 estimated species (extant and fossil species) (De Grave et al., 2009).

Table 2: Decapod classification after De Grave et al. (2009).

Order **Decapoda** Latreille, 1802
- Suborder **Dendrobranchiata** Bate, 1888
- Suborder **Pleocyemata** Burkenroa,. 1963
  - Infraorder **Stenopodidea** Bate, 1888
  - Infraorder **Caridea** Dana, 1852
  - Infraorder **Astacidea** Latreille, 1802
  - Infraorder **Glypheidea** Winkler, 1883
  - Infraorder **Axiidea** de Saint Laurent, 1979
  - Infraorder **Gebiidea** de Saint Laurent, 1979
  - Infraorder **Achelata** Scholtz & Richter, 1995
  - Infraorder **Polychelida** Scholtz & Richter, 1995
  - Infraorder **Anomura** MacLeay, 1838
  - Infraorder **Brachyura** Linnaeus, 1758

Also the relationships between the orders of Peracarida have long been debated, and also different hypotheses about which orders to include or exclude have been proposed (e.g. (Watling, 1981; Pires, 1987; Wagner, 1994; Hessler and Watling, 1999; Richter and Scholtz, 2001). Recently Peracarida are considered monophyletic, concordantly including amongst others Amphipoda, Isopoda, Cumacea and Tanaidacea (Poore, 2005; Wills et al., 2009). The phylogenetic position of the order Mysida is still unresolved. Mysida and Lophogastrida, however, are considered as sister taxa and currently regarded as the most basal clade (Poore, 2005). To get a general idea, table 1 shows the primary eumalacostran classification after Martin & Davis (2001) including extant peracarid orders and in table 2 the specified and currently accepted decapod suborders and infraorders are listed after De Grave et al. (2009). The early debates described above mainly were based on the results of morphological analyses, but also the today existing molecular evidence is often unable to resolve well-supported phylogenies.

Molecular analyses often concentrate on a phylogenetic classification at family level, like amongst others inside Anomura (Ahyong et al., 2009), Caridea (Bracken et al., 2009a; Li et al., 2011), Thalassinidea (Robles et al., 2009) or Isopoda (Wetzer, 2002; Wilson, 2009) and Mysida (Remerie et al., 2004).

An estimation of the relationships among all of the major decapod infraorders using molecular data is given in Porter et al. (2005) and concentrating on Pleocyemata in Tsang et al. (2008).

Combined molecular and morphological analyses also at higher levels recognized that Palinura are paraphyletic and placed Polychelida as sister to the remaining Reptantia (Ahyong and O'Meally, 2004) or questioned the monophyly of Thalassinidea (Bracken et al., 2009b). Conflicting data from different molecular markers as well as incongruences between morphological and molecular phylogenies complicate the effort to resolve a well-supported eumalacostracan phylogeny (Jenner et al., 2009; Wills et al., 2009). Future insights are likely to come from the development of new molecular markers, as well as hard-won data on internal anatomy and ultrastructure (Wills et al., 2009).

2.3. *History of larval research*

In contrast to the many diverse body forms and life styles during ontogeny all decapods have one thing in common: developing indirectly they produce pelagic larvae that may differ entirely in their morphology and habits from juvenile and adult conspecifics (Anger, 2001).

The larvae already drew the interest of the early zoological pioneers, like Linnaeus, who was the first to describe a larva (*Cancer germanus* (Linnaeus, 1767)). However, he believed it to be a new species and did not know that he was describing the larval phase of an already known adult. Later, Bosc (1802) introduced the term "zoea" in the description of a brachyuran larva (*Zoea pelagica*), also intending to establish the identification of a new species distinct from all other genera of the Crustacea known at this time and thus to define the genus *Zoea*. Only after witnessing the hatching of a zoea and the moulting of a megalopa into the first juvenile the remarkable dissimilarities between young and adult became obvious and the existence of larval phases in Decapoda was recognized. It was Thompson (1828), who made the first deliberate account of metamorphosis in Crustacea. In his work he initially cited and discussed Slabbers (1778) unaware discovery in a description of a larva which was designated to the species *Zoea taurus*. The studied morphological changes had been supposed to be the result of the inspection of an unknown specimen that had been introduced during the change of the sea water in his culture and the originally inspected zoea had been lost. Thompson then compared and discussed his own observations on moulting zoeae and together with the evidence from the observation of hatching *Cancer pagurus* larvae he proved that decapods undergo a metamorphosis. However, not everyone was convinced by Thompson's argumentation. Westwood (1835) criticized Thompsons statements and Milne-Edwards (1835) also maintained a controversy. Only some years later it was commonly accepted that there is both direct and indirect development in the

ontogeny of decapod species (see Ingle, 1992, for references). Since then the descriptions of many larval developments have been published and methods of catching planktonic larvae or hatching and rearing larvae in the laboratory have been developed further. Early standard works are Gurney´s "Bibliography of the larvae of the decapod Crustacea" (1939), reviewing all existing literature at the time and "Larvae of decapod Crustacea" (1942), that covers general morphology and taxonomy. Also there was Marie V. Lebour, another British carcinologist active at the same time, with her important contribution in several publications (see (Barnich, 1996) for a detailed reference list). Also to name are the descriptions of Rice (e.g. Rice, 1964; Rice et al., 1970; Rice, 1975; Rice, 1979; Rice, 1980a), the identification keys of Williamson (e.g. Williamson, 1957, 1969, 1976) or later the bibliographies of Bourdillon-Casanova (1960) and then González-Gordillo et al. (2001) and also the illustrated key of Ingle (1992) and the comprehensive work on general biology by Anger (2001). The previous paragraph does not intend to give a complete listing of the relevant literature but just to name a few important examples.

2.4. *General morphology and ontogeny*

As stated above, most Decapoda undergo an indirect development. In principle extant Decapoda show three distinguishable types of larvae after which the phases in larval development can be termed: the nauplius, the zoea and the decapodid. Only in the Dendrobranchiata the nauplius hatches from the egg as planktonic larval form, most Pleocyemata pass through this phase inside the egg and hatch as a zoea or as a prezoea. The nauplius is characterized by absent thoracic somites and absent or rudimentary posterior cephalic appendages. The anterior cephalic appendages, the antenna I and antenna II fulfill mainly natatory but also feeding functions. In the zoea functional thoracopods and paired compound eyes are present. Starting with the most anterior paired cephalic appendages there are the uniramous antennae I and the biramous antennae II followed by the mandibles which are the main masticating organs in early zoeae and the maxillae I and maxillae II. In first stage zoeas posteriorly may be the first, the second and sometimes the third maxillipeds present. The exopods of the maxillipeds are used for swimming while the endopods handle food. The larvae moult successively and accompanied by morphological changes they pass through several developmental stages. Interestingly, there can be an extreme intraspecific variability in the total number of zoeal stages. The final larval phase is the decapodid, preceding the metamorphosis to the first juvenile instar. It is characterized by

the presence of functional pleopods and a changed function of all cephalic and the anterior thoracic appendages as mouthparts. In strictly freshwater species, like most crayfish, development is direct, involving an eliminated pelagic larval phase and adult-like juveniles hatching from the egg (Anger, 2001).

In Peracarida the eggs are brooded and hatched in the marsupium. In contrast to Decapoda, the development is mostly direct and the juveniles hatch fully developed, already showing the complete set of segments and appendages. Only in Isopoda the hatching stage is a manca postlarva, characterized by the absence of the last pair of thoracic legs (Johnson et al., 2001; Barnes and Ruppert, 2004). In other peracarid taxa, like Caprellidae belonging to Amphipoda, however, also a slight derivation from a strict direct development was reported (Lang et al., 2007).

2.5. *The bearing of larval morphology on decapod phylogeny*

The biology of decapod larvae still holds a wealth of interesting aspects to study and furthermore results of appropriate morphological studies can have an important bearing on phylogenetic interpretations.

Older decapod systematics are mainly based on adult morphology but certain aspects underlying larval morphology proved the understanding of larvae to be helpful in classification and also in reconstructing decapod phylogeny (Clark, 2009). However, the problem is to recognize or exclude similarities that are product of convergences. Phenotypic characters are influenced by a genotype-environment interaction, and thus an exclusive analysis of adult characters of species that are adapted to different life styles may lead to wrong interpretations. In contrast the larvae of all decapod species are adapted to the same habitat, the pelagic zones of the seas. Thus, their features are subject to more or less constant selection pressures and may reflect relationships better than the morphology of the adults (see also Rice, 1980b; Williamson, 1982). An established feature, for example, is the setal pattern on certain appendages, appearing to be identical in brachyuran zoeas of congeneric species (see Clark, 2009). The appendage setation seems to be conservative and thus may reveal close relationships between larvae even if they differ markedly in general appearance (Rice, 1980b).

3. Aims of the thesis

3.1. *Background and general approach*

The main subjects of this cumulative dissertation project are morphological studies of larval decapod features and respective analyses in a representative peracarid species to contribute new sets of characters that are relevant in comparative analyses and phylogenetic considerations. Through integration of ontogenetic processes heterochronic events were analysed as motors of evolution. The initial project involved a comparative morphological study on the larval central nervous system (CNS) of first stage zoeas in three different decapod species. Next we conducted fine structural studies on the mandible development in successive larval stages in the decapod species *Palaemon elegans* Rathke, 1837. The analysis of new character sets involved two levels of organization of the body. The CNS study and the mandible ontogeny covered general structural organisation at the tissue and organ levels, while the cellular level was included by the analysis of internal ultrastructural features when revealing the sensory capacity of eumalacostracan mandibles and mandible evolution, with special reference to the lacinia mobilis. To achieve this in particular projects the ultrastructure of the mandibles of zoea I in *P. elegans* and the fine- and ultrastructure of the mandibles in the mysid *Neomysis integer* (Leach, 1814) were investigated.

Although many laboratories and scientists are working with decapod larvae and extensive literature has been published describing larval morphology and development (see e.g. González-Gordillo et al., 2001) there is still much to discover. For instance some Mediterranean species remain undescribed and others are just recently dealt with, such as *Periclimenes amethysteus* Risso, 1827, *Heterocarpus ensifer ensifer* A. Milne-Edwards, 1881 or *Gnathophyllum elegans* (Risso, 1816) (Geiselbrecht and Melzer, 2009; Maria Landeira et al., 2010; Meyer et al., 2014). Delicate issues are differences in the quality and standardization of larval descriptions. Sometimes crucial characters are omitted because they are not easily observable or require dissection, sometimes there are inconsistencies between the descriptions and the illustrations. Guidelines were published for improved standards in the description of crab zoeas (Rice, 1979; Clark et al., 1998), because early phylogenetic studies based on larval characters repeatedly had to be confined to only a few genera with adequate descriptions (e.g. Aikawa, 1937; Guinot, 1978). Figure 1 shows the lateral view of a zoea I in *Gnathophyllum elegans*, taken from the

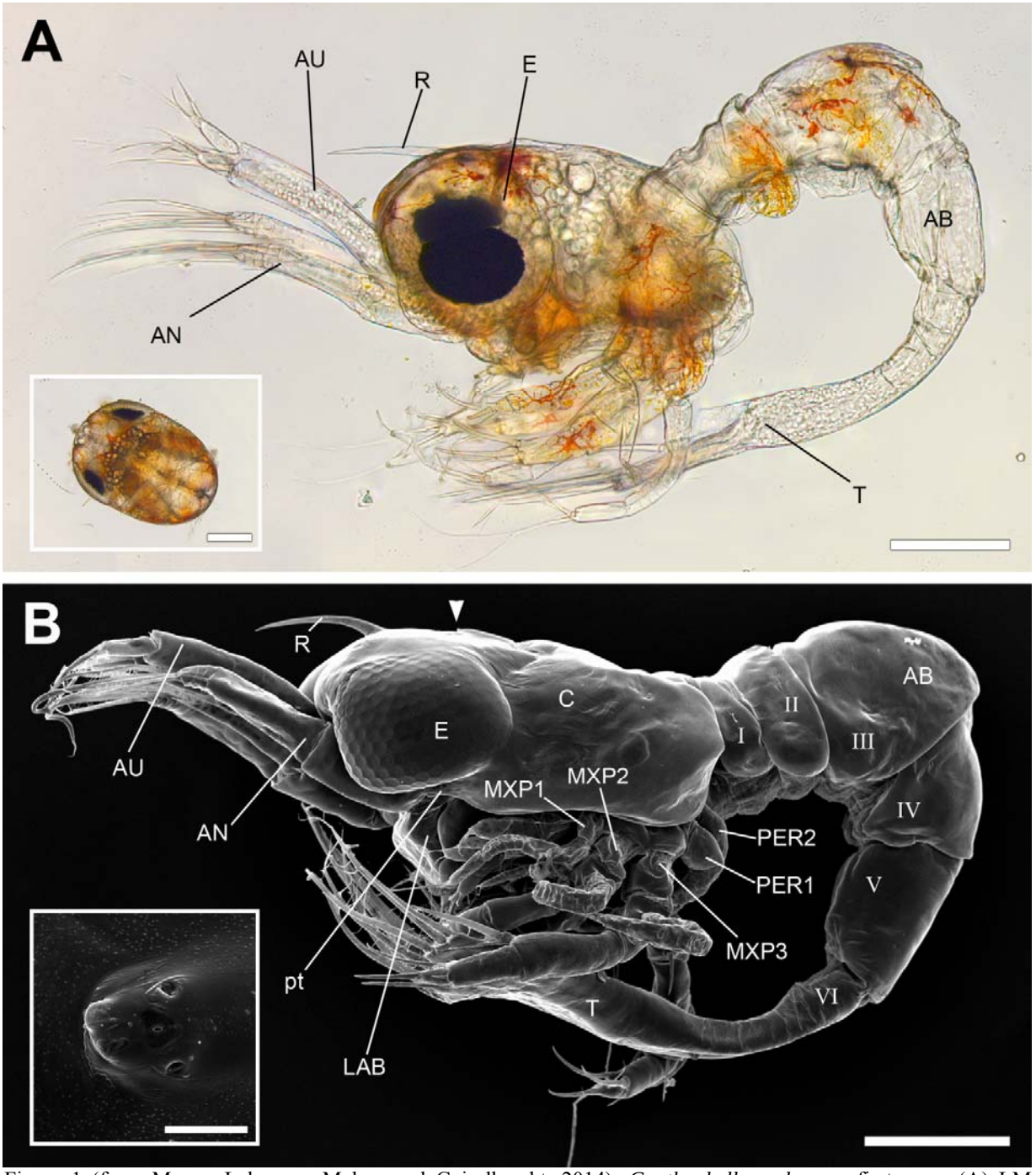


Figure 1 (from Meyer, Lehmann, Melzer and Geiselbrecht, 2014): *Gnathophyllum elegans*, first zoea: (A) LM image, lateral view, natural pigmentation visible; insert showing egg just before hatching; (B) SEM image, lateral view, arrowhead points to dorsal organ; insert showing detail of dorsal organ. Abbreviations: I-VI, abdominal segments; AB, abdomen; AN, antenna; AU, antennule; C, carapace; E, eye; LAB, labrum; MXP 1-3, first to third maxillipeds; PER1&2, pereiopods 1&2; PT, pterygostomial spine; R, rostrum; T, telson. Scale bars: A, 200µm; B, 200µm, insert 20µm.

larval description in Meyer et al. (2014). It is not only nicely visible the general habitus of a caridean zoea I but also the differences in details that can be viewed using on the one hand a

classical method like light microscopy (LM) and on the other hand a more modern but also more laborious method like scanning electron microscopy (SEM).

3.2. *Larval nervous systems in 3D*

The state of the art technique enabling detailed analyses of internal anatomical features of small specimen is the computer-aided 3D-reconstruction of histological section series. We applied this method to deal with the larval central nervous system (CNS) in Decapoda. With its complex structure and function the CNS is an organ that keeps scientists fascinated since long. The adult decapod nervous system has been studied in great detail (e.g. Bullock and Horridge, 1965; Sandeman et al., 1992; Sandeman et al., 1993). Because it can be dissected relatively easy and, prepared for electrophysiological measurements, it survives well in isolation, the decapod nervous system has been long used as model-organism to study various neurophysiological aspects (see e.g.Selverston and Moulins, 1987). In contrast surprisingly little is known about the larval CNS. Especially histological studies describing the whole CNS in early larval stages were not present. There are few histological studies on the ontogeny of larval osmoregulatory structures (Cieluch et al., 2005; Cieluch et al., 2007) and immunocytochemical studies on the development of neuroendocrine centers of larval European lobsters (Rotllant et al., 1993; Rotllant et al., 1994; Rotllant et al., 1995) or on neurogenesis in *Hyas araneus* (Linnaeus, 1758) larvae (Harzsch and Dawirs, 1994). A detailed histological analysis of the CNS of the megalopa in *Carcinus maenas* (Linnaeus, 1758) was published by Harzsch and Dawirs (1993) and Helluy et al. (1993) studied the brain ontogeny in *C. destructor* Clark, 1936 and *Homarus americanus* H. Milne Edwards, 1837. While there is a survey of the morphology of adult brains of 13 decapod species implicating representatives of all decapod taxa (Sandeman et al., 1993), no comparable study regarding the larval CNS is present. Many decapod taxa, like Anomura or Caridea, have not been studied in this regard at all.

Accordingly, our study is the first to present and compare the results of a histological analyses of the whole CNS of zoea I larvae in three species belonging to Caridea, Anomura and Brachyura, providing also basic data on innervation patterns to help understand the results of further projects presented in this thesis. Using serial semi-thin sections and digital 3D-reconstructions the aim was to investigate detailed features of the minute larval CNS. We analyse and compare the histological section images and the 3D-reconstructions with regard to general and species specific features. Describing the composition of the basic elements, like the segmental ganglia, their

neuropils and the segmental nerves, we pay special attention to the stage of development and structure of the particular ganglia during the ongoing differentiation process. We analyse many parts of the central nervous system, including the ganglia of the anterior CNS, ganglia of the mouthpart, maxilliped, pereion and pleon segments and the segmental nerves. Larvae undergo a sequential differentiation of body segments during ontogeny (Anger, 2001) and the results of the study should be analysed in relation to the question as to whether this is reflected in the development of the nervous system. Hence, does the development of the CNS correspond with the development of the respective segmental appendages?

The three studied species show a different set of developed segmental appendages. Thus, an interspecific comparison may reveal differences in the progress of the development of respective neuromeres. If such differences can be revealed it should be discussed whether they can be attributed to heterochronic mechanisms. Heterochrony refers to a change in the relative timing of developmental events in one species relative to an ancestral species (Smith, 2001). First defined by Haeckel (1866) the concept was repeatedly reviewed and the definition was changed (e.g. Russell, 1917; De Beer, 1940). However it was and still is always important in questions of evolutionary developmental biology (McNamara and McKinney, 2005). The most influential work on how the concept is used even today is probably Gould´s book *Ontogeny and Phylogeny* (Gould, 1977) followed by the shortly after published paper by Alberch et al. (1979).

3.3. *Mandible development in Palaemon elegans*

A heterochronic event also could best be considered as the evolutionary pattern underlying certain differences discovered in the study of the mandible development in two phylogenetically closely related species. We analyse the morphology and fine structure of the mandibles of the larval stages I-V in the two palaemonid shrimp species *P. elegans* and *Macrobrachium amazonicum* (Heller, 1862). This species pair is very interesting for a comparative ontogenetic study because they show different feeding modes in the zoea I. The zoea I in *P. elegans* is already feeding normally (Kumlu and Jones, 1995) and in contrast the zoea I in *M. amazonicum* is non-feeding (Anger and Hayd, 2009). Hence, it can be tested whether the mandible morphology in early zoeal stages depends on feeding habits only or whether a proposed hypothesis of an evolutionary ground pattern in mandible morphology is recognizable even in non-feeding species and thus maybe strengthen the validity of the hypothesis.

3.4. *Fine- and ultrastructure of mandibles in Decapoda and Peracarida*

Using a standardized terminology decapod larval descriptions should include particular characters, besides the carapace these are mainly the general features and setal patterns of the body appendages starting with the antenna I on the most anterior somite (Clark et al., 1998). Furthermore, in Brachyura the zoea of congeneric species may be inseparable using setal characters. This can complicate a conclusive classification and emphasizes the relevance of finding and using adequate characters (Christiansen, 1973; Rice, 1980b; Clark, 1983).

Compared to the maxillae, the mandibles were somewhat neglected in the past. Though early descriptions were given, e. g. in Gurney (1942), in many larval descriptions they are either not resolved in detail or ignored completely. However, mandibles are a key character for reconstructions of arthropod phylogenies (e.g. Bitsch and Bitsch, 2004; Edgecombe, 2010; Rota-Stabelli et al., 2011) and the Mandibulata concept is based on the assumption of a homology of the mandibles in Myripoda, Hexapoda and Crustacea (Bitsch, 2001; Edgecombe et al., 2003).

Decapod larval mandibles are certainly one of the most difficult structures to dissect out for detailed examination due to their minuteness, shape and way of insertion on the head. Nonetheless, Geiselbrecht & Melzer (2010) analysed the fine structure of mandibles of first stage zoeae in nine decapod species and indicated a significance of taxon-specific features in larval mandible morphology including the basic form, the form and orientation of the incisor and molar processes, and the shape, number and arrangement of certain appendages. A special mandibular appendage of peculiar interest is the lacinia mobilis. It is a movable, articulated protrusion on the mandible´s gnathal edge, described in various arthropods but, however, with no consensus about a homology or non-homology in the different taxa (reviewed in Dahl and Hessler, 1982; Richter et al., 2002; Richter and Kornicker, 2006; Mayer et al., 2013).

Although included in a phylogenetic analysis of the Malacostraca by Richter and Scholtz (2001) and scoring equally in all taxa which possess a lacinia mobilis as adults as well as in Caridea, which seemed to be the only decapod taxon in which it is present only on the larval mandible, a possible homology of the feature in Peracarida, Euphausiacea and Decapoda was questioned shortly after by Richter et al. (2002). Instead it was concluded that a lacinia mobilis which is present in adults and also asymmetrical on the left and right mandible may rather represent an autapomorphy of the Peracarida. In addition the critical arguments contradicting a homology in Peracarida, Euphausiacea and Decapoda were based on the knowledge of the presence of the feature only in euphausiacean and decapod larvae and also only on one mandible. However,

Geiselbrecht & Melzer (2010) could already devaluate two of the mentioned arguments, showing that in Caridea a 'lacinia mobilis' can be present on both mandibles and they can be dissimilar. But still conclusive arguments were missing since no studies were present revealing the nature and origin of the lacinia mobilis or respective similar structures.

3.4.1. The fine- and ultrastructure of a decapod larval mandible

A SEM-analyses already showed features on the larval mandibles in *P. elegans* and *P. amethysteus*, like the articulation on a basal ring and the presence of an ecdysial pore, suggesting that the 'lacinia mobilis' of decapod zoeas might be a sensillum (Geiselbrecht and Melzer, 2010). Studying the ultrastructure in addition to external features should reveal important findings for the understanding of the functional morphology and the origin of the lacinia mobilis by adding sets of characters referring to internal architecture to the available body of evidence based only on external inspection. Moreover, only little is known about the presence of sensilla on the gnathal lobe of arthropod mandibles in general, even less about their ultrastructure (e.g. Ong, 1969; Whitehead and Larsen, 1976; Tyson and Sullivan, 1981).

Hence, in one project we studied the ultrastructure of the gnathal lobe of the mandibles of zoea I larvae in *P. elegans* using transmission electron microscopy (TEM). Based on differences in ultrastructural and external features we aimed for a description and distinction of the different types of sensilla and innervating dendrites the presence of which was to be expected. Besides the 'lacinia mobilis' we analyse several sensillar structures with regard to their modality-specific structures, their distribution and external morphology. For each type we discuss the specific function, thus, give a comprehensive overview of the sensory equipment of the mandibles of a decapod zoea I larva. We also pay special attention to possible morphological specializations of the sensilla linked to the robust nature of the mandibles. Concerning the question of homology of the lacinia mobilis, the results might reveal features indicating that on the larval mandible in *P. elegans* it is a mechanosensitive sensillum and, thus, providing useful characters in the discussion of its origin.

3.4.2. *The fine- and ultrastructure and ecdysis of a peracarid mandible*

To follow a consistent argumentation elucidating the derivation of malacostracan mandibular appendages the next study should clarify whether the 'true' lacinia mobilis in Peracarida is an articulated mandibular structure with exclusively mechanical function, as stated by Richter et al.

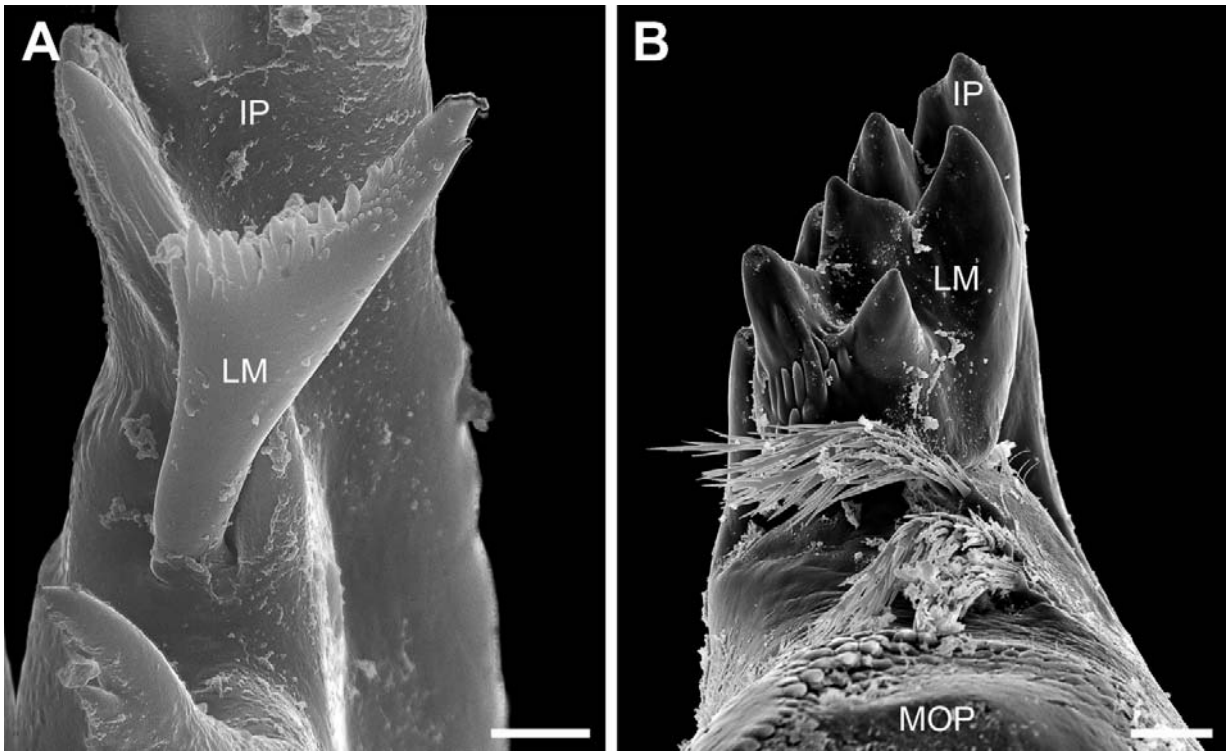


Figure 2: SEM images showing the left lacinia mobilis in (A) *Palaemon elegans*, zoea-I and (B) adult *Neomysis integer*. Abbreviations: IP, incisor process; LM, lacinia mobilis; MOP, molar process. Scale bars: A, 4µm; B, 20µm.

(2002), or whether it too has sensory capability. Thus, the fine- and ultrastructure of a peracarid mandible was studied and published in the final paper. The external features of the lacinia mobilis in Peracarida are well studied (De Jong-Moreau et al., 2001b; Richter et al., 2002; Mayer et al., 2013), but in no single peracarid species a TEM analysis of the internal features has been conducted to date. Therefore, we studied the lacinia mobilis of the mysid *Neomysis integer* with regard to external morphology and ultrastructural features. Using TEM, besides light microscopy (LM) and SEM, we conducted the first study providing insight into the sensory equipment of a peracarid mandible. The ultrastructural analyses should reveal if the lacinia mobilis on both mandibles is a structure innervated by sensory units. Analyzing the modality-specific features also the sensitivity of the receptors should be indicated. Furthermore we analyse histological changes inside the mandible involved in initiating the molting process. We classify the molting type and relate it to the molting of statocyst or aesthetasc sensilla, however with some distinctive characteristics. Bringing together the results of both the previous and the present study it should be possible to develop new conclusions about the derivation of the lacinia mobilis and draw further assumptions on a possible homology in Peracarida and Decapoda.

Table 3: List of species with collected zoea I larvae.	
Infraorder	Species
Dendrobranchiata	*Penaeus monodon* Fabricius, 1798
Caridea	*Palaemon elegans* Rathke, 1837 *Palaemonetes argentinus* (Nobili, 1901) *Hippolyte inermis* (Leach, 1815) *Lysmata seticaudata* (Risso, 1816) *Thoralus cranchii* (Leach, 1817) *Athanas nitiscens* (Leach, 1813) *Crangon crangon* (Linnaeus, 1758) *Gnathophyllum elegans* (Risso, 1816) *Macrobrachium amazonicum* (Heller, 1862)
Astacidea	*Homarus gammarus* (Linnaeus, 1758)
Thalassinidea	*Upogebia pusilla* (Petagna, 1792)
Brachyura	*Amarses miersii* (Rathbun, 1897) *Pachygrapsus marmoratus* (Fabricius, 1787) *Chiromantes eulimine* (de Man, in Weber, 1897) *Perisesarma fasciatum* (Lanchester, 1900) *Maja brachydactyla* Balss, 1922

3.5. *Specimen collection and methodological background*

During the early phase of the project I tried to collect the larvae from as many different species as possible. We made trips to Rovinj in Croatia and to the island Giglio in Italy to collect species by hand while snorkeling and with traps and fishing dredges. I also visited the Alfred Wegener Institute on Helgoland, where Dr. Klaus Anger maintained a rearing laboratory with several different species. The "easiest" way to get the zoea I of a distinct species is to catch an egg bearing female that one can identify and then keep the female isolated in an aquarium until the larvae hatch. To succeed you need the right timing and a proper amount of luck. However, I managed to gather the zoea I of 17 different species and the zoea I-V of two species (Tab. 3).

The project was partly supported by Sea Life Center München with a grant, including travel expenses, laboratory material and aquaristic equipment. Thus, besides collecting trips we could maintain an aquarium at the Bavarian State Collection of Zoology in Munich where rearing experiments were conducted.

In the particular projects we worked with the zoea I of *Palaemon elegans* Rathke, 1837, *Hippolyte inermis* (Leach, 1815), *Porcellana platycheles* (Pennant, 1777) and *Pachygrapsus*

marmoratus (Fabricius, 1787) and with the zoea I-V of *P. elegans* and *Macrobrachium amazonicum* (Heller, 1862) and also with adults of *Neomysis integer* (Leach, 1814).

The applied methods were scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM), light microscopy (LM) and digital 3D-Reconstruction with Amira® Software. The specimen dissection and preparation for microscopy was rather challenging and often required special treatment because of the minuteness of the mandibles and their way of insertion on the head.

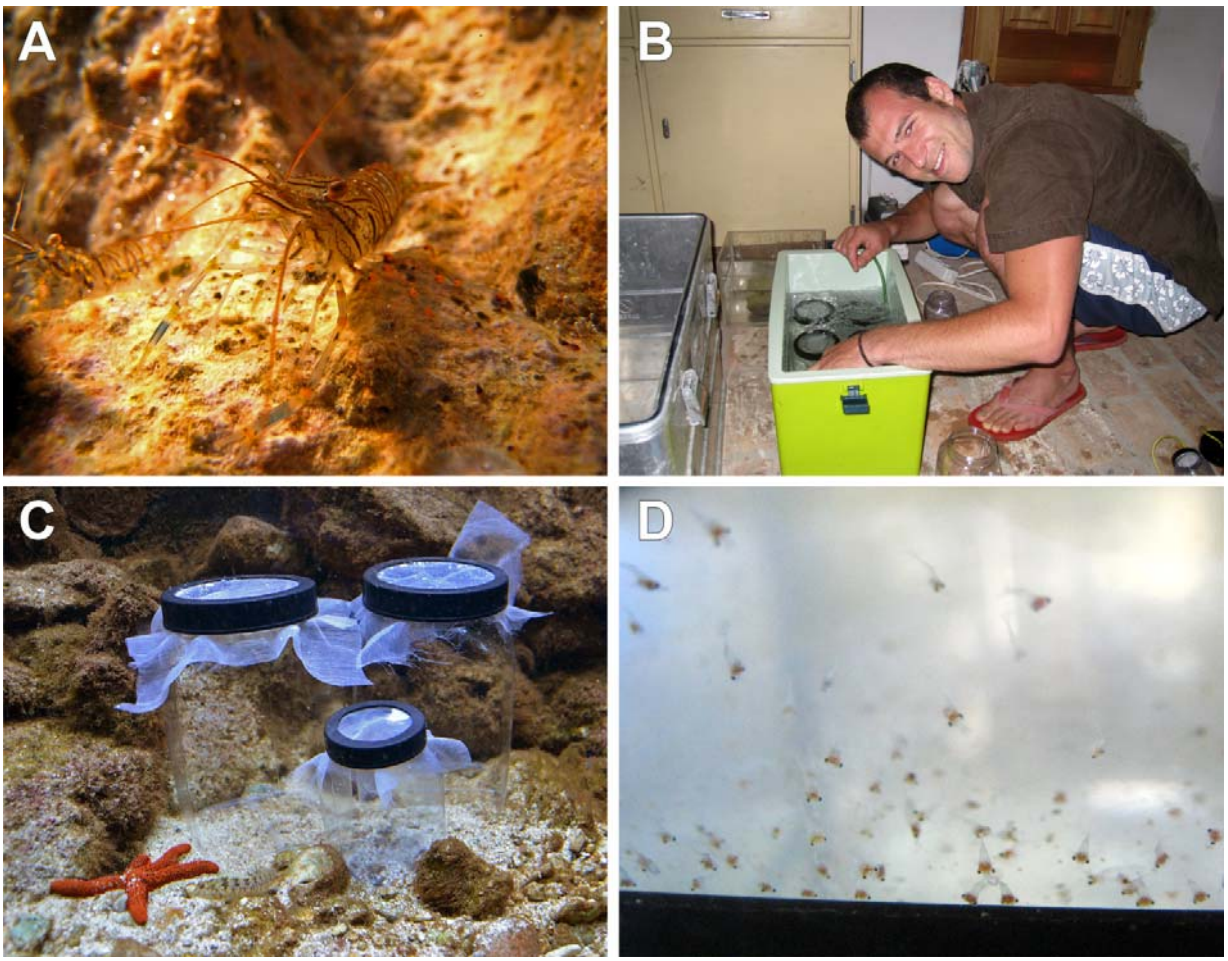


Figure 3: A: Adult *Palaemon elegans* in its natural habitat. B: Field work with improvised aquarium containing cautex vials to isolate collected egg bearing females. C: Cautex vials in the aquarium in Munich. D: Zoea I larvae of *P. elegans* in a separate small aquarium.

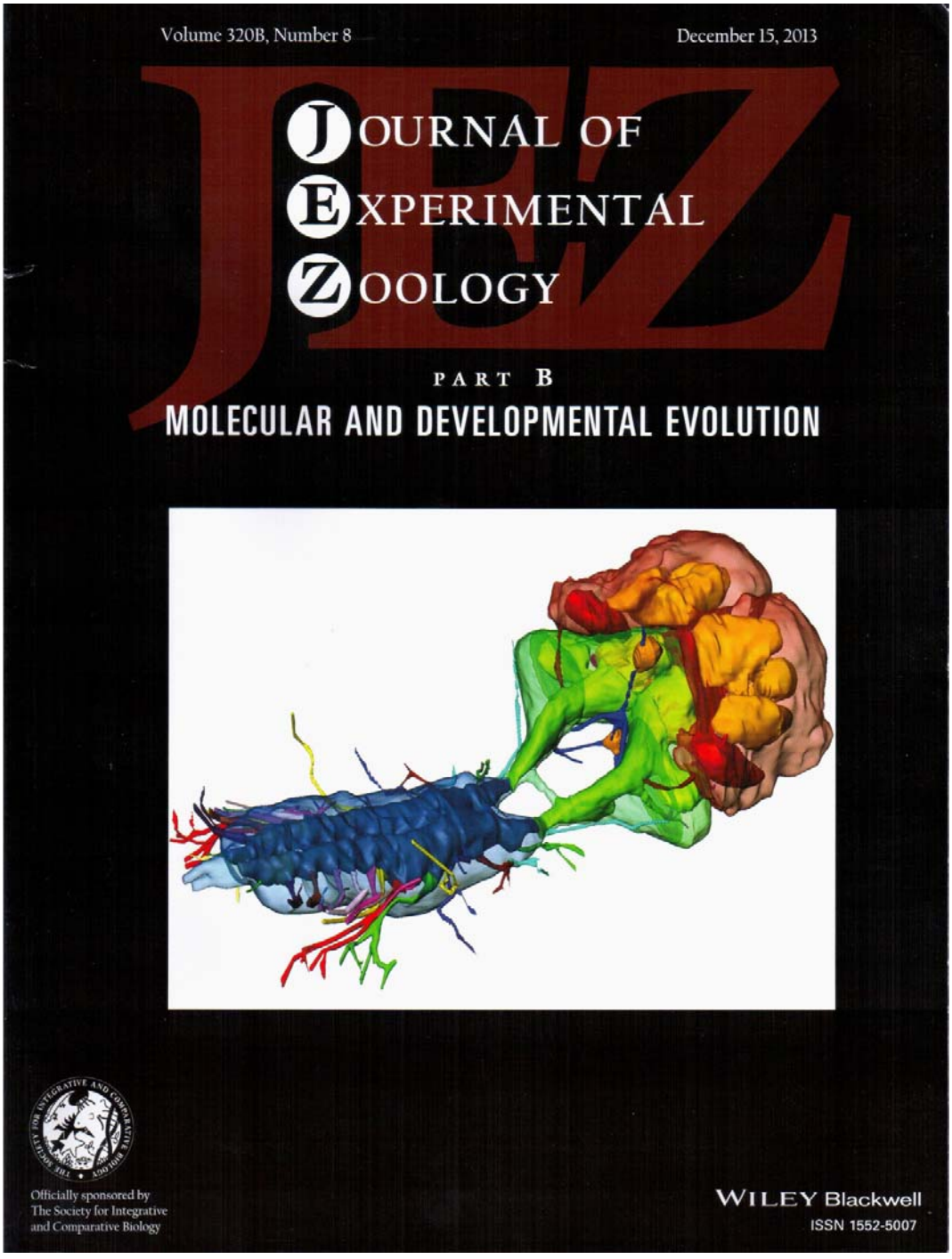
Studying the external morphology and fine structures of the larval mandibles I used SEM. It was not only quite difficult to dissect the small structures but also to keep them intact and not to lose some specimens during the preparation for the SEM. The dissection was necessary to get insight

into the otherwise covered structures on the medially oriented gnathal edges. I used thin tungsten wires mounted on glass tubes and sharpened with a fine grindstone to dissect the mandibles. During the critical point drying process the specimens were put in special tiny glass vials inside the usually used microporous specimen containers.

For the ultrastructural analyses at first I had to prepare series of sagittal ultrathin sections using a diamond knife and an ultramicrotome. The challenge was to cover an enormous distance comprising the critical region with a section thickness of only 60-70 nm. In *P. elegans* I had to cover about 100 µm and in *N. integer* about 400 µm, what was only manageable by alternating between ultrathin and semithin sections. Afterwards the section series were inspected in a TEM and the quantification of the structures was developed by inspecting consecutive sections.

4. Paper I

Geiselbrecht, H., Melzer, R.R., 2013a. Nervous systems in 3D: A comparison of caridean, anomuran, and brachyuran zoea-I (DECAPODA). Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 320, 511-524.



RESEARCH ARTICLE

Nervous Systems in 3D: A Comparison of Caridean, Anomuran, and Brachyuran Zoea-I (DECAPODA)

JOURNAL OF EXPERIMENTAL ZOOLOGY PART B MOLECULAR AND DEVELOPMENTAL EVOLUTION

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ABSTRACT

Using serial semi-thin sections and digital 3D-reconstructions we studied the nervous systems of zoea-I larvae in three decapod species, *Hippolyte inermis* (Leach, 1815), *Porcellana platycheles* (Pennant, 1777), and *Pachygrapsus marmoratus* (Fabricius, 1787). These taxa represent three decapod lineages, that is, Caridea, Anomura, and Brachyura, each characterized by specific zoea-I morphology. Special attention was paid to development of ganglia, neuropil composition, and segmental nerves. In all zoeae studied, the overall elements, for example, the segmental ganglia, their neuropils and most of the nerves of the adult decapod nervous system are present. Ongoing differentiation processes are observable as well, most obvious in segments with well-developed limbs the ganglia are in a more advanced stage of differentiation and more voluminous compared to segments with only limb buds or without externally visible limb *anlagen*. Intra- and interspecific comparisons indicate that neuromere differentiation thus deviates from a simple anterior–posterior gradient as, for example, posterior thoracic neuromeres are less developed than those of the pleon. In addition, the differences in the progress of the development of ganglia between the studied taxa can best be attributed to heterochronic mechanisms. Taxon and stage-specific morphologies indicate that neuronal architecture reflects both, morphogenesis to the adult stage and specific larval adaptions, and provides sets of characters relevant to understanding the corresponding phylogeny. *J. Exp. Zool. (Mol. Dev. Evol.) 320B:511–524, 2013.* © 2013 Wiley Periodicals, Inc.

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Abbreviations: ANT I, antenna I; AINv, antenna I nerve; ANT II, antenna II; AIINv, antenna II nerve; ANP, accessory neuropil; C, carapace; CB, central body; CC, cell-body cortex; CM, commissure; DCNP, deutocerebral neuropil; DC, dorsal commissure; DONP, dorsal optic neuropil; E, esophagus; EG, esophageal ganglion; LAB, labral nerve; L, lamina; LNv, lateral nerve; LO, lobula; LONP, lateral optic neuropil; M, medulla; ML, megalopa; MN, mandible; MNNv, mandibular nerve; MNNP, neuropil of mandibular ganglion; MONP, medial optic neuropil; MP, median protocerebrum; MXINv, maxilla I nerve; MXIINv, maxilla II nerve; MXP1–3, maxilliped 1–3; MXPNv1–3, nerve of maxilliped 1–3; NP, neuropil; OGT, olfactory globular tract; OL, olfactory lobe; OPL, optic lobe; PB, pereiopod bud; PEC, postesophageal commissure; P, pereiopod; PCT, protocerebral tract; PNv1–5, nerve of pereiopod 1–5; PGC, pereion ganglion commissure; PG, pereion ganglia; PGNP, pereion ganglion neuropil; PL, pleon; PLG1, pleon ganglion 1; R, retina; RS, rostral spine; SEG, subesophageal ganglia (mandible, maxilla I and II and maxilliped segments); STG, stomatogastric ganglion; TCNP, tritocerebral neuropil; TGN, tegumentary nerve; TM, terminal medulla; T, telson; TG, thoracic ganglia; VC, ventral commissure; Z, zoea.

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Larvae of animals with indirect development are of peculiar interest, since they exhibit sets of morphological characters that differ from those of the adults. These characters often represent ancestral, but sometimes also derived conditions, and therefore may be of high relevance for phylogeny reconstructions (see Anger, 2001; Clark, 2009). Furthermore they represent transitory stages in the development from embryo to adult, thus may be indispensable to evo-devo studies of the morphogenetic changes that indirectly developing species undergo during ontogeny. Unlike embryos, which are largely sealed off from external influences, many larvae live freely exposed to the environment, occupy their own niches and, therefore, stage-specific adaptive plateaus and structure–function relationships are manifested in their morphology (Anger, 2006).

The immature stages of bottom-dwelling decapods, representing the planktonic stages within the pelago-benthic lifecycles in this taxon, are classical examples of such larvae. They are found in three different forms, the nauplius (free larval stages in Decapoda are only present in Dendrobranchiata), zoea and megalopa (Anger, 2001). The zoea larvae exhibit distinct, species-specific features; therefore, the larvae of numerous species have been described and illustrated from classical preparations for compound light microscopes (Gurney, '42; Rice, '80), and recently also using the scanning EM (Meyer et al., 2006; Geiselbrecht and Melzer, 2010).

The structure and function of the nervous system of adult decapods has been studied in great detail (e.g., Bullock and Horridge, '65; Nässel and Elofsson, '87; Sandeman et al., '92, '93; Harzsch et al., 2012; Strausfeld, 2012). For a few species the knowledge of larval internal anatomy is comprehensive as well, comprising histological studies on the ontogeny of larval osmoregulatory structures (Cieluch et al., 2005; Cieluch et al., 2007) and immunocytochemical studies on the development of neuroendocrine centers of larval European lobsters (Rotllant et al., '93, '94, '95). The development of the decapod nervous systems regarding different larval stages has been thoroughly discussed in several studies. Harzsch and Dawirs ('94, '95, '96a, '96b) studied neurogenesis in *Hyas araneus* (Linnaeus, 1758) larvae mainly using immunocytochemical methods. A detailed histological analysis is available for *Carcinus maenas* (Linnaeus, 1758) in Harzsch and Dawirs ('93) and for *C. destructor* Clark, 1936 and *Homarus americanus* H. Milne Edwards, 1837 in Helluy et al. ('93). However, in those works the focus is either on later larval stages, for example, on the megalopa in Harzsch and Dawirs ('93), or only on particular areas of the nervous system, for example, on the brain in Helluy et al. ('93). And for many decapod taxa, amongst others the Anomura, such analyses have not been accomplished to date.

Therefore, the value in extending such studies to early zoeal stages of various decapods is obvious. Furthermore, recent micromorphological analyses of small organisms have benefited from novel computer-based reconstruction techniques using visualization software like Amira, analySIS or BioVis3D, which allow the processing of series of semi-thin sections to 3D views that display shape, steric arrangement of structural elements and their connections in great clarity.

We made serial semi-thin sections of various decapods and analyzed them with Amira to contribute to a wider basis of studied taxa that will allow more detailed comparisons between larvae, the corresponding adults, and outgroup representatives.

We studied the central nervous system (CNS) and segmental nerves of zoea-I larvae in three decapod taxa, *Hippolyte inermis* (Leach, 1815), *Porcellana platycheles* (Pennant, 1777), and *Pachygrapsus marmoratus* (Fabricius, 1787). These represent the three decapod lineages, Caridea, Anomura, and Brachyura, each characterized by specific zoea-I morphologies, larval lifestyles, and ontogenies.

The Caridean *H. inermis* hatches with the maxillipeds 1–3 fully developed. The 1st pereiopod is present as an embryonic bud, and the *anlagen* of the following appendages are not visible from the outside. In further postembryonic development eight zoeal stages are passed until the megalopa stage (Williamson, '57; Bourdillon-Casanova, '60; Zupo and Buttino, 2001). In the first zoeal stage of the anomuran *P. platycheles* only the 1st and 2nd maxillipeds are developed. The 3rd maxillipeds are present as biramous buds, the pereiopods as embryonic buds. *P. platycheles* possesses only two zoeal stages preceding the megalopa (Lebour, '43; González-Gordillo et al., '96). The brachyuran *P. marmoratus* also hatches with the 1st and 2nd maxillipeds developed and the 3rd maxillipeds are only present as embryonic buds. The *anlagen* of the pereiopods are not yet visible externally. In *P. marmoratus* there are six zoeal stages (Cuesta and Rodríguez, '94, 2000).

We show that many parts of the CNS, like the ganglia of the anterior CNS or ganglia of the mouthpart and pleon segments are well developed in the first-stage zoeae already, whereas other parts, like ganglia in particular maxilliped and pereion segments show a developmental lag. This partly reflects the sequential differentiation of body segments that larvae undergo during ontogeny, or corresponds well with the development of the respective segmental appendages. Comparing the species among each other differences in the progress of the development of neuromeres can be observed and revealed as possible heterochronic events, that is, changes in the timing or rate of developmental events compared to more ancestral relatives (De Beer, '40; Gould, '77; McKinney and McNamara, '91). These can be correlated with the different life histories and number of larval stages of the studied species.

MATERIALS AND METHODS

Ovigerous females of *H. inermis*, *P. platycheles*, and *P. marmoratus* were caught near Rovinj (Croatia) in shallow waters (1 m depth) inhabiting seagrass meadows (*H. inermis*) and the coastal littoral (*P. platycheles* and *P. marmoratus*), and kept in an aquarium at the laboratory of the Ruđer Bošković Institute until

larvae hatched (see also Meyer et al., 2004). The cuticulae of first stage zoea larvae were perforated with fine needles in cooled primary fixative (4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.1); afterwards the specimens were soaked in primary fixative for some hours in a refrigerator. Specimens were washed in sodium cacodylate buffer and then postfixed/postosmicated in 1% osmium tetroxide in buffer. After dehydration over graded acetone series specimens were embedded in epoxy resin according to standard procedures (Richardson et al., '60).

Serial semi-thin sections (1.5 μm) were cut with a Diatome HistoJumbo diamond knife on a Microm HM 360 microtome (Boeck, '84). Ribbons of semi-thin sections were obtained using contact cement as described in Henry ('77) and Ruthensteiner (2008), stained after Richardson et al. ('60), and embedded in DPX (Fluka, Buchs, Switzerland). One specimen of each species was cut throughout in transversal plane. Photographs of the sections were taken with a ProgRes® Speed XT core5 camera mounted on a Leica DM5000B microscope. After editing (resize, change to gray scale, unsharp mask) in Adobe Photoshop (Adobe Systems, Mountain View, CA, USA), photo stacks covering the volume of the CNS and containing the images of every single section were imported in Amira 5.2.0 software (Visage Imaging, Berlin, Germany) and aligned. Then, corresponding structures were extracted, labeled, and visualized by surface rendering. The program also provides the opportunity to conduct volume measurements of the labeled materials, what we used to measure the volume of the different neuropils. To display the whole CNS as well as the details in every species, two photo stacks of different magnification (*H. inermis*: 20× and 40×; *P. platycheles*: 5× and 20×; *P. marmoratus*: 10× and 40×) were processed separately.

The terminology in this manuscript is based on Sandeman et al. ('92) with a modification of the terminology of the optic neuropils as suggested by Harzsch (2002) and standard neuroanatomical terminology is adjusted to Richter et al. (2010). Generally several nerves leave the neuropils of each segment, containing sensory and motor neurons of the peripheral nervous system (Sandeman et al., '93). In this study, the 3D-reconstructions mainly depict the nerves innervating the segmental appendages, and these nerves are named either after the respective appendage, for example, antenna I nerve, or after a segmental nerve in more general context. For a better understanding we decided to distinguish the usually termed thoracic ganglia 1–8 in 1st to 3rd maxilliped ganglion and pereion ganglion 1–5 in reference to the segment appendage.

RESULTS

General Features of the Larval Central Nervous System

The anterior CNS of the studied first-stage zoeae is composed of well-developed ganglia, with neurites forming a central neuropil and a surrounding cell-body cortex. Within the brain the proto-, deuto, and tritocerebrum are prominent (Fig. 1); nerves or tracts can be found connecting these neuropils with sense organs and/or appendages of the head, like the compound eyes, antennae I, antennae II, and labrum (Figs. 1 and 2). Following the tritocerebrum, the esophageal connectives project posteriorly to the ganglia of the mandibular and maxilla I and maxilla II segments, followed again by the ganglia and/or *anlagen* of the three maxilliped and the five pereion segments (Figs. 1 and 3). By virtue of very short connectives ambilaterally joining consecutive ganglia, the neuropils are fused in anterior–posterior direction resulting in a composite structure that combines elements in various stages of differentiation (Figs. 1 and 3). By contrast, connectives between the posteriormost pereion and the 1st pleon ganglion and between the subsequent pleon ganglia are well distinguishable. Thus, the respective ganglia can be recognized individually and form a classical ventral nerve cord located in the pleon segments (Figs. 1 and 4).

The Protocerebrum. The supraesophageal neuropils are almost completely surrounded by a cell-body cortex (Figs. 5A–C, 6A, and 7A and B). Within the optic lobes, the optic neuropils form in the transversal plain obliquely arranged rows that taper medially (Fig. 2). The optic neuropils comprise—from exterior to interior—a well-developed lamina of convex form, a kidney-shaped medulla, a globular lobula, and a pronounced medulla terminalis (Figs. 2, 5A and B, 6A, and 7A and B). Postero-ventrally there follows the median protocerebrum, connected via the protocerebral tracts (Figs. 1C, 5B, and 7B). The median protocerebrum is also composed of units, the dorsal (Figs. 5B and 7B), lateral (Fig. 6C), and median optic neuropils (Figs. 2, 5C, and 6B). The most anterior parts are the paired dorsal optic neuropils, followed ventro-laterally by the also paired lateral optic neuropils. The two portions of the median optic neuropils are medially fused. In this region also the central body is located (Fig. 6B), a clearly distinguishable neuropil with transversally extending neurites.

The Deutocerebrum. Posterior to the protocerebral region the brain divides and the two hemiganglia of the deutocerebrum continue posteriorly on both sides (Fig. 1). The main elements are the laterally expanding globular olfactory lobes (Figs. 1C, 2A and C, 5C, 6C, and 7C). Connecting the deuto- and protocerebrum the olfactory globular tracts leave the olfactory lobes medially (Fig. 6C). Posteriorly ascending from the antennae I, the antenna I nerves enter the olfactory lobes (Figs. 2 and 7C).

The Tritocerebrum. Posteriorly to the deutocerebrum but without a distinct demarcation, the tritocerebrum is present (Fig. 1). The tritocerebral hemiganglia are oriented paralaterally to the esophagus (Fig. 5D). Three conspicuous nerves connect the tritocerebrum with various sections and appendages of the tritocerebral segment on both sides: ventro-laterally the antenna II nerve is inserted (Figs. 1C and 2A–C), anteriorly the labral

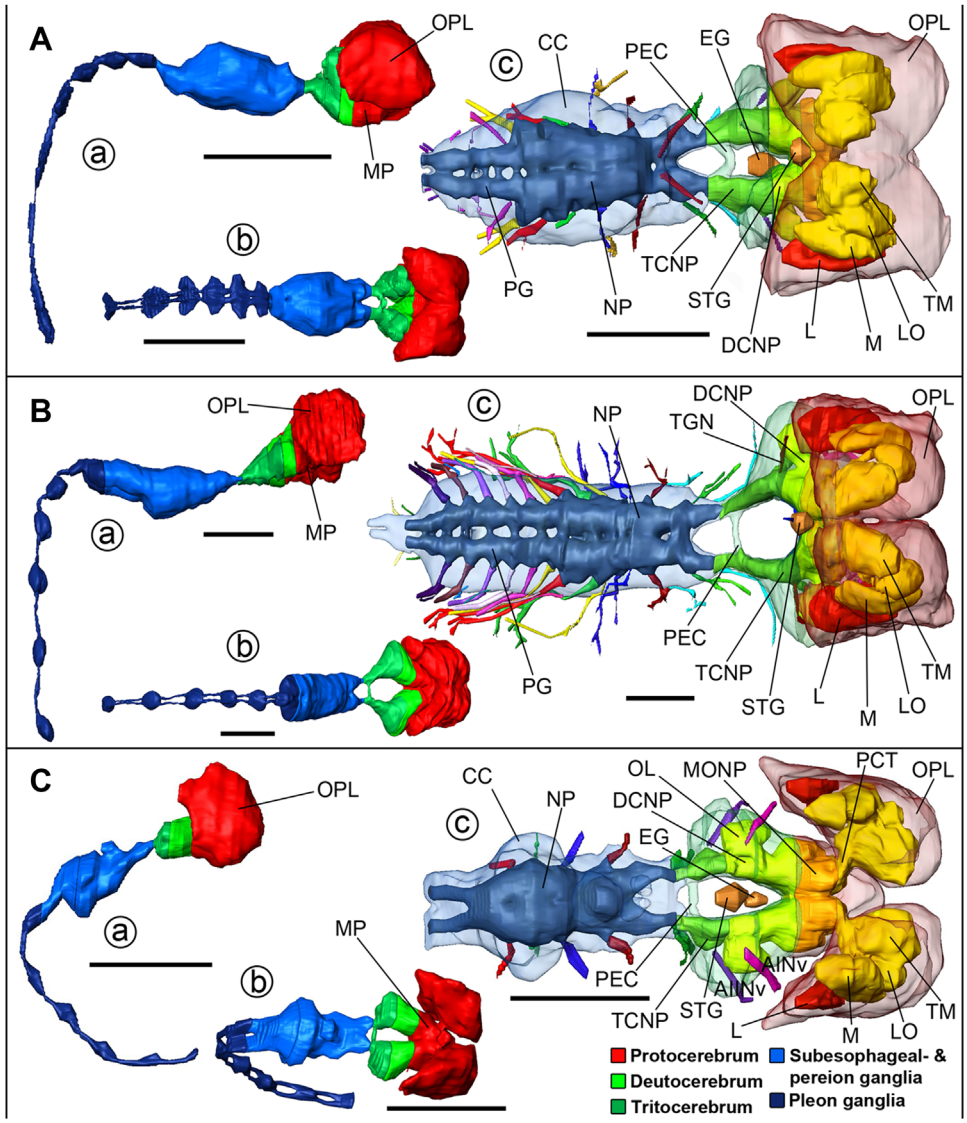

Figure 1. 3D-reconstructions of CNS. (A) *Hippolyte inermis*. (B) *Porcellana platycheles*. (C) *Pachygrapsus marmoratus*. (a) Lateral view, showing surface of cell-body cortex (bar = 200 μm). (b) Dorsal view, showing surface of cell-body cortex (bar = 200 μm). (c) Dorsal view, showing cell-body cortex in reduced transparency, and neuropils and nerves (bar = 100 μm).

nerve (Fig. 2B), and dorsally the tegmentum nerve (Fig. 1B). The small esophageal ganglion is situated antero-medially to the esophagus (Figs. 1A and C and 2A–C). It is connected to both hemiganglia of the tritocerebrum via lateral nerves (Fig. 2B), and the stomatogastric nerve is running dorsally forming the connection to the stomatogastric ganglion (Figs. 1A–C, 2A–C, 5C, and 6C), which is located anterio-medially to the stomach. Posterior to the esophagus the postesophageal commissure connects the two hemiganglia of the tritocerebrum (Figs. 1A–C and 6D).

The Ganglia of the Mandible, Maxilla I, Maxilla II and Maxilliped 1–3 Segments. The ganglia of these six segments are of a globular shape, they are longitudinally and transversally fused, and connectives as well as commissures are very short and not visible in reconstructed external view (Figs. 1 and 3). Sections in this area, however, show the presence of dorsal and ventral commissures connecting the segmental hemiganglia transversally (Fig. 6E) but the connectives cannot be distinguished due to the transversal sectioning plane. The cell-body cortex is most prominent ventrally (Figs. 6E and 7E). The ganglia receive input via lateral segmental

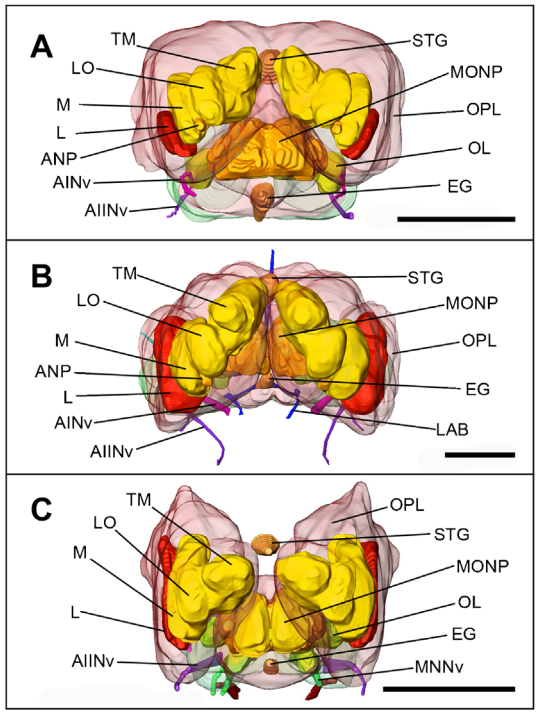

Figure 2. 3D-reconstruction of protocerebrum in frontal view, showing optic neuropils. Cell-body cortex displayed in reduced transparency (bars = 100 μm). **(A)** *Hippolyte inermis*. **(B)** *Porcellana platycheles*. **(C)** *Pachygrapsus marmoratus*.

nerves, that is, those of the mandibles, maxillae I, maxillae II, and the three maxillipeds (Fig. 3A, C, and E).

The Ganglia of the Pereion and Pleon Segments. The ganglia of the pereion segments do not show any feature common to all three species. Contrary to this, all six pleon segments in all species exhibit a distinct ganglion with a short commissure and long intersegmental connectives, but no segmental nerves can be detected (Fig. 1).

Species-Specific Characteristics

Hippolyte inermis. The cell-body cortex of the optic lobes is medially fused (Figs. 1A, 2A, 3A and B, and 5). The lamina is approximately half the size compared to the other species. A small accessory neuropil is located ventrally between the internal and external medulla. The ganglia of the 1st, 2nd, and 3rd maxilliped segments are well developed, each with a distinct segmental nerve projecting postero-laterally. The neuropil of the ganglion of the 1st pereion segment (VR = 2.72) is about one-fifth the size of the 3rd maxilliped neuropil (VR = 13.64) (Figs. 2B, 8A and B and Table 1), but the segmental nerve is also well developed. In the following pereion segments the segmental composition is obvious, as slender commissures are found between the neuropil portions but no distinct segmental nerves can be detected (Fig. 3A). The cell-body cortex of the pleon ganglia extends laterally, resulting in a wing-like shape.

Porcellana platycheles. The cell-body cortex of the optic lobes is also fused but with an observable medial demarcation (Figs. 1B, 2B, 3C, D, and 6). Also, a small accessory neuropil is located ventrally between the internal and external medulla. The ganglia of the 1st and 2nd maxillipeds are well developed, with a segmental nerve projecting postero-laterally. The neuropil as well as the nerve of the segment of the 3rd maxilliped is of distinctly smaller size (VR = 5.78), whereas the size of the neuropil of the 1st pereion segment (VR = 5.04) is similar to that of the 3rd maxilliped segment (Figs. 3D and 8C and D and Table 1). The ganglia of pereion segments 1–5 are evenly developed, each with a slender commissure connecting the two hemiganglia. A distinct nerve innervating the pereiopod buds can be detected in each segment (Fig. 3C). Displaying only very short connectives, the 1st pleon ganglion is closely spaced to that of the 5th pereion segment.

Pachygrapsus marmoratus. The median portions of the two optic lobes show no direct contact (Figs. 1C, 2C, 3E and F, and 7). The antenna I nerve enters the olfactory lobes dorsally (Figs. 1C and 7C). Following the mandible, maxilla I and maxilla II segments, only the ganglia of the segments of the 1st and 2nd maxillipeds are well developed and show a distinct segmental nerve. The cell-body cortex in this area is enlarged laterally and ventrally, resulting in a globular shape. The ganglion of the segment of the 3rd maxilliped is less developed and the respective nerve is not definable. The neuromeres of the future pereion segments 1–5 are not yet differentiated and not definable.

DISCUSSION

Methodological Approach

Various recent studies have shown that the method of computer-aided 3D-reconstruction of the inner organization of small animals using serial semi-thin sections has been developed to a level that makes it a useful method. Not only long-known facts can be depicted in a nice way, but also new insights into the forms, steric arrangement and structural connections of organs, organ systems and any kind of elements of a morphological structure can be gained (e.g., Neusser et al., 2006; Fritsch and Richter, 2010; Sombke et al., 2011). The application of the method to the nervous system of decapod larvae, as in the present article, seems to support this notion, although in many parts of the CNS we show the segmental composition of ganglia, neuropils and connecting tracts that is described for decapod adults and has been well known from zoology textbooks for a long time (e.g.,

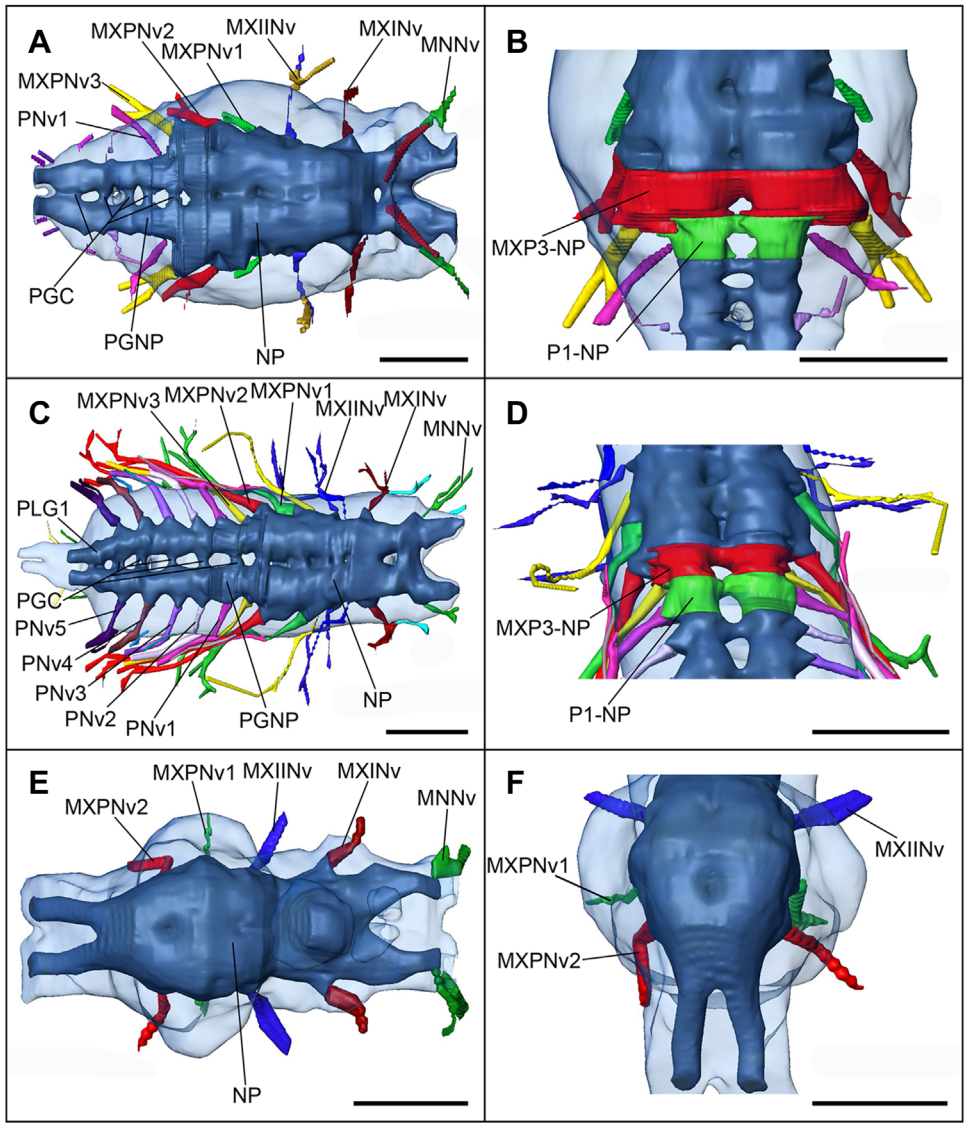

Figure 3. 3D-reconstruction of mandible, maxilla I and II and maxilliped ganglia in dorsal view, showing neuropils and segmental nerves. Cell-body cortex displayed in reduced transparency. (**A** and **B**) *Hippolyte inermis* (bars = 50 μm). (**C** and **D**) *Porcellana platycheles* (bars = 100 μm). (**E** and **F**) *Pachygrapsus marmoratus* (bars = 50 μm).

Hanström, '47; Bullock and Horridge, '65). Hence, many of the structures described here are miniature versions of the later adult configurations, and represent plesiomorphic character states at least for Decapoda, but partly even for Crustacea, Mandibulata, and/or Euarthropoda.

However, some of the observations that could be documented in great detail and clarity with our 3D-reconstructions advance the understanding of the specific aspects of larval morphogenesis summarized at the beginning. We will, thus, mainly discuss the points that are relevant in this context.

General Features of the Larval CNS

The basic elements of a typical adult decapod CNS, as shown in Sandeman et al. ('93), are already recognizable in all first-stage zoeae, for example, the segmental ganglia, their neuropils and the segmental nerves. The proto-, deuto-, and tritocerebrum are well differentiated; a stomatogastric ganglion and an esophageal ganglion could be detected as parts of the stomatogastric nervous system. Following posteriorly in all species the ganglia of the maxilla I, maxilla II, mandible and 1st and 2nd maxilliped segments are evenly developed and innervated by distinct

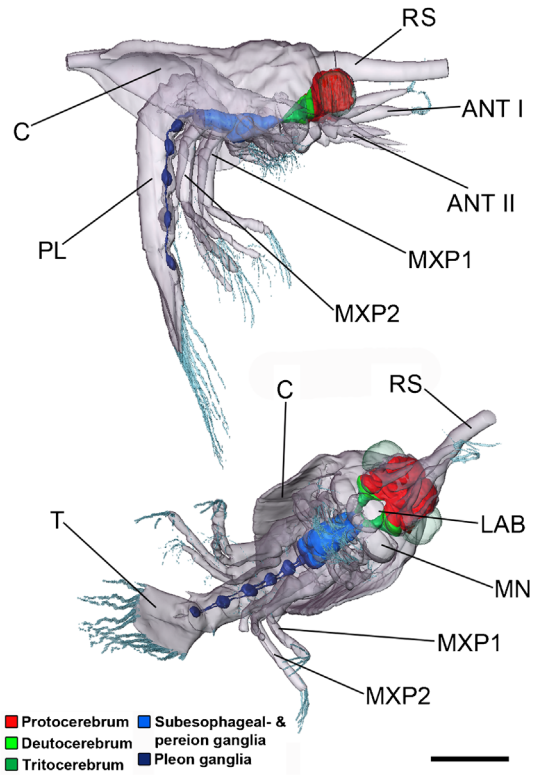

Figure 4. 3D-reconstruction of zoea I of *Porcellana platycheles* in lateral and ventral view, showing the CNS and its position within the body (bar = 400 μm). Note realistic display of body shape and its segmental appendages. Surface of body displayed with reduced transparency.

segmental nerves. The general and structural organization of the CNS of a first-stage zoea also corresponds well with that described for later developmental stages, for example, for the megalopa in *C. maenas* (see Harzsch and Dawirs, '93). Both developmental stages, the zoea-I and the megalopa, are characterized by a coherent cell-body cortex surrounding the neuropil, in contrast to the adult situation, where the cell somata in the brain are arranged in clearly recognizable clusters (Sandeman et al., '92). However, in the first-stage zoea the protocerebral tracts are short and the optic lobes are located close to the median part of the protocerebrum. In the *C. maenas* megalopa the protocerebral tracts stretch laterally and the optic lobes move away from the brain in association with the growth of the larval eye stalks. The differences in development between the 3rd maxilliped and/or the pereion ganglia detected in the first-stage zoeae studied here, and discussed in detail below, are not observable in the megalopa (Harzsch and Dawirs, '93).

Furthermore, we could not identify the median connective that is found in many malacostracans and some other crustaceans (see, e.g., Harzsch et al., '97; Stegner and Richter, 2011; and revision in Harzsch, 2003). We assume that it was not detectable in our transversal semi-thin sections due to its longitudinal orientation and the small body volume of the first-stage zoea. Very delicate nerves such as the labral and tegmentum nerves only could be clearly distinguished in *P. platycheles*. The ganglia, however, connected by these nerves were observed in all three species.

In contrast, we could find structures that are not described from other species or from adult decapods, such as the small accessory optic neuropils in *H. inermis* and *P. platycheles*. The connections of these paired neuropils remain unclear, but similar structures are described from other arthropods, for example, from *Chaoborus crystallinus*, a dipteran species (Melzer, 2009), or from *Nebalia herbstii*, a leptostracan (Kenning et al., 2013). In Hexapoda, the accessory neuropils are connected with stemmata or larval eyes. In the crustaceans studied so far, no such connections have been found. The accessory neuropils might be a shared character of the Tetraconata, but this question remains to be addressed by a detailed comparative study (see discussion in Melzer, 2009).

Inter- and Intraspecific Comparison of Morphogenesis

On the neuronal level segmental ganglia and nerves reflect different developmental plateaus of the larval body segments and tagmata; for example, segments with already well-developed appendages possess well-developed ganglia as well, whereas in segments without limbs or limb buds the morphogenesis of ganglia is also at a less advanced stage. Different sets of nervous system characters are thus revealed for the studied species, correlated with different types of external zoea-I morphology (Table 1). Furthermore, the occurring taxon-specific differences are relevant under the aspects of (1) comparing different taxa and (2) analysis of ontogeny.

In *H. inermis* maxillipeds 1–3 are well developed and the 1st pereiopod is present as an embryonic bud; accordingly the ganglia and nerves of the three maxilliped segments are evenly developed and the ganglion of the 1st pereiopod segment is proportionally less. In *P. platycheles* a similar developmental divergence among the ganglia is observable when the 1st and 2nd maxilliped segments are compared with the 3rd. Here the 3rd maxilliped is only present as a biramous bud, but the pereiopods are already present as embryonic buds as well. Also the corresponding ganglia and nerves of these segments are evenly developed, but less so compared to the 1st and 2nd maxilliped segments. In *P. marmoratus* the 3rd maxilliped is only present as an embryonic bud and pereiopods are completely undeveloped, which is reflected in rather undeveloped neuromeres of the respective segments. Studying *H. araneus* and *H. americanus* Harzsch et al. ('98) described the effects of a correlation between the degree of maturation and the use of segmental appendages and by comparing the neurogenesis in the ventral nerve cords of brachyuran larvae, lobster larvae and crayfish larvae, which exhibit different modes of embryonic and larval development,

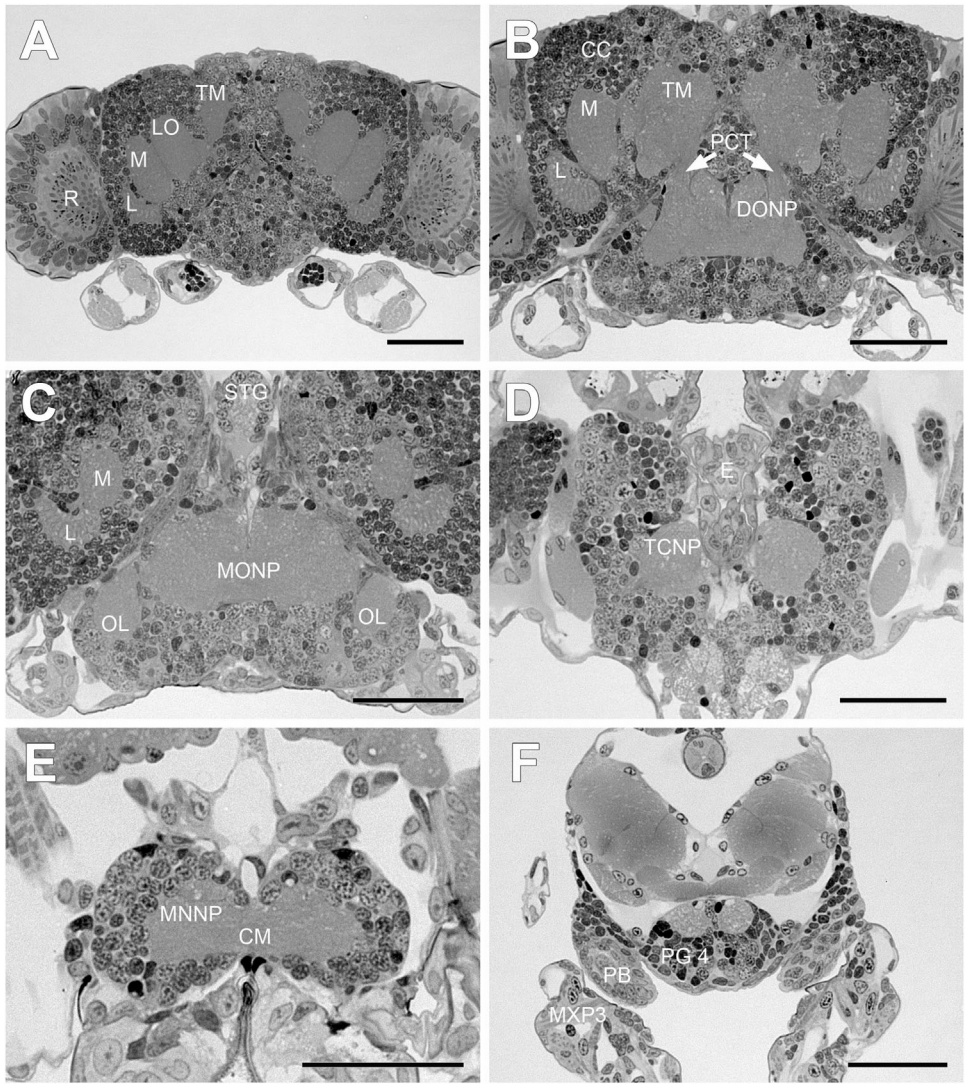

Figure 5. Transversal semi-thin sections of zoea I of *Hippolyte inermis* (bars = 50 μm). (A) Optic neuropils within optic lobe, (B) optic lobe and median protocerebrum, (C) median protocerebrum, (D) tritocerebrum, (E) ganglion of mandibular segment, (F) ganglion of 4th pleon segment.

temporal patterns of neurogenesis could be detected (Harzsch, 2003). Together, these results strengthen the claim that the patterns of neurogenesis in the ventral ganglia of decapod crustaceans are intimately related to the development of the segmental appendages and maturation of motor behaviors. In *H. araneus*, which hatches with thoracic segments 3–8 bearing only embryonic limbs, neurogenic activity persists into the larval stages. In *H. americanus*, which hatches with functional appendages on all eight thoracic segments, the neuroblast proliferation already ceases when embryogenesis is 80% complete (Harzsch and Dawirs, '94; Harzsch et al., '98). The species studied here also show differences in the number of functional appendages at hatching, which appear to be related to variation in state of neuromere differentiation and/or size in the ventral ganglia. It seems that the temporal patterns of neurogenesis as observed by Harzsch et al. ('98) and by Sullivan and MacMillan (2001), and the differences observed here, are two facets of the same phenomenon, that is, that timing of developmental processes is an important factor forming segment-specific adaptions during ontogeny, and also differences between taxa.

Usually during development of the ventral nerve cord additional neuromeres and their commissures emerge in an

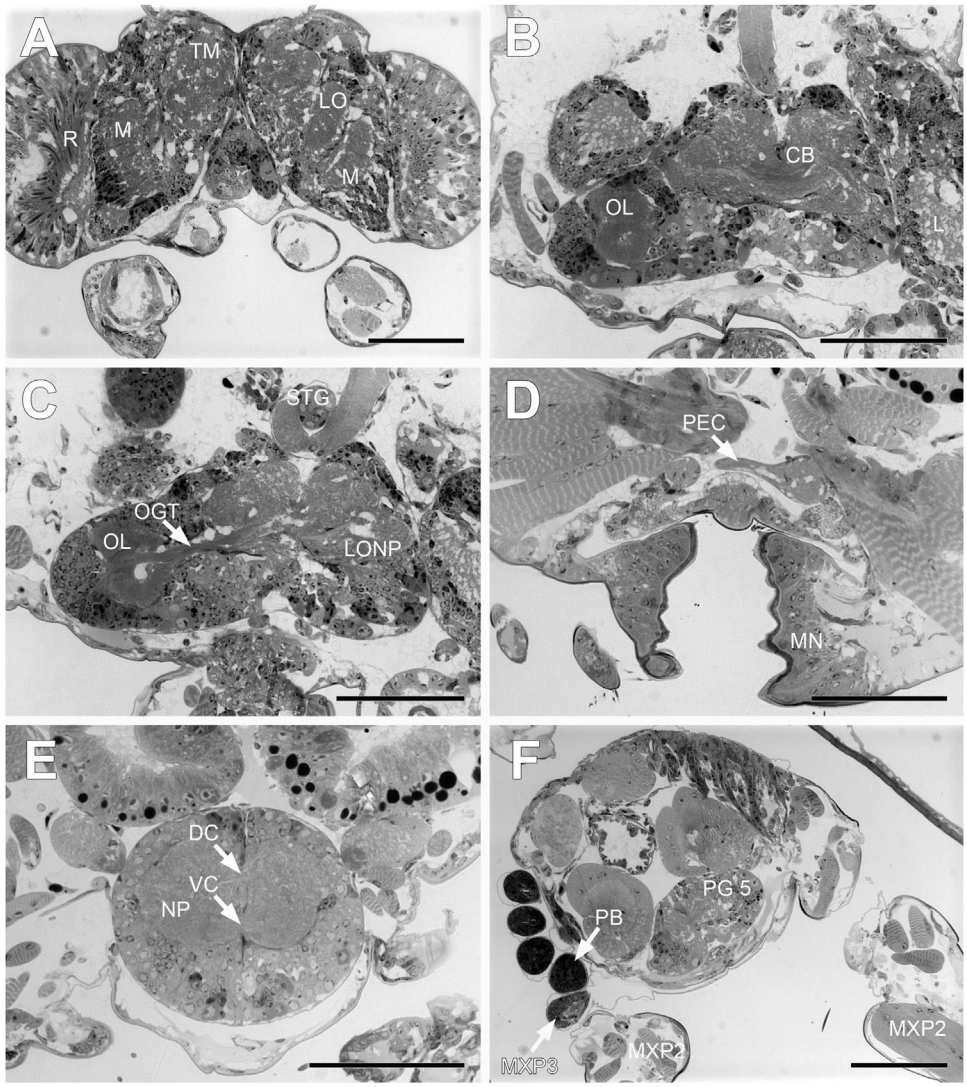

Figure 6. Transversal semi-thin sections of zoea I of *Porcellana platycheles* (bars = 100 μm). **(A)** Optic neuropils within optic lobe. **(B)** Median protocerebrum and olfactory lobe. **(C)** Fiber bundle of olfactory globular tract leaving olfactory lobe. **(D)** Postesophageal commissure. **(E)** Ganglion of maxilla II segment. **(F)** Ganglion of 5th pleon segment and pereiopod buds.

anterior–posterior gradient (Vilpoux et al., 2006; Fritsch and Richter, 2010; Ungerer et al., 2011). This is well reflected here in the progress of neuromere development of the successive thoracic segments, but not in the whole body: While the ganglia in the posteriormost pereion segments are the least developed, all species show a well-developed ventral nerve cord in the pleon segments; therefore, in our zoeae the anterior–posterior gradient is interrupted in the pereion neuromeres of segments with underdeveloped limbs. This, however, can be correlated with the life style of the planktonic larvae: while swimming with the exopods of the present maxillipeds (Gurney, '42), they additionally all show an escape behavior through a complex mechanism of rapid strokes of the pleon (Dahl, '83). Hence it is obvious that both the maxilliped and the pleon musculature and its innervating nervous system should be well developed, and similarly so in all species, while the remaining thoracic neuromeres can be retained at a less differentiated state until the corresponding limbs are differentiated.

Conspicuous interspecific differences are found when ganglia, neuropils and segmental nerves of the 3rd maxilliped segment are compared. The different external morphologies, as described above, are reflected in different developmental stages of the

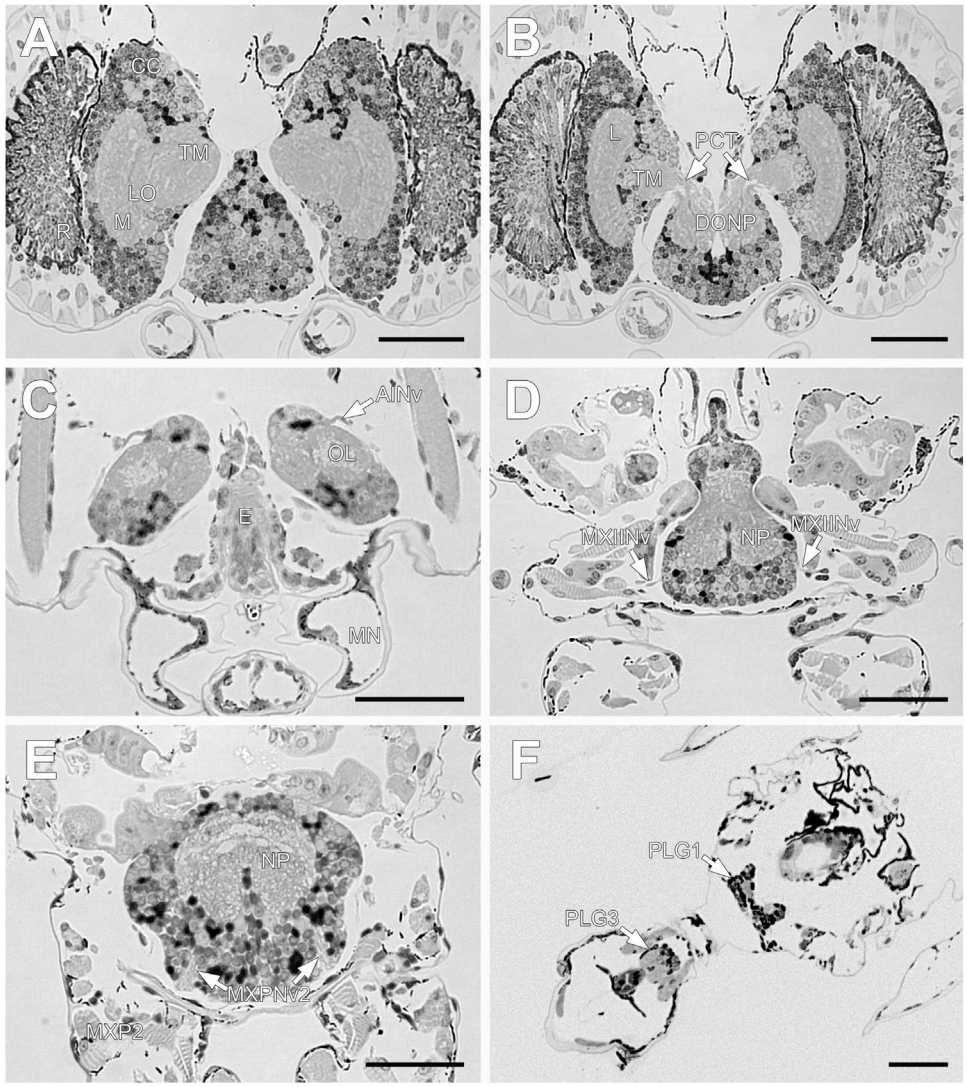

Figure 7. Transversal semi-thin sections of zoea I of *Pachygrapsus marmoratus* (bars = 50 μm). **(A)** Optic neuropils within optic lobe. **(B)** Optic lobe and median protocerebrum. **(C)** Olfactory lobe. **(D)** Ganglion of maxilla II segment. **(E)** Ganglion of 2nd maxilliped segment. **(F)** Ganglion of 1st and 3rd pleon segment.

nervous system (Table 1). Correspondingly, the 3rd maxilliped ganglion, neuropil and segmental nerve are well developed in *H. inermis*, whereas in *P. platycheles* they are also well developed but proportionally smaller and in *P. marmoratus* this neuromere is not definable, that is, undeveloped. Comparison of the pereion segments reveals a similar correlation between limb and neuromere development. Only in *P. platycheles* the pereiopods 2–5 are present as embryonic buds, what is also reflected by well-developed ganglia and segmental nerves in the respective segments. In *H. inermis* and *P. marmoratus*, ganglia and nerves are developed to a much lesser extent in segments lacking pereiopod buds.

A Case of Heterochrony?

The differences observed within and between species concerning the stage of development of neuromeres and nerves are paralleled by the respective appendage development. This variation can be explained best by shifts in the timing of morphogenetic events, for example, like the early appearance and rapid maturation of the accessory lobes in the freshwater crayfish *C. destructor* compared with the lobster *H. americanus* (Helluy et al., '93). Another such example is the stage-specific timing of appearance and rate of development of certain setae and other characters occurring during development in different species of pilumnine crabs (Clark, 2005).

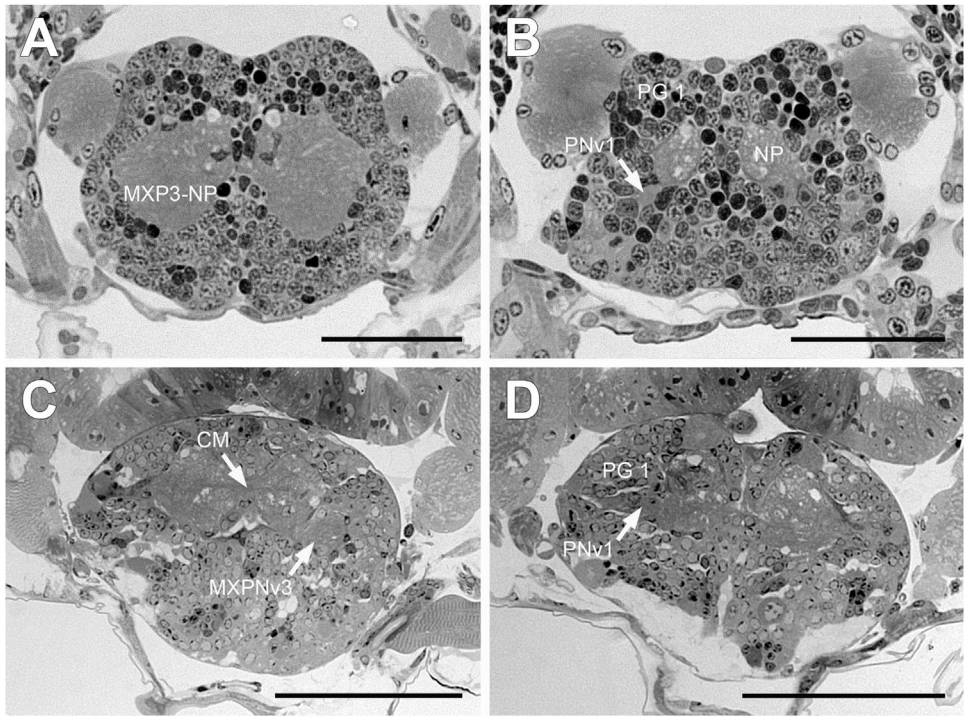

Figure 8. Transversal semi-thin sections of zoea I of *Hippolyte inermis* (**A** and **B**, bars = 50 μm) and *Porcellana platycheles* (**C** and **D**, bars = 100 μm), showing respective proportions of 3rd maxilliped neuropils and 1st pereion neuropils.

In a few other decapod species, detailed analyses have shown that these differences in ganglion/limb development disappear during later development. In *H. araneus*, *H. americanus*, and *C. destructor* (Sullivan and MacMillan, 2001; Harzsch, 2003), neurogenic activity ceases during or before molting to the stage in which the appendage of a segment is first used in coordinated movements. Considering the species studied here, all the limbs that are underdeveloped in the first-stage zoea are well developed and

Table 1. Taxon-specific features of zoea-I morphologies and neuropil volumes (x, present; o, absent).

	Hippolyte inermis	*Porcellana platycheles*	*Pachygrapsus marmoratus*
Appendages			
MXP1	x	x	x
Mxp2	x	x	x
Mxp3	x	Biramous bud	Embryonic bud
PER1	Embryonic bud	Embryonic bud	o
PER2	o	Embryonic bud	o
Pleon (somites)	5 + T	5 + T	5 + T
Volume (μm^3)			
SEG + TG-NP	334905	1571547	179676
MXP3-NP	45673	90771	–
PER1-NP	9098	79202	–
Volume ratio (%)			
MXP3-NP	13.64	5.78	–
PER1-NP	2.72	5.04	–
Larval stages	ZI-VIII, M	ZI-II, M	ZI-VI, M

become functional during subsequent larval development. In *H. inermis* this concerns the 1st pereiopod in zoea III (Zupo and Buttino, 2001), in *P. platycheles* the 3rd maxilliped and the 1st pereiopod in zoea II (González-Gordillo et al., '96), and in *P. marmoratus* the 3rd maxilliped and the 1st pereiopod in zoea VI (Cuesta and Rodríguez, 2000). It can be expected that the underdeveloped ganglia of the first-stage larvae accordingly develop in the later stages.

Since the decapod lineages of our studied species, represent both ancestral and derived character states (Bracken et al., 2009), plausible explanations for the phenomena are differences in the timing of morphogenesis, that is, heterochrony, a classical concept developed by Haeckel (1866) (review in Smith, 2001) and proposed as a general evolutionary pattern (e.g., Richardson and Oelschläger, 2002; Maxwell et al., 2010), as well as in various studies used to explain how ontogenetic shifts can result in stage-specific adaptions and evolutionary diversification (e.g., Helluy et al., '93; Clark, 2005; Tills et al., 2011).

Relevance for Reconstruction of Phylogeny

Comparison of the three taxa studied here indicates that clear taxon-specific differences can be detected, and even in the first zoeal stage the studied species can be distinguished according to the specific configuration of the nervous system. It seems that phylogenetically relevant signal may be found not only in limb development, but also in the morphogenesis of segmental ganglia, for example, in different segment- or tagma-specific ways of delayed ganglion and limb development at the zoea I stage. Nevertheless, it is not possible at this time to recognize an unambiguous evolutionary trend from more basally branching lineages like the Caridea to more derived ones like the Anomura and Brachyura (e.g., see Bracken et al., 2009 and older works cited therein), since the delay in neuromere differentiation seems long in Caridea, shorter in Anomura, and greatest in Brachyura. The picture is complicated even more by the fact that in Astacideans like *Homarus*, the first free-living larval stage (the mysis 1) hatches with all pereiopods and respective segmental ganglia well developed (Helluy and Beltz, '91) and therefore the delay seems to be at zero. Thus, other groups need to be studied, for example, the Stenopodidea and Thalassinidea, to get a better idea of the evolutionary processes.

With respect to the ontogenetic dimension one can say that ganglia undergo development at different tempos depending on general larval morphogenesis, and only those elements seem to be fully differentiated that are actually "needed" at a given developmental stage (Harzsch et al., '98; Sullivan and MacMillan, 2001). This leads to differences between segments and/or tagmata within single species, but also between different taxa. On the one hand the CNS of decapod zoea-I larvae is a stage-specific system reflecting adaptations to larval life, on the other hand a transitory stage to the adult organization. Constraints superimposed by both aspects can be detected in the zoea-I CNS.

AUTHORS' CONTRIBUTIONS

Herewith I certify that my coauthor Roland R. Melzer has accurately read the article and agrees to have his name listed as author. The same applies accordingly for colleagues who are acknowledged as having contributed to or criticized the article.

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LITERATURE CITED

Anger K. 2001. The biology of decapod crustacean larvae. Lisse: A.A. Balkema Publishers.

Anger K. 2006. Contributions of larval biology to crustacean research: a review. Invertebr Reprod Dev 49:175–205.

Boeck P. 1984. Der Semiduennschnitt. Muenchen: Bergmann.

Bourdillon-Casanova L. 1960. Le méroplancton du golfe de Marseille: les larves de crustacés décapodes. Recueil des travaux de la Station marine d'Endoume fasc 30 = bull 18:1–286.

Bracken HD, Toon A, Felder DL, Martin JW, Finley M, Rasmussen J, Palero F, Crandall KA. 2009. The decapod tree of life: compiling the data and moving toward a consensus of decapod evolution. Arthropod Syst & Phyl 67:99–116.

Bullock TH, Horridge GA. 1965. Structure and function in the nervous systems of invertebrates. San Francisco Cal: W.H. Freeman.

Cieluch U, Charmantier G, Grousset E, Charmantier-Daures M, Anger K. 2005. Osmoregulation, immunolocalization of Na+/K+-ATPase, and ultrastructure of branchial epithelia in the developing brown shrimp, *Crangon crangon* (Decapoda, Caridea). Physiol Biochem Zool 78:1017–1025.

Cieluch U, Anger K, Charmantier-Daures M, Charmantier G. 2007. Osmoregulation and immunolocalization of Na+/K+-ATPase during the ontogeny of the mitten crab, *Eriocheir sinensis* (Decapoda, Grapsoidea). Mar Ecol Progress Ser 329:169–178.

Clark PF. 2005. The evolutionary significance of heterochrony in the abbreviated zoeal development of pilumnine crabs (Crustacea: Brachyura: Xanthoidea). Zool J Linn Soc 143:417–446.

Clark PF. 2009. The bearing of larval morphology on brachyuran phylogeny. In: JW Martin, KA Crandall, FL Darryl, editors. Decapod crustacean phylogenetics. London: Taylor & Francis, Crustacean Issues 18. p 221–241.

Cuesta JA, Rodríguez A. 1994. Early zoeal stages of *Pachygrapsus marmoratus* (Fabricius), *P. transversus* (Gibbes) and *P. maurus* (Lucas) (Decapoda, Brachyura, Grapsidae) reared in the laboratory. Sci Mar 58:323–327.

Cuesta JA, Rodríguez A. 2000. Zoeal stages of the intertidal crab *Pachygrapsus marmoratus* (Fabricius, 1787) (Brachyura, Grapsidae) reared in the laboratory. Hydrobiologia 436:119–130.

Dahl E. 1983. Malacostracan phylogeny and evolution. In: FR Schram, editor. Crustacean phylogeny crustacean issues. Rotterdam: A.A. Balkema. p 189–212.

De Beer G. 1940. Embryos and ancestors. Oxford: The Clarendon Press.

Fritsch M, Richter S. 2010. The formation of the nervous system during larval development in *Triops cancriformis* (Bosc) (Crustacea, Branchiopoda): an immunohistochemical survey. J Morphol 271:1457–1481.

Geiselbrecht H, Melzer RR. 2010. Mandibles of zoea-I-larvae of nine decapod species. A scanning EM analysis. Spixiana 33:27–47.

González-Gordillo JI, Cuesta JA, Rodríguez A. 1996. Studies on the larval development of northeastern Atlantic and Mediterranean Procellanidae (Decapoda, Anomura). I—Redescription of the larval stages of *Porcellana platycheles* (Pennant, 1777) reared under laboratory conditions. Helgoländer Meeresuntersuch 50:517–531.

Gould SJ. 1977. Ontogeny and phylogeny. Cambridge, MA: The Belknap Press of Harvard University Press.

Gurney R. 1942. Larvae of decapod Crustacea. London: Printed for the Ray Society, sold by B. Quaritch.

Haeckel E. 1866. Generelle Morphologie der Organismen: allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformierte Deszendenz-Theorie, Vol. 2. Berlin: George Reimer.

Hanström B. 1947. The brain, the sense organs, and the incretory organs of the head in the Crustacea Malacostraca. Lund: C.W.K. Gleerup.

Harzsch S. 2002. The phylogenetic significance of crustacean optic neuropils and chiasmata: a re-examination. J Comp Neurol 453:10–21.

Harzsch S. 2003. Ontogeny of the ventral nerve cord in malacostracan crustaceans: a common plan for neuronal development in Crustacea, Hexapoda and other Arthropoda? Arthropod Struct Dev 32:17–37.

Harzsch S, Dawirs RR. 1993. On the morphology of the central nervous system in larval stages of *Carcinus maenas* L. (Decapoda, Brachyura). Helgoland Mar Res 47:61–79.

Harzsch S, Dawirs RR. 1994. Neurogenesis in larval stages of the spider crab *Hyas araneus* (Decapoda, Brachyura): proliferation of neuroblasts in the ventral nerve cord. Roux's Arch Dev Biol 204:93–100.

Harzsch S, Dawirs RR. 1995. A developmental study of serotonin-immunoreactive neurons in the larval central nervous system of the spider crab *Hyas araneus* (Decapoda, Brachyura). Invertebr Neurosci 1:53–65.

Harzsch S, Dawirs RR. 1996a. Development of neurons exhibiting FMRF amide-related immunoreactivity in the central nervous system of spider crab larvae *Hyas araneus* L. (Decapoda, Majidae). J Crust Biol 16:10–19.

Harzsch S, Dawirs RR. 1996b. Neurogenesis in the developing crab brain: postembryonic generation of neurons persists beyond metamorphosis. J Neurobiol 29:384–398.

Harzsch S, Anger K, Dawirs RR. 1997. Immunocytochemical detection of acetylated alpha-tubulin and *Drosophila* synapsin in the embryonic crustacean nervous system. Int J Dev Biol 41:477–484.

Harzsch S, Miller J, Benton J, Dawirs RR, Beltz B. 1998. Neurogenesis in the thoracic neuromeres of two crustaceans with different styles of metamorphic development. J Exp Biol 201:2465–2479.

Harzsch S, Sandeman D, Chaigneau J. 2012. Morphology and development of the central nervous system. In: J Forest, JC von Vaupel Klein, editors. Treatise on zoology—Crustacea. Leiden: Koninklijke Brill Academic Publishers. p 9–236.

Helluy SM, Beltz BS. 1991. Embryonic development of the American lobster (*Homarus americanus*): quantitative staging and characterization of an embryonic molt cycle. Biol Bull 180:355–371.

Helluy S, Sandeman R, Beltz B, Sandeman D. 1993. Comparative brain ontogeny of the crayfish and clawed lobster: implications of direct and larval development. J Comp Neurol 335:343–354.

Henry EC. 1977. A method for obtaining ribbons of serial sections of plastic embedded specimens. Biotech Histochem 52:59–60.

Kenning M, Müller C, Wirkner CS, Harzsch S. 2013. The Malacostraca (Crustacea) from a neurophylogenetic perspective: New insights from brain architecture in *Nebalia herbstii* Leach, 1814 (Leptostraca, Phyllocarida). Zool Anz 252:319–336.

Lebour MV. 1943. The larvae of the genus *Porcellana* (Crustacea Decapoda) and related forms. J Mar Biol Assoc UK 25:721–737.

Maxwell EE, Harrison LB, Larsson HCE. 2010. Assessing the phylogenetic utility of sequence heterochrony: evolution of avian ossification sequences as a case study. Zoology 113:57–66.

McKinney ML, McNamara K. 1991. Heterochrony: the evolution of ontogeny. New York: Plenum Press.

Melzer RR. 2009. Persisting stemma neuropils in *Chaoborus crystallinus* (Diptera: Chaoboridae): development and evolution of a bipartite visual system. J Morphol 270:1524–1530.

Meyer R, Melzer RR. 2004. Scanning EM diagnosis of marine decapoda larvae: a comparison of preparation techniques. Crustaceana 77:883–886.

Meyer R, Wehrtmann IS, Melzer RR. 2006. Morphology of the first zoeal stage of *Portunus acuminatus* Stimpson, 1871 (Decapoda: Portunidae: Portuninae) reared in the laboratory. Sci Mar 70:261–270.

Nässel DR, Elofsson R. 1987. Comparative anatomy of the crustacean brain. In: AP Gupta, editor. Arthropod brain. New York: Wiley. p 111–133.

Neusser TP, Hess M, Haszprunar G, Schrödl M. 2006. Computer-based three-dimensional reconstruction of the anatomy of *Microhedyle remanei* (Marcus, 1953), an interstitial acochlidian gastropod from Bermuda. J Morphol 267:231–247.

Rice AL. 1980. Crab zoeal morphology and its bearing on the classification of the Brachyura. Trans Zool Soc Lond 35:271–372.

Richardson MK, Oelschläger HHA. 2002. Time, pattern, and heterochrony: a study of hyperphalangy in the dolphin embryo flipper. Evol Dev 4:435–444.

Richardson KC, Jarett L, Finke EH. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Biotech Histochem 35:313–323.

Richter S, Loesel R, Purschke G, et al. 2010. Invertebrate neurophylogeny—suggested terms and definitions for a neuroanatomical glossary. Front Zool 7:29.

Rotllant G, De Kleijn D, Charmantier-Daurès M, Charmantier G, Van Herp F. 1993. Localization of crustacean hyperglycemic hormone (CHH) and gonad-inhibiting hormone (GIH) in the eyestalk of *Homarus gammarus* larvae by immunocytochemistry and in situ hybridization. Cell Tissue Res 271:507–512.

Rotllant G, Charmantier-Daurès M, Trilles JP, Charmantier G. 1994. Ontogeny of the sinus gland and the organ of Bellonci in larvae and postlarvae of the European lobster *Homarus gammarus*. Invert Reprod Dev 26:13–22.

Rotllant G, Charmantier-Daurès M, De Kleijn D, Charmantier G, Van Herp F. 1995. Ontogeny of neuroendocrine centers in the eyestalk of *Homarus gammarus* embryos: an anatomical and hormonal approach. Invert Reprod Dev 27:233–245.

Ruthensteiner B. 2008. Soft Part 3D visualization by serial sectioning and computer reconstruction. In: Geiger DLRB, editor. Micromolluscs: methodological challenges—exciting results: proceedings from the micromollusc symposium of the 16th Unitas Malacologica World Congress of Malacology. p 63–100.

Sandeman DC, Sandeman RE, Derby C, Schmidt M. 1992. Morphology of the brain of crayfish, crabs, and spiny lobsters: a common nomenclature for homologous structures. Biol Bull 183:2304–2326.

Sandeman DC, Scholtz G, Sandeman RE. 1993. Brain evolution in decapod Crustacea. J Exp Zool 265:112–133.

Smith KK. 2001. Heterochrony revisited: the evolution of developmental sequences. Biol J Linn Soc 73:169–186.

Sombke A, Harzsch S, Hansson BS. 2011. Organization of deutocerebral neuropils and olfactory behavior in the centipede *Scutigera coleoptrata* (Linnaeus, 1785) (Myriapoda: Chilopoda). Chem Senses 36:43–61.

Stegner MEJ, Richter S. 2011. Morphology of the brain in *Hutchinsoniella macracantha* (Cephalocarida, Crustacea). Arthropod Struct Dev 40:221–243.

Strausfeld NJ. 2012. Arthropod brains. Evolution, functional elegance, and historical significance. Cambridge, MA: The Belknap Press of Harvard University Press.

Sullivan JM, MacMillan DL. 2001. Embryonic and postembryonic neurogenesis in the ventral nerve cord of the freshwater crayfish *Cherax destructor*. J Exp Zool 290:49–60.

Tills O, Rundle SD, Salinger M, et al. 2011. A genetic basis for intraspecific differences in developmental timing? Evol Dev 13:542–548.

Ungerer P, Geppert M, Wolff C. 2011. Axogenesis in the central and peripheral nervous system of the amphipod crustacean *Orchestia cavimana*. Integr Zool 6:28–44.

Vilpoux K, Sandeman R, Harzsch S. 2006. Early embryonic development of the central nervous system in the Australian crayfish and the Marbled crayfish (Marmorkrebs). Dev Genes Evol 216:209–223.

Williamson DI. 1957. Crustacea Decapoda: Larvae. V. Caridea: Family Hippolytidae. Charlottenlund slot: Conseil International pour l'Exploration de la Mer.

Zupo V, Buttino I. 2001. Larval development of decapod crustaceans investigated by confocal microscopy: an application to *Hippolyte inermis* (Natantia). Mar Biol 138:965–973.

5. Paper II

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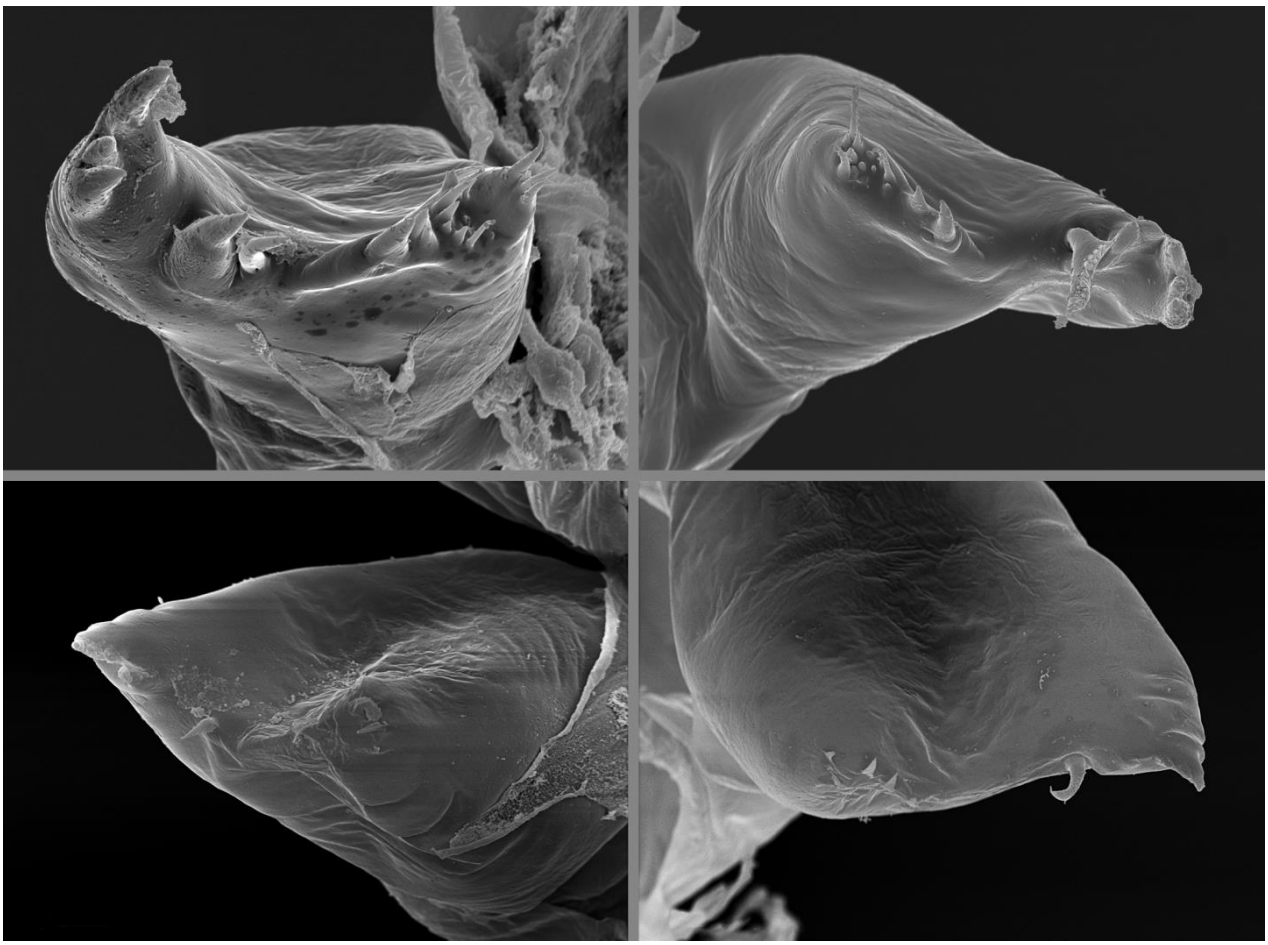

Heterochrony in Mandible Development of Larval Shrimp (Decapoda: Caridea) - a Comparative Morphological SEM Study of Two Carideans

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Running title: Heterochrony in mandible development

ABSTRACT Mandible development in the larval stages I-V of two palaemonid shrimp species, *Palaemon elegans* and *Macrobrachium amazonicum*, was analysed mainly with scanning electron microscopy and additionally with light microscopy and confocal laser scanning microscopy. In contrast to the zoea I of *P. elegans*, first-stage larvae of *M. amazonicum* are non-feeding. At hatching, the morphology of the mandibles is fully expressed in *P. elegans*, while it appears underdeveloped *in M. amazonicum*, presenting only small precursors of typical caridean features. In successive zoeal stages, both species show similar developmental changes, but the mandibular characters of the larvae in *M. amazonicum* were delayed compared to the equivalent stages in *P. elegans*, especially in the development of submarginal setae and mandible size. In conclusion, our results indicate heterochrony (postdisplacement) of mandible development in *M. amazonicum* compared to that in *P. elegans*, which is related to initial lack of mandible functionality or planktivorous feeding at hatching, respectively. This conclusion is supported by comparison with other palaemonid zoeae exhibiting different feeding modes. Our data suggest that an evolutionary ground pattern of mandible morphology is present even in species with non-feeding first-stage larvae.

Keywords: *Macrobrachium amazonicum*, *Palaemon elegans*, heterochrony, larval development, mandible morphology, zoea

INTRODUCTION

Among the body appendages of the zoea larvae of decapod crustaceans, the mandibles have been least studied, mainly due to their minuteness and disadvantageous position on the head capsule, which render their microscopical examination technically difficult. In morphological descriptions of zoeae, the mandibles are therefore often omitted or shown in insufficient detail (for review, see Rice 1979, 1980; Ingle, 1983, 1992; and older literature cited therein). For brachyurans, Clark et al. (1998) considered the mandibular palp and the setation on the margin in the megalopa as the only relevant feature of larval mandibles. In consequence, detailed light-microscopic analyses of zoeal mandibles exist for only a limited number of species (e.g., Martin and Goy 2004; dos Santos et al. 2004; Bolaños et al. 2005). Scanning electron microscopy (SEM), which allows more detailed analyses of small structures and their steric arrangement at high resolution, has hardly been used in studies of zoeal mandibles (Greenwood and Fielder 1979; Minagawa and Takashima 1994). Such analyses are needed, however, not only for complete morphological descriptions but also in phylogenetic reconstructions using "mandibulate" mandibles as key characters (e.g., Dahl and Hessler 1982; Bitsch 2001; Richter et al 2002; Edgecombe et al. 2003; Bitsch and Bitsch 2004; Rota-Stabelli et al 2010).

In recent studies, Meyer et al. (2006) and Geiselbrecht and Melzer (2009) used SEM analyses for detailed descriptions of zoeal mandibles, and Geiselbrecht and Melzer (2010) compared the mandibles of nine decapod species with different types of zoeal morphology, and found distinct taxon-specific features. The authors concluded that certain sets of zoeal mandible characters, including the basic form and the presence, structure and position of certain appendages, provide phylogenetically relevant signals. Moreover, they analysed the ultrastructure of zoeal mandibles in *Palaemon elegans* Rathke, 1837 using for the first time transmission electron microscopy (TEM), and demonstrated eleven sensillar units on the gnathal edge of the mandibles (Geiselbrecht and Melzer 2013). These findings allow a differentiation between innervated setae, innervated spines, and non-innervated spines, which helps to improve the terminology for different mandible structures.

However, the morphology and development of crustacean mandibles depends also on feeding habits (Mekhanikova 2010). This implies that different food sources may be associated with differences in zoeal mandible structures of closely related species, veiling phylogenetically relevant characters, and contradicting hypotheses presented by Geiselbrecht and Melzer (2010).

In the present study, zoeae of two phylogenetically closely related species are compared to study the influence of differential modes of feeding on mandible structure. Being part of the plankton, zoea larvae are generally known to feed on a vast variety of particulate food sources such as zooplankton (including other larvae, De Araujo and Valenti 2007; Paul et al. 1989), detritus (Schembri 1982) and phytoplankton (Paul et al. 1989). Thus, the mandibles of the earliest zoea larvae are used as main masticating organs for processing a broad spectrum of food with different qualities.

In some taxa, non-feeding zoeae are known, e.g. in the palaemonid genera *Macrobrachium*, *Pseudopalaemon* and *Palaemonetes* (Anger 2001). In these limnic shrimps, early zoeae may migrate or disperse downstream, so that the zoea II can start feeding in plankton-rich estuarine or coastal waters. In the most Palaemonidae, e.g. in the mostly marine genus *Palaemon*, already the newly hatched zoea I is a feeding stage (Kumlu and Jones 1995).

The Palaemonidae are thus ideal for testing the above mentioned hypotheses. In the present study we therefore analyzed the mandible development in zoeal stages I to V of two palaemonid shrimp, *Palaemon elegans* and *Macrobrachium amazonicum* (Heller, 1862), which show different life styles and feeding modes. *Palaemon elegans* is a marine species that lives in shallow coastal European waters, whereas *M. amazonicum* inhabits rivers and estuaries along the northeastern coasts of South America (Hayd and Anger 2013). During the zoea I stage, the larvae of *M. amazonicum* are lecithotrophic (non-feeding; Anger and Hayd 2009), whereas those of *P. elegans* are planktivorous (Fincham 1977).

M. amazonicum passes through an extended larval development with 9-11 stages (Magalhães 1985; Anger and Hayd 2009), which is a typical pattern in estuarine species of *Macrobrachium* (for review, see Anger 2013). Its larvae need higher salinities than the adults, indicating an early larval transport to estuarine or coastal marine habitats (Charmantier and Anger 2011). During the downstream transport in fast-flowing river water, the larvae are presumably faced with food limitation due to low or unpredictable mesozooplankton production in lotic environments (Anger and Hayd 2009). In the initial postembryonic stage, the non-feeding larvae of *M. amazonicum* utilize internal energy reserves that originate from an enhanced maternal investment into egg production. In *P. elegans*, a variable number of 4-9 zoeal stages has been reported (Fincham 1977; Fincham and Williamson 1978; Sanders et al. 2005), however, the onset of larval feeding occurs already at hatching (Fincham 1977; Kumlu and Jones 1995). Extended and fully planktivorous patterns of larval development are generally considered as the ancestral state in decapod crustaceans, whereas tendencies

towards an abbreviation and lecithotrophy are believed to represent derived conditions (for discussion, see Jalihal et al. 1993; Anger 2013).

Different life-history traits observed in two closely related species raise the question if they are paralleled by modifications in the patterns of morphological development. Shifts in the timing of morphogenetic events compared to more ancestral relatives are known as heterochrony (Haeckel 1866; McKinney and McNamara 1991; for recent discussion, see Tills et al. 2011). Heterochrony appears to be a pervasive evolutionary feature (Gould 1979), especially in vertebrate phylogeny (Richardson and Oelschläger 2002; Maxwell et al. 2010; Tills et al. 2011). It was also shown that abbreviated zoeal development can affect the timing of appearance and rates of character development in brachyuran zoeae (Clark 2005).

The principal aim of the present study was to examine whether early zoeal mandibles of *P. elegans* and *M. amazonicum* differ significantly in morphology and development, reflecting different modes of feeding at hatching. Besides differential adaptive traits, we expected to find features representing common developmental patterns in Caridea or Palaemonidae. Since our findings are based on only two species and other SEM studies on zoeal mandible development are lacking, we compare our results not only between these two species, but also with earlier light-microscopical results on other palaemonid zoeae with differing feeding habits, in order to avoid the "two-species-trap" (Garland and Adolph 1994).

MATERIAL AND METHODS

Larval rearing, fixation and staging

Larvae of *Palaemon elegans* Rathke, 1837 and *Macrobrachium amazonicum* (Heller, 1862) were reared in the laboratory to obtain different stages of larval development. Rearing experiments with *P. elegans* were conducted at the Bavarian State Collection of Zoology and the Sea Life Center in Munich. Newly hatched larvae were individually transferred to and subsequently reared in non-aerated 100ml plastic vials kept at room temperature (24-26°C) and a constant salinity of 35PSU. Larvae were fed newly hatched nauplii of *Artemia* sp., and water was changed every second day. *Macrobrachium amazonicum* originating from the Amazon Delta were mass-reared at the Helgoland Marine Biological Laboratory (BAH) using aerated 1-L beakers, a constant temperature of 29°C, salinity 10, and an artificial 12:12h light:dark cycle (for more details of rearing techniques, see Anger et al. 2009; Charmantier and Anger 2011).

Every two to three days, larvae were separated from the cultures and fixed in a graded ethanol series following Meyer and Melzer (2004): 30% EtOH for 30 min, 50% for 7 hours and 70%,

with repeated rinsing to remove remnants of salts. Fixed larvae of both species were microscopically checked and staged using the larval descriptions provided by Fincham (1977) and Magalhães (1985), respectively.

Fixed reference larvae are deposited at the ZSM under the following registration numbers: *Macrobrachium amazonicum:* ZSM A20120307-311; *Palaemon elegans*: ZSM A20080754. SEM preparations are deposited under following numbers: *Palaemon elegans*: ZSM A20080755-757 and ZSM A20120316-319; *Macrobrachium amazonicum*: ZSM A20120320-324.

SEM preparation and microscopy

Specimens were dissected under a stereo microscope using thin tungsten wires and forceps. Mandibles were isolated completely or attached to the carapace in order to facilitate SEM preparation. Mandibles and whole zoeae were incubated for 30 min in 36% hydrogen-peroxide to remove dirt particles. For SEM preparation, specimens were dehydrated in a graded acetone series (70%, 80% and 90% each for 10 min, 3 times 20 min in 100%) and then critical-point-dried in a Baltec CPD 030. Dried specimens were mounted on SEM stubs with self-adhesive carbon stickers and sputtered with gold on a Polaron E 5100. Whole larvae and mandibles were studied with a LEO 1430VP SEM at 15 kV. Left and right mandibles (n = 10-20) of each stage were studied and compared with focus on length, number of teeth, denticles, spines and setae. Every specimen was scanned under different angles.

Morphometric analyses

Mandible size (length of gnathal edges), the sizes of incisor process, 'lacinia mobilis' and the first small spine on the molar process of *P. elegans* and *M. amazonicum* were measured on the left and right mandible. Based on SEM pictures with lateral or frontal views, mandible size was measured using automatically inserted scales and measuring function in Adobe Photoshop. Data on total length (TL) of larval stages was taken from Fincham (1977) and Anger et al. (2009). Data was analyzed and graphs produced with Excel 2010. [*P. elegans*; mandible size in stages: I (n=19), II (n=10), III (n=16), IV (n=14), V (n=5); 'lacinia mobilis' in stages: I (n=11), II (n=9), III (n=14), IV (n=13), V (n=2); incisor process in stages: I (n=9), II (n=11), III (n=11), IV (n=9), V (n=2); first small spine in stages: I (n=10), II (n=11), III (n=10), IV (n=12), V (n=2); *M. amazonicum*; mandible size in stages: I (n=6), II (n=10), III (n=23), IV (n=11), V (n=6); 'lacinia mobilis' in stages: I (n=9), II (n=7), III (n=14), IV (n=10), V (n=7); incisor process in stages: I (n=6), II (n=3), III (n=6), IV (n=8), V (n=4); first small spine in stages: I (n=7), II (n=8), III (n=11), IV (n=8), V (n=6); see also table 1].

Terminology

Cuticular processes on the mandibles are basically named according to definitions given in Watling (1989) and Garm (2004). In a recent ultrastructural study using TEM, however, Geiselbrecht and Melzer (2013) showed for *Palaemon elegans* that not only slender processes, in particular the 'lacinia mobilis', with a distinct movable socket are innervated (consistently termed "setae"). In some cases, an innervation was detected also in stout processes lacking a movable socket. Therefore an updated terminology is used here, referring to such structures as "sensory spines". Moreover, appendages that were seta shaped, but on which we could not detect an unequivocal basal ring, are referred to as processes. The line drawing in figure 1 is a schematic showing the different types of mandibular appendages described in this study.

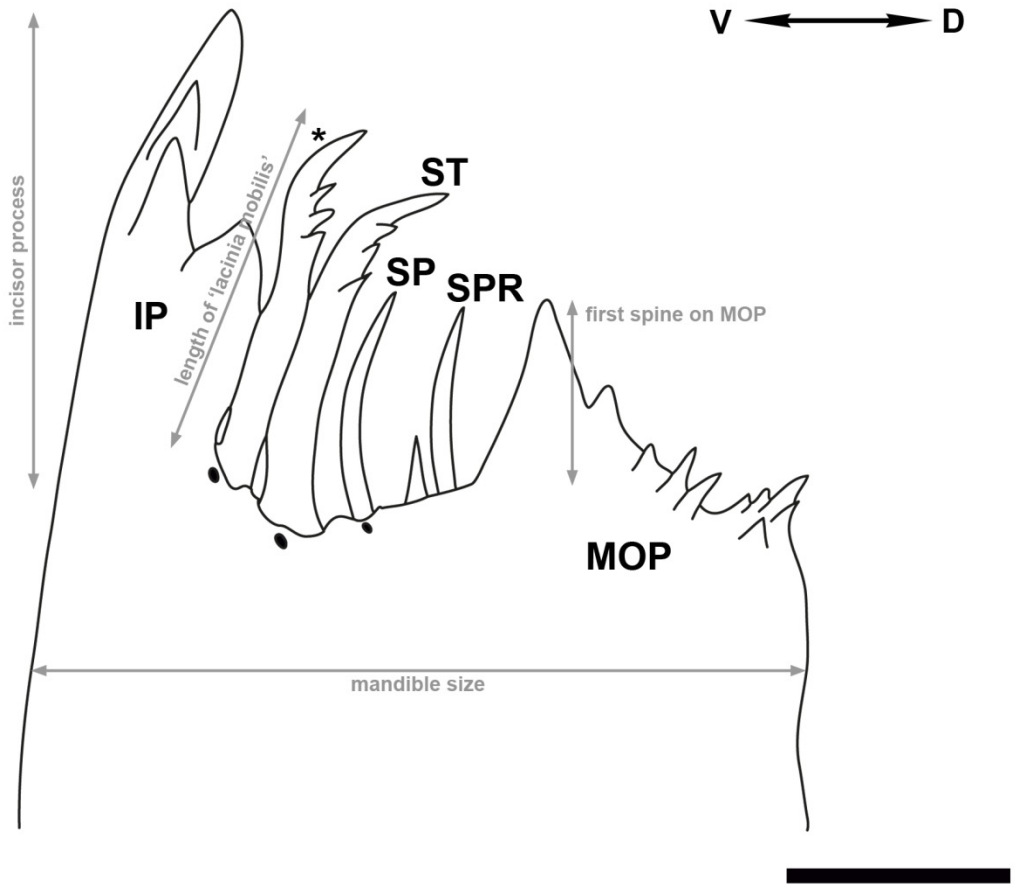

Fig. 1. Gnathal edge of a larval mandible in schematic drawing. Illustrated are different types of appendages independently of actual position (bar 20 µm). Abbreviations: Asterisk, 'lacinia mobilis'; D, dorsal; IP, incisor process; MOP, molar process; SP, sensory spine; SPR, submarginal process; ST, submarginal seta; V, ventral.

***Palaemon elegans* Rathke, 1837**

Zoea I (Fig. 2A, B)

The basic form of the mandible is a distally flattened and bent tube. Molar and incisor process well developed, nearly merging into each other. Both processes oriented medially. Incisor process forming a fork-like structure with several spines. Size measured on gnathal edges 53 µm (SD=4.6), incisor process 30 µm (SD=2.2), length of 'lacinia mobilis' 21 µm (SD=1.6), first ventral small spine on molar process 11 µm (SD=1.9).

Right mandible (Fig. 2A): Fork-like incisor process with a ventral row of 3 acute spines. One submarginal robustly built sensory spine (SP) between incisor process and a spine-like 'lacinia mobilis'. 'Lacinia mobilis' articulated on a basal ring and a pore at the base. Molar process with 9-11 small spines, medial spine bigger than the following ones, on the dorsal margin a row of 3 spines.

Left mandible (Fig. 2B): Incisor process with a ventral row of 5 spines, a fan-shaped, serrated 'lacinia mobilis' located nearby. 'Lacinia mobilis' articulated on a basal ring and a pore at the base. Molar process with a dorsal row of 4 marginal spines and 6-8 small submarginal spines, medially two spines bigger than the other ones.

Zoea II (Fig. 2C, D)

Basic form as in zoea I. Size measured on gnathal edges 57 µm (SD=4.8), incisor process 34 µm (SD=5.4), length of 'lacinia mobilis' 21 µm (SD=2.75), first ventral small spine on molar process 11 µm (SD=0.96).

Right mandible (Fig. 2C): Fork-like incisor process with a ventral row of 3 acute spines. One submarginal sensory spine between incisor process and a spine-like 'lacinia mobilis'. Molar process with 9-11 small spines, medially spine bigger than the following ones, on the dorsal margin a row of 3 spines.

Left mandible (Fig. 2D): Incisor process with a ventral row of 5 spines. Nearby a fan-shaped, serrated 'lacinia mobilis' and a proximate submarginal serrated seta (ST). Molar process on the dorsal margin with a row of 4 spines and 6-8 small submarginal spines, medially two spines bigger than the other ones.

For the following stages, only changes in structure are mentioned.

Zoea III (Fig. 2E, F)

Length of gnathal edge 64 µm (SD=4.48), incisor process 42 µm (SD=2.83), 'lacinia mobilis' 23 µm (SD=2.8), first ventral spine on molar process 11 µm (SD=1.83).

Left mandible (Fig. 2F): The serrated submarginal seta (ST) located dorsally to the 'lacinia mobilis' is larger and well developed. In some specimens (12.5%) a second submarginal seta (SST) was observed dorsally to the serrated seta.

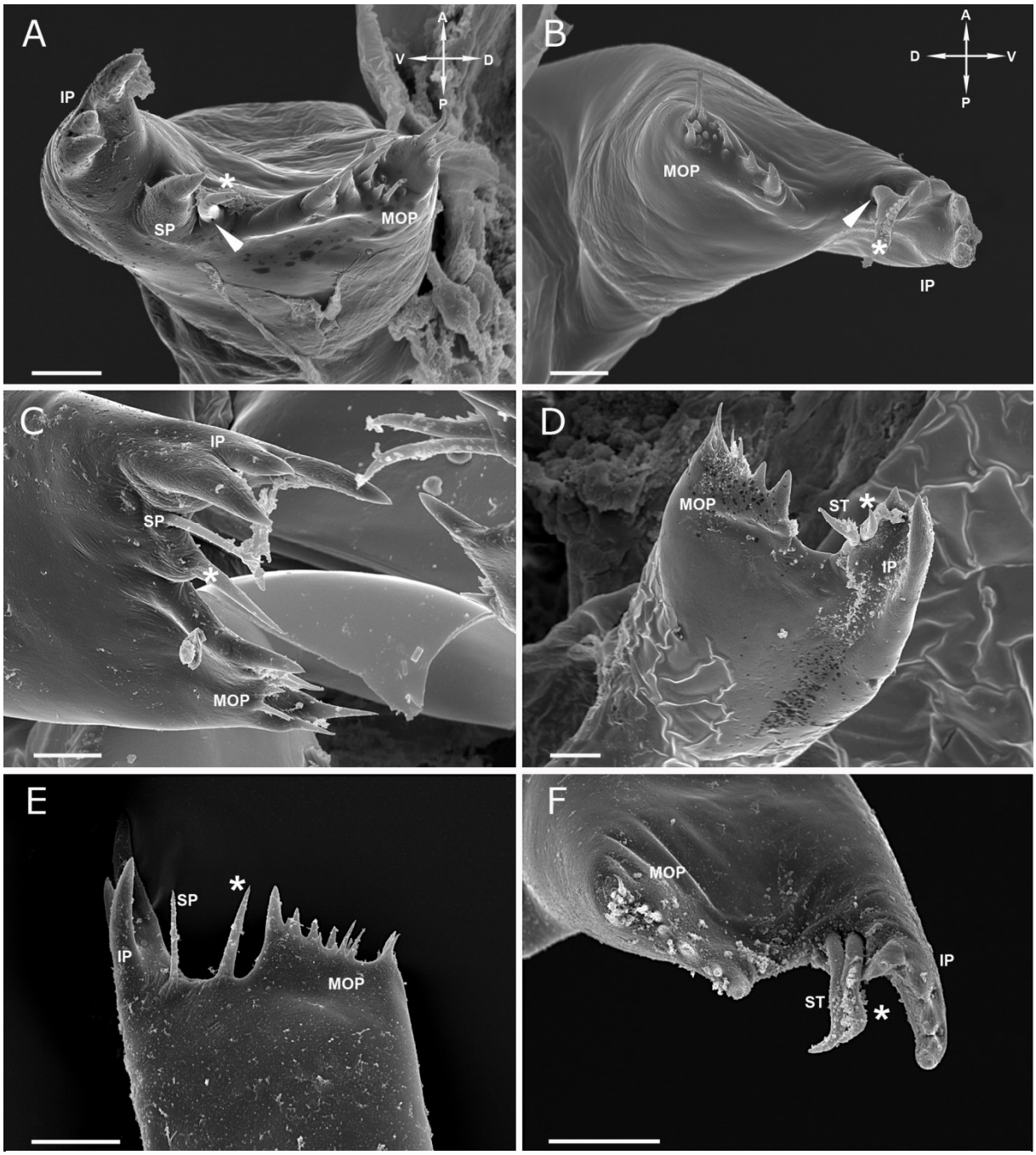

Fig. 2 *Palaemon elegans*, zoea I - III. **A**. Zoea I; frontal view of right mandible (bar 10 µm). **B**. Zoea I; frontal view of left mandible (bar 10 µm). **C**. Zoea II; outer posterior view of right mandible (bar 20 µm). **D**. Zoea II; outer posterior view of left mandible (bar 20 µm). **E**. Zoea III; outer posterior view of right mandible (bar 20 µm). **F**. Zoea III; inner anterior view of left mandible (bar 20 µm). Abbreviations: A, anterior; arrowhead, pore; asterisk, 'lacinia mobilis'; D, dorsal; IP, incisor process; MOP, molar process; P, posterior; SP, sensory spine; ST, submarginal seta; V, ventral.

Zoea IV (Fig. 3A, B)

Length of gnathal edge 68 µm (SD=3.52), 'lacinia mobilis' 27 µm (SD=4.52), incisor process 46 µm (SD=4.19), first small ventral spine on molar process 11 µm (SD=1.49).

Left mandible (Fig. 3B): In about one half of our specimens (45%), a second submarginal seta (SST) was observed, in some mandibles well developed.

Zoea V (Fig. 3C,D)

Length of gnathal edge 77 µm (SD=3.32), 'lacinia mobilis' 25 µm (SD=5), incisor process 49 µm (SD=1), first small ventral spine on molar process 11 µm (SD=1).

Right mandible (Fig. 3C): A second submarginal process (SPR) develops between the 'lacinia mobilis' and the molar process (observed in 77% of our specimens).

Left mandible (Fig. 3D): Most specimens (83%) showed a second submarginal seta (SST) near the first one (Fig. 6F).

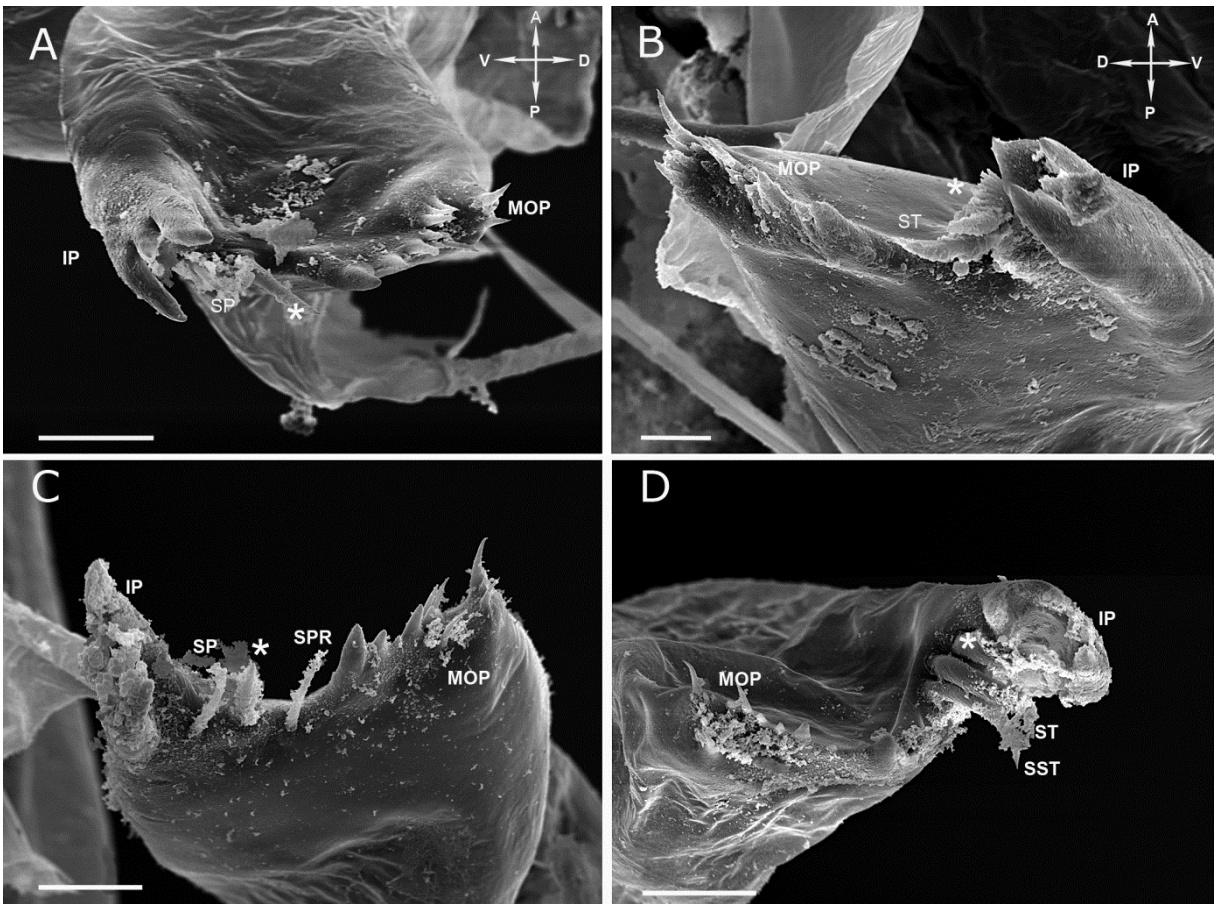

Fig. 3 *Palaemon elegans*, zoea IV - V. **A**. Zoea IV; frontal view of right mandible. **B**. Zoea IV; frontal view of left mandible. **C**. Zoea V; frontal view of right mandible. **D**. Zoea V; frontal view of left mandible (bars 20 µm). Abbreviations: A, anterior; asterisk, 'lacinia mobilis'; D, dorsal; IP, incisor process; MOP, molar process; P, posterior; SP, sensory spine; SPR, submarginal process; ST, submarginal seta; SST, second submarginal seta; V, ventral.

***Macrobrachium amazonicum* (Heller, 1862)**

Zoea I (Fig. 4A, B)

The basic form of the mandibles is mitten-like. Incisor process and molar process can be distinguished, being separated by a thin central notch. Both show processes such as spines and a 'lacinia mobilis'. Length of gnathal edge 38 µm (SD=1.67), incisor process of both mandibles 16 µm (SD=1.37), 'lacinia mobilis' 9 µm (SD=0.8), first small ventral spine on molar process 5 µm (SD=0.45).

Right mandible (Fig. 4A): Incisor process ventrally to molar process, with three small spines. Small 'lacinia mobilis' between incisor process and molar process. Molar process wide, with 6 small spines (SS).

Left mandible (Fig. 4B): Incisor process with 5 small spines, a 'lacinia mobilis' in the form of an articulated simple seta with a basal pore (Fig. 7F) on the lower edge of the incisor process. Molar process wider than incisor process, with 6 small spines.

Zoea II (Fig. 4C, D)

Flattened anterior-posteriorly to its distal edge, with a central notch. Molar process located dorsally, wider than the incisor process. Incisor process forming a thin, fork-like structure with several spines. Length of gnathal edge 52 µm (SD=2.72), incisor process 28 µm (SD=2.05), 'lacinia mobilis' 19 µm (SD=1.12), first small ventral spine on molar process 8 µm (SD=1.21).

Right mandible (Fig. 4C): Fork-like incisor process with a ventral row of 3 spines, central spine smaller than the outer ones. One submarginal small sensory spine (SP) with basal pore between a spine-like 'lacinia mobilis' and the incisor process. Molar process with 6-7 small spines, two spines at the dorsal margin, 4-5 at the anterior margin.

Left mandible (Fig. 4D): Incisor process with a ventral row of 5 spines and a fan-like, serrated 'lacinia mobilis' nearby. Molar process with 6-7 small spines, two spines at the dorsal margin, 4-5 at the anterior margin.

For the following stages, only changes in structure are mentioned.

Zoea III (Fig. 4E, F)

Length of gnathal edge 58 (SD=2.48), incisor process 30 µm (SD=2.98), 'lacinia mobilis' 22 µm (SD=1.66), first small ventral spine on molar process 11 µm (SD=1.31).

Left mandible (Fig. 4F): All left mandibles with a second bristly process (SPR) between the 'lacinia mobilis' and the molar process.

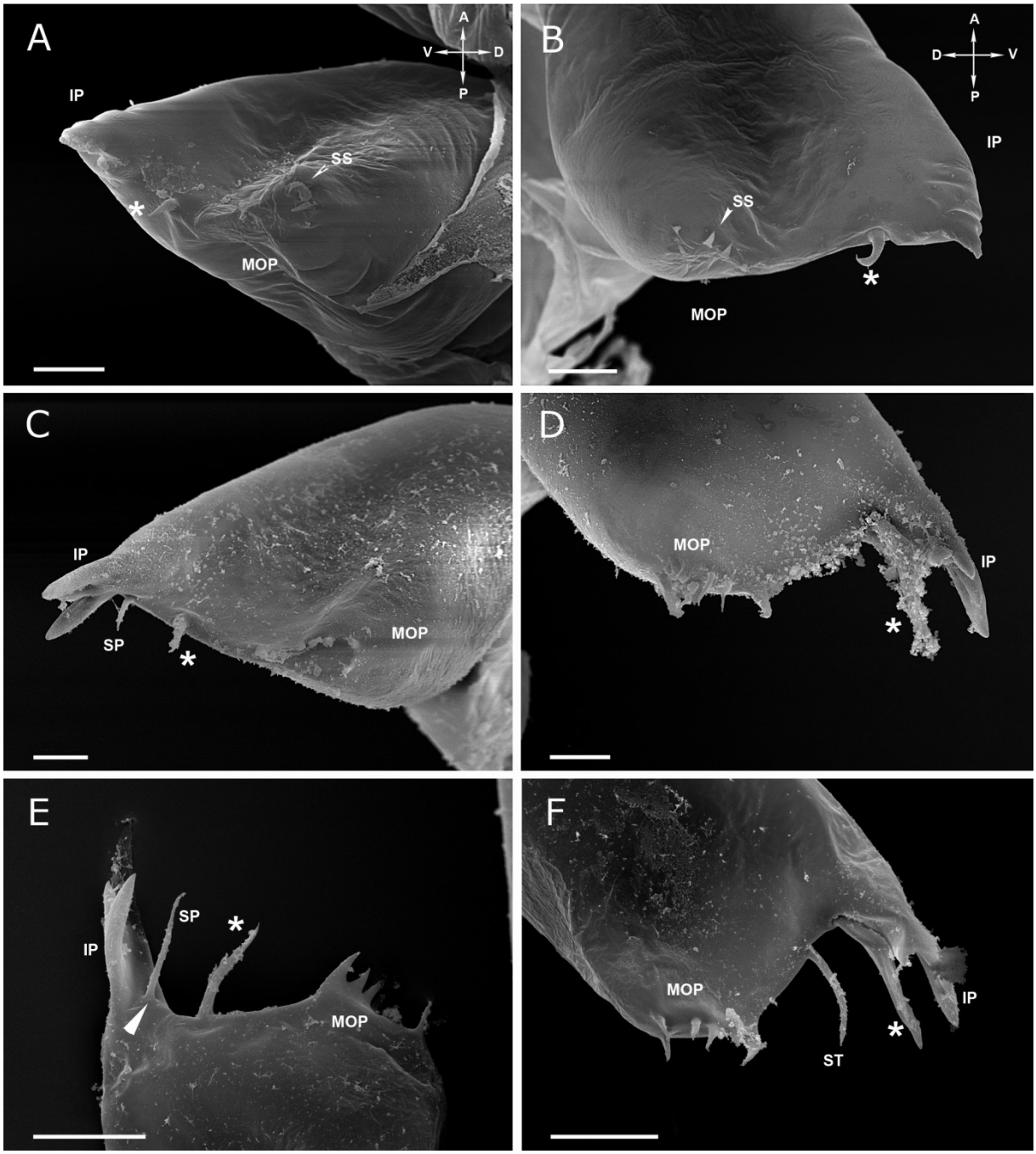

Fig. 4 *Macrobrachium amazonicum*, zoea I - III. **A**. Zoea I; frontal view of right mandible. **B**. Zoea I; frontal view of left mandible. **C**. Zoea II; frontal view of right mandible. **D**. Zoea II; Inner anterior view of left mandible. **E**. Zoea III; outer posterior view of left mandible. **F**. Zoea III; inner anterior view of left mandible (bars 20 µm). Abbreviations: A, anterior; asterisk, 'lacinia mobilis'; D, dorsal; IP, incisor process; MOP, molar process; P, posterior; SP, sensory spine; SS, small spines of molar process; ST, submarginal seta; V, ventral.

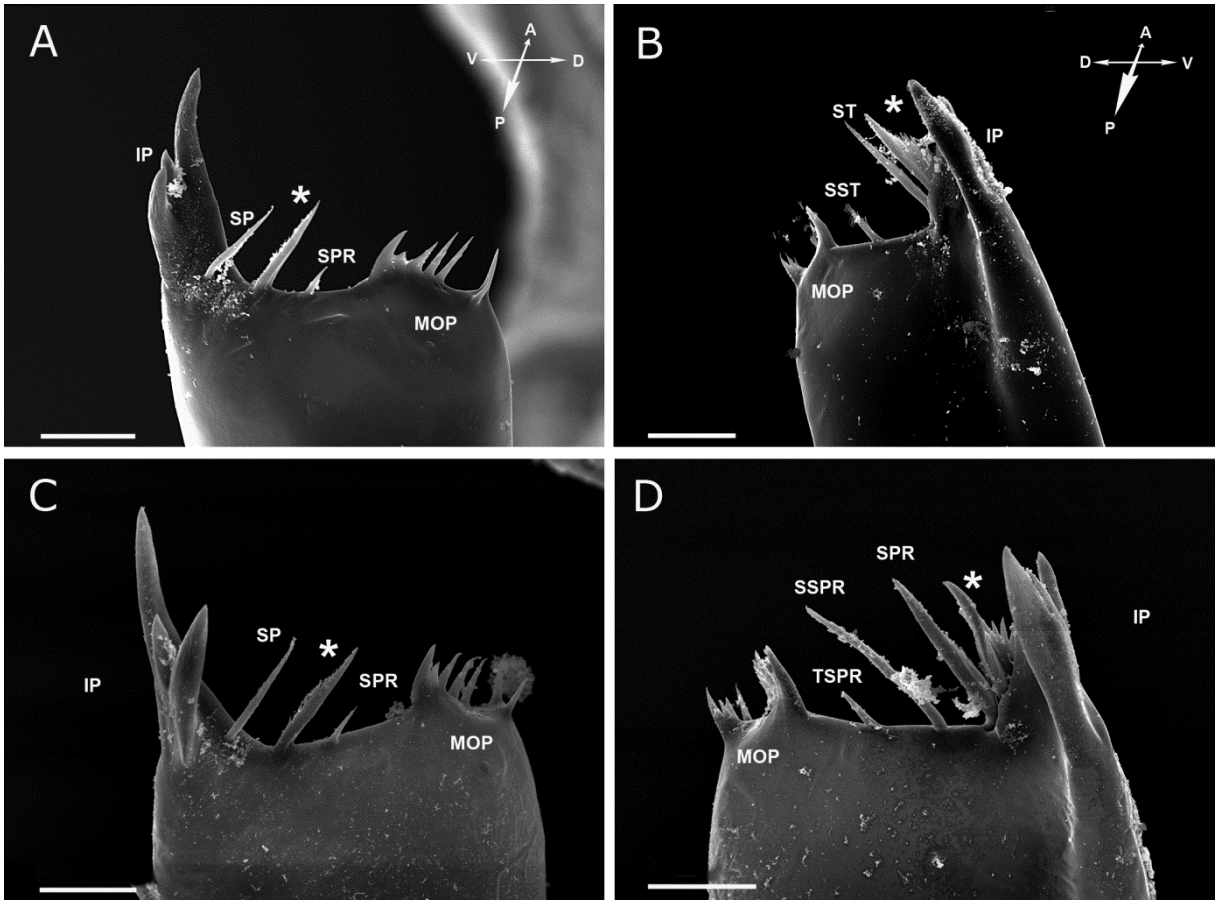

Fig. 5 *Macrobrachium amazonicum*, zoea IV – V. **A**. Zoea IV; outer posterior view of right mandible. **B**. Zoea IV; outer posterior view of left mandible. **C**. Zoea V; outer posterior view of right mandible. **D**. Zoea V; outer posterior view of left mandible (bars 20 µm). Abbreviations: A, anterior; asterisk, 'lacinia mobilis'; D, dorsal; IP, incisor process; MOP, molar process; P, posterior; SP, sensory spine; SPR, submarginal process; ST, submarginal seta; SSPR, second submarginal process; SST, second submarginal seta; TSPR, third submarginal process; V, ventral.

Zoea IV (Fig. 5A, B)

Length of gnathal edge 63 µm (SD=2.12), incisor process 39 µm (SD=1.39), 'lacinia mobilis' 22 µm (SD=1.66), first small ventral spine on molar process 11 µm (SD=1).

Right mandible (Fig. 5A): Most mandibles (60%) with a second small process (SSPR) between the 'lacinia mobilis' and the molar process.

Left mandible (Fig. 5B): one third of our specimens with a second process (SSPR) between the 'lacinia mobilis' and the molar process.

Zoea V (Fig. 5C, D)

Length of gnathal edge 71 µm (SD=4.78), incisor process 40 µm (SD=1.48), 'lacinia mobilis' 30 µm (SD=5.01), first small ventral spine on molar process 13 µm (SD=1.79).

Right mandible (Fig. 5C): All mandibles with a second small process between the 'lacinia mobilis' and the molar process.

Left mandible (Fig. 5D): 62% of specimens with a third process (TSPR), 12% with a fourth process.

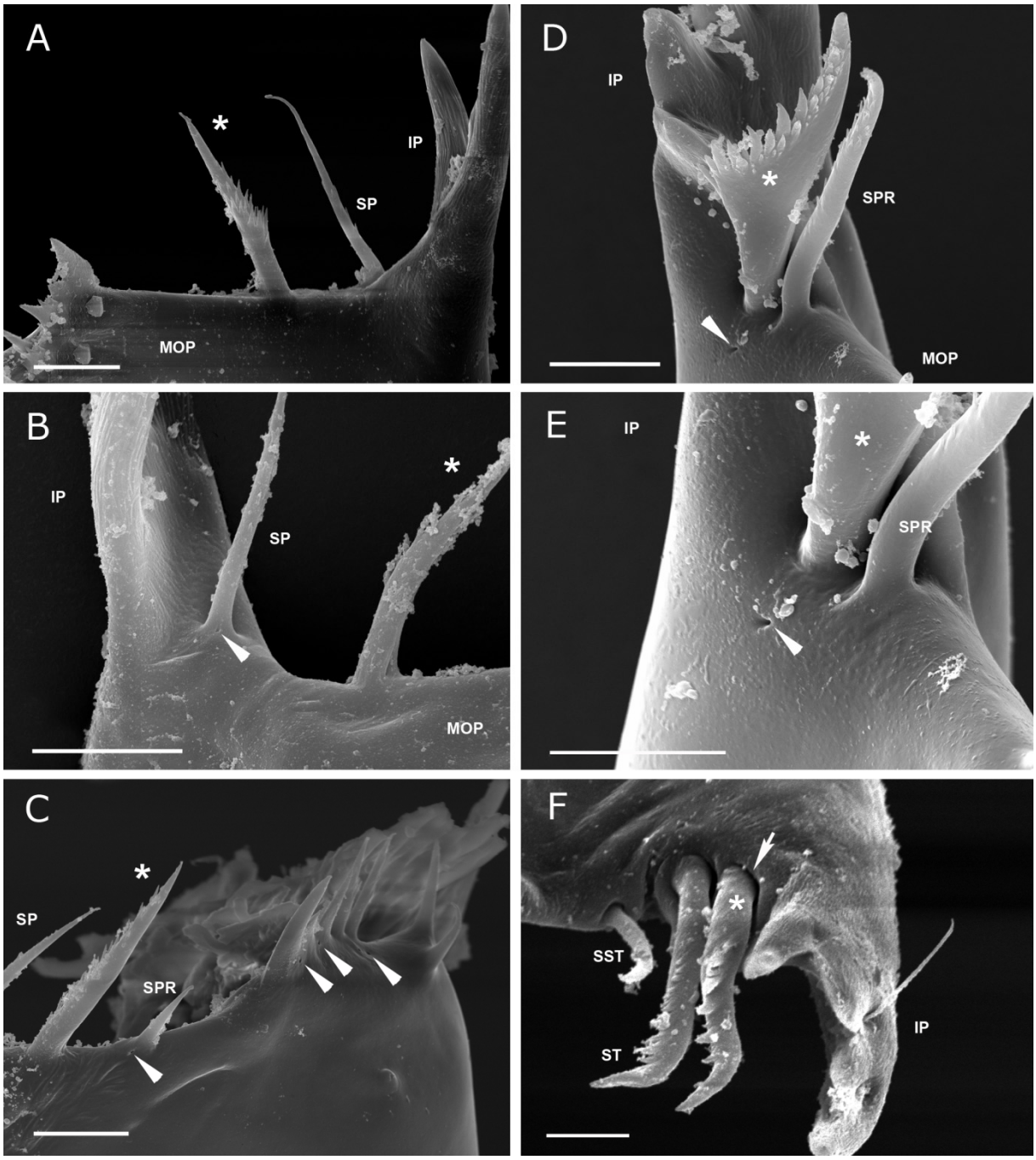

Fig. 6 Detailed views of mandibles in *P. elegans* and *M. amazonicum*. **A**. *M. amazonicum* stage IV; inner view of right mandible. **B**. *M. amazonicum* stage III; outer view of incisor process of right mandible. **C**. *M. amazonicum* stage IV; outer view of molar process of right mandible. **D**. *M. amazonicum* stage IV; frontal view of incisor process of left mandible. **E**. *M. amazonicum* stage IV; detail of pore at 'lacinia mobilis' of left mandible. **F**. *P. elegans* stage V; frontal view of incisor process of left mandible with basal articulation of 'lacinia mobilis' (arrow); (bars 10 µm). Abbreviations: Arrowhead, pore; asterisk, 'lacinia mobilis'; IP, incisor process; MOP, molar process; SP, sensory spine; SPR, submarginal process; ST, submarginal seta; SST, second submarginal seta.

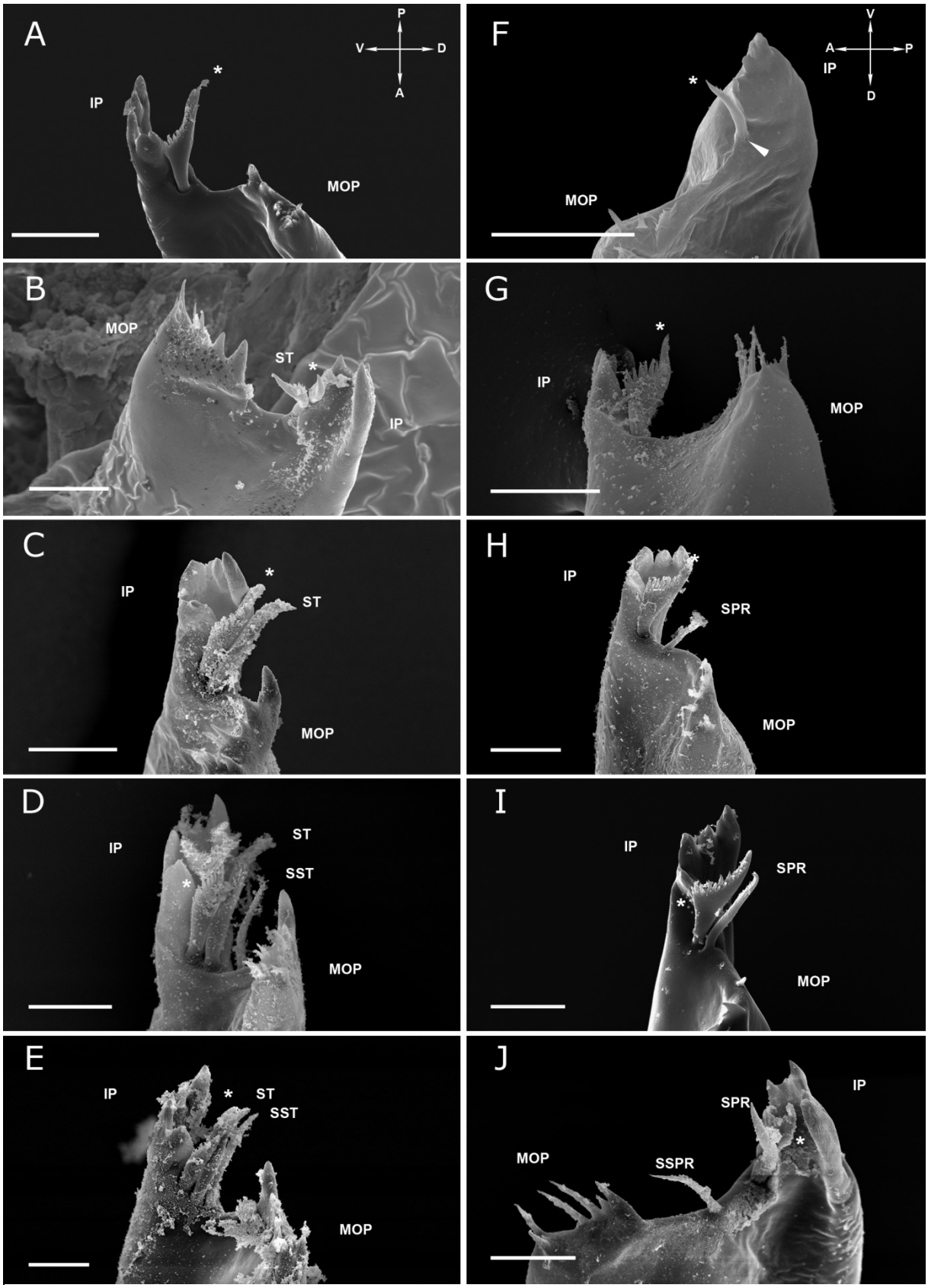

Fig. 7 Comparison of development of left mandibles in *P. elegans* and *M. amazonicum*. **A-E**. *P. elegans* stage I (A) to stage V (E) (A, from Geiselbrecht and Melzer (2010)). **F-J**. *M. amazonicum* stage I (F) to stage V (J) (bars 20 µm). Abbreviations: A, anterior; asterisk, 'lacinia mobilis'; D, dorsal; IP, incisor process; MOP, molar process; P, posterior; SPR, submarginal process; SSPR, second submarginal process; ST, submarginal seta; SST, second submarginal seta; V, ventral.

Analysis of morphogenetic development of *M. amazonicum* and *P. elegans*

In order to determine characteristic features for each larval stage, special attention was given to the morphogenetic development of the mandibles, measuring in both mandibles the length of the gnathal edges, the incisor process, the 'lacinia mobilis', and the first ventral spine on the molar process (see above, and Table 1).

The direction of the morphogenetic development of the mandibles, e.g., growth of the gnathal edges, the 'lacinia mobilis', and the incisor process as well as the development of submarginal setae, is similar in both species. However, this development is initially retarded in *M. amazonicum*, lagging behind that observed in *P. elegans*. In later larval stages, a similar level is eventually reached, reflecting a faster rate of developmental change in *M. amazonicum* (Figs. 7, 8, 9). This is indicated in the graphs by different gradients of the length-to-stage curves in early zoeae that later become parallel lines.

Table 1: Summary of morphogenetic development of several features of zoea I-V in *P. elegans* and *M. amazonicum* (n = number of mandibles measured)

Larval stage	Species	Size in µm (n) mandible	Size in µm (n) 'lacinia mobilis' left	Size in µm (n) 'lacinia mobilis' right	Size in µm (n) Pars incisivus	Size in µm (n) first ventral spine at pars molaris	Mandible with first submarginal seta	Mandible with second submarginal seta
Zoea I	*P. elegans*	53 (19)	22 (7)	21 (4)	30 (9)	11 (10)	0%	0%
	M. amazonicum	38 (6)	10 (5)	9 (5)	16 (6)	5 (7)	0%	0%
Zoea II	*P. elegans*	57 (10)	23 (5)	19 (4)	34 (9)	11 (10)	100%	0%
	M. amazonicum	52 (10)	19 (3)	19 (4)	28 (3)	8 (8)	0 % left, 100 % right	0%
Zoea III	*P. elegans*	64 (16)	26 (15)	22 (9)	42 (11)	11 (10)	100%	13 % left, 0 % right
	M. amazonicum	58 (23)	23 (9)	20 (5)	30 (6)	11 (11)	100%	0%
Zoea IV	*P. elegans*	68 (14)	30 (7)	23 (6)	46 (09)	11 (12)	100%	44 % left, 0 % right
	M. amazonicum	63 (11)	24 (3)	21 (7)	39 (8)	11 (8)	100%	33 % left, 60 % right
Zoea V	*P. elegans*	77 (5)	30 (1)	20 (1)	49 (2)	11 (2)	100%	83 % left, 78 % right
	M. amazonicum	71 (6)	32 (4)	25 (3)	40 (4)	13 (6)	100%	63 % left, 100 % right

Macrobrachium amazonicum reached the mandibular size of *P. elegans* zoea I only in stage III. At stage V, however, the two species showed similar lengths of the gnathal edges. Similarly, the 'lacinia mobilis' in first-stage *P. elegans* showed twice this size than that of *M. amazonicum*. At larval stage II, however, size difference had disappeared. The same accounts

for the first spine on the molar process. In stage I *P. elegans* it already had its final size, while in *Macrobrachium amazonicum* it was fully developed in stage III. The incisor process of stage I in *M. amazonicum* was less than half the length of that observed in *P. elegans*. The subsequent rate of growth per stage, however, was faster in *M. amazonicum*. Nevertheless, during the period of larval development studied here (stages I-V), the incisor process in *M. amazonicum* reached never the same size as in *P. elegans*. The appearance of submarginal setae did not always correspond to a specific zoeal stage. In larvae being in the same stage, some mandibles showed submarginal setae, while others did not. But in all cases the development followed the same sequence. A summary of the morphological features of zoeal stages I-V of both species is given in Table 2.

To make clear that the observed size differences are not correlated with the overall growth rates of the larval body, we used total body length of zoea larvae in the two species as reference size. Figures 10 and 11 show the ratio of length of mandible appendages (lacinia mobilis and spine on molar process) to total length (TL) in zoea stage I-V. These graphs show that relative size of the appendages and growth rate strongly differ between *M. amazonicum* and *P. elegans* until zoea II and III, respectively. Values of relative appendage lengths of the subsequent stages indicate a more or less parallel development in the two species, which means that the degree of underdevelopment in *M. amazonicum* decreased.

Table 2: Summary of morphological features of mandibles of *P. elegans* and *M. amazonicum* (abbreviations: IP, incisor process; MOP, molar process; Sp MOP, spine on molar process)

		Zoea I	**Zoea II**	**Zoea III-V**
P. elegans	IP	fork-like	fork-like	fork-like
	MOP	slender	slender	slender
	Sp MOP	9-11 right, 6-8 left, small	9-11 right, 6-8 left, small	9-11 right, 6-8 left, small
	Dorsal row at MOP	3 small spines	3 small spines	3 small spines
	Fan-like structures	only 'l. mobilis'	only 'l. mobilis'	only 'l. mobilis'
M. amazonicum	IP	mitten form	fork-like	fork-like
	MOP	mitten form	slender	slender
	Sp MOP	6 small spines	6 -7, small spines	6 -7, small spines
	Dorsal row at MOP	2 small spines	2 small spines	2 small spines
	Fan-like structures	only 'l. mobilis'	only 'l. mobilis'	only 'l. mobilis'

Discussion

General and taxon-specific features of mandibles

The larval mandibles of *P. elegans* and *M. amazonicum* show similar features as previously described for larval mouth parts in other caridean shrimp (e.g., Fielder 1970; Dos Santos et al. 2004; Yang 2005; Geiselbrecht and Melzer 2010). This includes in particular an absence of mandibular palps and the presence of an incisor process, molar process, as well as particular spines, teeth, and a 'lacinia mobilis'. Also, an asymmetrical morphology of left and right mandibles seems to be common.

Further clarification of terminology is required for the 'lacinia mobilis' and the additional processes on the left mandible, which also show a basal articulation and cuticular pores (Fig. 6D-F). The structural similarity of these features, corresponding to the innervated structures shown in Geiselbrecht and Melzer (2013), indicates presence of respective sensory equipment. The same accounts for respective structures in the successive larval stages and in *M. amazonicum*. These processes are therefore also referred to as "setae" rather than "spines". The part of the mandible referred to as 'lacinia mobilis' is a conspicuous, with an innervated setal structure that was found also in previous studies of decapod zoeal mandibles (Konishi 2007; Geiselbrecht and Melzer 2010, 2013). The debate is, whether this should be referred to as "movable element" or "lacinia mobilis"? This is dependent on whether or not it is considered as a homologue of the lacinia mobilis in the Peracarida (Dahl and Hessler 1982; Richter et al. 2002; Geiselbrecht and Melzer 2010). In the present study of *M. amazonicum*, we describe a zoea with a 'lacinia mobilis' on both mandibles. This contradicts one of the non-homology arguments stressed by Richter et al. (2002), saying that a 'lacinia mobilis' can only be found on a single mandible in decapods. In both species studied here, additional processes in form of sensory spines and setae appear during the course of larval development in a position near the base of the incisor process. Similar situations were shown for *Periclimenes sagittifer* (dos Santos et al. 2004) and *Crangon uritai* (Li and Hong 2004), suggesting that this may be common in the mandible development of caridean larvae. These structures might represent developing parts of the spine-row, a synapomorphic feature of the Caridoida (Eumalacostraca excl. Stomatopoda; e.g., Hessler 1983; Richter and Scholtz 2001). This also accounts for the peracarid lacinia mobilis on the right mandible, while the origin of the lacinia mobilis on the left mandible is as yet not clear (Dahl and Hessler 1982; Richter et al. 2002, Geiselbrecht and Melzer 2014). Hence, we here use the term 'lacinia mobilis', for the time being, in quotation marks.

The results of this study, together with those previously presented by Geiselbrecht and Melzer (2009, 2010), reveal through SEM analysis new features for first-stage larval mandibles of three palaemonid species, *P. elegans*, *M. amazonicum* and *Periclimenes amethysteus*. The mandibles of these species show great similarities including the same basic form, a fork-like incisor process, a fan-like 'lacinia mobilis' on the left mandible, and a slender molar process comprising a number of small spines with a row of spines at the dorsal edge. Comparing zoeae of three species belonging to different genera within the same family, common mandibular features appear to show taxon-specificity on the family level. Furthermore, we show here that such taxon-specific features do not change during the early zoeal development of *P. elegans* and *M. amazonicum*.

Comparison of mandible development in *P. elegans*, *M. amazonicum* and other palaemonids

Compared to *P. elegans* and other Palaemonidae, the zoea I of *M. amazonicum* shows untypical mandible structures. Its mitten form with small processes was also described by Magalhães and Walker (1988) and Queiroz et al. (2011). During the subsequent larval development, the main changes comprise an increase in the size of the gnathal edges and processes and an appearance of additional submarginal setae. In *M. amazonicum* these developmental changes lag initially one stage behind those observed in *P. elegans*. Retarded morphogenesis is also visible in length measurements of the gnathal edges, the 'lacinia mobilis', incisor process, and the first molar spines. Our results show that the mandibles of stage-I larval *M. amazonicum* are conspicuously smaller than in *P. elegans*, but the subsequent growth rate is higher in *M. amazonicum*, resulting in a similar size and developmental condition at stage V in both species.

In conventional light-microscopical analyses of zoeae of *Macrobrachium*, mandibles of the non-feeding zoea I stage have been described as rudimentary (review in Queiroz et al. 2011). This does not mean that appendages on the gnathal edge are absent, as they are found in the form of minute precursors of the feeding stages appendages. Figures 1 and 2 in Queiroz et al (2011) show that the mandibular appendages develop after the moult from zoea I (non-feeding) to zoea II (feeding). This fits well with the results of the present study.

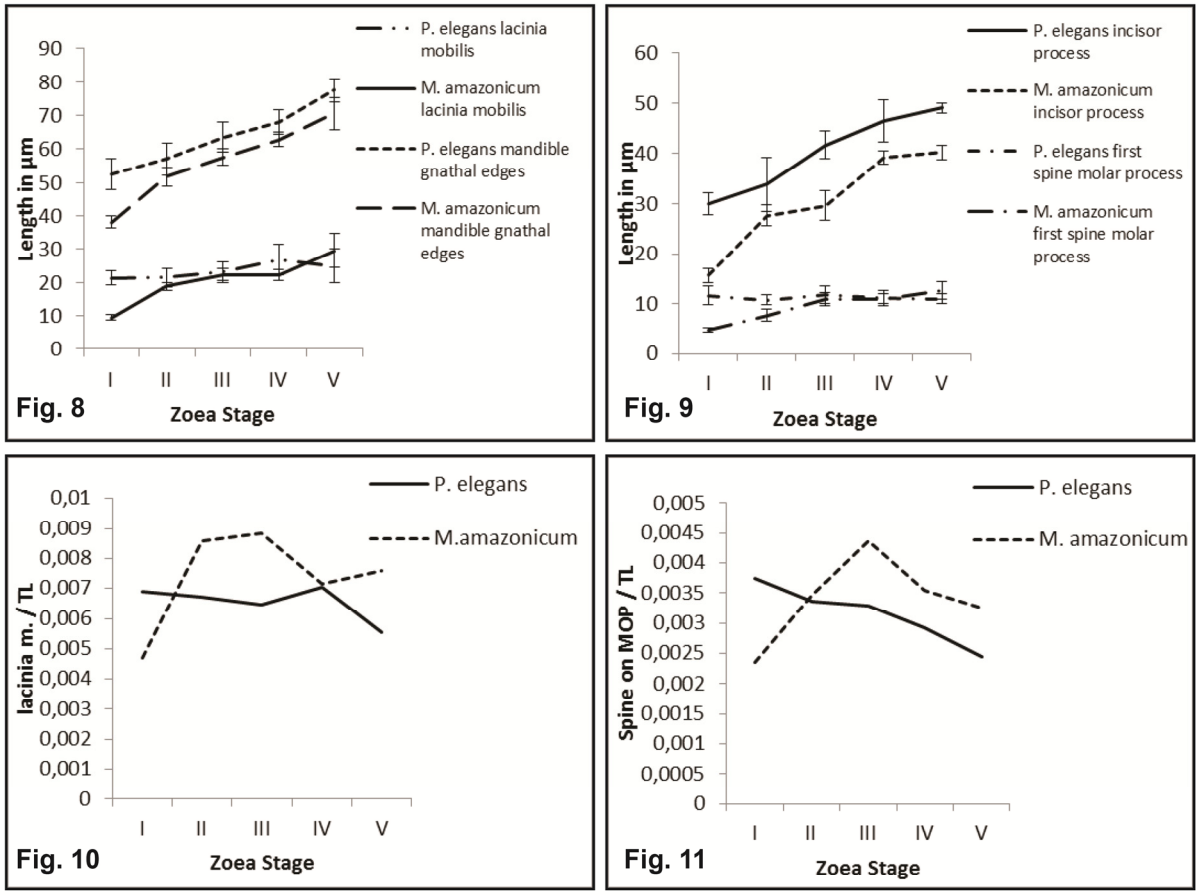

Fig. 8 Mandible size and 'lacinia mobilis' size in *P. elegans* and *M. amazonicum* during zoeal stages I to V.

Fig. 9 Size of incisor process and first ventral spine at molar process in *P. elegans* and *M. amazonicum* during development from zoeal stage I to V.

Fig. 10 Ratio of length of lacinia mobilis to total length in stages I-V in *P. elegans* and *M. amazonicum.*

Fig. 11 Ratio of length of first spine on molar process to total length in stages I-V in *P. elegans* and *M. amazonicum.*

For more than 200 species of adult *Macrobrachium* described to date, different developmental modes have been observed ranging from an amphidromous strategy with a lecithotrophic zoea I or abbreviated larval to benthic and fully lecitotrophic development (Anger 2013). Only *M. intermedium* is fully marine and has a feeding, non-lecitotrophic zoea I (Williamson 1972). This species has the same mandible appendages as *M. niloticum*, a limnic species, but figures 1 and 6 show that the mandible appendages in the latter species are much smaller than in *M. intermedium*. This supports our observations and conclusions. In addition to species of *Macrobrachium*, migratory strategies are found in species of *Pseudopalaemon* and *Palaemonetes* (Anger 2001). Conversely, in *Palaemon* spp., which are almost exclusively marine, first-stage zoeae have well developed mandibles (e.g., Fincham 1983, 1985).

Although detailed mandible analysis in palaemonid zoeae are available for a few species only, we suggest that different feeding modes are reflected in zoeal mandible morphology as described in the present study. The mandibles differ in size, but not in the presence of gnathal appendages. It seems that a well developed zoea I mandible is found in marine forms, while it is underdeveloped in limnic forms. Phylogenetic relationships among *Macrobrachium*, *Palaemon* and *Palaemonetes* have been studied by Murphy and Austin (2004). They suggest that large-scale dispersal rather than regional adaptive radiation is the main motor of evolutionary diversification in *Macrobrachium*. Of peculiar interest here is the outgroup comprising species of *Palaemon*, *Palaemonetes*, and *Macrobrachium intermedium*. The latter is the only species of *Macrobrachium* that is fully marine and has well-developed zoea I mandibles (Williamson 1972). The zoea I of the freshwater inhabiting species *Palaemon pandaliformis* also seems to show an underdeveloped mandible (Gamba 1998; see Fig. 2), and Ashelby et al. (2012) place this species outside of *Palaemon* and closer to *Macrobrachium*. The underdevelopment of zoeal mandibles therefore might be a morphological correlate of the marine-limnic transition, and therefore represent a key character for the understanding of palaemonid phylogeny.

Our comparative study indicates heterochrony in mandible morphogenesis of *M. amazonicum* when compared to *P. elegans* and, in a more general way, between feeding and non-feeding palaemonid zoeae. Heterochrony is defined as an altered timing in phenotypic development compared to ancestors or close relatives (Haeckel 1866; Gould 1979; McKinney and McNamara 1991; Smith 2001; Horder 2001). Recent phylogenetic studies propose heterochrony as a general evolutionary pattern (e.g., Richardson et al. 2002; Maxwell et al. 2010). Tills et al. (2011), for example, analyzed intraspecific heterochrony in a gastropod, *Radix balthica*, and reported significant differences in the genetic distance between individuals with altered timing of phenotypic developmental events. This suggests that heterochrony, associated with genetic diversification and natural selection, may eventually lead to speciation. Of the two closely related species compared here, *P. elegans* may be considered as being closer to the ancestral mode of development, with planktotrophic behavior beginning at hatching, while tendencies towards lecithotrophy (as in *M. amazonicum*, see Anger and Hayd 2010) are considered as a derived condition (for discussion and references, see e.g., Greenwood et al. 1976; Jalihal et al. 1993; Signoret et al. 2000; Ashelby et al. 2012; Anger 2013). However, also an opposite view has been proposed (Murphy and Austin 2004). The delayed development of mandible structures observed in *M. amazonicum* shows thus the characteristics of two heterochronic processes referred to as

postdisplacement (i.e. later onset of appearance) and acceleration (development at a higher rate; for definitions of terms, see McKinney and McNamara 1991; Horder 2001). While the former phenomenon causes an initial delay in mandible development of *M. amazonicum*, the latter is responsible for an increasing similarity between equivalent successive larval stages of the two species compared here.

It is possible that heterochronic shifts may also play a role in the diversification within the *Macrobrachium amazonicum* species complex, in which ongoing speciation is currently under debate (Hayd and Anger 2013; Anger 2013; dos Santos et al. 2013). The postdisplacement of mandible development in *M. amazonicum* saves energy invested in embryonic morphogenesis, as the first larval stage does not use its mandibles for feeding (Odinetz Collart and Magalhães 1994; de Araujo and Valenti 2007; Anger and Hayd 2010). In later zoeal stages of this species, which feed on zooplankton, the mandibles develop rapidly, showing both family-specific and species-specific features. This supports the hypothesis of an evolutionary ground pattern of spines and protrusions (Geiselbrecht and Melzer 2010). In order to detect and analyze the presumably ongoing diversification among the various estuarine, coastal and fully limnic inland populations assigned to the *M. amazonicum* complex, future comparative research on mandible development in larvae from different regions (e.g. upper vs. lower Amazon) may add further evidence for speciation.

Conclusions

Our study supports the hypothesis proposing that larval mandible morphology provides phylogenetically important information (Geiselbrecht and Melzer 2010). Underlying species- and stage-specific adaptations to mandibular function, an evolutionary ground pattern in the basic mandibular form and armature is present even in species with non-feeding larval stages such as *M. amazonicum*. In a comparison of larval mandibles in *P. elegans* and *M. amazonicum* using exclusively first-stage zoeae, great morphological differences associated with differential modes of feeding would obscure the apomorphous features of palaemonids. These become conspicuous only in an analysis of mandible development through various successive larval stages (here: zoea I-V).

Heterochrony seems to be a mechanism that can bring about differential evolutionary adaptations to specific selection pressures such as food limitation. This may be most simply described by the notion that animals differentiate a structure only when it is needed. Thus, in a phylogenetic analysis of two closely related taxa showing heterochronic shifts, it may not be useful to compare the same developmental stage (e.g., the zoea I). In our case we find the

relevant phylogenetic information when we compare the zoea I of *P. elegans* with the zoea II or III of *M. amazonicum*. This, however, requires unequivocal identification of larval stages and an identification of morphogenetic hallmarks allowing for comparing different ontogenies. It presents an example for understanding holomorphology (Hennig 1966) of a given taxon, viz. the characters found in all stages of mandible development, and the underlying mechanisms that result in different developmental speed in related taxa.

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Conflict of interest

All authors of this article confirm that no conflict of interest of any potential source is present.

References

Anger K. 2001. The biology of decapod crustacean larvae. Crustacean Issues 14. A.A. Balkema, Lisse. 419 p.

Anger K. 2013. Neotropical *Macrobrachium* (Caridea: Palaemonidae): on the biology, origin, and radiation of freshwater-invading shrimp. Journal of Crustacean Biology 33(2):151-183.

Anger K, Hayd L. 2009. From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*. Aquatic Biology 7(1):19-30.

Anger K, Hayd L. 2010. Feeding and growth in early larval shrimp *Macrobrachium amazonicum* from the Pantanal, southwestern Brazil. Aquatic Biology 9(3):251-261.

Anger K, Hayd L, Knott J, Nettelmann U. 2009. Patterns of larval growth and chemical composition in the Amazon River prawn, *Macrobrachium amazonicum*. Aquaculture 287(3):341-348.

Ashelby CW, Page TJ, De Grave S, Hughes JM, Johnson ML. 2012. Regional scale speciation reveals multiple invasions of freshwater in Palaemoninae (Decapoda). Zoologica Scripta 41(3):293-306.

Bitsch C, Bitsch J. 2004. Phylogenetic relationships of basal hexapods among the mandibulate arthropods: a cladistic analysis based on comparative morphological characters. Zoologica Scripta 33(6):511-550.

Bitsch J. 2001. The arthropod mandible: morphology and evolution. Phylogenetic implications. Société entomologique de France. p 305-321.

Bolaños J, Rivero W, Hernández J, Magán I, Hernández G, Cuesta JA, Felder DL. 2005. Abbreviated larval development of the pea crab *Orthotheres barbatus* (Decapoda: Brachyura: Pinnotheridae) described from laboratory-reared material, with notes on larval characters of the Pinnotherinae. Journal of Crustacean Biology 25(3):500-506.

Charmantier G, Anger K. 2011. Ontogeny of osmoregulatory patterns in the South American shrimp *Macrobrachium amazonicum*: Loss of hypo-regulation in a land-locked population indicates phylogenetic separation from estuarine ancestors. Journal of Experimental Marine Biology and Ecology 396(2):89-98.

Clark PF. 2005. The evolutionary significance of heterochrony in the abbreviated zoeal development of pilumnine crabs (Crustacea : Brachyura : Xanthoidea). Zoological Journal of the Linnean Society 143(3):417-446.

Clark PF, Calazans DK, Pohle GW. 1998. Accuracy and standardization of brachyuran larval descriptions. Invertebrate Reproduction and Development 33(2-3):127-144.

Dahl E, Hessler RR. 1982. The crustacean lacinia mobilis: a reconsideration of its origin, function and phylogenetic implications. Zoological Journal of the Linnean Society 74(2):133-146.

de Araujo MC, Valenti WC. 2007. Feeding habit of the Amazon river prawn *Macrobrachium amazonicum* larvae. Aquaculture 265(1):187-193.

dos Santos A, Calado R, Bartilotti C, Narciso L. 2004. The larval development of the partner shrimp *Periclimenes sagittifer* (Norman, 1861)(Decapoda: Caridea: Palaemonidae: Pontoniinae) described from laboratory-reared material, with a note on chemical settlement cues. Helgoland Marine Research 58(2):129-139.

dos Santos A, Hayd L, Anger K. 2013. A new species of *Macrobrachium* Spence Bate, 1868 (Decapoda, Palaemonidae), *M. pantanalense*, from the Pantanal, Brazil. Zootaxa 3700(3): 534–546.

Edgecombe GD, Richter S, Wilson GD. 2003. The mandibular gnathal edges: homologous structures throughout Mandibulata? African Invertebrates 44(1):115-135.

Fielder D. 1970. The larval development of *Macrobrachium australiense* Holthuis, 1950 (Decapoda, Palaemonidae), reared in the laboratory. Crustaceana 18(1):60-74.

Fincham A. 1977. Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). I. Laboratory methods and a review of *Palaemon (Paleander) elegans* Rathke, 1837. Bulletin of the British Museum and national History (Zool.) 32:1-28.

Fincham A. 1983. Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). 4. *Palaemon (Palaemon) serratus* (Pennant, 1777) and functional morphology of swimming. Bulletin of the British Museum (Natural History) Miscellanea Zoology Series 44:125-161.

Fincham A. 1985. Larval development of British prawns and shrimps (Crustacea, Decapoda, Natantia). V. *Palaemon (Palaemon) adspersus* (Rathke, 1873). Bulletin of the British Museum (Natural History) Zoology 48:215-231.

Fincham A, Williamson DI. 1978. Crustacea decapoda: larvae, 6: Caridea, families: Palaemonidae and Processidae. Fiches d'identification du zooplancton.

Gamba AL. 1998. The Larval Development of a Fresh-Water Prawn, *Palaemon pandaliformis* (Stimpson, 1871), under Laboratory Conditions (Decapoda, Palaemonidae). Crustaceana 71(1):9-35.

Garland T, Adolph SC. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. Physiological Zoology 64(4):797-828.

Garm A. 2004. Revising the definition of the crustacean seta and setal classification systems based on examinations of the mouthpart setae of seven species of decapods. Zoological Journal of the Linnean Society 142:233-252.

Geiselbrecht H, Melzer RR. 2009. Morphology of the first zoeal stage of the partner shrimp *Periclimenes amethysteus* Risso, 1827 (Decapoda: Caridea: Palaemonidae: Pontoniinae) studied with the Scanning EM. Zootaxa 2140:45-55.

Geiselbrecht H, Melzer RR. 2010. Mandibles of zoea-I-larvae of nine decapod species. A scanning EM analysis. Spixiana 33(1):27-47.

Geiselbrecht H, Melzer RR. 2013. How do mandibles sense?-The sensory apparatus of larval mandibles in *Palaemon elegans* Rathke, 1837 (DECAPODA, PALAEMONIDAE). Arthropod Structure and Development 42:1-16.

Geiselbrecht H, Melzer RR. 2014. Fine structure and ecdysis of mandibular sensilla associated with the lacinia mobilis in *Neomysis integer* (Leach, 1814) (CRUSTACEA, MALACOSTRACA, PERACARIDA). Arthropod Structure and Development (published online 7 February 2014).

Gould SJ. 1979. On the Importance of Heterochrony for Evolutionary Biology. Systematic Zoology 28(2):224-226.

Greenwood J, Fielder D. 1979. The zoeal stages and megalopa of *Portunus rubromarginatus* (Lanchester)(Decapoda: Portnnidae), reared in the laboratory. J Plankton Res 1(2):191-205.

Greenwood J, Fielder D, Thorne M. 1976. The larval life history of *Macrobrachium novaehollandiae* (De Man, 1908) (Decapoda, Palaemonidae), reared in the laboratory. Crustaceana:252-286.

Haeckel E. 1866. Generelle Morphologie der Organismen: allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformirte Descendenz-Theorie: G. Reimer.

Hayd L, Anger K. 2013. Reproductive and morphometric traits of *Macrobrachium amazonicum* (Decapoda: Palaemonidae) from the Pantanal, Brazil, suggests initial speciation. Revista de Biología Tropical 61(1):39-57.

Hennig W. 1966. Phylogenetic systematics. Translated by Davis DD and Zangerl, R. Univ of Illinois Press: Urbana, Chicago and London. 263 p.

Hessler RR. 1983. A defense of the cardioid facies: wherein the early evolution of the Eumalacostraca is discussed. In: Schram, F. R. (ed.) Crustacean Phylogeny. Crustacean Issues 1. A.A. Balkema, Rotterdam, p. 145-164.

Horder T. 2001. Heterochrony. eLS: John Wiley and Sons, Ltd.

Ingle R. 1983. A comparative study of the larval development of *Monodaeus couchi* (Couch), *Xantho incisus* Leach and *Pilumnus hirtellus* (Linnaeus)(Crustacea: Brachyura: Xanthidae). Journal of Natural History 17(6):951-978.

Ingle R. 1992. Larval stages of Northeastern Atlantic crabs. An illustrated key. Hall Ca, editor. London. 388 p.

Jalihal D, Sankolli K, Shenoy S. 1993. Evolution of larval developmental patterns and the process of freshwaterization in the prawn genus *Macrobrachium* Bate, 1868 (Decapoda, Palaemonidae). Crustaceana:365-376.

Klaus A, Kulasekera V, Schawaroch V. 2003. Three-dimensional visualization of insect morphology using confocal laser scanning microscopy. Journal of Microscopy 212(2):107-121.

Konishi K. 2007. Morphological notes on the mouthparts of decapod crustacean larvae, with emphasis on palinurid phyllosomas. Bulletin fisheries research agency Japan 20:73.

Kumlu M, Jones DA. 1995. Feeding and digestion in the caridean shrimp larva of *Palaemon elegans* Rathke and *Macrobrachium rosenbergii* (De Man) (Crustacea: Palaemonidae) on live and artificial diets. Aquaculture Nutrition 1(1):3-12.

Li H and Hong S. 2004. Larval development of *Crangon uritai* (Decapoda: Crangonidae) reared in the laboratory. Journal of Crustacean Biology 24(4):576-591.

McKinney ML, McNamara KJ. 1991. Heterochrony: the evolution of ontogeny. New York [etc.]: Plenum.

Magalhães C. 1985. Desenvolvimento larval obtido em laboratorio de palaemonideos da Região Amazônica. I. *Macrobrachium amazonicum* (Heller, 1862)(Crustacea, Decapoda). Amazoniana 9(2):247-274.

Magalhães C, Walker I. 1988. Larval development and ecological distribution of central Amazonian palaemonid shrimps (Decapoda, Caridea). Crustaceana:279-292.

Martin JW, Goy JW. 2004. The first larval stage of *Microprostema semilaeve* (Von Martens, 1872) (Crustacea: Decapoda: Stenopodidea) obtained in the laboratory. Gulf and Caribbean Research 16:19-25.

Maxwell EE, Harrison LB, Larsson HC. 2010. Assessing the phylogenetic utility of sequence heterochrony: evolution of avian ossification sequences as a case study. Zoology 113(1):57-66.

Mekhanikova IV. 2010. Morphology of mandible and lateralia in six endemic amphipods (Amphipoda, Gammaridea) from Lake Baikal, in relation to feeding. Crustaceana 83(7):865.

Meyer R, Melzer RR. 2004. Scanning EM Diagnosis of Marine Decapoda Larvae: A Comparison of Preparation Techniques. Crustaceana 77(7):883-886.

Meyer R, Wehrtmann IS, Melzer RR. 2006. Morphology of the first zoeal stage of *Portunus acuminatus* Stimpson, 1871 (Decapoda : Portunidae : Portuninae) reared in the laboratory. Sci Mar 70(2):261-270.

Minagawa M, Takashima F. 1994. Developmental changes in larval mouthparts and foregut in the red frog crab, *Ranina ranina* (Decapoda: Raninidae). Aquaculture 126(1):61-71.

Murphy NP and Austin CM. 2004. Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxonomy and the convergent evolution of abbreviated larval development. Zoologica Scripta 34:187–197.

Odinetz Collart O, Magalhães C. 1994. Ecological constraints and life history strategies of palaemonid prawns in Amazonia.

Paul A, Paul J, Coyle K. 1989. Energy sources for first-feeding zoeae of king crab *Paralithodes camtschatica* (Tilesius)(Decapoda, Lithodidae). Journal of Experimental Marine Biology and Ecology 130(1):55-69.

Queiroz LD, Abrunhosa FA, Maciel CR. 2011. Ontogenesis and functional morphology of the digestive system of the freshwater prawn, *Macrobrachium amazonicum* (Decapoda: Palaemonidae). Zoologia (Curitiba) 28:395-402.

Rice A. 1979. A plea for improved standards in descriptions of crab zoeae. Crustaceana 37(2):214-218.

Rice AL. 1980. Crab zoeal morphology and its bearing on the classification of the Brachyura. The Transactions of the Zoological Society of London 35(3):271-372.

Richardson MK, Oelschläger HH. 2002. Time, pattern, and heterochrony: a study of hyperphalangy in the dolphin embryo flipper. Evolution and Development 4(6):435-444.

Richter S, Edgecombe GD, Wilson GDF. 2002. The lacinia mobilis and Similar Structures – a Valuable Character in Arthropod Phylogenetics? Zoologischer Anzeiger - A Journal of Comparative Zoology 241(4):339-361.

Richter S, Scholtz G. 2001. Phylogenetic analysis of the Malacostraca (Crustacea). Journal of Zoological Systematics and Evolutionary Research 39(3):113-136.

Rota-Stabelli O, Campbell L, Brinkmann H, Edgecombe GD, Longhorn SJ, Peterson KJ, Pisani D, Philippe H, Telford MJ. 2011. A congruent solution to arthropod phylogeny: phylogenomics, microRNAs and morphology support monophyletic Mandibulata. Proceedings of the Royal Society B: Biological Sciences 278(1703):298-306.

Sanders MB, Billinghurst Z, Depledge MH, Clare AS. 2005. Larval development and vitellin-like protein expression in *Palaemon elegans* larvae following xeno-oestrogen exposure. Integrative and Comparative Biology 45(1):51-60.

Schembri P. 1982. Locomotion, feeding, grooming and the behavioural responses to gravity, light and hydrostatic pressure in the stage I zoea larvae of *Ebalia tuberosa* (Crustacea: Decapoda: Leucosiidae). Mar Biol 72(2):125-134.

Signoret G, Ortega A, Brailovsky D. 2000. Partially abbreviated larval development in an undescribed freshwater palaemonid prawn of the genus *Macrobrachium* from Chiapas, Mexico. Crustaceana 73:273-282.

Smith KK. 2001. Heterochrony revisited: the evolution of developmental sequences. Biological Journal of the Linnean Society 73(2):169-186.

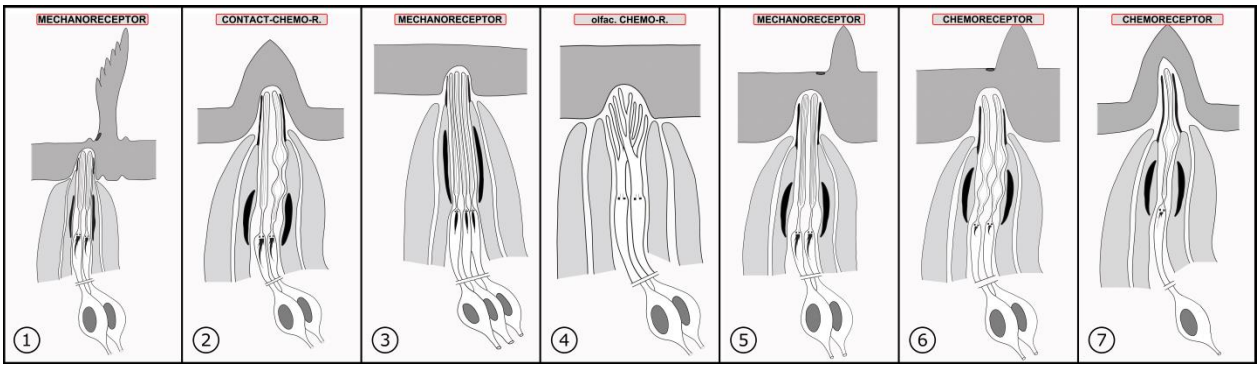
Tills O, Rundle SD, Salinger M, Haun T, Pfenninger M, Spicer JI. 2011. A genetic basis for intraspecific differences in developmental timing? Evolution and Development 13(6):542-548.

Watling L. 1989. A classification system for crustacean setae based on the homology concept. Crustacean Issues 6:15-26.

Yang HJ. 2005. Larval development of *Latreutes anoplonyx* (Decapoda: Hippolytidae) reared in the laboratory. Journal of Crustacean Biology 25(3):462-479.

6. Paper III

Geiselbrecht, H., Melzer, R.R., 2013b. How do mandibles sense? The sensory apparatus of larval mandibles in *Palaemon elegans* Rathke, 1837 (DECAPODA, PALAEMONIDAE). Arthropod Structure & Development 42, 1-16.



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How do mandibles sense? – The sensory apparatus of larval mandibles in *Palaemon elegans* Rathke, 1837 (Decapoda, Palaemonidae)

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ABSTRACT

The mandibles of decapod zoea-I larvae are robustly built masticating mouthparts equipped with several processes and spines. Superficial examination of these sturdy, inflexible structures can suggest that they are lacking sensory receptors. However, detailed TEM analysis of their ultrastructure revealed up to 11 sensillar cell clusters on the gnathal edges of the mandibles of the zoea-I in *Palaemon elegans* Rathke, 1837. Based on ultrastructural criteria we distinguish 7 types of sensilla: mechanoreceptors, chemoreceptors and mechano- and chemoreceptors. One sensory unit located at the base of the 'lacinia mobilis' exhibits the typical features of a crustacean mechanosensitive sensillum with an external seta and corresponding ultrastructure. Another unit shows features indicating bimodal contact chemosensitivity. A third one is similar to known olfactory chemoreceptors.

Using the concept of modality-specific structures we analyse the structure and functional morphology of each sensillum, and give a comprehensive overview of the sensory abilities of zoea mandibles. We take a closer look at the ultrastructure of the 'lacinia mobilis', providing further features to trace its evolutionary history in Decapoda, and thus contributing to a better understanding of malacostracan phylogeny.

1. Introduction

Crustacean sensory structures, with their distinct and variously shaped external and internal ultrastructure, are well studied features in the field of crustacean morphology (e.g. Ball and Cowan, 1977; Tyson and Sullivan, 1979; Altner et al., 1983; Schmidt and Gnatzy, 1984; Schmidt, 1990; Garm and Høeg, 2006; Hallberg and Skog, 2011). Besides a multitude of cuticular structures, setal morphology and steric arrangement of sensilla are important features in crustacean taxonomy (e.g. Watling, 1989; Ingle, 1992; Garm, 2004b). Nevertheless, one still can make new discoveries in this area, as we aim to show in the present work by studying the ultrastructure of the mandibles of the zoea-I larva in *Palaemon elegans*, a caridean decapod shrimp.

In this species, the gnathal edges of the larval mandibles are equipped with various forms of appendages, described as acute spines or denticles (Geiselbrecht and Melzer, 2010), that are non-articulated outgrowths of the cuticle (Ingle, 1992). This observation at first does not suggest these structures to be sensilla. However, Factor (1978) stated that mandibular 'teeth' of first-stage larvae of *Homarus americanus* have a lumen and thick, cuticularized walls, and in this regard appear similar to some types of setae, viz. sensory receptors. Recently a comparative SEM analysis of the mandibles of zoea-I larvae in various decapod taxa indicated the presence of sensillar structures. For example, cuticular pores similar to the ends of ecdysial canals were found, either located on the mandible's surface or associated with seta-like cuticular protrusions (Geiselbrecht and Melzer, 2010).

Of peculiar interest in this context is the 'lacinia mobilis', a movable appendage of the larval mandible in ancestral decapods. The question of homology between this structure and the laciniae in other malacostracan crustaceans is still unresolved (Richter et al., 2002). However, our SEM analyses of *P. elegans* and *Periclimenes amethysteus* showed features (articulation on a basal ring, presence of an ecdysial pore) suggesting that the 'lacinia mobilis' of decapod zoeas might be a sensillum. Therefore, studying the lacinia's ultrastructure in addition to external features is important for the understanding of its functional morphology and evolutionary origin. Moreover, it would be interesting to know if there are even more sensory structures on the mandibles, how they are distributed, and what their sensory modalities might be.

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Very little is known about the presence, and even less about the ultrastructure, of sensilla located on the gnathal lobe of arthropod mandibles (Ong, 1969; Friedman and Strickler, 1975; Whitehead and Larsen, 1976; Tyson and Sullivan, 1981; Hunter and Ullman, 1992). Most works on this topic either cover external setal morphology only (Factor, 1978; Chauvin and Faucheux, 1981; Lavalli and Factor, 1992; Faucheux, 1995; Garm et al., 2004; Garm, 2004a; Davoodi et al., 2009) or do not analyse the gnathal lobe and its processes (Sutcliffe and McIver, 1982; Honomichl, 1982, 2008; Sinitsina et al., 2003; Garm and Høeg, 2006).

It is hardly possible to detect and conclusively document a sensillum by means of external examination only. Arthropod sensilla have cuticular components, usually expressed as hairs (setae) or hair-derived structures, with distinct properties indicating a common origin (Watling, 1989). These features can be hard to detect, however, and derived structures can be extremely modified and difficult to interpret correctly. Using SEM, the only observable characters are pores and the basal rings of setae. Thus, to prove that a certain structure has a sensory function an ultrastructural analysis is required. Here, the scientific concepts of necessary and sufficient conditions come into play. In many SEM studies a sensillum is assumed based on the presence of an articulated hair arising above a pore, but these features are necessary but not sufficient here. The only feature that is both necessary and sufficient is the presence of a bipolar neuron with a dendrite that is a modified cilium. In this regard, too, the present study is meant as a contribution to our knowledge on sensory capacities of arthropod mandibles in general.

We studied the ultrastructure of the gnathal lobe of the mandibles of zoea-I larvae in *P. elegans* using transmission electron microscopy. Several sensillar structures were analysed with regard to their modality-specific structures, their distribution and external morphology, and to morphological specialisations of the sensilla linked to the robust nature of the mandibles. Based on differences in ultrastructural and external features we distinguish 7 types of sensilla that are innervated by 4 different types of dendrites. For each type we discuss the specific function, thus give a comprehensive overview of the sensory equipment of the mandibles of a decapod zoea-I larva.

2. Material & methods

2.1. Animals

Ovigerous females of *P. elegans* were collected from intertidal rock pools in Cross Bay, Rovinj (Croatia), during a student excursion to Ruđer Bošković Institute in August/September 2009. Animals were kept individually in 500 ml Cautex vials. The caps of the vials were replaced with gauze, and all vials placed together in a 250 l tank supplied with fresh seawater. Larvae hatched overnight and were fixed immediately.

2.2. Transmission electron microscopy (TEM)

After dissection of antennae and telson the animals were fixed in 4% glutardialdehyde in 0.1 M cacodylate buffer at 4 °C, post-osmicated in 1% OsO_4 in buffer, and embedded in epoxy resin. Ultra-thin sections of 60–70 nm thickness were made with a diamond knife on a RMC-MTXL ultramicrotome. Sections were double-stained with uranyl acetate and lead citrate, and inspected in a FEI Morgagni transmission EM at 80 kV. For quantitation of the single features of each sensillum we prepared complete saggittal serial sections of both mandibles of one specimen and inspected the whole series. We sectioned two more specimens and only inspected critical sections to check the number and quality of the sensilla.

2.3. Light microscopy (LM)

Specimens were dissected, fixed and embedded in epoxy resin as described above for TEM. Semi-thin sections of 1.5 μm were made with a diamond knife on a RMC MT-XL ultramicrotome. Sections were stained with Richardson's stain (Richardson et al., 1960), covered with cover glasses with DPX, and photographed with a digital camera mounted on a Leica stereomicroscope.

2.4. Scanning electron microscopy (SEM)

Dissected mandibles were dehydrated in a graded acetone series (70%, 80%, 90% for 10 min each, plus 3 times 100% for 20 min each), then critical point-dried in a Baltec CPD 030. Because most mandible dimensions were below 100 μm, specimen containers with smaller pore dimension were used. Dried specimens were mounted on SEM stubs with self-adhesive carbon stickers, and sputtered with gold on a Polaron E 5100. Mandibles were studied with a LEO 1430VP SEM at 15 kV (left mandible: $n = 12$; right mandible: $n = 13$)

2.5. Sensillum terminology

We named each sensillum (S) according to the assigned sensillum type in random order, and denoted the number of sensory cells by a subscript. The $S1_2$ sensillum, for example, is a type 1 sensillum that has two sensory cells.

3. Results

3.1. External morphology and general ultrastructure of mandibular sensilla

We found surprisingly high numbers of sensillar structures located on the gnathal lobe of the larval mandibles. Fig. 1 shows an SEM overview of the mouth region illustrating the position and structure of the mandibles. Using the TEM we detected 10 sensillar cell clusters on the left mandible and 11 on the right mandible in equal measure in all studied specimens ($n = 3$). Correlations with external structures viewed by SEM indicate that these sensilla (1) are of articulated, solid seta-like structure, i.e. constitute 'laciniae mobiles', or (2) are found in the form of non-articulated, solid spines, or (3) do not show special external cuticular structures. An overview of the respective positions of the external structures as well as the corresponding innervating cells is given in Figs. 2 and 3.

Each sensillum is innervated by 1–3 ciliary dendrites, each inserted on one bipolar sensory cell respectively. The perikarya of the neurons lie near the proximal part of the gnathal lobe (Fig. 3E). Dendrites are composed of an inner (IDS), a ciliary (CDS) and an outer (ODS) dendrite segment. Each IDS is approximately 25 μm long, extends distally from the respective cell body, and independently from each other in the direction of the gnathal edge. In its apical region an accumulation of mitochondria (Figs. 4F, 7F) and small vesicles is conspicuous (Fig. 7E, F). At the distal tip of each IDS a single basal body can be found (Figs. 7E, 9D). Usually it consists of 9 microtubule triplets embedded in an osmiophilic matrix (McIver, 1975; Schmidt and Gnatzy, 1984; Grünert and Ache, 1988). Except in type 4 sensilla, a proximal ciliary rootlet originates from the basal body. Each inner segment gives rise to one outer segment connected by a ciliary segment. Within this ciliary region microtubules are arranged in 9 peripheral doublets forming the typical $9 \times 2 + 0$ pattern. Central tubules are lacking (Figs. 4D, 6C and 7E, 8C, 9D). Basally, microtubules connect with the basal body; distally they extend into the ODSs with no distinct pattern, and are more or less densely packed.

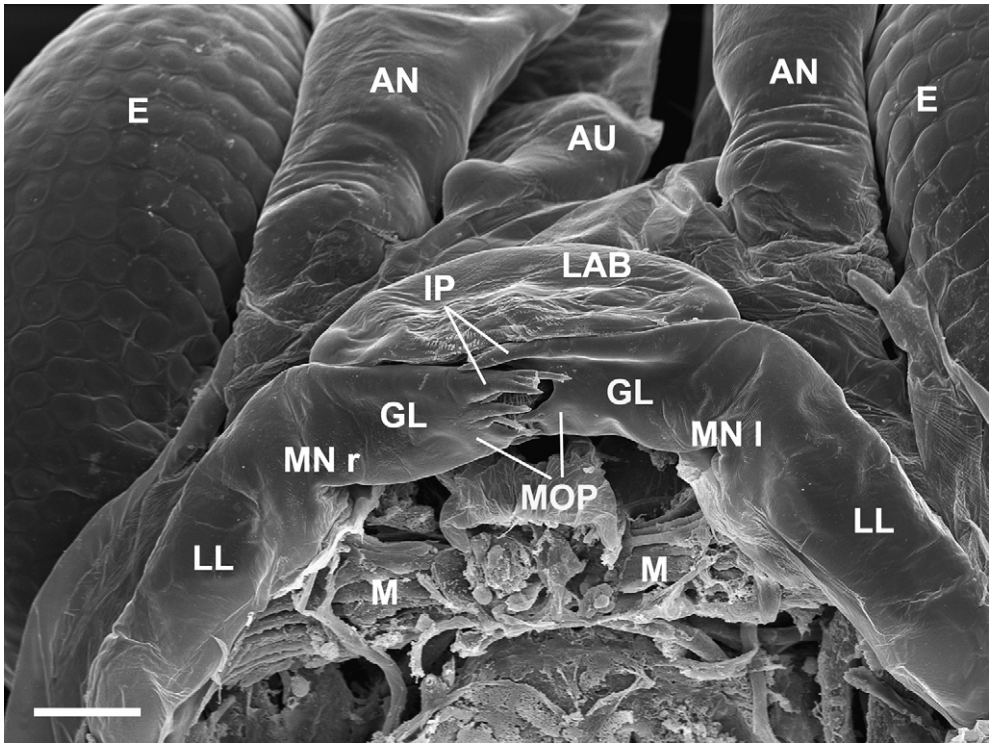

Fig. 1. Scanning electron micrograph showing overview of mouth region and position and structure of mandibles of zoea-I-larva in *Palaemon elegans*. To provide better insight specimen has been dissected and following mouthparts like paragnaths and maxillae have been removed. Abbreviations: AN, antenna; AU, antennule; E, eye; GL, gnathal lobe; IP, incisor process; LAB, labrum; LL, lateral lobe; M, mandibular muscle; MNl, left mandible; MNr, right mandible; MOP, molar process.

According to further ultrastructural criteria four types of dendrites can be distinguished. The features that characterize a dendrite as type I (Figs. 4, 5 and 8) are the presence of a microtubule accumulation in the ODS, A-tubules with a dense core and arms in the CDS, and a strong ciliary rootlet. A type II dendrite (Figs. 5, 9 and 10) is characterized by a short CDS, A-tubules without arms, and by a more delicate ciliary rootlet (terminology after Schmidt and Gnatzy, 1984). The type III dendrite (Fig. 6) is characterized by a relatively thin ODS with just a few microtubules, A-tubules without arms, and by a short but compact ciliary rootlet. Type IV dendrites (Fig. 7) have branched ODS, short CDS, A-tubules without arms, and no distinct ciliary rootlet. Besides microtubules no other organelles were noticeable throughout the ODSs.

Two inner enveloping cells (EC) closely surround the inner and outer dendrite segments (Figs. 4–10). Except in type 4, the innermost EC forms a tubular envelope enclosing the dendrites and an extracellular space, the receptor lymph cavity (RLC). The RLC has its largest extension in the ciliary region of the dendrites (Figs. 4D, 5E and 8C). Distally the innermost EC forms finger-like evaginations that extend beyond the base of the dendrite sheath (Figs. 4B, 5B). The second EC is completely wrapped around the innermost one. Prevalent organelles in the distal part of the EC are numerous microtubules and vesicles. Mitochondria were discovered only proximal of the transitional region. The EC adhere to each other by numerous septate junctions (Figs. 4B, 5B, 7B and 7D); the IDS are connected to the innermost EC by well developed desmosomal junctions (Figs. 4E, 5E, 6D, 8D and 9E). In the examined region no endoplasmatic reticulum or Golgi apparatus could be detected inside the EC.

3.2. The different types of sensilla

3.2.1. The type 1 sensillum ($S1_2$) ('lacinia mobilis')

This sensillum exhibits a hair-like cuticular structure, and is articulated on a basal ring with a basal pore. It appears as a serrate seta on the left mandible (Fig. 3D) and as a simple seta on the right mandible (Fig. 2C); both setae are solid, i.e. lack an inner lumen, and are composed of 2 cuticle layers, an outer epicuticle and an inner exocuticle. $S1_2$ is innervated by two equal sensory cells with one type I dendrite each. ODSs measure about 16.4 μm in length and about 0.25 μm in diameter. The two unbranched dendrites extend to the blind ending of a canal in the cuticle of the hair base, where they terminate (Fig. 4A). No structures connecting the dendrite membranes and the canal wall could be seen. A plug of electron-dense material overlaps the tips of the dendrites and fills the ending of the canal. Accumulations of about 25 microtubules each are present all along the ODSs. Proximally dendrites are enclosed in a dendrite sheath, consisting of homogeneous, electron-dense material (Fig. 4B). The dendrite sheath extends over about 3.2 μm and ends approximately 13 μm above the ciliary bases. CDSs measure about 1.5 μm. The nine microtubule doublets are composed of an A-tubule, with an electron-dense core and two small dynein arms, and a B-tubule (Fig. 4D). Both dendrites have a strong ciliary rootlet reaching about 2.5 μm into the IDSs (Fig. 4E). A scolopale, an intracellular structure composed of longitudinally oriented microtubules that are enclosed by electron-dense material, is present in the innermost enveloping cell. In cross section it appears discontinuous, like columns spaced all around the cell body; extending between the transitional region and the base of the dendrite sheath it is most prominent in the ciliary region (Fig. 4C, D).

3.2.2. The type 2 sensillum ($S2_2$)

The external appearance of this sensillum is that of an inflexible spine. Located on the processus incisivus (IP) of the left and the right mandible (Figs. 2A, 3B and 3D), the spine is solid at its apical end, whereas proximally it consists of 3 cuticular layers and a lumen (Fig. 5A, B). Fine processes of the second EC project far into the distal part of the spine (Fig. 5A). The $S2_2$ is innervated by two

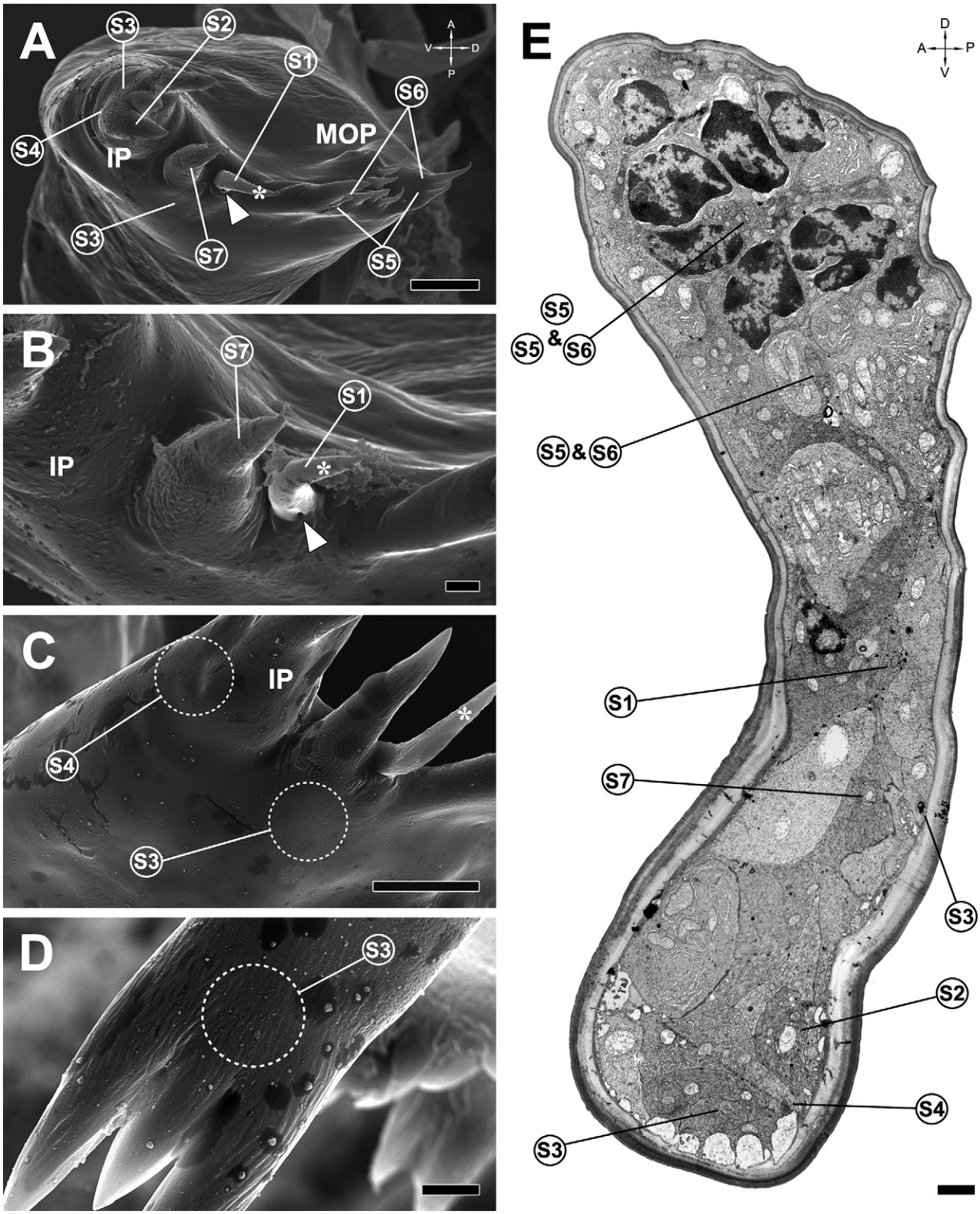

Fig. 2. Scanning (A–D) and transmission (E) electron micrographs showing external morphology and overview of sensillar arrangement of right mandible. A: Inner view (bar = 10 μm) B: Detail of incisor process showing the S1 and S7 sensilla (bar = 2 μm). C: Posterior view showing termination regions (dashed circles) of S3 and S4 (bar = 10 μm). D: Ventral view of incisor process showing termination region of S3 (bar = 3 μm). E: Cross section of mandible (bar = 2 μm). Specimen orientation: A, anterior; D, dorsal; P, posterior; V, ventral. Pins labelled "S1" to "S7" indicate positions of sensilla. Asterisk, 'lacinia mobilis'; arrowhead, pore. Abbreviations: IP, incisor process; MOP, molar process.

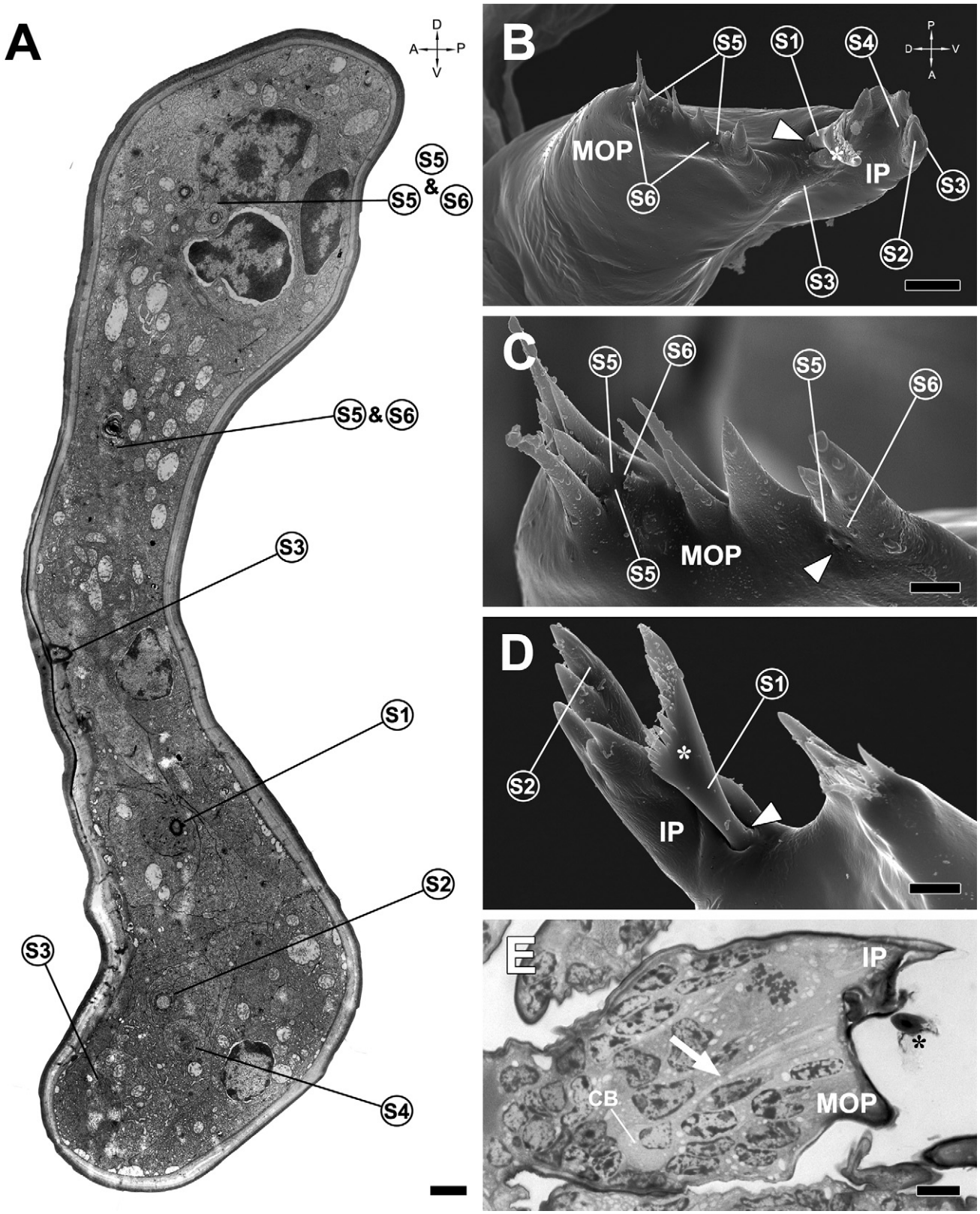

Fig. 3. Transmission (A), scanning electron (B–D), and light (E) micrographs showing external morphology and overview of sensillar arrangement of left mandible. A: Cross section of mandible (bar = 2 μm). B: Inner view (bar = 10 μm). C: Detail of molar process showing S5 and S6 (bar = 3 μm). D: Dorsal view showing S1 and S2 (bar = 6 μm). E: Longitudinal semithin section showing a dendrite (arrow) and cell body (bar = 10 μm). Specimen orientation: A, anterior; D, dorsal; P, posterior; V, ventral. Pins labelled "S1" to "S7" indicate positions of sensilla. Asterisk, 'lacinia mobilis'; arrowhead, pore. Abbreviations: CB, cell body; IP, incisor process; MOP, molar process.

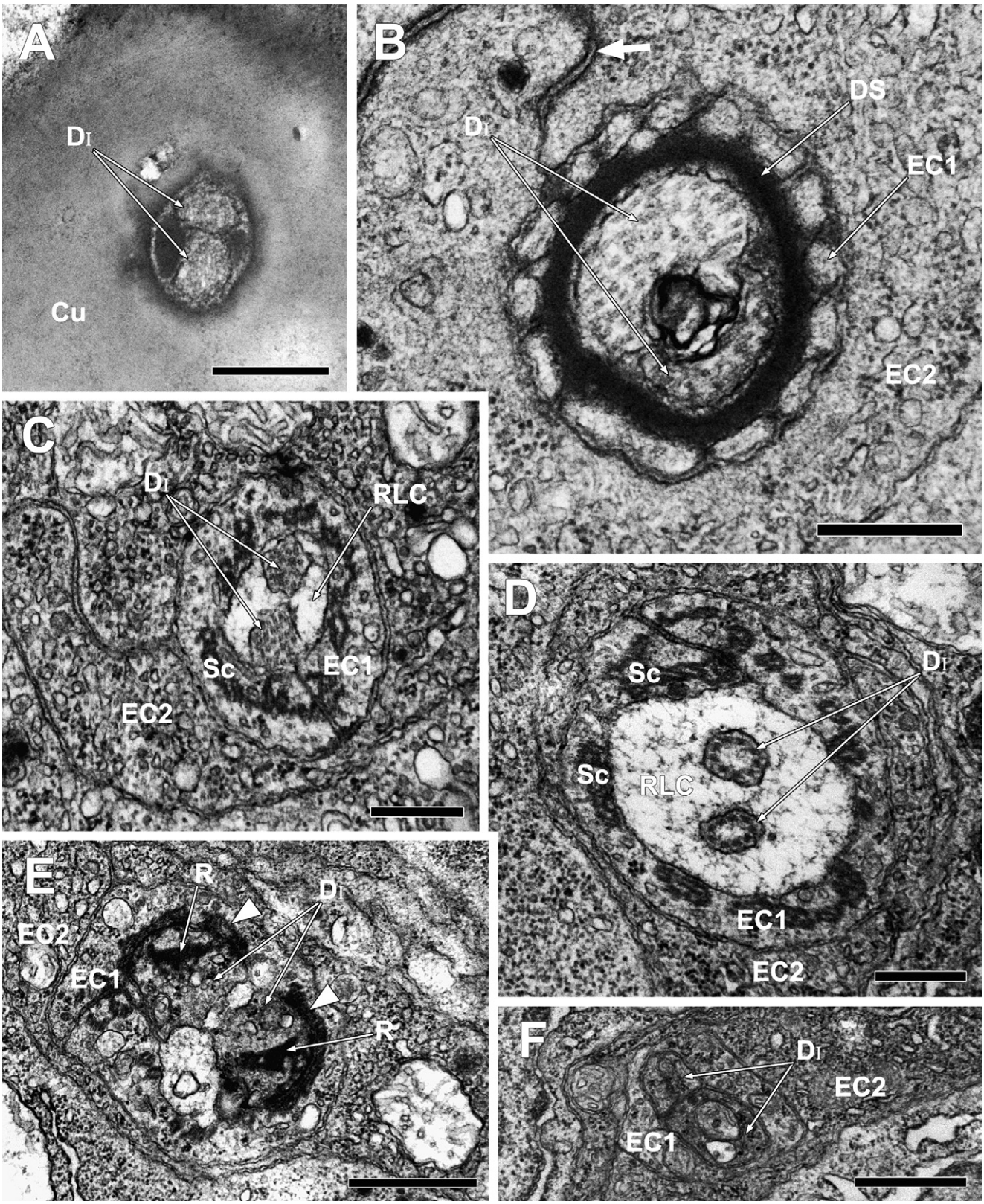

Fig. 4. The $S1_2$ sensillum; cross sections of mandible at different levels. A: Termination region of two type I dendrites in cuticular canal near the hair base (bar = 0.5 μm). B: ODSs proximal to hair base surrounded by dendrite sheath and evaginations of EC1 (bar = 0.5 μm). C: ODSs distal to transitional region (bar = 0.5 μm). D: Ciliary dendrite segments in receptor lymph cavity (bar = 0.5 μm). E: Transitional region with ciliary rootlets of dendrites and desmosomal junctions (arrowheads) with inner enveloping cell (bar = 1 μm). F: IDSs proximal to transitional region (bar = 1 μm). Arrow, septate junction; Cu, cuticle; DI, type I dendrite; DS, dendrite sheath; EC1, EC2, enveloping cell 1, 2; R, rootlet; RLC, receptor lymph cavity; Sc, scolopale.

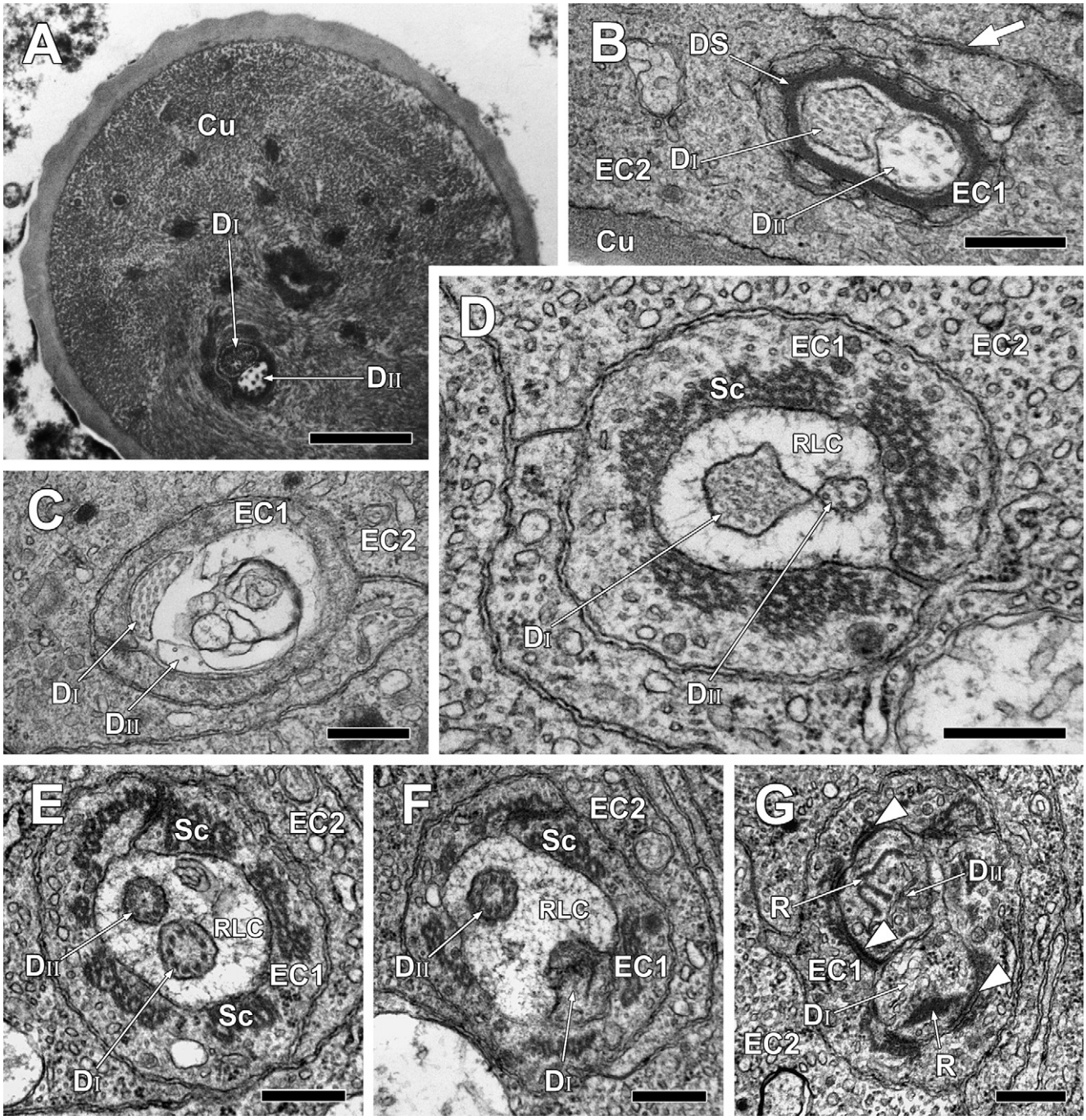

Fig. 5. The $S2_2$ sensillum; cross sections of mandible at different levels. A: Termination region of one type I and one type II dendrite in cuticular canal in distal part of spine (bar = 1 μm). B: Two ODSs with different densities of microtubules in proximal part of spine surrounded by dendrite sheath and evaginations of EC1 (bar = 0.5 μm). C: ODSs distal to transitional region; dendrite swelling of type II dendrite (bar = 0.5 μm). D: ODSs in receptor lymph cavity distal to transitional region (bar = 0.5 μm). E: Ciliary dendrite segments distal to transitional region (bar = 0.5 μm). F: CDS of DII and DI already in transitional region (bar = 0.5 μm). G: Transitional region with ciliary rootlets of dendrites and desmosomal junctions (arrowheads) with inner enveloping cell (bar = 1 μm). Arrow, septate junction; Cu, cuticle; DI, DII, dendrite of type I, II; DS, dendrite sheath; EC1, EC2, enveloping cell 1, 2; R, rootlet; RLC, receptor lymph cavity; Sc, scolopale.

types of sensory cells differing in the number of microtubules present in the ODSs; type I cells have about 23 microtubules, type II cells have around 10. The two unbranched ODSs terminate in the proximal part of the spine enclosed in a dendrite sheath (Fig. 5A, B). No conspicuous pore could be detected in this region, neither with the TEM nor by scanning electron microscopy. The dendrite sheath extends proximally for about 19 μm. Compared to the $S1_2$ sensillum the ODSs are approximately twice as long, each measuring about 30 μm in length and 0.3 μm in diameter. Between the CDSs and the part that is enclosed by the dendrite sheath, the ODS of the type II dendrite sequentially shows two extreme dilatations of the dendrite membrane (Fig. 5C). The CDS of the type I dendrite is approximately 4.3 μm long, the microtubule doublets have A-tubules with an electron-dense core and dynein arms (Fig. 5E), and the ciliary rootlet is compact, strong and about 3.4 μm long (Fig. 5G). The CDS of the type II dendrite is approximately 0.6 μm long, the microtubule doublets are composed of two equal tubules with no electron-dense core and no dynein arms (Fig. 5F). The

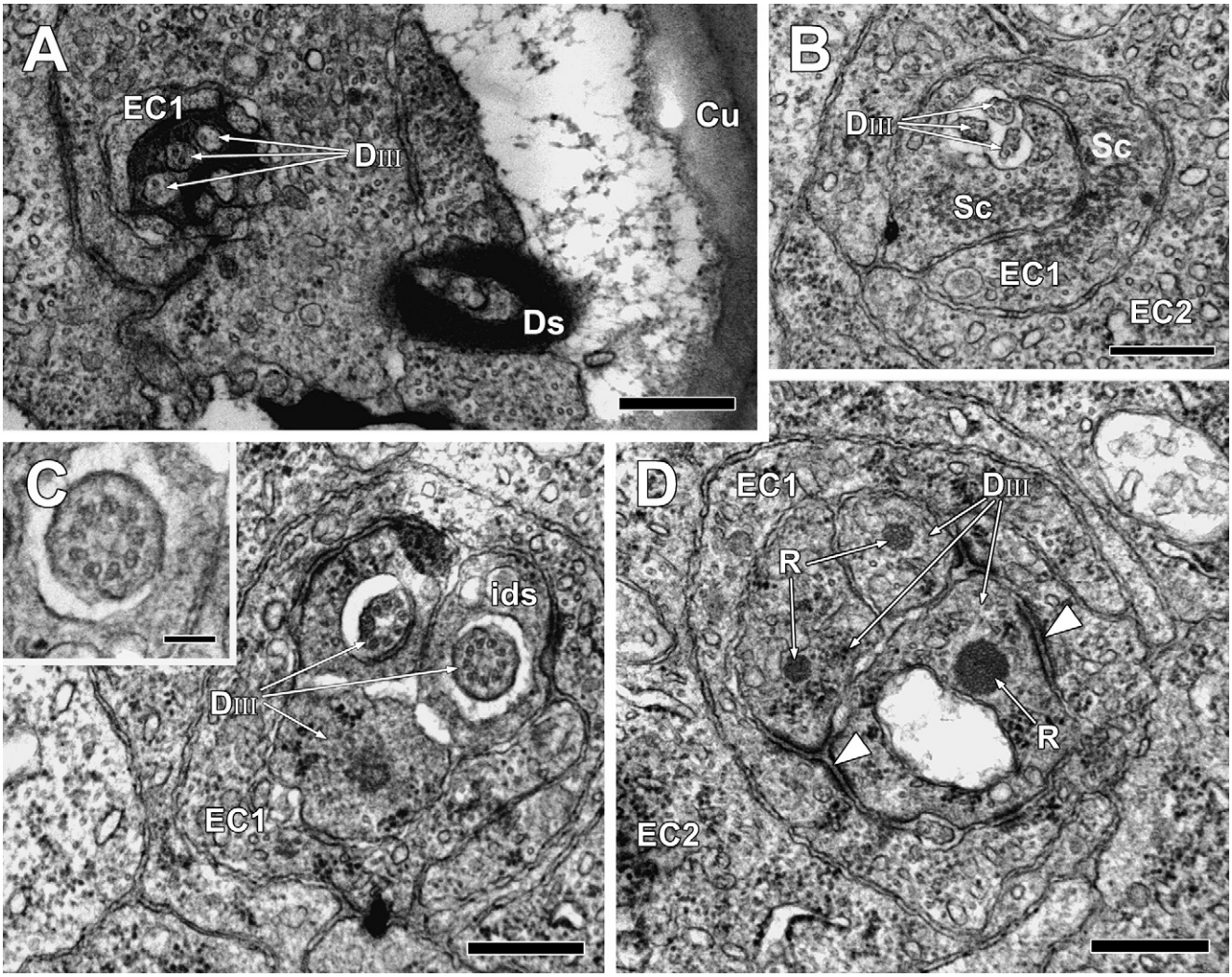

Fig. 6. The $S3_{2/3}$ sensillum; cross sections of mandible at different levels (bars = 0.5 μm). A: Termination region of three type III dendrites ventrally on the IP. B: ODSs surrounded by EC1. C: CDSs and evaginations of IDSs; insert: CDS with 9 × 2 + 0 pattern of microtubules and A-tubules lacking arms (bar = 0.125 μm). D: IDSs with ciliary rootlets and desmosomal junctions (arrowheads) proximal to transitional region. Cu, cuticle; DIII, type III dendrite; DS, dendrite sheath; EC1, 2, enveloping cell 1, 2; ids, inner dendrite segment; R, rootlet; Sc, scolopale.

ciliary rootlet is delicate, branched and about 3.0 μm long (Fig. 5G). Somewhat overlapping the ciliary region there is a distinct scolopale in the EC1 (Fig. 5D).

3.2.3. *The type 3 sensillum ($S3_{2/3}$)*

No special external cuticular structure is associated with the $S3_{2/3}$ sensillum (Figs. 2A, 3B). It may be innervated by either two or three sensory cells, but all the latter have the same ultrastructure and type III dendrites. On both mandibles the $S3_{2/3}$ sensillum with three dendrites appears ventrally on the IP, and the $S3_{2/3}$ sensillum with two dendrites appears posteriorly on the IP. The dendrites extend longitudinally in the direction of the gnathal edge, but the distalmost part is curved outwards and terminates directly under the lateral cuticle (Fig. 2C, D). The ODSs are accompanied by several processes of the EC1, and the distal part is surrounded by a dendrite sheath (Fig. 6A). The TEM analysis showed that the dendrite sheath and the cuticle are in contact, but no conspicuous pores could be detected in the particular regions by SEM (Fig. 2C, D). The EC1 is virtually curled up around the ODSs (Fig. 6B). ODSs measure about 20 μm in length and about 0.15 μm in diameter. 3–5 microtubules pass longitudinally through the ODSs. CDSs extend for about 1.7 μm, A-tubules lack dynein arms (Fig. 6C), and the ciliary rootlets are compact, round and about 1.1 μm long (Fig. 6D). There are annular protuberances of the IDSs respectively overlapping the proximal parts of the CDSs (Fig. 6C).

3.2.4. *The type 4 sensillum ($S4_2$)*

As with the $S3_{2/3}$, no special external cuticular structure is associated with this sensillum (Fig. 2A). The $S4_2$ is innervated by two sensory cells with one type IV dendrite each. The ODSs of the dendrites branch repetitively, resulting in about 7–8 fine branches per dendrite that terminate ventrally on the IP on both mandibles (Fig. 7B). The terminal ends of the branches contact an invagination of the cuticle, but no pore could be detected (Figs. 2C, 7A). The ODSs measure about 6.5 μm in length, their diameter is about 0.5 μm proximally, and distally the fine branches measure about 0.15 μm. The EC1 and EC2 are wrapped around the dendrites from proximal to the CDSs to the distal end of the ODSs. No dendrite sheath is present, and the dendrites nearly fill the entire volume of the RLC (Fig. 7D). Only a small number of scattered microtubules can be found throughout the ODSs. The very short CDSs of about 180 nm are embedded inside the dendrites. The microtubule doublets lack

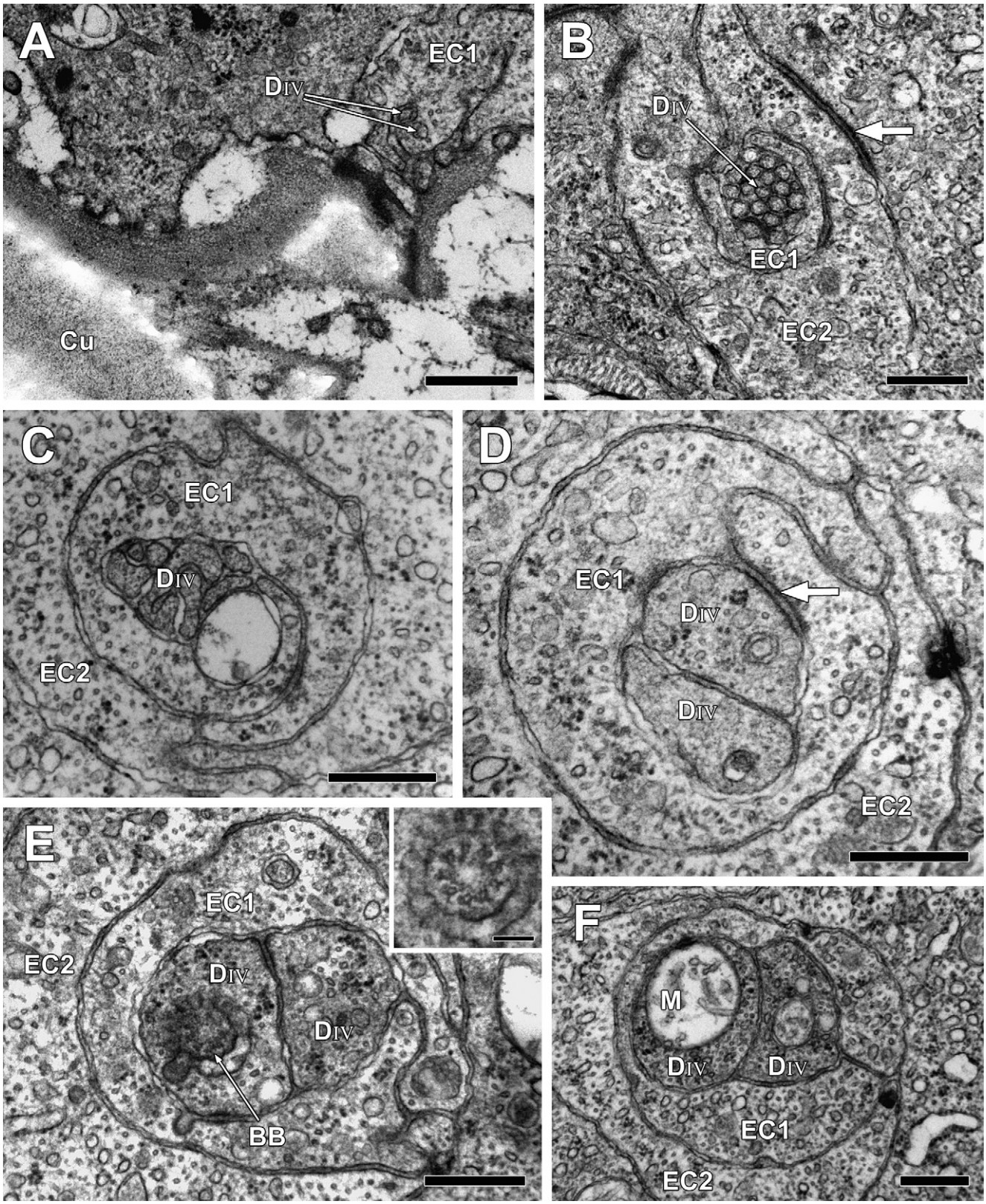

Fig. 7. The $S4_2$ sensillum; cross sections of mandible at different levels (bars = 0.5 μm). A: Termination region of two ODSs near cuticular invagination ventral on the IP. B: two branched ODSs. C: ODSs at the level of initial branching. D: ODSs distal to transitional region. E: Transitional region with very short CDS (insert; bar = 0.125 μm) and basal body. F: IDSs proximal to transitional region. Arrow, septate junction; BB, basal body; Cu, cuticle; DIV, type IV dendrite; EC1, 2, enveloping cell 1, 2; M, mitochondrium.

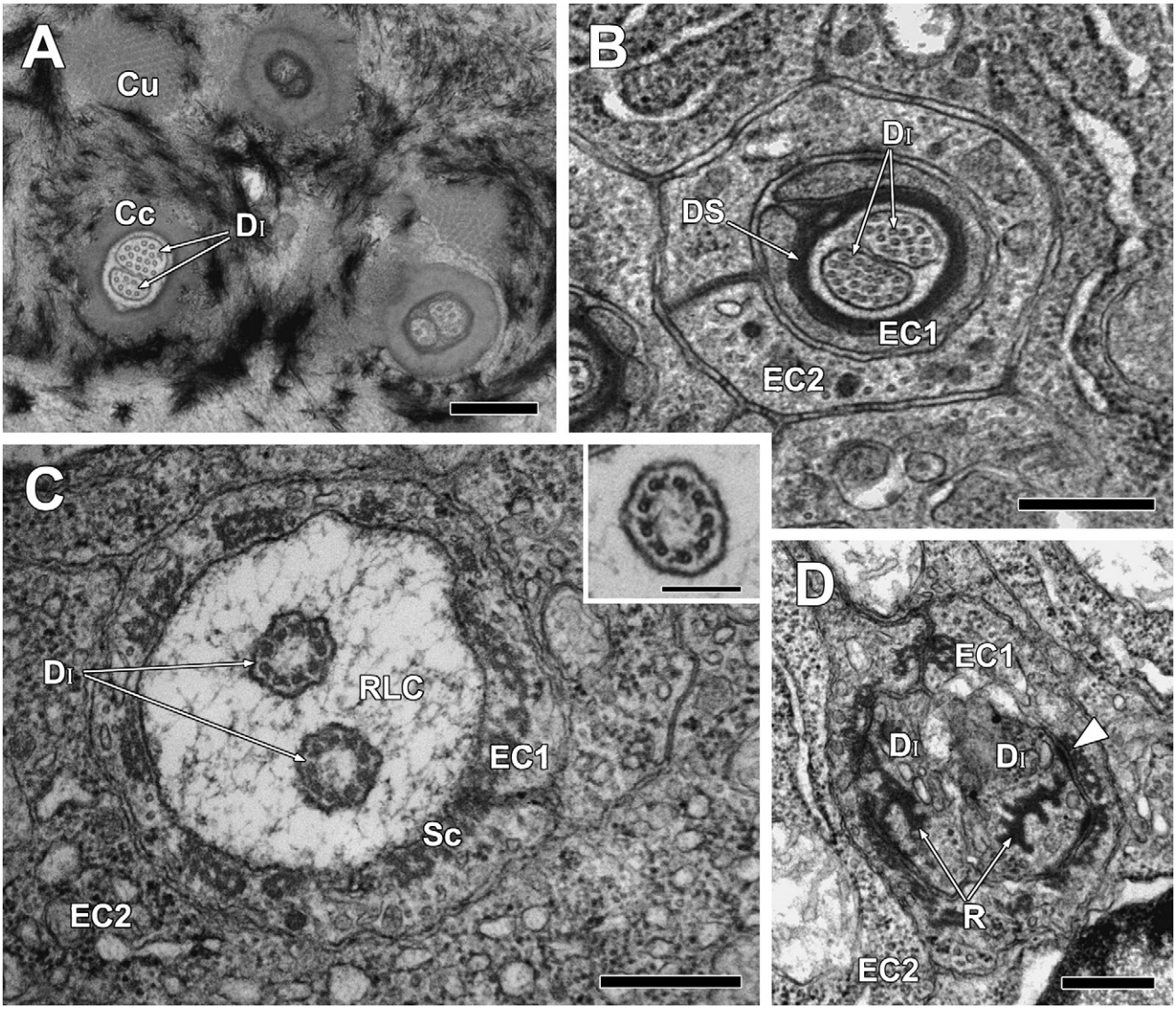

Fig. 8. The $S5_2$ sensillum; cross sections of mandible at different levels (bars = 0.5 μm). A: Triplet of sensilla on dorsal part of MOP; termination region of two type I dendrites in cuticular canal near the spine base; note comb-like structure of cuticle. B: ODSs proximal to spine base surrounded by dendrite sheath and two ECs. C: CDSs distal to transitional region; insert: CDS with 9 × 2 + 0 pattern of microtubules and A-tubules with arms (bar = 0.125 μm). D: Transitional region with ciliary rootlets of dendrites and desmosomal junctions (arrowheads) with inner enveloping cell. Cc, cuticular canal; Cu, cuticle; DI, type I dendrite; DS, dendrite sheath; EC1, 2, enveloping cell 1, 2; R, rootlet; RLC, receptor lymph cavity; Sc, scolopale.

dynein arms, but each doublet is connected to the membrane by a fine fibrillary structure (Schmidt and Gnatzy, 1984: "ciliary necklace structure") (Fig. 7E). No distinct ciliary rootlets could be detected. Apart from a high amount of vesicles no prominent intracellular structure is present in the enveloping cells (Fig. 7B–F).

3.2.5. *The type 5 sensillum ($S5_2$)*

This type of sensillum can be found in two positions on the processus molaris (MOP) of each mandible (Figs. 2A, 3B and 3C). The MOP is equipped with a group of inflexible small spines. There is a pair of sensilla on the ventral part of the MOP of which one is a $S5_2$ sensillum; more dorsally located there is a triplet of sensilla also including one $S5_2$ sensillum (Fig. 8A). Two ecdysial pores could be found in the termination region on the ventral part of the MOP (Fig. 3C). The two sensory cells each display a type I dendrite that terminates below the base of the corresponding inflexible spine (Fig. 8A). The unbranched ODSs measure about 24 μm in length and about 0.25 μm in diameter, and reach into blind cuticular canals. Proximally the dendrites are enclosed in a dendrite sheath (Fig. 8B) that extends for about 3.2 μm and ends approximately 14.5 μm above the ciliary bases. Accumulations of about 20 microtubules are present in the ODSs. CDSs measure about 1.5 μm. The nine microtubule doublets are composed of an A-tubule, with an electron-dense core and two small dynein arms, and a B-tubule (Fig. 8C). Both dendrites have a strong ciliary rootlet reaching about 2.3 μm into the IDSs (Fig. 8D). A scolopale is present in the innermost enveloping cell and most prominent in the ciliary region (Fig. 8C).

3.2.6. *The type 6 sensillum ($S6_2$)*

This type of sensillum can be found in the same two positions on the processus molaris (MOP) of each mandible as the $S5_2$ sensilla (Figs. 2A, 3B and 3C). The pair of sensilla is composed of a $S5_2$ and a $S6_2$; the more dorsally located triplet includes two $S6_2$ sensilla. Two sensory cells each display a type II dendrite. These terminate in

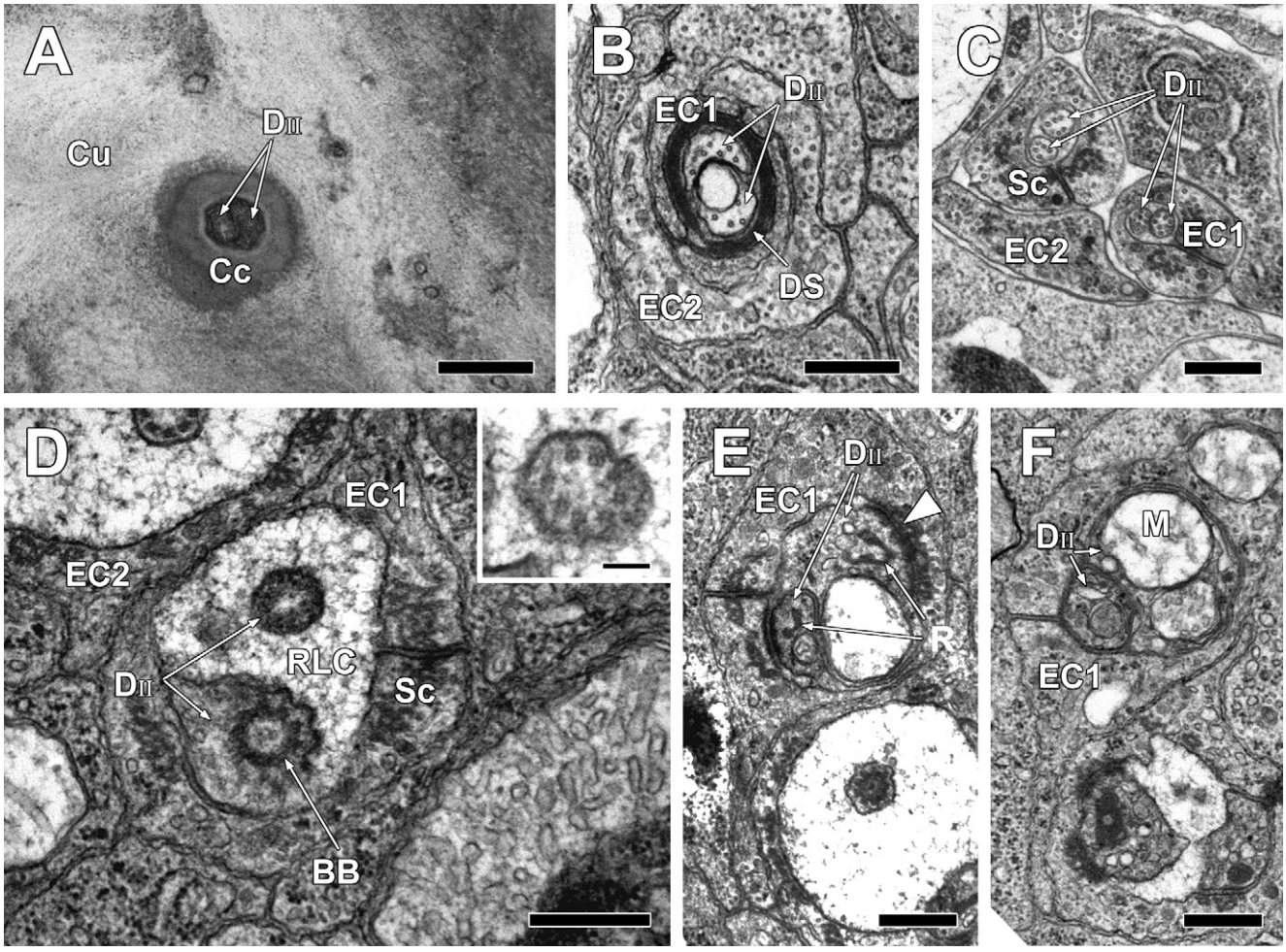

Fig. 9. The $S6_2$ sensillum; cross sections of mandible at different levels (bars = 0.5 μm). A: Termination region of two type II dendrites in cuticular canal near the spine base. B: ODSs proximal to spine base surrounded by dendrite sheath and two ECs. C: ODSs distal to transitional region. D: CDS of one dendrite, the other one already in transitional region; insert: CDS with 9 × 2 + 0 pattern of microtubules and A-tubules lacking arms (bar = 0.125 μm). E: Transitional region with ciliary rootlets of dendrites and desmosomal junctions (arrowheads) with inner enveloping cell. F: IDSs proximal to transitional region. BB, basal body; Cc, cuticular canal; Cu, cuticle; DII, type II dendrite; DS, dendrite sheath; EC1, 2, enveloping cell 1, 2; M, mitochondrium; R, rootlet; RLC, receptor lymph cavity; Sc, scolopale.

the same regions as with the $S5_2$ sensilla. The unbranched ODSs measure about 20 μm in length and about 0.2 μm in diameter; these also reach into blind cuticular canals (Fig. 9A). Proximally dendrites are enclosed in a dendrite sheath extending for about 3.2 μm (Fig. 9B). Only about 6–8 microtubules are present in the ODSs (Fig. 9C), and similarly to the $S2_2$ sensillum the dendrites sometimes show extreme dilatations of the dendrite membrane in the region between the CDSs and the part enclosed by the dendrite sheath. CDSs measure about 1.1 μm. The nine microtubule doublets lack dynein arms (Fig. 9D), and both dendrites have a delicate and branched ciliary rootlet reaching about 0.7 μm into the IDSs (Fig. 9E). As with the $S5_2$, a scolopale is present in the innermost enveloping cell.

3.2.7. The type 7 sensillum ($S7_1$)

We found this type of sensillum only on the right mandible, associated with an inflexible spine on the IP that has no counterpart on the contralateral mandible (Fig. 2A, B). The spine has a solid cusp and a lumen in the proximal part (Fig. 10B). No conspicuous pore could be detected. The sensillum is innervated by one sensory cell displaying a type II dendrite that is unbranched and reaches the proximal part of the spine (Fig. 10A). The ODS measures about 25 μm in length and 0.3 μm in diameter; about 10 microtubules are present (Fig. 10C). The distal part is surrounded by a dendrite sheath (Fig. 10B). The CDS is very short, though its exact dimensions could not be resolved (Fig. 10D). The ciliary rootlet is delicate and branched (Fig. 10E); again the exact dimensions could not be resolved. A scolopale is present in the innermost enveloping cell (Fig. 10D).

4. Discussion

The sensory units show ultrastructural features that allow us to distinguish 7 types of sensilla on the gnathal lobe of the mandibles of the studied specimens. Their differences are interpreted as reflecting the mechanisms of stimulation the respective sensory unit is adapted for. A summary of the character sets of the different types of sensilla is given in Table 1.

Combined morphological and physiological analyses have shown that structural features can be good indicators of the modality of sensory neurons innervating arthropod sensilla (e.g. Altner et al., 1983; Schmidt and Gnatzy, 1984; Altner et al., 1986; Cate and Derby, 2002). In some sensilla we did find combinations of structures that strongly suggest a specific modality, but others were lacking such unequivocal features. We did not perform physiological experiments and could not find corresponding references.

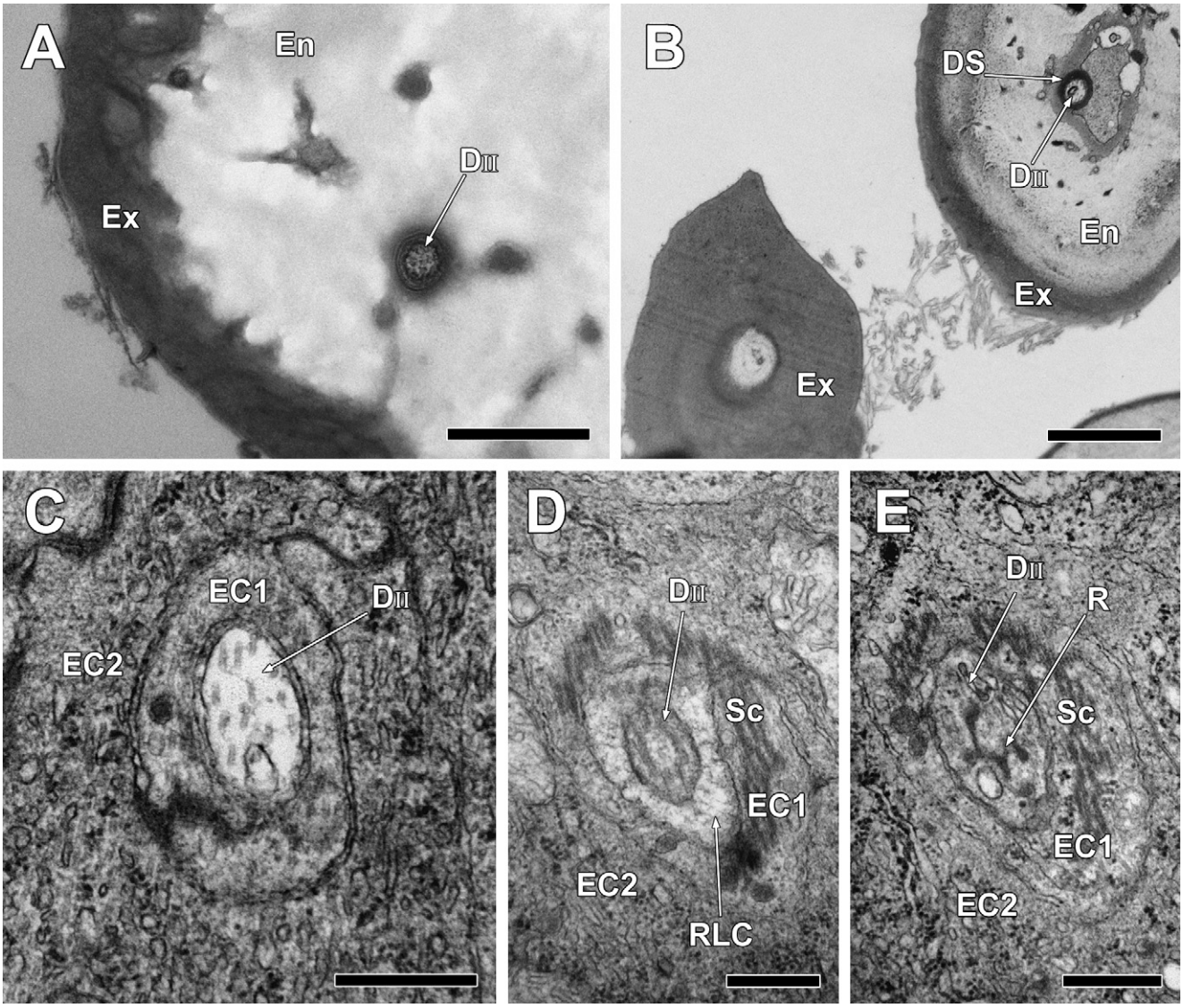

Fig. 10. The $S7_1$ sensillum; cross sections of mandible at different levels. A: Termination region of one type II dendrite in cuticular canal in distal part of spine (bar = 0.5 μm). B: ODS in proximal part of spine surrounded by dendrite sheath (bar = 2 μm). C: ODS distal to transitional region (bar = 0.5 μm). D: CDS in receptor lymph cavity distal to transitional region (bar = 0.5 μm). E: Transitional region with ciliary rootlet of the dendrite (bar = 0.5 μm). En, endocuticle; Ex, exocuticle; DII, type II dendrite; DS, dendrite sheath; EC1, 2, enveloping cell 1, 2; R, rootlet; RLC, receptor lymph cavity; Sc, scolopale.

Therefore, where the evidence is insufficient for definitive assignment of the respective sensillum, we can only discuss assumptions of its modality. Fig. 11 presents a schematic illustration of the specific features of each type of sensillum.

The sensory units we found all show the internal features typical of arthropod sensilla (McIver, 1975; Hallberg and Hansson, 1999).

$S1_2$ (the 'lacinia mobilis') exhibits the characteristics of a crustacean mechanoreceptive hair sensillum (Crouau, 1997, 2001). Features that have been correlated with mechano-sensitivity are the dense packing of microtubules in the outer dendrite segment, the presence of dense A-tubules with two arms (most probably 'dynein') in the ciliary segment, the distinct ciliary rootlet within the IDS, the scolopale in the innermost enveloping cell, and the tight connection of the IDS to this cell by desmosomal junctions (Schmidt and Gnatzy, 1984; Altner et al., 1986, 1983). Both dendrites show these features, representing the type I cell. Hence, the $S1_2$ sensillum seems to be a mechanoreceptor. Only one type of chemoreceptors is known to have type I cells, in the horseshoe crab *Limulus polyphemus* (Hayes, 1971).

The ultrastructural arrangement of the $S2_2$ exhibits features suggestive of a bimodal contact chemoreceptor. The innervation of the sensillum by one type I and one type II cell, and the particular ultrastructural features of the dendrites agree well with the descriptions of crustacean bimodal chemo- and mechanoreceptors (Altner et al., 1983; Schmidt and Gnatzy, 1984; Cate and Derby, 2002). On the other hand we found no signs of a single pore at the tip or the base of the setal spine or of any multiporous cuticular structure in the termination region of the dendrites; at least one of these features has been recorded from several studied crustacean contact chemoreceptors (Altner et al., 1983; Schmidt and Gnatzy, 1984; Schmidt, 1989; Cate and Derby, 2002; Garm and Høeg, 2006). However, the type II cell also shows features that could indicate a mechanosensory rather than a chemosensitive function, i.e. the ciliary rootlet and the desmosomal junction to the inner enveloping cell in this region. The presumed chemosensory neurons in crustacean bimodal sensilla usually do not show this connection (Altner et al., 1983; Schmidt and Gnatzy, 1984; Cate and Derby, 2002). Assuming that both neurons in the $S2_2$ sensillum are

Table 1
Morphological features of mandibular sensilla of the zoea-I larva in *Palaemon elegans*. CDS, ciliary dendrite segment; CR, ciliary rootlet; IP, incisor process; MN r & l, right and left mandible; MOP, molar process; MT, microtubule; ODS, outer dendrite segment.

	$S1_2$	$S2_2$	$S3_{2/3}$	$S4_2$	$S5_2$	$S6_2$	$S7_1$
Position	Dorsal of IP of MN r & l	IP of MN r & l	Ventral and posterior on IP of MN r & l	Ventral on IP of MN r & l	MOP of MN r & l	MOP of MN r & l	Dorsal of IP of MN r
External structure	Articulated seta	Inflexible spine	None	None	Inflexible spines	Inflexible spines	Inflexible spine
Pore	Basal pore	??	??	Yes	Yes	Yes	??
Sensory cells	2	2	3/2	2	2	2	1
Dendrite number	2	2	3/2	2	2	2	1
Dendrite type	I	I and II	III	IV	I	II	II
Dendrites equal/diff.	Equal	Different	Equal	Equal	Equal	Equal	–
ODS							
Termination	Hair base	Proximal part of spine	Ventral/posterior on IP	Ventral on IP	Spine base	Spine base	Proximal part of spine
Dimension	c. 16.4 μm	c. 30 μm	c. 20.5 μm	c. 6.5 μm	c. 24 μm	c. 20 μm	c. 25 μm
MT in apical region	c. 25	DI = 23; DII = 11	3–5	Few	c. 20	6–8	9–11
Branching yes/no	No	No	No	Yes	No	No	No
CDS							
Dimension (BB + cil. seg.)	c. 1.5 μm	DI = c. 4.3 μm; DII = c. 0.6 μm	c. 1.7 μm	c. 180 nm	c. 3.6 μm	c. 1.1 μm	Very short
MT pattern	9 × 2 + 0 with a rms	DI = 9 × 2 + 0 with arms; DII = 9 × 2 + 0 no arms	9 × 2 + 0 no arms	9 × 2 + 0 no arms	9 × 2 + 0 with arms	9 × 2 + 0 no arms	–
CR dimension	c. 2.5 μm	DI = c. 3.4 μm; DII = c. 3 μm	c. 1.1 μm	–	c. 2.3 μm	c. 700 nm	–
CR morpho	Compact strong	DI = compact strong; DII = branched	Compact round	No distinct rootlet	Compact strong	Branched	Branched
Enveloping cells							
Dendrite sheath	Yes	Yes	Yes	No	Yes	Yes	Yes
Scolopale	Yes	Yes	Yes	No	Yes	Yes	Yes
Putative physiolog. function	Mechanoreceptor	Contact-chemoreceptor	Mechanoreceptor	Unimodal chemoreceptor	Mechanoreceptor	Mechano- or chemoreceptor	Mechano- or chemoreceptor

mechanosensitive, the lower density of microtubules throughout the ODS of one dendrite could indicate a lower sensitivity to mechanical stress. Ciliary microtubules are thought to play a central role in signal transmission in crustacean mechanoreceptors (Crouau, 2001), and dense microtubules in the ODS are believed to enhance the sensitivity (Garm and Høeg, 2006). Furthermore, our findings resemble very much the description of the sensory cells of a chordotonal organ, which is thought to be strictly mechanosensitive, in the legs of *Carcinus maenas* (Whitear, 1962). Therefore, although it seems more likely that the $S2_2$ is a contact chemoreceptor, we cannot exclude the possibility that it is a mere mechanoreceptor and that the structural differences between the dendrites indicate different sensitivity to mechanical stress. Dilatations of the ODS similar to those in the type II cells have been described from the funnel-canal organs of *C. maenas* (Schmidt and Gnatzy, 1984) and as spindle-shaped swellings in the aesthetasc sensilla of *Panulirus argus* (Grünert and Ache, 1988) and *Panulirus interruptus* (Spencer and Linberg, 1986). While the functional significance of those dilatations remains unknown, the possibility that they are fixation artefacts has been ruled out (Frisch and Everingham, 1972).

In the $S3_{2/3}$ sensillum we could find both features that are correlated with mechanosensitivity, but also features that rather indicate chemosensitivity. The scolopale in the inner EC and the distinct ciliary rootlets are modality-specific features of crustacean mechanoreceptors (Altner et al., 1983; Schmidt and Gnatzy, 1984). While the shape and dimension of the ciliary rootlets clearly are different, the dimensions of the CDSs rather correspond to the previously described mechanoreceptors (see Table 1). However, further features indicating mechanosensitivity, such as densely packed microtubules in the ODS and dense A-tubules with arms in the CDS, are lacking. The absence of these features is reported for chemoreceptive cells (Altner et al., 1983; Grünert and Ache, 1988; Cate and Derby, 2002). With this combination of features, and in the absence of physiological data, the evidence on the modality of the $S3_{2/3}$ sensillum is insufficient. No description of any similar crustacean sensillum has been found; thus the type III dendrite might exhibit an unknown character until now.

Our results on the $S4_2$ sensillum suggest the presence of a sensillum located ventrally on the incisor process of both mandibles with features, such as the branched ODSs, mostly resembling those of olfactory sensilla (Spencer and Linberg, 1986; Grünert and Ache, 1988). But in Crustacea, olfactory sensilla, with a unimodal chemosensitivity, are considered to comprise only the antennal aesthetascs and male-specific sensilla (Hallberg et al., 1997). Also there are certain differences of the $S4_2$ sensillum from the aesthetasc structure, like the absence of an external cuticular structure and a ciliary rootlet, and the presence of only one basal body and one branched ODS per sensory cell. In our cases the apical ends of the dendrites stay in contact with the cuticle in some way, but no distinct pore or spongy area was found. This could be due to dirt particles obscuring the critical cuticle areas. However, there would have to be some kind of permeable structure allowing adequate molecules to pass the cuticular barrier and be detected by the sensory cells, but in crustacean this spongy structure can hardly be detected by SEM. Features that are indirectly correlated with chemosensitivity are the absence of a dendrite sheath, the absence of a scolopale, and the very short CDS (Grünert and Ache, 1988). Unlike Schmidt and Mellon (2011) we see 'olfaction' as term referring to function of sensilla and do not restrict its use to describe those sensilla that innervate the "olfactory lobe" of the brain. From our functional perspective we refer to the $S4_2$ sensillum as olfactory chemosensillum. And furthermore this unimodal chemosensillum is located uncharacteristically in Crustacea on the mandible and precisely not on the antennule.

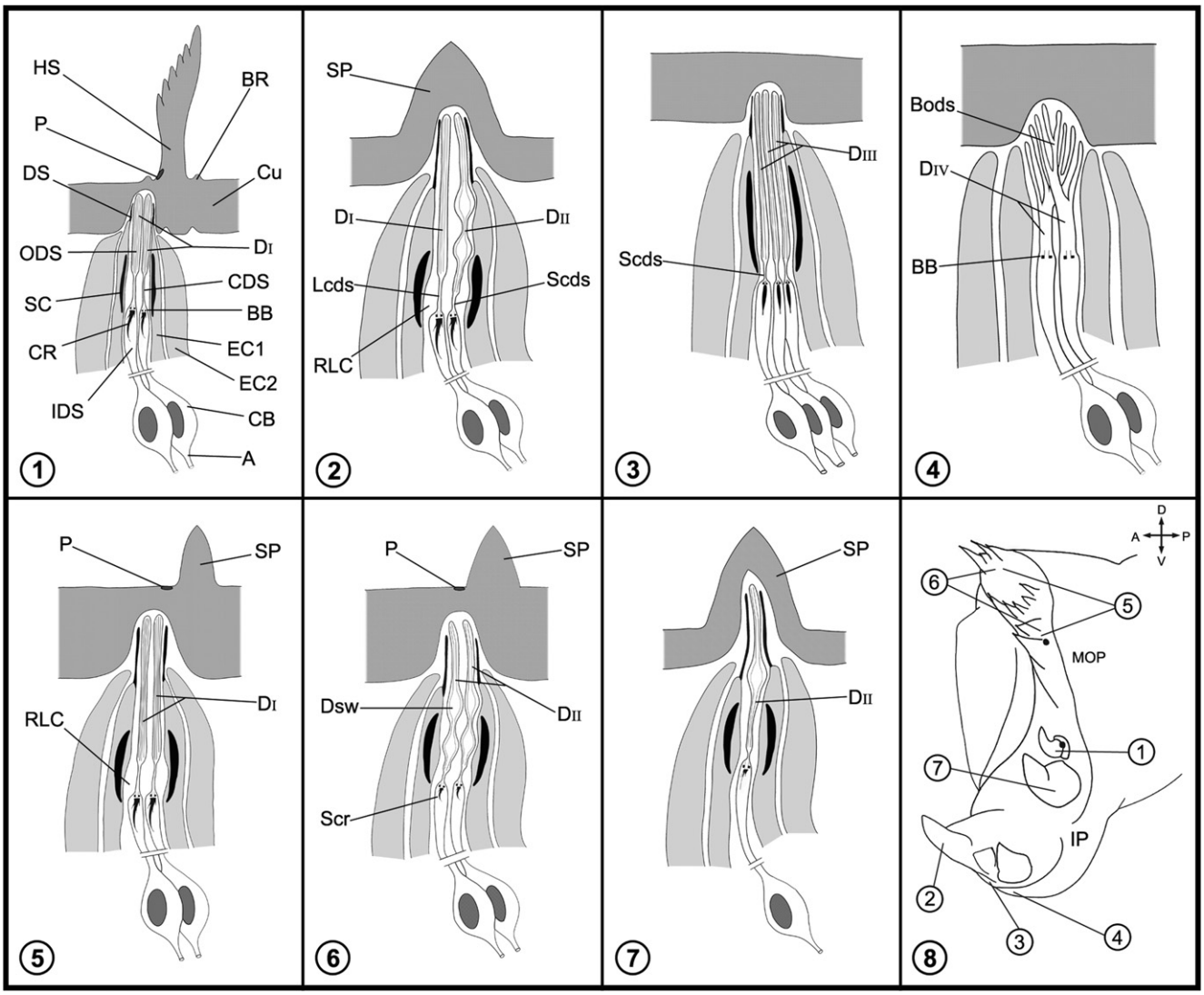

Fig. 11. Schematic drawings showing the 7 different types of sensilla. 1: $S1_2$, the 'lacinia mobilis' (depicted here like on the left mandible), a mechanoreceptive hair-sensillum. 2: $S2_2$, putative contact-chemo-receptor. 3: $S3_{2/3}$, mechanosensitive sensillum with possible proprioceptive function, without external structure. 4: $S4_2$, unimodal chemoreceptor without external structure. 5: $S5_2$, mechanoreceptor with inflexible spine. 6: $S6_2$, chemoreceptor or mechanoreceptor with inflexible spine. 7: $S7_1$, chemoreceptor or mechanoreceptor with inflexible spine. 8: diagram showing external morphology and arrangement of sensillar types 1–7 on right mandible (Specimen orientation: A, anterior; D, dorsal; P, posterior; V, ventral). A, Axon; BB, basal body; Bods, branched outer dendrite segment; BR, basal ring; CB, cell body; CDS, ciliary dendrite segment; CR, ciliary rootlet; Cu, cuticle; DI–DIV, dendrites of types I–IV; DS, dendrite sheath; Dsw, dendrite swelling; EC1, inner enveloping cell; EC2, second enveloping cell; HS, hair shaft; IDS, inner dendrite segment; IP, incisor process; Lcds, long ciliary segment; MOP, molar process; ODS, outer dendrite segment; P, pore; RLC, receptor lymph cavity; SC, scolopale; Scds, short ciliary segment; Scr, short ciliary rootlet; SP, spine.

Both dendrites innervating the $S5_2$ sensillum are type I dendrites showing the typical features that are modality-specific for mechanosensitivity. Therefore, the $S5_2$ seems to be a mechanoreceptor. In contrast to the $S1_2$ sensillum the cuticular structure that seems to be part of the sensillar unit here is a non-articulated, small and robust spine (see Table 1). The pore we found near the base of the spine is an ecdysial pore that plays a role during moulting (Kouyama and Shimozawa, 1984).

The accompanying structures of the $S6_2$ sensillum correspond approximately to the ones discussed for the $S5_2$ sensillum, but in contrast the two dendrites are both type II dendrites (see Table 1). Concerning the functional properties of this sensillum the same dilemma occurs as in the $S2_2$. The $S6_2$ could either be a mechanoreceptor sensitive to a different stimulus intensity like the $S5_2$, or the former is strictly chemosensitive and a bimodality results from the combination of the $S5_2$ and $S6_2$ sensilla.

The structure of the $S7_1$ sensillum presents a new aspect in the discussion of the functional properties of the type II dendrites. The single dendrite innervating the $S7_1$ shows features classifying it as a type II dendrite. If the modality of all type II dendrites examined in this study is chemosensitive as suggested by Schmidt and Gnatzy (1984) for similar dendrites in funnel-canal organs of the shore crab, *C. maenas*, then the presence of a scolopale is of interest. The scolopale is thought to be associated with mechanosensitivity (Schmidt and Gnatzy, 1984; Altner et al., 1986, 1983); therefore its presence in this dendrite either contradicts the other features or a scolopale can be similarly correlated with both, mechano- and chemosensitivity. Therefore, our argument that a type II dendrite could also be mechanosensitive could hold true for the $S7_1$ sensillum as well. All features correlated with mechanosensitivity can be found, except for a dense packing of microtubules in the ODS. According to the literature the relatively short CDS is the main

feature indicating chemosensitivity (Schmidt and Gnatzy, 1984; Altner et al., 1986). However, we could not detect any pore or porous structure in the cuticule at the tip or base of the spine in this case either. But as already mentioned above, this is no argument against a possible chemosensitivity. Hence, the respective functional significance of the $S7_1$ sensillum being innervated by one type II dendrite, of all other sensilla having type II dendrites, and of the $S3_{2/3}$ sensillum with its type III dendrites is not obvious at this point.

The different number of sensilla on the left and the right mandible, hence a cellular difference, is connected to an also external dissimilarity between the left and the right mandible known in decapod larvae (Ingle, 1992). The submarginal spine, that is associated with the S7-sensillum on the right mandible, is not present on the left mandible.

In summary, we could show that the zoea-I mandibles studied here are equipped with a relatively high number of diverse sensory structures. The latter include not only classical movable setae with a basal ring ($S1_2$), but also unarticulated spines ($S2_2$, $S5_2$, $S6_2$, $S7_1$) and other cuticular structures ($S3_{2/3}$, $S4_2$). In earlier studies these appendages have been seen as massive cuticular ornamentations functioning as teeth without any sensory capacities (Ingle, 1992). We show here that they are in fact connected to sensory units. Since the mandibles are the main masticating organs of the larvae (Factor, 1989), the obvious interpretation is that their equipment with sensilla allows the animal to monitor food quality and the mechanical forces occurring during the masticating process. The adequate stimuli for the mechanoreceptors most likely are the deflection of the hair ($S1_2$) and pressure or tension on the surface and the resulting deformation of the cuticle. The $S3_{2/3}$ sensillum seems a good candidate for proprioceptor-like function. The presumed chemoreceptors in the $S2_2$, $S6_2$ and $S7_1$ sensilla might detect direct chemical stimuli of food components, and the $S4_4$ sensillum might detect odour molecules from a distance.

It can be assumed that the main function of the mandibles leads to their robustness, and brings about uncommon cuticular outer structures of sensilla that are hard to detect by SEM. There is a multitude of cuticular projections in Crustacea that are referred to as setae under various definitions of the latter term. The presence of an articulated basis with a basal ring is generally implied (Watling, 1989). Garm (2004) added some defining internal characteristics – a continuous lumen and semicircular sheath cells – and proposed innervation and sensory function as additional important elements of all setae. However, in our case of the $S1_2$ sensillum in *P. elegans* the connected seta does not show a lumen, whereas on the other hand we found cuticular structures associated with sensory structures ($S2_2$, $S5_2$, $S6_2$, $S7_1$) that would not be classified as setae when observed externally because of their extreme modification. This 'robust' version of cuticular structures of sensilla further increases the already multifaceted wealth of seta-derived sensilla known from arthropods.

To date there is no comprehensive and well documented concept of the sensory capacity of crustacean mandibles. For certain appendages, e.g. the lacinia mobilis, homology and function are still being discussed. In our studies, what was presumed initially due to external examination (Geiselbrecht and Melzer, 2010) now could be proven by analysis of the ultrastructure: The 'lacinia mobilis' on the larval mandible in *P. elegans* is a mechanosensitive sensillum. Though, it is impossible to decide with certainty whether a seta or cuticular structure is a primary sensillum that always has been connected to sensory cells or the connection to a sensillar cell cluster happened secondarily. It also remains uncertain whether the 'true' lacinia mobilis in Peracarida is an articulated mandibular structure with exclusively mechanical function, or whether it too has sensory capability. To answer this question should be the next step, and the combined results of the present and such future studies should elucidate the derivation of malacostracan mandibular appendages such as the lacinia mobilis.

In conclusion, the present findings contribute to our knowledge of the functional morphology of zoeal mouthparts, and provide a complex set of characters, including external and internal ultrastructural features, of decapod larval mandibles. They can help along the discussion of the homology of the 'lacinia mobilis' and other mandibular characters with respect to decapod phylogeny. Using the TEM, a much more detailed comparison of mandibular structure in different species will be possible than with the SEM alone.

Acknowledgements

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References

Altner, H., Hatt, H., Altner, I., 1986. Structural and functional properties of the mechanoreceptors and chemoreceptors in the anterior oesophageal sensilla of the crayfish, *Astacus astacus*. Cell and Tissue Research 244, 537–547.

Altner, I., Hatt, H., Altner, H., 1983. Structural properties of bimodal chemo- and mechanosensitive setae in the pereiopod chelae of crayfish, *Austropotamobius torrentium*. Cell and Tissue Research 228, 357–374.

Ball, E.E., Cowan, A.N., 1977. Ultrastructure of the antennal sensilla of *Acetes* (Crustacea, Decapoda, Natantia, Sergestidae). Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 277, 429–457.

Cate, H.S., Derby, C.D., 2002. Ultrastructure and physiology of the hooded sensillum, a bimodal chemo-mechanosensillum of lobsters. The Journal of Comparative Neurology 442, 293–307.

Chauvin, G., Faucheux, M., 1981. Les pieces buccales et leurs recepteurs sensoriels chez l'imago de *Micropterix calthella* L. (Lepidoptera: Micropterigidae). International Journal of Insect Morphology and Embryology 10, 425–439.

Crouau, Y., 1997. Comparison of crustacean and insect mechanoreceptive setae. International Journal of Insect Morphology & Embryology 26, 181–190.

Crouau, Y., 2001. Mechanosensitive cells of hexapods, crustaceans and myriapod setae: a comparison under phylogenetic aspects. Annales de la Societé Entomologique de France 37, 233–242.

Davoodi, P., Rahimian, H., Agh, N., 2009. SEM study of the early development of the mandible of *Artemia urmiana* and *A. parthenogenetica* (Branchiopoda, Anostraca). Crustaceana 82, 257–266.

Factor, J.R., 1978. Morphology of the mouthparts of larval lobsters, *Homarus americanus* (Decapoda: Nephropidae), with special emphasis on their setae. The Biological Bulletin 154, 383–408.

Factor, J.R., 1989. Development of the feeding apparatus in decapod crustaceans. In: Felgenhauer, B.E. (Ed.), Functional Morphology of Feeding and Grooming in Crustaceans. Crustacean Issues 6. A. A. Balkema, Rotterdam, pp. 173–184.

Faucheux, M.J., 1995. Sensilla on the larval antennae and mouthparts of the European sunflower moth, *Homoeosoma nebulella* Den. and Schiff. (Lepidoptera: Pyralidae). International Journal of Insect Morphology and Embryology 24, 391–403.

Friedman, M.M., Strickler, J.R., 1975. Chemoreceptors and feeding in calanoid copepods (Arthropoda: Crustacea). Proceedings of the National Academy of Sciences 72, 4185–4188.

Frisch, D., Everingham, J.W., 1972. Fine structure of crab olfactory cilia: non-chemical fixation; environmental effects. In: Schneider, D. (Ed.), Olfaction and Taste IV. Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp. 5–12.

Garm, A., 2004a. Mechanical functions of setae from the mouth apparatus of seven species of decapod crustaceans. Journal of Morphology 260, 85–100.

Garm, A., 2004b. Revising the definition of the crustacean seta and setal classification systems based on examinations of the mouthpart setae of seven species of decapods. Zoological Journal of the Linnean Society 142, 233–252.

Garm, A., Derby, C.D., Høeg, J.T., 2004. Mechanosensory neurons with bend- and osmo-sensitivity in mouthpart setae from the spiny lobster *Panulirus argus*. The Biological Bulletin 207, 195–208.

Garm, A., Høeg, J.T., 2006. Ultrastructure and functional organization of mouthpart sensory setae of the spiny lobster *Panulirus argus*: new features of putative mechanoreceptors. Journal of Morphology 267, 464–476.

Geiselbrecht, H., Melzer, R.R., 2010. Mandibles of zoea-I larvae of nine decapod species. A scanning EM analysis. Spixiana 33, 27–47.

Grünert, U., Ache, B.W., 1988. Ultrastructure of the aesthetasc (olfactory) sensilla of the spiny lobster, *Panulirus argus*. Cell and Tissue Research 251, 95–103.

Hallberg, E., Hansson, B.S., 1999. Arthropod sensilla: morphology and phylogenetic considerations. Microscopy Research and Technique 47, 428–439.

Hallberg, E., Johansson, K.U.I., Wallén, R., 1997. Olfactory sensilla in crustaceans: morphology, sexual dimorphism, and distribution patterns. International Journal of Insect Morphology and Embryology 26, 173–180.

Hallberg, E., Skog, M., 2011. Chemosensory sensilla in crustaceans. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, New York, pp. 103–121.

Hayes, W.F., 1971. Fine structure of the chemoreceptor sensillum in *Limulus*. Journal of Morphology 133, 205–239.

Honomichl, K., 1982. Fine structure of a mandibular stretch receptor in *Thrips physapus* L. (Thysanoptera, Insecta). Zoomorphology 101, 177–186.

Honomichl, K., 2008. Muscle receptors at the mandible of insects. Entomologia Generalis 31, 173–191.

Hunter, W.B., Ullman, D.E., 1992. Anatomy and ultrastructure of the piercing-sucking mouthparts and paraglossal sensilla of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). International Journal of Insect Morphology and Embryology 21, 17–35.

Ingle, R., 1992. Larval Stages of Northeastern Atlantic Crabs. An Illustrated Key, first ed.. London.

Kouyama, N., Shimozawa, T., 1984. The ecdysis of hair mechanoreceptors in crayfish. Cell and Tissue Research 236, 339–343.

Lavalli, K.L., Factor, J.R., 1992. Functional morphology of the mouthparts of juvenile lobsters, *Homarus americanus* (Decapoda: Nephropidae), and comparison with the larval stages. Journal of Crustacean Biology 12, 467–510.

McIver, S.B., 1975. Structure of cuticular mechanoreceptors of arthropods. Annual Review of Entomology 20, 381–397.

Ong, J.E., 1969. The fine structure of the mandibular sensory receptors in the brackish water calanoid copepod *Gladioferens pectinatus* (Brady). Cell and Tissue Research 97, 178–195.

Richardson, K.C., Jarett, L., Finke, E.H., 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Biotechnic & Histochemistry 35, 313–323.

Richter, S., Edgecombe, G.D., Wilson, G.D.F., 2002. The lacinia mobilis and similar structures – a valuable character in arthropod phylogenetics. Zoologischer Anzeiger 241, 339–361.

Schmidt, M., 1989. The hair-peg organs of the shore crab, *Carcinus maenas* (Crustacea, Decapoda): ultrastructure and functional properties of sensilla sensitive to changes in seawater concentration. Cell and Tissue Research 257, 609–621.

Schmidt, M., 1990. Ultrastructure of a possible new type of crustacean cuticular strain receptor in *Carcinus maenas* (Crustacea, Decapoda). Journal of Morphology 204, 335–344.

Schmidt, M., Gnatzy, W., 1984. Are the funnel-canal organs the 'campaniform sensilla' of the shore crab, *Carcinus maenas* (Decapoda, Crustacea)? II. Ultrastructure. Cell and Tissue Research 237, 81–93.

Schmidt, M., Mellon, D.F., 2011. Neuronal processing of chemical information in crustaceans. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, New York, pp. 123–147.

Sinitsina, E.E., Chaika, S.Y., Ochirov, I.V., 2003. Sensory organs in larvae of Symphyta (Hymnoptera, Symphyta) species. Zoologichesky Zhurnal 82, 1201–1210.

Spencer, M., Linberg, K.A., 1986. Ultrastructure of aesthetasc innervation and external morphology of the lateral antennule setae of the spiny lobster *Panulirus interruptus* (Randall). Cell and Tissue Research 245, 69–80.

Sutcliffe, J.F., McIver, S.B., 1982. Innervation and structure of mouth part sensilla in females of the black fly *Simulium venustum* (Diptera: Simuliidae). Journal of Morphology 171, 245–258.

Tyson, G.E., Sullivan, M.L., 1979. Antennular sensilla of the brine shrimp *Artemia salina*. The Biological Bulletin 156, 382–392.

Tyson, G.E., Sullivan, M.L., 1981. A scanning electron microscopic study of the molar surfaces of the mandibles of the brine shrimp (Cl. Branchiopoda: O. Anostraca). Journal of Morphology 170, 239–251.

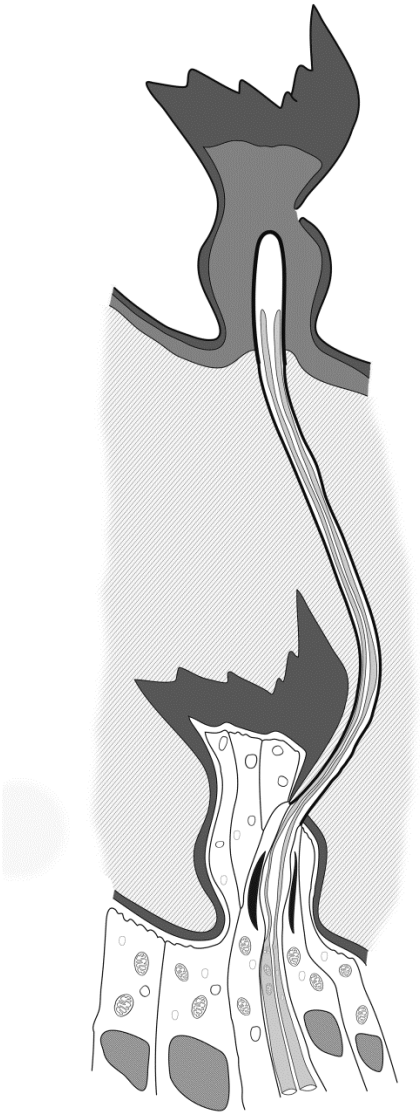
Watling, L., 1989. A classification system for crustacean setae based on the homology concept. Crustacean Issues 6, 15–26.

Whitear, M., 1962. The fine structure of crustacean proprioceptors. The chordotonal organs in the legs of the shore crab, *Carcinus maenas*. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 245, 291–324.

Whitehead, A.T., Larsen, J.R., 1976. Ultrastructure of the contact chemoreceptors of *Apis mellifera* L. (Hymenoptera: Apidae). International Journal of Insect Morphology and Embryology 5, 301–315.

7. Paper IV

Geiselbrecht, H., Melzer, R.R., 2014. Fine structure and ecdysis of mandibular sensilla associated with the lacinia mobilis in *Neomysis integer* (Leach, 1814) (CRUSTACEA, MALACOSTRACA, PERACARIDA). Arthropod Structure & Development (published online 7 February 2014).



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Highlights

- Mandibles of adult *Neomysis integer* were studied using light and electron microscopy.
- We describe the external and internal structure with special reference to the lacinia mobilis and characteristics of the ecdysis.
- Our analysis showed the presence of sensory cells revealing that both laciniae mobiles are mechanosensory organs.
- We discuss the classification of the lacinia mobilis as a sensillar appendage and a possible homology in Peracarida and Decapoda.

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Fine structure and ecdysis of mandibular sensilla associated with the lacinia mobilis in *Neomysis integer* (Leach, 1814) (Crustacea, Malacostraca, Peracarida)

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ABSTRACT

The external and internal structures of adult *Neomysis integer* mandibles were studied using light and electron microscopy with special reference to the lacinia mobilis, a highly specialized appendage on the gnathal edge of many crustaceans. The right and left lacinia mobilis were equipped with ciliary primary sensory cells revealing that both laciniae are also mechanosensory organs in addition to their mechanical function during mastication. A detailed character analyses indicated that the right lacinia was probably a highly derived sensory seta, whereas two alternative interpretations were considered for the left lacinia; it could be a sensillar appendage equipped with two mechanosensory units, or it could be a movable appendage of the incisor process containing two sensilla deprived of external appendages. The ecdysis of the lacinia mobilis corresponded very well to type I sensillar ecdysis, suggesting classification as a sensillar appendage. These features support a possible homology of the right lacinia mobilis in Peracarida and Decapoda, tracing them to an origin as a member of the setal row. Whether the left lacinia mobilis is a sensillum or an appendage with sensilla cannot be resolved presently.

1. Introduction

The lacinia mobilis is a distinctive structure among various appendages arming the gnathal edges of mandibles in Peracarida. The lacinia mobilis on the left mandible is described as an extremely developed, jointed structure resembling the toothed shape of the adjacent incisor process. The lacinia on the right mandible seems to be smaller and variously shaped but is also articulated (De Jong-Moreau et al., 2001; Mayer et al., 2013). Similar seta-like structures have been described in other eumalacostracan taxa such as Euphausiacea and Decapoda, but only in larvae (i.a. Weigmann-Haass, 1977; Maas and Waloszek, 2001; Yang, 2005; Dupré et al., 2008). Hypotheses about the origins of these structures, including assumptions about their possible homology, are still not discussed (Dahl and Hessler, 1982; Richter et al., 2002; Geiselbrecht and Melzer, 2010). A wealth of studies has analyzed the external features of the lacinia mobilis, but a transmission electron microscopic (TEM) analysis of the internal features has not been conducted. Richter et al. (2002) presumed that the incisor process, the setal row and the lacinia mobilis, are simple cuticular outgrowths and indicated that detailed histological or electron microscopic comparisons would have questionable value. However, they considered a different origin for the left and the right lacinia mobilis. This is of peculiar interest as mandibles of decapod zoea have a large number of sensory structures as detected by Geiselbrecht and Melzer (2013) and the gnathal edge appendage, also referred to as "lacinia mobilis", has been shown to be among these sensory structures in these larvae. Given the unresolved question of homology, an electron microscopic analysis of the ultrastructure of a peracarid lacinia mobilis should resolve some of these issues. Therefore, we studied the lacinia mobilis of *Neomysis integer* with regard to external morphology and ultrastructural features. We also studied histological changes inside the mandible involved in initiating the molting process.

2. Materials and methods

2.1. Animals

Living adult specimens of *N. integer* (Leach, 1814) were purchased from a commercial supplier (Aquarium-Center Wildenauer,

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Munich, Germany). The animals were collected in the area around the Elbe estuary, North Sea, Germany. Specimens were identified according to Makings (1977). Mandibles of specimens in the intermolt stage and mandibles of specimens showing signs of imminent ecdysis were studied. The molting process can be assigned to stage D_0 and D_1, indicating early or late apolysis, or D_3 and D_4, which are distinguishable by visible secretion of the new exocuticle molting stages after Drach and Tchernigovtzeff (1967). This classification was carried out individually in each studied specimen corresponding to these criteria because the animals had not been staged previously.

2.2. Light microscopy (LM) and TEM

After dissecting the antennae and whole posterior body following the maxillary segments, the specimens were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer at 4 °C, post-osmicated in 1% OsO_4 in buffer, and embedded in epoxy resin (Glycidether 100). A series of semi- and ultra-thin sagittal sections were cut alternately in the area covering the gnathal edges. Semi-thin sections of 1.5 μm were made with a diamond knife on a RMC MT-XL ultramicrotome. The sections were stained with Richardson's stain (1960), embedded in DPX, covered with cover glass, and photographed with a digital camera mounted on a Leica stereomicroscope. Up to five pictures with different focal depth were combined into a single respective image with a greater field of depth using Syncroscopy Auto Montage software. Ultra-thin sections of 60–70 nm thickness were made with a diamond knife on an RMC MT-XL ultramicrotome. The sections were double-stained with 4% uranyl acetate and 0.7% lead citrate and inspected using an FEI Morgagni transmission electron microscope at 80 kV (LM and TEM: right mandible: $n = 4$; left mandible: $n = 2$; plus LM only: left mandible: $n = 1$).

2.3. Scanning electron microscopy (SEM)

The dissected mandibles were dehydrated in a graded acetone series (70, 80, and 90% for 10 min each, plus three times in 100% for 20 min each), then critical point-dried in a Baltec CPD 030. The dried specimens were mounted on SEM stubs with self-adhesive carbon stickers and sputtered with gold on a Polaron E 5100. The mandibles were studied with a LEO 1430VP SEM at 15 kV (right mandible: $n = 4$; left mandible: $n = 5$).

2.4. Terminology

Cuticular processes on the mandibles were named according to definitions given in Watling (1989) and Garm (2004). This applied to use of the term "setal row" instead of the formerly used "spine row" (see also Mayer et al., 2013). In a recent ultrastructural study, we showed that the "lacinia mobilis", a structure with a distinct movable socket and basal pore, is innervated (consistently termed "seta") in larvae of the decapod *Palaemon elegans* (Geiselbrecht and Melzer, 2013). However, innervation was also detected in stout processes lacking a movable socket. According to Watling's and Garm's classical morphological definition, these processes would be named "spines". Considering the new findings, updated terminology is used here, referring to such structures as "sensory spines".

3. Results

3.1. External structure of the mandibles

The mandibles of *N. integer* are composed of two distinct main portions, the mandibular palp and a medially extending coxal endite forming the gnathal edge (Fig. 1). The gnathal edge is armed with different processes and appendages (Figs. 2A and 4A). The molar process is dorsally situated closest to the mouth. It is a flattened oval structure densely covered with small setae. The margin of the gnathal edge becomes narrower ventrally and a series of setae form the "setal row". The lacinia mobilis is located between the "setal row" and the incisor process, a ventral marginal protrusion armed with a row of four acute sensory spines (Fig. 2A and C). The completely different shape of the left and the right lacinia mobilis is a key feature distinguishing the left and right mandibles.

3.2. Fine structure of the right and left lacinia mobilis

The lacinia mobilis on the right mandible is an articulated cuticular structure with a short shaft on a broader basis but becomes comb-shaped distally owing to two rows of small spine-like extensions (Fig. 2B). A pore is located proximally on the shaft. The right lacinia mobilis appears solid and strongly sclerotized when viewed in cross section in the shaft region (Fig. 2C and D). The cuticle is composed of three layers, and the endocuticle shows areas with aligned filaments and areas with more diffuse filaments, resulting in sponge-like tissue. No cellular processes reach into the shaft except a sensillar cell cluster. The unbranched outer dendritic segments (ODSs) of two ciliary dendrites terminate proximally in the central region of the lacinia shaft (Fig. 2D) and are enclosed in a cuticular canal (Fig. 3B). The surrounding tissue becomes cellular below the lacinia base and the ODSs are enclosed in a dendrite sheath, consisting of homogenous, electron-dense material (Fig. 3C) which is closely surrounded by two enveloping cells. The ODSs measure about 55 μm in length and about 0.25 μm in diameter. Numerous microtubules run longitudinally along the ODSs. The microtubules are arranged in nine peripheral doublets in the ciliary dendritic segment (CDS), which are composed of an A-tubule with small dynein arms and a B-tubule (Fig. 3F). The typical $9 \times 2 + 0$ pattern is displayed with the absence of central tubules. The receptor lymph cavity (RLC) is wider in this region and filled with electron-dense material. A scolopale is present in the innermost enveloping cell (Fig. 3D and F). The scolopale is composed of longitudinally oriented microtubules embedded in actin filament bundles and extends between the transitional region and the proximal end of the dendrite sheath. Each ODS arises from one inner dendritic segment (IDS) connected by the CDS. An accumulation of mitochondria is found in the apical region of the IDS (Fig. 3G).

The lacinia mobilis on the left mandible is also a movable, articulated cuticular structure (Fig. 4A and B). It is clearly bigger than the right one because of its massive, claw-like shape almost reaching the size of the incisor process. The left lacinia bears four strong and acute, spine-like extensions (Fig. 4C). It also appears strongly sclerotized when viewed in cross section (Fig. 4D). However, in the proximal region of the lacinia (Fig. 4D), the endocuticle seems to have a demarcation inwards, leaving a central lumen. Cellular processes reach proximally into the lumen, but perikarya are located below the lacinia base (Fig. 4E). No conspicuous pore was found on external examination. However, ultrastructural analyses showed that two sensillar cell clusters are associated with the left lacinia mobilis.

Cluster 1 shows two unbranched ciliary dendrites terminating proximally in the central region of the lacinia enclosed in a cuticular canal (Figs. 4D and 5A). The ODSs measure about 69 μm in length and about 0.3 μm in diameter. Longitudinally extending microtubules are present in the ODSs, and a dendrite sheath encloses the distal region (Fig. 5A and B). The short CDSs stretch over about 5.2 μm, and the microtubules are arranged in the $9 \times 2 + 0$ pattern composed of A-tubules with small dynein arms and B-tubules. The

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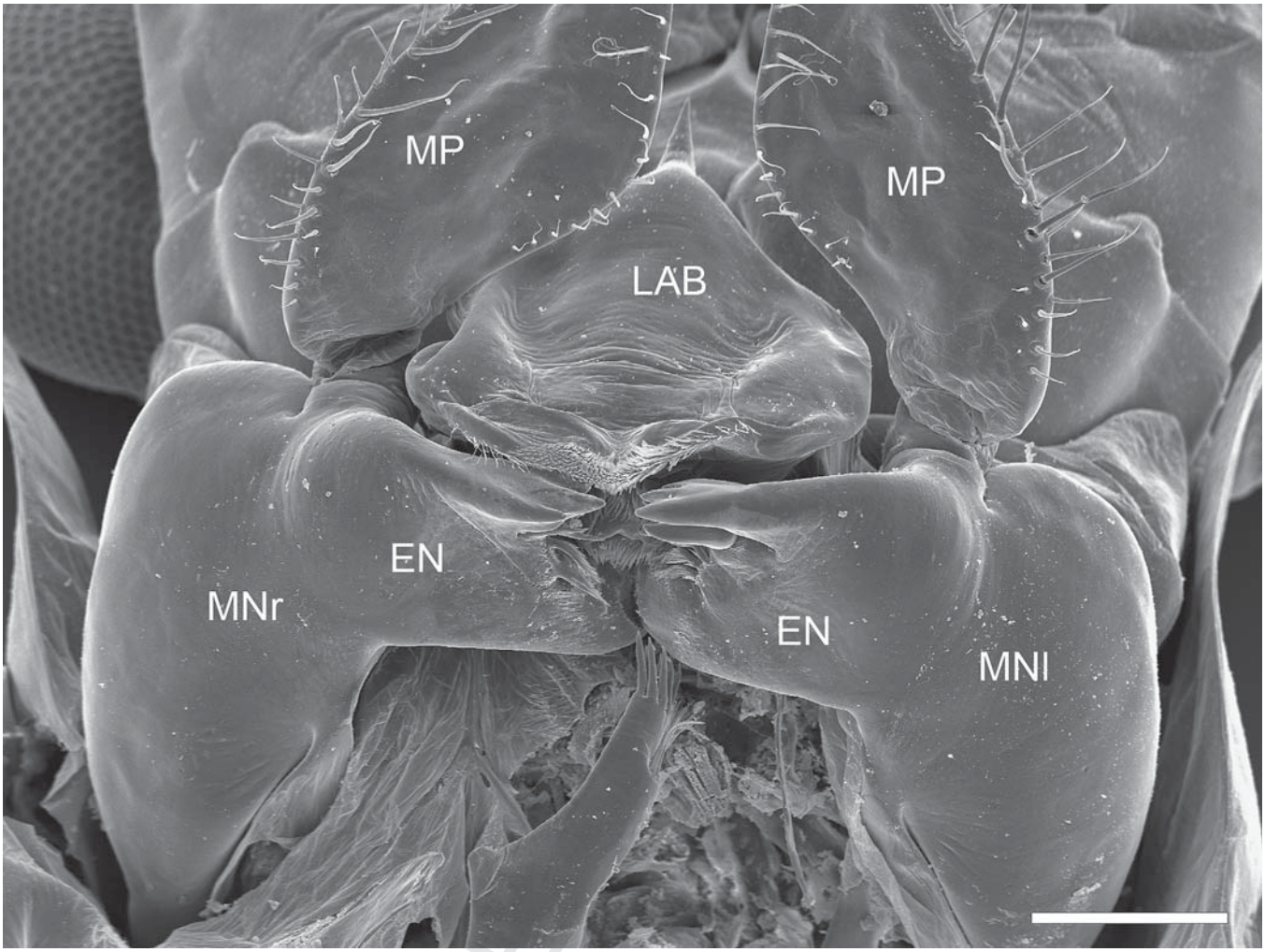

Fig. 1. Mouthpart region of *Neomysis integer*. Scanning electron micrograph showing ventral view of the mandibles from a dissected specimen (scale, 200 μm). Abbreviations: EN, coxal endite; LAB, labrum; MNl, left mandible; MNr, right mandible; MP, mandibular palp.

RLC is wider in the region of the CDSs and filled with electron-dense material. A prominent scolopale is present in the innermost enveloping cell (Fig. 5G). Each ODS arises from one IDS where an accumulation of mitochondria are found in the apical region (Fig. 5H).

Cluster 2 shows three unbranched ciliary dendrites terminating proximally near the dendrites of cluster 1. The ODSs measure about 53 μm in length and about 1.5 μm in diameter. Longitudinally extending microtubules in the ODSs and an enclosing dendrite sheath in the distal region are present (Fig. 5B). The short CDSs extend only about 2.5 μm but also show the $9 \times 2 + 0$ microtubule pattern with armed A-tubules. The inner enveloping cell encloses a wide RLC filled with electron-dense material and shows a prominent scolopale (Fig. 5E and F).

3.3. Ecdysis

During the intermolt phase the epidermal tissue of the mandible directly touches the endocuticle inwards. The region proximally to the bases of the mandibular processes is completely filled with cell bodies of the epidermis and sheath cells when viewed in cross section (Fig. 6A). During molting (stage D_0–D_1, sensu (Drach and Tchernigovtzeff, 1967)) the cellular tissue is initially withdrawn from the old cuticle, an extensive exuvial space expands, and is filled with exuvial fluid (Fig. 6B). The condensed cellular areas, not yet clearly demarcated, are distinguished and correlated with the respective external structures. In the later phase (stage D_3–D_4) a new cuticle is secreted by the epidermal and sheath cells, and the future lacinia mobilis, incisor process, and members of the setal row lie underneath the old external structures already assuming their definitive shape (Fig. 6C–E). No back-folded structures of the new cuticle were recognized. ODSs penetrating the new cuticle of the future left and right lacinia mobilis are detectable on their ascending path in specimens that document this phase (Fig. 6H). The ODSs stay connected with the old cuticular structures running through the exuvial space within the dendrite sheaths (Figs. 6E, G and 7). However, it seems that the ODSs are slightly retracted because they do not reach the distal region of the cuticular canal, at least in the right lacinia (Figs. 6F and 7). These cuticular structures in the proximal shaft region of the right lacinia look the same during the molting phase. However, the lumen of the left lacinia seems to be entirely filled with exuvial fluid. Members of the "setal row" also show associated sensory cell clusters that can be detected penetrating the new cuticle (Fig. 6D, insert), in which two uniform ODSs filled with longitudinally oriented microtubules become visible and can be viewed in detail. The ODSs also seem to be ascending through the exuvial space enclosed by a dendrite sheath (Fig. 6D).

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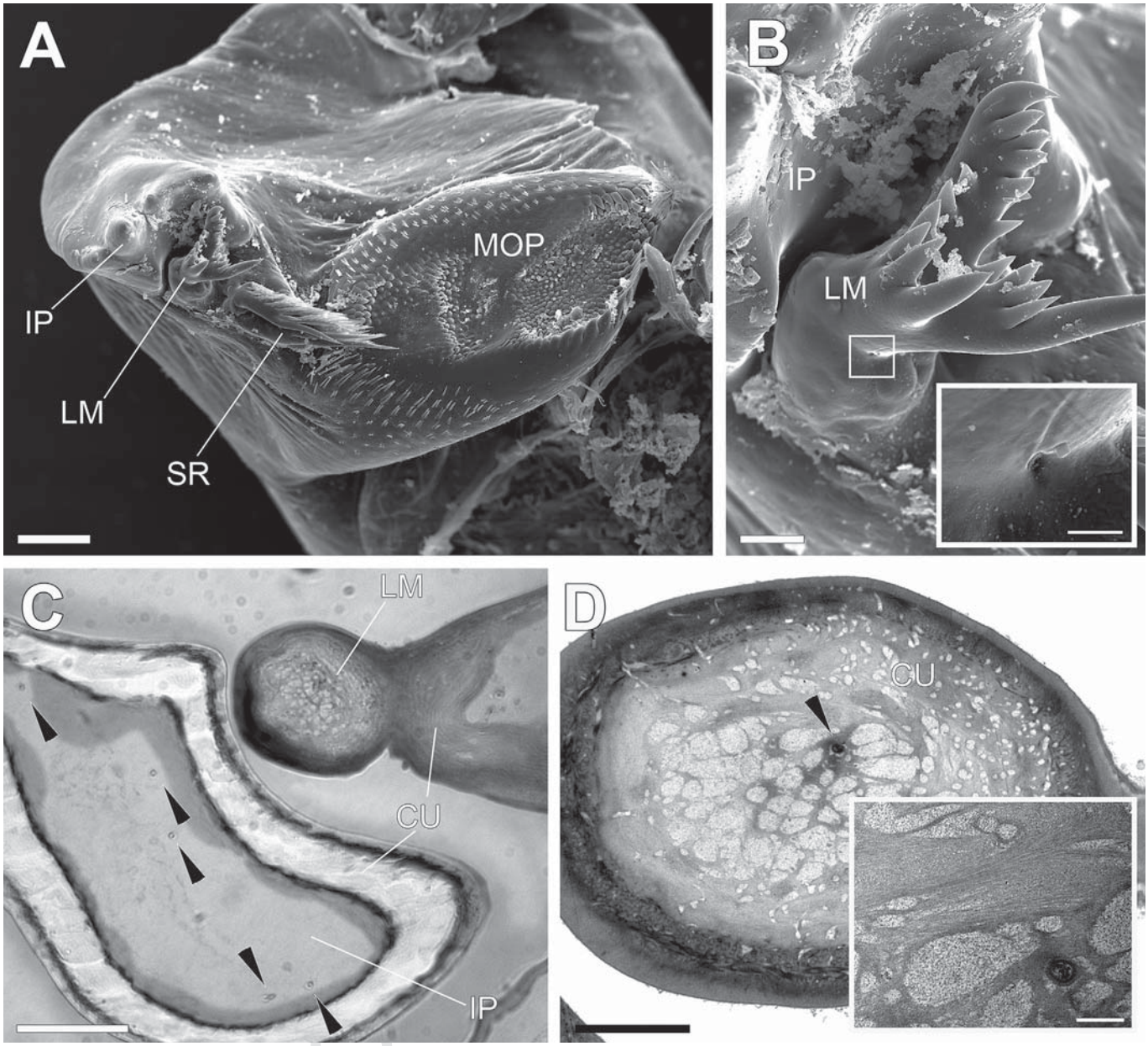

Fig. 2. Right mandible of *Neomysis integer*. **A**: Scanning electron micrograph (SEM) showing the inner view of the gnathal edge (scale, 40 μm). **B**: SEM showing the lacinia mobilis (scale, 10 μm) and detail of the pore (insert; scale, 2 μm). **C**: Light micrograph of a semi-thin section showing proximal region of the lacinia mobilis and five sensory cell clusters associated with the incisor process (arrowheads) (scale, 20 μm). **D**: Transmission electron micrographs showing the termination region of dendrites (arrowhead) and cuticular structure in the proximal region of the lacinia mobilis. Abbreviations: CU, cuticle; IP, incisor process; LM, lacinia mobilis; MOP, molar process; SR, 'setal row'.

4. Discussion

The basic form of the mandibles and laciniae mobiles as well as their asymmetry correspond well with the descriptions for *Boreomysis inermis* and *Hemimysis speluncola* (De Jong-Moreau et al., 2001) and also for some gammaridean species (Mayer et al., 2013). The strong toothed structure of the lacinia mobilis on the left mandible and a smaller and seta-like structure on the right mandible seem common. Our results agree with most of the features described by Richter et al. (2002) who studied the external morphology of the mandibles in *N. integer*, such as position, orientation, and shape of the laciniae on both mandibles or the similar look of the left lacinia and the left incisor process. However, the position of the right lacinia appeared slightly separate from the setal row in our results and we rather rate the right lacinia as separately articulated, as it is shaped with more complexity but a certain similarity.

The sensory capability of crustacean mandibles is surprisingly poorly studied. Ong (1969) first described the mandibular sensory receptors in the copepod *Gladioferens pectinatus* and recently a detailed survey of the sensory apparatus of larval mandibles in *P. elegans* was published by Geiselbrecht and Melzer (2013). This study now follows up the latter and provides insight into the sensory equipment of a peracarid mandible. We clearly show that the lacinia mobilis on both mandibles in *N. integer* is a structure innervated by sensory units. The ultrastructural analyses of the sensillar cell clusters showed features indicating the modality of the respective sensory neurons. Because of the alternating ultra-

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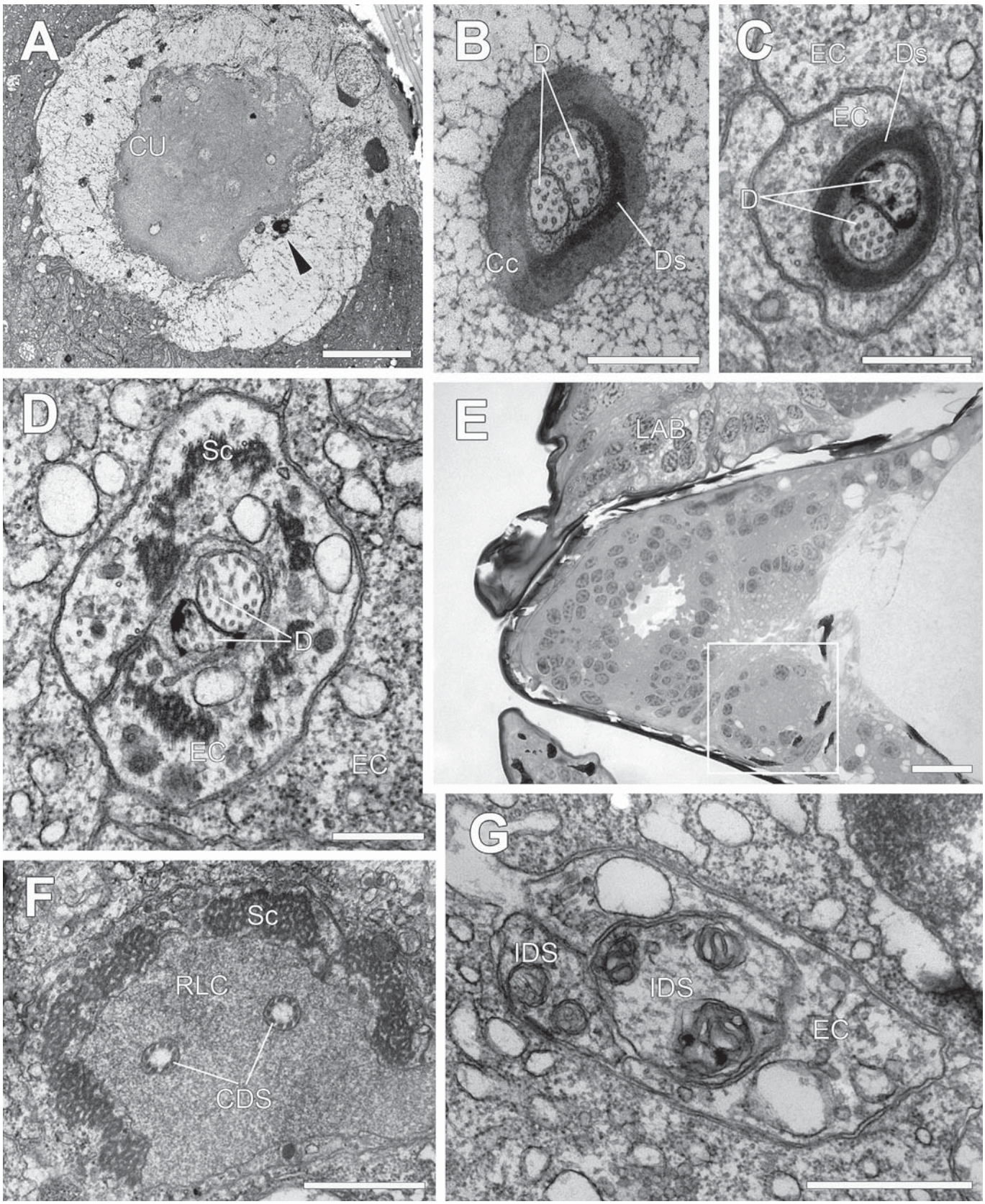

Fig. 3. Transmission electron and light micrographs showing ultrastructure of the lacinia mobilis on the right mandible. **A**: Lacinia basis with sensillar cell cluster (arrowhead) (scale, 5 μm). **B**: Distal region of the ODSs enclosed in the cuticular canal (scale, 0.5 μm). **C**: ODSs proximal to the lacinia basis surrounded by a dendrite sheath and enveloping cells (scale, 0.5 μm). **D**: ODSs distal to the transition region (scale, 0.5 μm). **E**: Cross section of the mandible showing epidermal tissue and enveloping cells with cut perikarya (square mark indication) (scale, 20 μm). **F**: Ciliary dendritic segments in receptor lymph cavity (scale, 1 μm). **G**. IDSs proximal to the transition region (scale 1 μm). Abbreviations: Cc, cuticular canal; CDS, ciliary dendritic segment; CU, cuticle; D, dendrite; Ds, dendrite sheath; EC, enveloping cell; IDS, inner dendritic segment; ODS, outer dendritic segment; LAB, labrum; RLC, receptor lymph cavity; Sc, scolopale.

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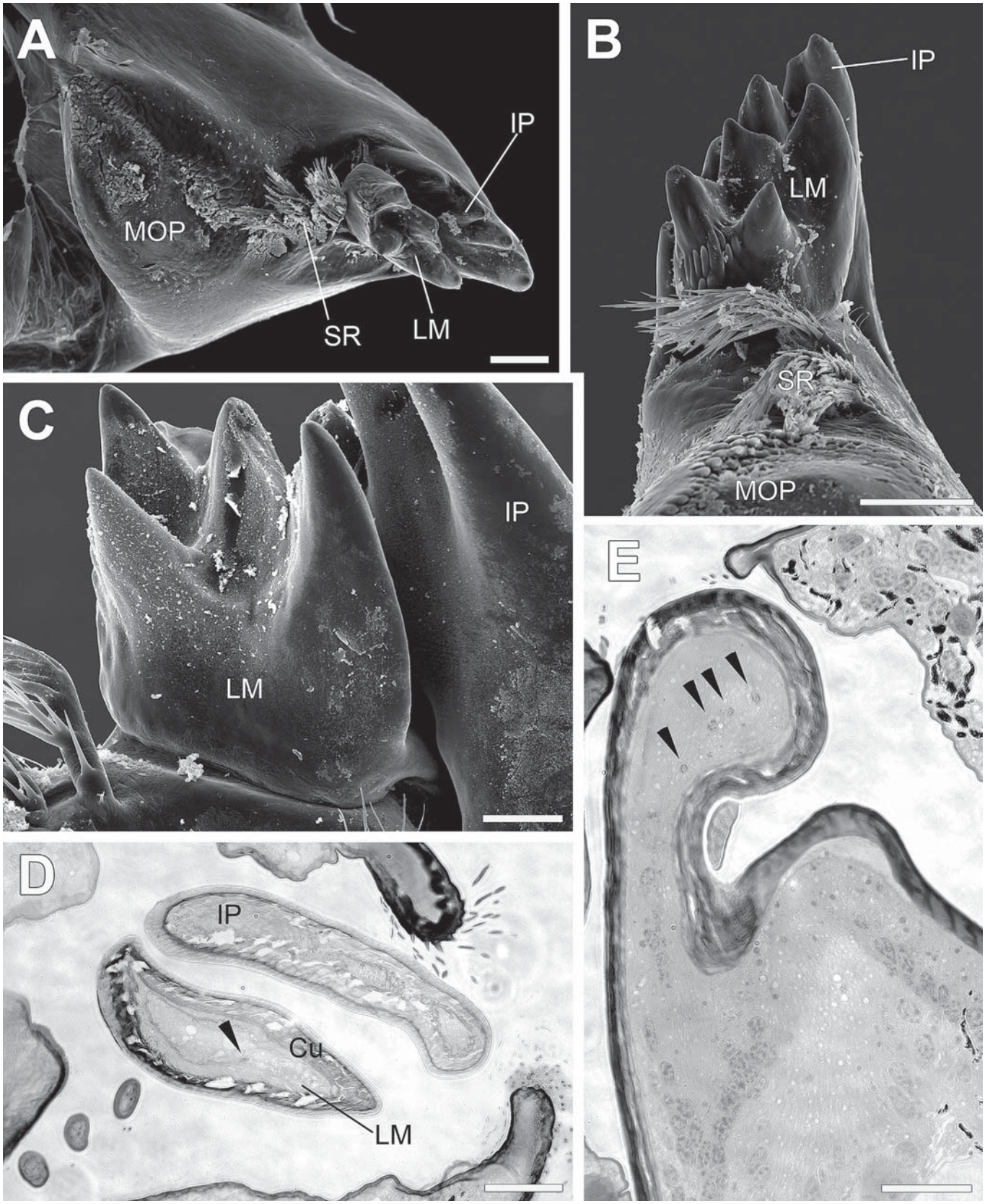

Fig. 4. Left mandible of *Neomysis integer*. **A**: Scanning electron micrograph (SEM) showing inner view of the gnathal edge (scale, 40 μm). **B**: SEM showing lacinia mobilis and "setal row" (scale, 40 μm). **C**: SEM micrograph showing lacinia mobilis (scale, 20 μm). **D**: Light micrograph showing cross section of the lacinia mobilis and incisor process (scale, 40 μm). **E**: Cross section of the mandible showing epidermal tissue and enveloping cells proximal to lacinia basis and sensillar cell clusters in the incisor process (arrowheads) (scale, 40 μm). Abbreviations: Cu, cuticle; IP, incisor process; LM, lacinia mobilis; MOP, molar process; SR, 'setal row'.

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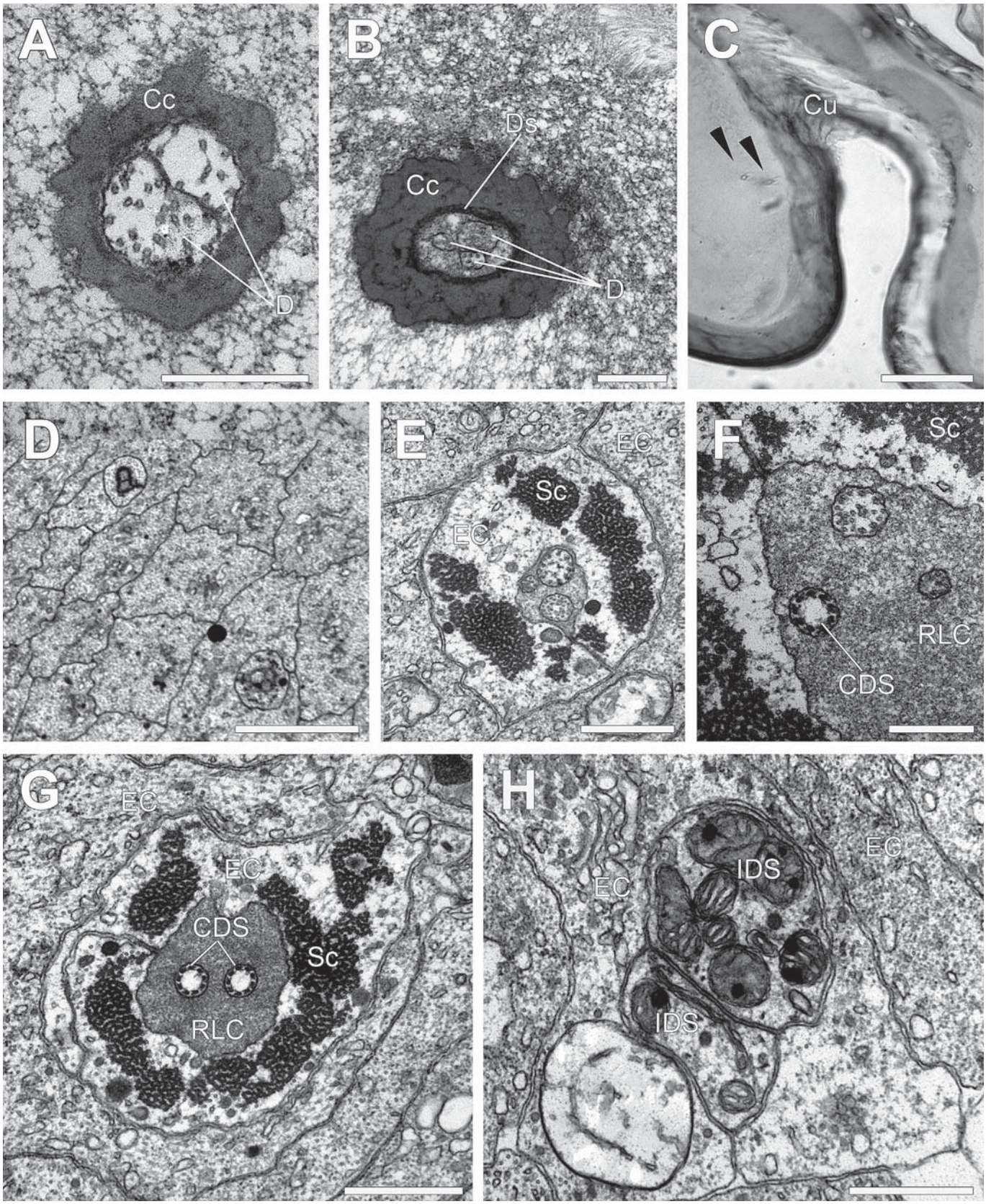

Fig. 5. Transmission electron and light micrographs showing ultrastructure of the lacinia mobilis on the left mandible. **A**: ODSs of cluster 1 in the termination region enclosed in the cuticular canal (scale, 0.5 μm). **B**: ODSs of cluster 2 in the termination region enclosed in the cuticular canal (scale, 0.5 μm). **C**: Cross section of the mandible proximal to the lacinia basis showing the position of sensillar cell clusters (arrowheads) (scale, 20 μm). **D**: ODSs and enveloping cells distal to transition region (scale, 2.5 μm). **E**: ODSs of cluster 2 distal to the transition region (scale, 1 μm). **F**: Ciliary dendritic segment of cluster 2 in the receptor lymph cavity (scale, 0.5 μm). **G**: Ciliary dendritic segments of cluster 1 in the receptor lymph cavity (scale, 1 μm). **H**: Inner dendritic segments of cluster 1 proximal to the transition region (scale, 1 μm). Abbreviations: Cc, cuticular canal; CDS, ciliary dendritic segment; CU, cuticle; D, dendrite; Ds, dendrite sheath; EC, enveloping cell; IDS, inner dendritic segment; ODS, outer dendritic segment; RLC, receptor lymph cavity; Sc, scolopale.

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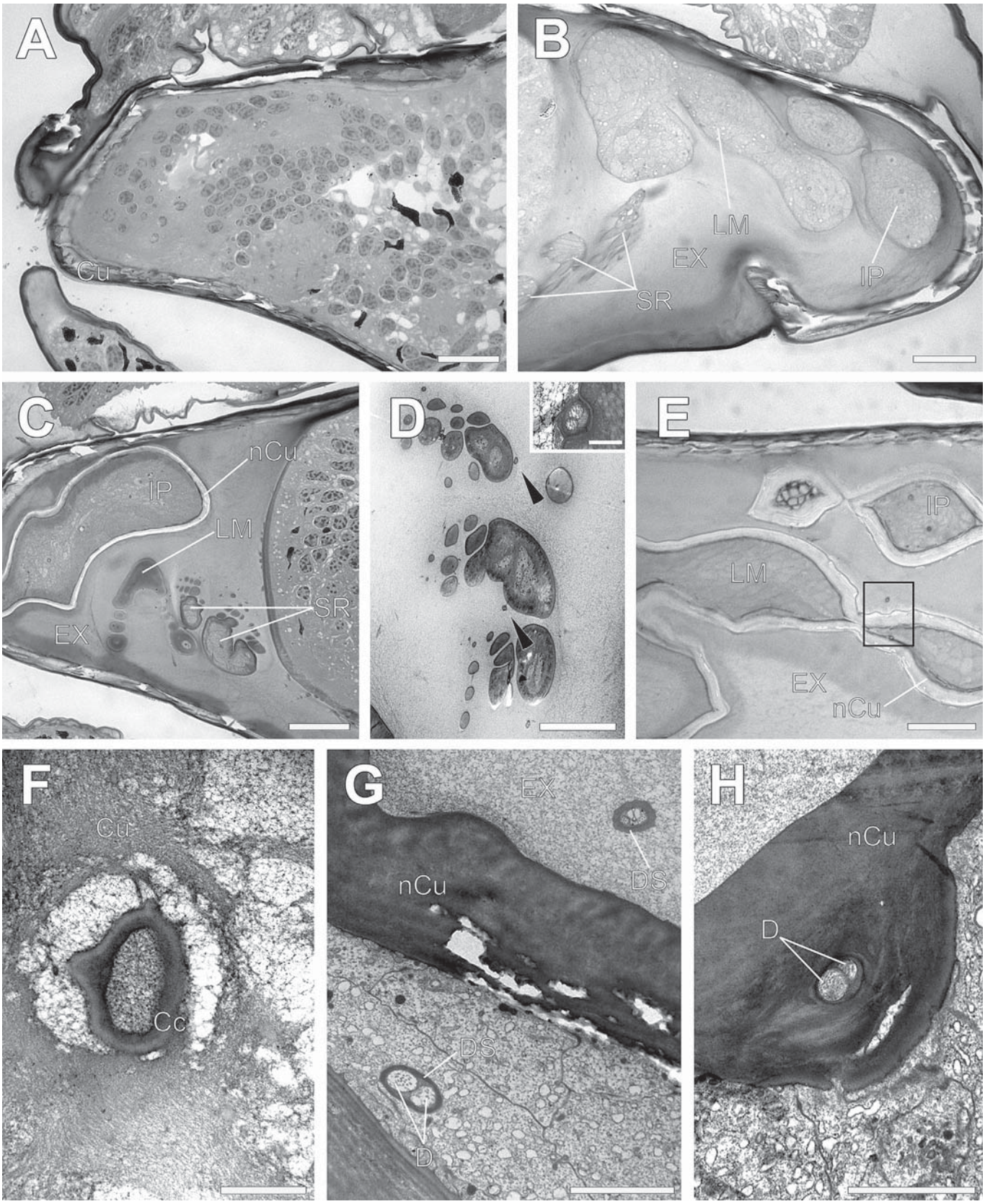

Fig. 6. Light and transmission electron micrographs showing the mandible of *Neomysis integer* during different phases of ecdysis. **A**: Right mandible during the intermolt phase (scale, 20 μm). **B**: Left mandible in stage D_0–D_1 (scale, 20 μm). **C**: Right mandible in stage D_3–D_4; future lacinia mobilis, incisor process, and members of the "setal row" lie underneath the old external structures (scale, 20 μm). **D**: Future "setal row" of the right mandible and associated sensory cell clusters (arrowheads) (scale, 10 μm); insert: two ODSs penetrating the new cuticle (scale, 0.5 μm). **E**: Left mandible in stage D_3–D_4; square mark indicates picture detail of G. **F**: Distal region of the cuticular canal in the right lacinia mobilis (scale, 0.5 μm). **G**: Left mandible, ODSs of cluster 1 inside newly secreted cuticle and cluster 2 outside the exuvial space (scale, 2 μm). **H**: ODSs of cluster 1 penetrating the new cuticle on their ascending path (scale 2 μm). Abbreviations: Cc, cuticular canal; Cu, cuticle; D, dendrite; DS, dendrite sheath; EX, exuvial space; IP, incisor process; LM, lacinia mobilis; ODS, outer dendritic segment; nCu, new cuticle; SR, "setal row".

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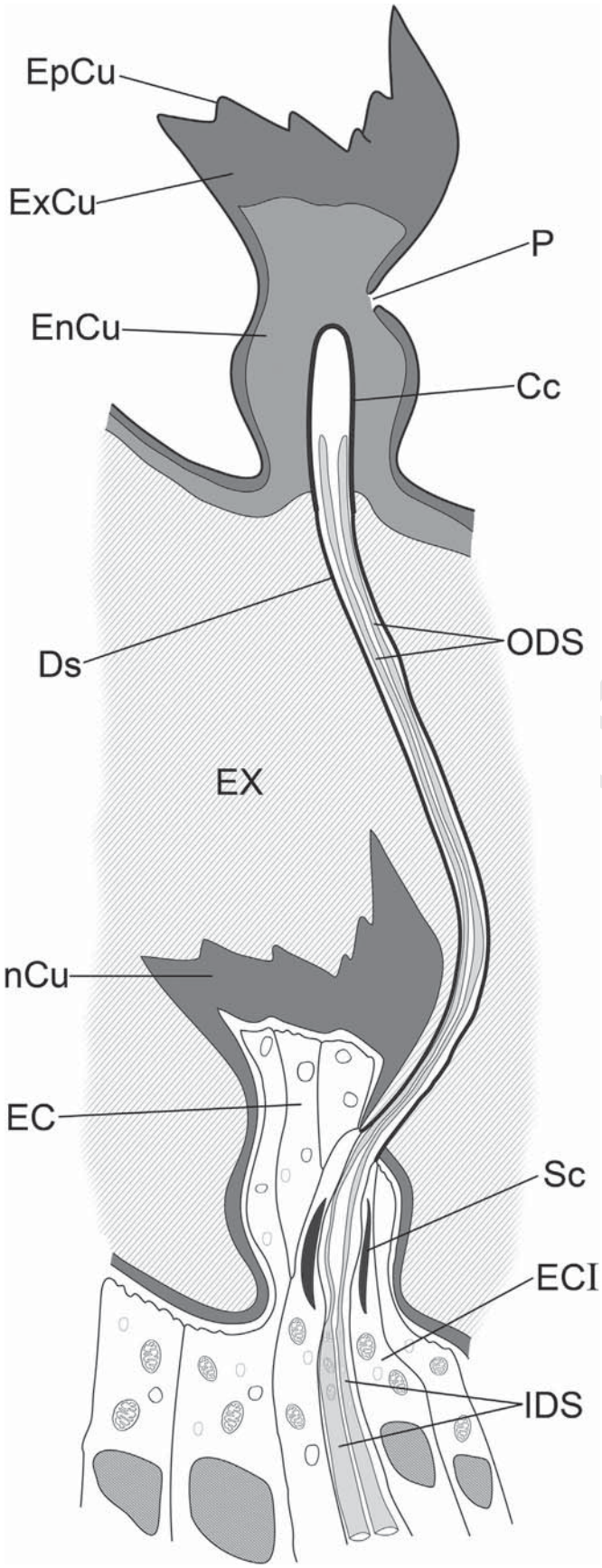

Fig. 7. Schematic drawing of the right lacinia mobilis during ecdysis. Abbreviations: Cc, cuticular canal; Ds, dendrite sheath; EC, enveloping cell; ECI, inner enveloping cell; EnCu, endocuticle; EpCu, epicuticle; EX, exuvial space; ExCu, exocuticle; IDS, inner dendritic segment; nCu, new cuticle; ODS, outer dendritic segment; P, pore; Sc, scolopale.

and semi-thin sectioning, we could not show the overall features of each sensory neuron. The combined features of all units that have been correlated with mechanosensitivity are the dense packing of microtubules in the ODSs, the presence of A-tubules with two arms in the CDS, and the prominent scolopale in the innermost enveloping cell (Schmidt and Gnatzy, 1984; Altner et al., 1986). Hence, these sensilla seem to be mechanoreceptors exclusively. Also the features agree with chordotonal-type cells, typical mechanosensitive neurons in aquatic crustaceans (Crouau, 1997). The stimulatory mechanism may be related with articulation of the external structures. Mayer et al. (2013) discussed the functional aspects of the lacinia mobilis in six gammaridean species. An important part of the biting mechanism is attributed to the laciniae mobiles with an interaction between the left and right lacinia involving extreme deflections. Because the external morphology of the gammaridean laciniae mobiles and those studied here are comparable, the primary stimuli of the sensilla are most likely the tilt of structures. The capability of sensing the position or the degree of deflection is consequently of high value.

Separate considerations are necessary concerning the comparative discussion of ecdysis and the origin or derivation of the laciniae mobiles. Two molting types have been described in Arthropoda differing in general characteristics of the ecdysis. The first type is characterized by sensory cells that stay connected with the old cuticular structure. Encased within a dendrite sheath, elongated ODSs penetrate the newly secreted cuticle and run through the exuvial space to their termination region. Thus, the sensilla remain functional until ecdysis. Originally described in insects (e.g. Altner and Thies, 1972; Moran et al., 1976; Gnatzy and Tautz, 1977), this type is also reported for molting of the statocyst and aesthetasc sensilla in *N. integer* (Guse, 1980a; Espeel, 1986). In the second type, the dendrites lose the connection and withdraw together with the sheath cells and epidermis during apolysis (Altner and Thies, 1972). Thus, the condition during molting of the lacinia mobilis can be attributed to type I, but there are certain differences compared with molting of the statocyst or aesthetasc sensilla. A specific characteristic is the enormous expansion of the exuvial space in the region of the incisor process, the lacinia mobilis and the setal row. Such an expansion was not found in other sections of the body in our specimens. Concerning the lacinia mobilis in particular, we did not notice an invagination within the epidermal tissue in the studied molting stages as generally described during the sensilla molting process (Guse, 1980b, 1983; Kouyama and Shimozawa, 1984; Espeel, 1986). It can be assumed that the mode of molting provoked the unusual extreme expansion of the exuvial space, with the new structure lying underneath the old cuticle in its definitive shape, and this shape was complex.

Underlying the preceding considerations is the question of whether the lacinia mobilis is derived from a classical seta or not, which leads us to the crucial point of the discussion. We showed that the lacinia mobilis on both mandibles is a sensory structure. The lacinia on the right mandible exhibits many features that define an appendage derived from a classical sensory seta: (1) the structure is articulated on a basal ring; (2) ciliary sensory dendrites given rise by primary neurons terminate distally of the base; (3) the sensory unit is accompanied by enveloping cells; (4) the condition of the ODSs and the enveloping cells during the molt; and (5) and the presence of an ecdysial pore. By comparing external features, Dahl and Hessler (1982) and also Richter et al. (2002) believed that the origin of the right lacinia was most likely from the setal row. A similar association with sensory cell clusters and the similar molting type presented

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here provide additional support for this idea. Furthermore, some of the features of the right lacinia mobilis listed above correspond well with those described for the decapod zoea of *P. elegans* (Geiselbrecht and Melzer, 2013) and thus also support the view that these structures might be homologous. The situation is more complex with the left lacinia mobilis. The basal articulation, the presence of sensory structures and ecdysial pores, here indicated by the ODSs penetrating the new cuticle during the molting process, and the positions of the sheath cell body and nucleus below the base support the conclusion that the left lacinia mobilis is also a very high derived seta. However, the finding that there are two discrete sensory units is very uncommon. Complex sensilla with more than one sensory unit are usually found in chemoreceptors such as the chemosensory cone of insect larvae (Nicastro et al., 1998), and not in mechanoreceptors. Thus, an alternative explanation could be that the left lacinia mobilis is a non setal, movable appendage with two associated sensory units without extra external structures, or the innervation could suggest that the left lacinia is a compound structure that became movable and is armed with two sensory spines, considering evolution from the incisor process (Richter et al., 2002). This point cannot be resolved currently and needs developmental studies, investigating the formation of the structure and further external and internal studies of features in other peracarid species as well as in Eumalacostraca in general.

4.1. Conclusions

With the present and our earlier study (Geiselbrecht and Melzer, 2013) we show that the mandible is a masticating organ with multifaceted sensory equipment, including highly aberrantly structured sensilla, e.g., stout sensory spines of considerable size optimized for robustness. Second, the lacinia mobilis in *N. integer* is also a sensory structure with highly distinct features that are very helpful for comparative analyses at the histological level. This study of the lacinia mobilis demonstrates that histological features and their comparative analysis can bring crucial arguments into the study and classification of crustacean body appendages. Future studies should be conducted by including more taxa and the ontogeny in addition to external analyses that will help understand the evolution and homologies of the laciniae mobiles.

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References

Altner, H., Hatt, H., Altner, I., 1986. Structural and functional properties of the mechanoreceptors and chemoreceptors in the anterior oesophageal sensilla of the crayfish, *Astacus astacus*. Cell Tissue Res. 244, 537–547.

Altner, H., Thies, G., 1972. Reizleitende Strukturen und Ablauf der Häutung an Sensillen einer euedaphischen Collembolenart. Z. Zellforsch. Mikrosk. Anat. 129, 196–216.

Crouau, Y., 1997. Comparison of crustacean and insect mechanoreceptive setae. Int. J. Insect Morphol. Embryol. 26, 181–190.

Dahl, E., Hessler, R.R., 1982. The crustacean lacinia mobilis: a reconsideration of its origin, function and phylogenetic implications. Zool. J. Linn. Soc. 74, 133–146.

De Jong-Moreau, L., Casanova, B., Casanova, J., 2001. Detailed comparative morphology of the peri-oral structures of the Mysidacea and Euphausiacea (Crustacea): an indication for the food preference. J. Mar. Biol. Assoc. U. K. 81, 235–242.

Drach, P., Tchernigovtzeff, C., 1967. Sur la méthode de détermination des stades d'intermue et son application générale aux crustacés. Vie Milieu 18, 595–609.

Dupré, E., Flores, L., Palma, S., 2008. Larval development of the rock shrimp *Rhynchocinetes typus* Milne Edwards, 1937 (Decapoda, Caridea) reared in the laboratory. Rev. Chil. Hist. Nat. 81, 155–170.

Espeel, M., 1986. Morphogenesis during molting of the setae in the statocyst sensilla of the mysid shrimp *Neomysis integer* (Leach, 1814) (Crustacea, Mysidacea). J. Morphol. 187, 61–68.

Garm, A., 2004. Revising the definition of the crustacean seta and setal classification systems based on examinations of the mouthpart setae of seven species of decapods. Zool. J. Linn. Soc. 142, 233–252.

Geiselbrecht, H., Melzer, R.R., 2010. Mandibles of zoea-I-larvae of nine decapod species. A scanning EM analysis. Spixiana 33, 27–47.

Geiselbrecht, H., Melzer, R.R., 2013. How do mandibles sense?-The sensory apparatus of larval mandibles in *Palaemon elegans* Rathke, 1837 (Decapoda, Palaemonidae). Arthropod Struct. Dev. 42, 1–16.

Gnatzy, W., Tautz, J., 1977. Sensitivity of an insect mechanoreceptor during moulting. Physiol. Entomol. 2, 279–288.

Guse, G.-W., 1980a. Development of antennal sensilla during moulting in *Neomysis integer* (Leach) (Crustacea, Mysidacea). Protoplasma 105, 53–67.

Guse, G.-W., 1980b. Fine structure of sensilla during moulting in *Neomysis integer* (Leach) (Crustacea, Mysidacea). Experientia 36, 1382–1384.

Guse, G.-W., 1983. Ultrastructure, development, and moulting of the aesthetascs of *Neomysis integer* and *Idotea baltica* (Crustacea, Malacostraca). Zoomorphology 103, 121–133.

Kouyama, N., Shimozawa, T., 1984. The ecdysis of hair mechanoreceptors in crayfish. Cell Tissue Res. 236, 339–343.

Maas, A., Waloszek, D., 2001. Larval development of *Euphausia superba* Dana, 1852 and a phylogenetic analysis of the Euphausiacea. Hydrobiologia 448, 143–169.

Makings, P., 1977. A guide to the British coastal Mysidacea. Field Stud. 4, 575–595.

Mayer, G., Haug, J.T., Maas, A., Waloszek, D., 2013. Functional aspects of the gammaridean mandibles with special reference to the lacinia mobilis (Crustacea, Amphipoda). Zool. Anz. 252, 536–547.

Moran, D.T., Rowley, J.C., Zill, S.N., Varela, F.G., 1976. The mechanism of sensory transduction in a mechanoreceptor. Functional stages in campaniform sensilla during the molting cycle. J. Cell Biol. 71, 832–847.

Nicastro, D., Melzer, R.R., Hruschka, H., Smola, U., 1998. Evolution of small sense organs: sensilla on the larval antennae traced back to the origin of the Diptera. Naturwissenschaften 85, 501–505.

Ong, J., 1969. The fine structure of the mandibular sensory receptors in the brackish water calanoid copepod *Gladioferens pectinatus* (Brady). Z. Zellforsch. Mikrosk. Anat. 97, 178–195.

Richardson, K.C., Jarett, L., Finke, E.H., 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Biotech. Histochem. 35, 313–323.

Richter, S., Edgecombe, G.D., Wilson, G.D.F., 2002. The lacinia mobilis and similar structures – a valuable character in arthropod phylogenetics? Zool. Anz. – J. Comp. Zool. 241, 339–361.

Schmidt, M., Gnatzy, W., 1984. Are the funnel-canal organs the 'campaniform sensilla' of the shore crab, *Carcinus maenas* (Decapoda, Crustacea)? II. Ultrastructure. Cell Tissue Res. 237, 81–93.

Watling, L., 1989. A classification system for crustacean setae based on the homology concept. In: Felgenhauer, B.E., Watling, L., Thistle, A.B. (Eds.), Crustacean Issues 6. A. A. Balkema, Rotterdam, pp. 15–26.

Weigmann-Haass, R., 1977. Die Calyptopis-und Furcilia-stadien von *Euphausia hanseni* (Crustacea: Euphausiacea). Helgol. Wiss. Meeresunters. 29, 315–327.

Yang, H.J., 2005. Larval development of *Latreutes anoplonyx* (Decapoda: Hippolytidae) reared in the laboratory. J. Crustacean Biol. 25, 462–479.

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8. General discussion

8.1. *Methodological approach*

The present thesis provides previously unknown insights into general decapod larval morphology and morphogenesis, and a detailed view on the sensory capacity of a larval decapod and an adult peracarid mandible. The applied methods offer comprehensive tools for morphological analyses. Compared to the LM, the usually applied method for larval descriptions, the SEM technique is advantageous due to a higher maximum of possible magnification and the high resolution power. Besides the usually described characters it is thus possible to analyse additional significant fine structural features, like also shown in some previous larval studies (Greenwood and Fielder, 1979; Meyer et al., 2006; Geiselbrecht and Melzer, 2009). The external features that suggested a sensillar nature of the 'lacinia mobilis' in the *P. elegans* zoea I could only be detected and depicted with the SEM and also the small precursors of the typical caridean features of the zoea I in *M. amazonicum*. However, the sensillar nature of an articulated appendage can only be proven by an ultrastructural analysis using TEM.

The 3D visualization of internal features by surface rendering is a useful method for comparative and descriptive studies of small specimens. It allows not only to depict and compare certain morphological features in detail but also to calculate and compare volumes and surfaces. Although the method is complex in the required laboratory equipment and the application is quite time consuming the results speak in favor of it.

8.2. *The decapod larval CNS*

The decapod nervous system in general shows the typical arthropod structure with well-developed segmental ganglia, neurites forming a central neuropil and a surrounding cell-body cortex (Hanström, 1947). It includes the central nervous system (CNS), consisting of the brain and ventral nerve cord, and the peripheral nervous system (PNS), consisting of the segmental nerves connecting muscles and sensory organs with the CNS (Barnes and Ruppert, 2004). The main elements of a typical adult decapod CNS can already be recognized in the zoea I. The comparative analysis of the histological sections of the zoea I clearly showed that the larval CNS also bears taxon specific characters. The studied species, belonging to three different decapod main lineages, i.e. Caridea, Anomura and Brachyura, can be clearly distinguished according to the specific configuration of the nervous system. Another phenomenon that could be observed is

a differing stage of development of neuromeres and nerves within and between species. Ganglia develop at different tempos depending on general larval morphogenesis. The differences in the developmental stages of certain ganglia are paralleled by respective peculiarities in the development of the segmental appendages. This is most obvious in segments with well-developed limbs, where the ganglia are in a more advanced stage of differentiation and more voluminous compared to segments with only limb buds or without externally visible limb *anlagen*. Observing variations like this in the comparison of closely related species, representing both ancestral and derived characters states, can most plausibly be explained being effected by heterochronic events, in the meaning of shifts in the timing of morphogenesis (review in Smith, 2001). With the present results it is not possible to recognize an unambiguous evolutionary trend from basally branching lineages like the Caridea to more derived ones like the Anomura and Brachyura (Bracken et al., 2009b). However, this should be accomplishable by including all remaining decapod main taxa, like e.g. Stenopodidea, Astacidea, Glypheidea or Achelata. A phylogenetically relevant signal may then be found in the morphogenesis of the segmental ganglia.

8.3. *Mandible development in decapod zoea larvae*

Studying the larval mandibular fine structure in different decapod species Geiselbrecht and Melzer (2010) suggested the hypotheses of significant phylogenetic signal present in certain sets of zoeal mandible characters and an evolutionary trend from a slender mandible with ‘lacinia mobilis’ in Caridea to a more oval or even massive and rotund mandible without ‘lacinia mobilis’ in Anomura and Brachyura. However, the morphology of crustacean mandibles depends also on feeding habits, Mekhanikova (2010) reported this for six amphipod species with different food sources and De Jong-Moreau et al. (2001a) in several species of Mysidacea and Euphausiacea. Certainly, this influence was not investigated in decapod larvae until now. The individual features in different taxa may indeed represent morphological adaptations to food preferences produced by functional constraints but in the present study in the non-feeding species small precursors of the typical caridean features as well as the according basic mandibular form could be demonstrated, corroborating the hypothesis of an evolutionary ground pattern in mandible morphology. The morphological differences between the zoea I in the two species associated with different feeding modes obscured the apomorph features of palaemonids. These become conspicuous only in the detailed analysis of the developing mandible. Showing a somewhat

retarded appearing mandible in zoea I, in *M. amazonicum* the developmental changes lag always one stage behind the equivalent in *P. elegans*. This variation can also be best attributed to heterochronic effects. Related to the initial lack of mandible functionality, the mandible development in *M. amazonicum* is postdisplaced compared to that in *P. elegans*.

8.4. *How do mandibles sense?*

8.4.1. *Decapoda*

The mandibles of the zoea I in *P. elegans* are equipped with a relatively high number of diverse sensory structures. Among these are mechanoreceptive hair-sensilla, putative contact-chemoreceptors, mechanosensitive sensilla and unimodal chemoreceptors without external structure and mechano- and chemoreceptors associated with inflexible spines. Besides classical seta-like structures with a movable socket also unarticulated spines are proved to be sensory structures. This refutes the so far claimed assumption that the mandibular armature are just simple teeth deprived of a sensory function (Ingle, 1992). The function of the larval mandibles as masticating organ requires a robust building and this robustness brings about uncommon structures or extreme modifications in the sensory equipment. These 'robust' versions increase the already multifaceted wealth of seta-derived sensilla known from arthropods. The results provide new insights in the functional morphology of zoeal mandibles and also constitute a complex set of fine- and ultrastructural characters. Thus, it could be proven that the 'lacinia mobilis' on the larval mandible in *P. elegans* is a mechanosensitive sensillum, providing new evidence in the question of homology of the 'true' lacinia mobilis in Peracarida and the structure found on larval caridean mandibles.

8.4.2. *Peracarida*

Primarily considered as a diagnostic feature of the Peracarida, the lacinia mobilis for a long time attracted the attention of quite a few morphological studies. These included functional considerations as well as reviewing surveys and detailed discussions of its origin and possible homology with similar structures e.g. found in Decapoda (Dahl and Hessler, 1982; Richter et al., 2002; Mayer et al., 2013). Surprisingly no ultrastructural analyses revealing the sensory capacity were present so far. This knowledge gap could be filled now. The lacinia mobilis in the mysid *N. integer* is a sensory structure on both mandibles. The different external structure on the left and right mandible was already known, now in addition also ultrastructural differences could be

found. Accordingly the right lacinia is probably a highly derived sensory seta, whereas two alternative interpretations have to be suggested for the left lacinia; it could be also a derived sensillar appendage equipped with two mechanosensory units, or it could be a movable appendage of the incisor process containing two sensilla deprived of external appendages. Hence, these results support a possible homology of the right lacinia mobilis in Peracarida and Decapoda with a common origin as a member of the setal row. Concerning the left lacinia mobilis questions on the origin and possible homology remain unsettled, because whether it is also a derived sensillum or an appendage with two sensilla cannot be resolved presently.

9. Conclusions and outlook

The examination of the sensory system included an overall study of the larval central nervous system and the peripheral nerves, followed by a more detailed description of the presence and distribution of sensory structures on a larval decapod and an adult mysid mandible. Finally the external finestructure and the internal ultrastructure of single sensilla were analysed. Differences in the modality specific structures could be presented and accordingly various types of receptors could be described and compared. In addition ontogenetic considerations could be made in the analysis of the CNS and a developmental study was conducted investigating and comparing the mandibular morphology in different larval stages. The main results and conclusions present themselves in the following way.

The CNS in decapod zoea I larvae is in a transitory stage to the adult organization. The basic main elements of a typical adult decapod nervous system can already be clearly identified, however, certain differences are present; (1) in the cell body cortex, whereas it completely surrounds the neuropil in the young, in the adult there are cell body clusters, (2) in the position of the optic neuropils, that stretch in adults into the eyestalks, are located close to the median part of the protocerebrum in the larvae, and (3) in the complete set of ganglia that is not entirely present or partially less developed in the larvae. The larval CNS is also a stage specific system reflecting adaptations to larval life. The morphogenesis of ganglia can be in a different stage of development. An anterior-posterior gradient is recognizable in the progress of neuromere development of the successive thoracic segments. But considering the whole body an interruption of the anterior-posterior gradient can be recognized: While the ganglia in the posteriormost pereion segments are the least developed, all species show a well-developed ventral nerve cord in the pleon segments; therefore, in our zoeae the gradient is interrupted in the pereion neuromeres

of segments with underdeveloped limbs. This can be correlated with the life style of the planktonic larvae: while swimming with the exopods of the present maxillipeds (Gurney, 1942), they additionally all show an escape behavior through a complex mechanism of rapid strokes of the pleon (Dahl, 1983).

The studied species belong to three of the decapod main lineages, i.e. Caridea, Anomura and Brachyura. They represent closely related taxa but with more ancestral character states in Caridea, that are considered as the most basal taxon of the three, and more derived character states in Anomura and Brachyura (Bracken et al., 2009b). The differences observed within and between species concerning the stage of development of neuromeres and nerves can best be explained by shifts in the timing of morphogenetic events. In the different species the zoea I larvae show a different set of appendages and in segments with well-developed limbs the ganglia are in a more advanced stage of differentiation compared to segments with only limb buds or without externally visible limb *anlagen.* Relating these phenomena to the phylogeny of the taxa, representing both ancestral and derived characters states, a plausible explanation is heterochrony in ganglion development. These specific features indicate that also in the larval decapod CNS phylogenetic relevant signal can be found. With a broader data basis of studied taxa it should be possible to classify decapod infraorders by reference to the specific shape of larval neuronal structures. The relevance of brain architecture in understanding of arthropod phylogeny was already early explored by the Swedish pioneers Nils Holmgren (1916) and Bertil Hanstrom (1928) and currently also treated by Strausfeld (2012) (see also Harzsch, 2006; Harzsch 2007; and specifically for Decapoda Sandeman et al., 1993).

Heterochrony effects can also be recognized in the development of the mandibles in the comparison of two closely related decapod species showing different feeding modes. The mandibles in the zoea I of the non-feeding species *M. amazonicum* appear retarded compared to the ones of the already feeding zoea I in *P. elegans*. During the progressing development both species show comparable morphological changes in the mandibular finestructure but with *M. amazonicum* larvae always lagging one stage behind. Hence, in a comparison of the mandible morphology limited to the zoea I of a species pair, adaptations to food preference can obscure the taxon specific characters. However, in a detailed analyses the hypothesis suggesting phylogenetic relevant signal sets of characters on the larval mandible (Geiselbrecht and Melzer, 2010) could be strengthened by proving the presence of basic features representing caridean apomorphies even in species with aberrant feeding modes in the zoea I stage.

The common notion of the two studies at first is that animals differentiate a structure only when it is needed. But, looking closely it is indicated, that early larval stages already bear taxon and possibly even species specific characters, on the one hand in the composition and the developmental stage of the CNS and also in the finestructure and basic form of the mandibles. In the future more species of the current taxa and also representatives of the remaining decapod main lineages like i.a. Dendrobranchiata, Stenopodidea, Astacidea, or Polychelida to strengthen this notion have to be analysed.

Furthermore, when comparing the ontogeny of two closely related species representing ancestral and derived conditions the timing of the appearance of certain characters can be shifted. These shifts can be attributed to heterochronic mechanisms like predisplacement or postdisplacement (McNamara, 1986; McKinney and McNamara, 1991) and can bring about differential evolutionary adaptations to specific selection pressures such as food limitation.

All this advocates the importance of a holomorphological approach in comparative studies. The concept of 'holomorphology', first introduced by Hennig (1966), incorporates the sum total of an organism's morphological information, over its entire anatomy and life history (Kaplan, 2001). Thus, comparable larval characters can appear in different stages and relationships only become intelligible by comparing the appropriate stages.

When it comes to certain characters, like sensory structures, also the ultrastructure should be part of a holomorphological approach. The cellular elements of arthropod sensilla are bipolar neurons with an axon and a dendrite derived from a cilium plus surrounding auxiliary cells. Mechanosensitive neurons are mostly associated with external structures, i.e. hair-derived setae (McIver, 1975). In the course of evolution arthropods developed sensilla comprised of these elements on every imaginable position on the body and in the separate main lineages sensilla underwent stepwise changes in their ultrastructural features (Nicastro et al., 1998; Crouau, 2001). In Crustacea some features in the ultrastructure of mechanosensitive sensilla are conservative, like the axoneme-like structure without central tubules (9 x 2 + 0), or the presence of a scolopale in the inner sheath cell (Crouau, 1997). In case of suggested homologies based on location or distribution pattern and ultrastructural features, phylogenetic conclusions can be made using these characters (Nicastro et al., 1998).

The sensilla on the mandibles of the zoea I in the decapod *P. elegans* and of the adult mysid *N. integer* showed common features that allow certain conclusions. Eumalacostracan mandibles are masticating organs with multifaceted sensory equipment. Adaptations to the function of the

mandibles can be observed in the presence of highly aberrantly structured sensilla showing a robust spine-like external structure. ‘Sensory spine’ is a new term that was introduced describing these types of sensilla. The results of both studies showing a variety of sensilla associated with the incisor process suggest the stout and sharp protrusions formerly described as simple cuticular outgrowth and termed ‘spines’ or ‘teeth’ (e.g. Factor, 1978; Ingle, 1992) are in many cases sensory structures and in the future should be termed ‘sensory spines’. This demonstrates that histological features and their comparative analysis can bring crucial arguments into the study and classification of crustacean body appendages.

The ultrastructural analyses also brought new arguments into the discussion of a possible homology between the ‘true’ lacinia mobilis in Peracarida and the ‘lacinia mobilis’ found on larval decapod mandibles. In both taxa studied in this project the structures on the right mandibles exhibit features that define appendages derived from classical sensory setae: (1) the articulation on a basal ring and the presence of an ecdysial pore; (2) the presence of ciliary dendrites given rise by primary sensory cells terminating distally of the base; (3) the sensory unit is accompanied by enveloping cells. These features also provide additional support for the idea of an origin of the right lacinia as a member of the setal row (Dahl and Hessler, 1982; Richter et al., 2002). Furthermore, in both studied taxa the position at the base of the incisor process is the same and also corresponding modality specific features of the sensilla respectively suggest mechanosensitivity. Consequently, the view that these structures might be homologous gained strong support. Because the origin and the derivation of the left lacinia mobilis could not be evaluated clearly, no according conclusions can be made at this point. Here, further developmental and comparative studies, also in other peracarid and eumalacostracan species need to be accomplished.

It astonishes that in Mandibulata, the largest of all animal groups, to date nearly nothing is known in detail about the sensory capabilities and the ultrastructure of the mandibular ganthal edges. Even though the presence of mandibles has been regarded as such important characteristic, morphologically and functionally, that it has been used to define a clade Mandibulata (Bitsch, 2001). Concerning Crustacea, there are some studies describing the presence of sensory receptors on the mandible, sometimes with more (Ong, 1969), sometimes with less notes on ultrastructural features (Friedman and Strickler, 1975), but mostly only including external examinations limited to the mandibular palp (e.g. Garm, 2004; Garm and Høeg, 2006). Also in Hexapoda and

Myriapoda knowledge is limited (see e.g. Zacharuk and Albert, 1978; Albert, 1980; Stoffolano and Yin, 1983; LeSage, 1984; Masuko, 1986). In this thesis at first a comprehensive description of the ultrastructure of mandibular sensilla and the overall sensory capability of the mandibles in a decapod larva could be presented. The analysis of the modality specific structures revealed seven different types of sensilla, including mechanoreceptors, chemoreceptors and bimodal mechano-chemoreceptors, presenting the mandible as a masticating organ with multifaceted sensory equipment and a set of characters relevant also in a phylogenetic context. Positional and structural correspondence in general features and in structural details of incisor and molar processes already supported a general homology of the mandibular gnathal edges throughout Mandibulata (Edgecombe et al., 2003). The ongoing discussion about the homology of the lacinia mobilis, a distinctive mandibular feature in several arthropod taxa, now could be added with new conclusions. A possible homology of the feature in Decapoda and Peracarida gained further support based on the analyses of ultrastructural features. Hence, new features could be described that, placed on a broader basis by the analyses of more taxa, can strengthen the phylogenetic position of Peracarida and Decapoda. This again shows the importance and phylogenetic relevance of such character analyses.

After a period with excessive practice of partly exclusive molecular phylogeny Wheeler (2008) and Sudhaus (2007) emphasized the importance of morphology and of comparative morphological studies, that provide complex information-rich characters. . Besides the application of powerful new digital tools, like computer aided 3D-reconstruction, still the combination of these modern with classical methods contributes to a progress in our knowledge of species, phylogeny and classification. Combining classical morphology and molecular analyses in the concept of integrative taxonomy will undoubtedly result in better supported phylogenies and in most profound science (Dayrat, 2005; Will et al., 2005; Valdecasas et al., 2008). For that reason new morphological characters must be explored and documented further on and like indicated by the results presented here there is still a wealth of unknown features to discover, to describe, and to compare.

10. References

Ahyong, S., Schnabel, K., Maas, E., 2009. Anomuran phylogeny: new insights from molecular data. Decapod Crustacean Phylogenetics (Crustacean Issues 18), 391-408.

Ahyong, S.T., O'Meally, D., 2004. Phylogeny of the Decapoda Reptantia: resolution using three molecular loci and morphology. The Raffles Bulletin of Zoology 52, 673-693.

Aikawa, H., 1937. Further notes on brachyuran larvae. Records of Oceanographic Works of Japan 9, 87-162.

Alberch, P., Gould, S.J., Oster, G.F., Wake, D.B., 1979. Size and Shape in Ontogeny and Phylogeny. Paleobiology 5, 296-317.

Albert, P.J., 1980. Morphology and innervation of mouthpart sensilla in larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Canadian Journal of Zoology 58, 842-851.

Anger, K., 2001. The biology of decapod crustacean larvae. A.A. Balkema Publishers, Lisse.

Anger, K., Hayd, L., 2009. From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*. Aquatic Biology 7, 19-30.

Barnes, R.D., Ruppert, E.E., 2004. Invertebrate zoology. Brooks/Cole, Belmont.

Barnich, R., 1996. The larvae of the Crustacea Decapoda (excl. Brachyura) in the plankton of the French Mediterranean coast: identification keys and systematic review. Cuvillier Verlag.

Bethe, A., 1895. Studien über das Centralnervensystem von Carcinus Maenas nebst Angaben über ein neues Verfahren der Methylenblaufixation. Archiv für Mikroskopische Anatomie 44, 579-622.

Bitsch, C., Bitsch, J., 2004. Phylogenetic relationships of basal hexapods among the mandibulate arthropods: a cladistic analysis based on comparative morphological characters. Zoologica Scripta 33, 511-550.

Bitsch, J., 2001. The arthropod mandible: morphology and evolution. Phylogenetic implications, Annales De La Societe Entomologique De France. Société entomologique de France, pp. 305-321.

Boas, J.E.V., 1880. Studier over Decapodernes Slaegtskabsforhold. Kongelzge Danske Videmkabernes Selskabs Skriftr. Kjobenhaun 6, 26-210.

Bosc, L.A.G., 1802. Histoire naturelle des Crustaces, contenant leur description et leurs moeurs, avec figures dessinees d'apres natur. Detervill, Paris.

Bourdillon-Casanova, L., 1960. Le méroplancton du golfe de Marseille: les larves de crustacés decapodes. Aix-en-Provence.

Bowman, T.E., Abele, L.G., 1982. Classification of the recent Crustacea. The Biology of Crustacea 1, 1-27.

Bracken, H., De Grave, S., Felder, D., 2009a. Phylogeny of the infraorder Caridea based on mitochondrial and nuclear genes (Crustacea: Decapoda). Decapod crustacean phylogenetics, 281-305.

Bracken, H.D., Toon, A., Felder, D.L., Martin, J.W., Finley, M., Rasmussen, J., Palero, F.,

Crandall, K.A., 2009b. The Decapod Tree of Life: Compiling the Data and Moving toward a Consensus of Decapod Evolution. Arthropod Systematics & Phylogeny 67, 99-116.

Bullock, T.H., Horridge, G.A., 1965. Structure and function in the nervous systems of invertebrates. W.H. Freeman, San Francisco.

Burkenroad, M.D., 1963. The evolution of the Eucarida (Crustacea, Eumalacostraca), in relation to the fossil record. Tulane Studies in Geology 2, 1-17.

Burkenroad, M.D., 1981. The higher taxonomy and evolution of decapod crustacea. Transactions of the San Diego Society of Natural History 19, 251-268.

Calman, W., 1904. On the classification of the Crustacea Malacostraca. The Annals and Magazine of Natural History 7, 144-158.

Chan, T.-Y., 2010. Annotated checklist of the world's marine lobsters (Crustacea: Decapoda: Astacidea, Glypheidea, Achelata, Polychelida). The Raffles Bulletin of Zoology 23, 153-181.

Christiansen, M., 1973. The complete larval development of *Hyas araneus* (Linnaeus) and *Hyas coarctatus* Leach (Decapoda, Brachyura, Majidae) reared in the laboratory. Norwegian journal of zoology 21, 63-89.

Cieluch, U., Anger, K., Charmantier-Daures, M., Charmantier, G., 2007. Osmoregulation and immunolocalization of Na+/K+-ATPase during the ontogeny of the mitten crab *Eriocheir sinensis* (Decapoda, Grapsoidea). Marine Ecology Progress Series 329, 169-178.

Cieluch, U., Charmantier, G., Grousset, E., Charmantier-Daures, M., Anger, K., 2005. Osmoregulation, immunolocalization of Na+/K+- ATPase, and ultrastructure of branchial epithelia in the developing brown shrimp, *Crangon crangon* (Decapoda, Caridea). Physiological and Biochemical Zoology 78, 1017-1025.

Clark, P.F., 1983. The larval and first crab stages of three *Inachus* species (Crustacea: Brachyura: Majidae); a morphological and statistical analysis. Bulletin of the Natural History Museum (Zoology) 44, 179-190.

Clark, P.F., 2009. The bearing of larval morphology on brachyuran phylogeny, In: Martin, J.W.,

Crandall, K.A., Darryl, F.L. (Eds.), Crustaceans Issues 18. Decapod Crustacean Phylogenetics. Taylor & Francis, London, pp. 221-241.

Clark, P.F., Calazans, D.K., Pohle, G.W., 1998. Accuracy and standardization of brachyuran larval descriptions. Invertebrate Reproduction & Development 33, 127-144.

Crouau, Y., 1997. Comparison of crustacean and insect mechanoreceptive setae. International Journal of Insect Morphology & Embryology 26, 181-190.

Crouau, Y., 2001. Mechanosensitive cells of hexapods, crustaceans and myriapods setae: A comparison under phylogenetic aspects. Annales De La Societe Entomologique De France 37, 233-242.

Dahl, E., 1983. Malacostracan phylogeny and evolution, In: Schram, F.R. (Ed.), Crustacean Phylogeny. Crustacean Issues 1. A. A. Balkema, Rotterdam, pp. 189-212.

Dahl, E., Hessler, R.R., 1982. The crustacean lacinia mobilis: a reconsideration of its origin, function and phylogenetic implications. Zoological Journal of the Linnean Society 74, 133-146.

Dayrat, B., 2005. Towards integrative taxonomy. Biological Journal of the Linnean Society 85, 407-415.

De Beer, G., 1940. Embryos and ancestors. The Clarendon press, Oxford.

De Grave, N., Pentcheff, D., Ahyong, S.T., 2009. A classification of living and fossil genera of decapod crustaceans. Raffles Bulletin of Zoology, 1-109.

De Grave, S., Fransen, C., 2011. Carideorum catalogus: the recent species of the dendrobranchiate, stenopodidean, procarididean and caridean shrimps (Crustacea: Decapoda). NCB Naturalis.

De Jong-Moreau, L., Casanova, B., Casanova, J.-P., 2001a. Detailed comparative morphology of the peri-oral structures of the Mysidacea and Euphausiacea (Crustacea): an indication for the food preference. Journal of the Marine Biological Association of the United Kingdom 81, 235-241.

De Jong-Moreau, L., Casanova, B., Casanova, J., 2001b. Detailed comparative morphology of the peri-oral structures of the Mysidacea and Euphausiacea (Crustacea): an indication for the food preference. JMBA-Journal of the Marine Biological Association of the United Kingdom 81, 235-242.

Edgecombe, G.D., 2010. Arthropod phylogeny: an overview from the perspectives of morphology, molecular data and the fossil record. Arthropod Structure & Development 39, 74-87.

Edgecombe, G.D., Richter, S., Wilson, G.D., 2003. The mandibular gnathal edges: homologous structures throughout Mandibulata? African Invertebrates 44, 115-135.

Factor, J.R., 1978. Morphology of the mouthparts of larval lobsters, *Homarus americanus* (decapoda: nephropidae), with special emphasis on their setae. The Biological Bulletin 154, 383-408.

Friedman, M.M., Strickler, J.R., 1975. Chemoreceptors and feeding in calanoid copepods (Arthropoda: Crustacea). Proceedings of the National Academy of Sciences 72, 4185-4188.

Garm, A., 2004. Mechanical functions of setae from the mouth apparatus of seven species of decapod crustaceans. Journal of Morphology 260, 85-100.

Garm, A., Høeg, J.T., 2006. Ultrastructure and functional organization of mouthpart sensory setae of the spiny lobster Panulirus argus: New features of putative mechanoreceptors. Journal of Morphology 267, 464-476.

Geiselbrecht, H., Melzer, R.R., 2009. Morphology of the first zoeal stage of the partner shrimp *Periclimenes amethysteus* Risso, 1827 (Decapoda: Caridea: Palaemonidae: Pontoniinae) studied with the Scanning EM. Zootaxa 2140, 45-55.

Geiselbrecht, H., Melzer, R.R., 2010. Mandibles of zoea-I-larvae of nine decapod species. A scanning EM analysis. Spixiana 33, 27-47.

Giesbrecht, W., 1913. Crustacea, In: Lang, A. (Ed.), Handbuch der Morphologie der Wirbellosen Tiere, Arthropoden. Gustav Fisher, Jena, pp. 9-252.

González-Gordillo, J., Santos, A.d., Rodríguez, A., 2001. Checklist and annotated bibliography of decapod crustacean larvae from the Southwestern European coast (Gibraltar Strait area). Scientia Marina 65(4), 275-305.

Gould, S.J., 1977. Ontogeny and phylogeny. The Belknap Press of Harvard Univ. Press, Cambridge, Mass. [u.a.].

Greenwood, J., Fielder, D., 1979. The zoeal stages and megalopa of *Portunus rubromarginatus* (Lanchester)(Decapoda: Portnnidae), reared in the laboratory. Journal of Plankton Research 1, 191-205.

Grobben, K., 1919. Uber die Muskulatur des Vorderkopfes der Stomatopoden und die systematische Stellung dieser Malakostrakengruppe. Sitzungsberichte der Kaiserlichten Academie der Wissenschaften in Wien 128, 185-214.

Guinot, D., 1978. Principes d'une classification évolutive des Crustacés Décapodes Brachyoures. Bulletin Biologique de la France et de la Belgique 112, 211-292.

Gurney, R., 1939. Bibliography of the larvae of the decapod Crustacea. Ray Society, London.

Gurney, R., 1942. Larvae of decapod Crustacea. Printed for the Ray Society, sold by B. Quaritch, London.

Haeckel, E., 1866. Generelle Morphologie der Organismen: allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformirte Descendenz-Theorie. G. Reimer.

Hanstrom, B., 1928. Vergleichende Anatomie des Nervensystems der wirbellosen Tiere unter Berücksichtigung seiner Funktion. Springer, Berlin, Heidelberg.

Hanström, B., 1947. The brain, the sense organs, and the incretory organs of the head in the Crustacea Malacostraca. C.W.K. Gleerup, Lund.

Harzsch, S., 2006. Neurophylogeny: Architecture of the nervous system and a fresh view on arthropod phyologeny. Integrative and Comparative Biology 46, 162-194.

Harzsch, S., 2007. The architecture of the nervous system provides important characters for phylogenetic reconstructions: examples from the Arthropoda. Species, Phylogeny and Evolution 1, 33-57.

Harzsch, S., Dawirs, R., 1993. On the morphology of the central nervous system in larval stages *Carcinus maenas* L. (Decapoda, Brachyura). Helgoland Marine Research 47, 61-79.

Harzsch, S., Dawirs, R., 1994. Neurogenesis in larval stages of the spider crab Hyas araneus (Decapoda, Brachyura): proliferation of neuroblasts in the ventral nerve cord. Roux's archives of developmental biology 204, 93-100.

Helluy, S., Sandeman, R., Beltz, B., Sandeman, D., 1993. Comparative brain ontogeny of the crayfish and clawed lobster: Implications of direct and larval development. The Journal of Comparative Neurology 335, 343-354.

Hennig, W., 1966. Phylogenetic systematics. Univ. of Illinois Press: Urbana, Chicago & London, 263.

Hessler, R.R., 1983. A defense of the caridoid facies; wherein the early evolution of the Eumalacostraca is discussed. Crustacean issues 1, 145-164.

Hessler, R.R., Watling, L., 1999. Les peracarides: un groupe controverse, In: Forest, J. (Ed.), Traite de zoologie. Anatomie, systematique, biologie. Memoires de l'lnstitut Oceanographique, Monaco, pp. 1-10.

Holmgren, N.F., 1916. Zur vergleichenden Anatomie des Gehirns von Polychaeten, Onychophoren, Xiphosuren, Arachniden, Crustaceen, Myriapoden und Insekten: Vorstudien zu einer phylogenie der anthropoden. Kungl. Svenska Vetenskapsakademiens Handligar.

Holthuis, L., 1993. The recent genera of the caridean and stenopodidean shrimps (Crustacea, Decapoda), with an appendix on the order Amphionidacea. Leiden: National Naturhistorisches Museum, 328 pp.

Huxley, T.H., 1884. The crayfish: an introduction to the study of zoology. Kegan Paul Trench.

Ingle, R., 1992. Larval stages of Northeastern Atlantic crabs. An illustrated key., 1 ed, London.

Jenner, R.A., Dhubhghaill, C.N., Ferla, M.P., Wills, M.A., 2009. Eumalacostracan phylogeny and total evidence: limitations of the usual suspects. Bmc Evolutionary Biology 9.

Johnson, W.S., Stevens, M., Watling, L., 2001. Reproduction and development of marine peracaridans, Advances in Marine Biology. Academic Press, pp. 105-260.

Kaplan, P., 2001. Morphology and Disparity through Time, eLS. John Wiley & Sons, Ltd.

Kumlu, M., Jones, D.A., 1995. Feeding and digestion in the caridean shrimp larva of *Palaemon elegans* Rathke and *Macrobrachium rosenbergii* (De Man) (Crustacea: Palaemonidae) on live and artificial diets. Aquaculture Nutrition 1, 3-12.

Lang, C., Krapp, T., Melzer, R.R., 2007. Postembryonal development in Caprellidae: SEM description and comparison of ready-to-hatch juveniles and adults of two Mediterranean skeleton shrimps (Crustacea: Amphipoda). Zootaxa 1605, 1-32.

LeSage, L., 1984. Immature stages of canadian *Neochlamismus karren* (COLEOPTERA: CHRYSOMELIDAE). The Canadian Entomologist 116, 383-409.

Li, C.P., De Grave, S., Chan, T.-Y., Lei, H.C., Chu, K.H., 2011. Molecular systematics of caridean shrimps based on five nuclear genes: implications for superfamily classification. Zoologischer Anzeiger-A Journal of Comparative Zoology 250, 270-279.

Linnaeus, C., 1767. Systema naturae, ed. 12, vol. 2. Salvii, Holmiae.

Maria Landeira, J., Lozano-Soldevilla, F., Almansa, E., Ignacio Gonzalez-Gordillo, J., 2010. Early larval morphology of the armed nylon shrimp *Heterocarpus ensifer ensifer* A. Milne-Edwards, 1881 (Decapoda, Caridea, Pandalidae) from laboratory culture. Zootaxa, 1-14.

Martin, J.W., Davis, G.E., 2001. An updated classification of the recent Crustacea. Natural History Museum of Los Angeles County Science Series, i.

Masuko, K., 1986. Motor innervation and proprioceptors of the mouthparts in the worker honey bee, Apis mellifera. I. Mandibular nerve. Journal of Morphology 188, 51-67.

Mayer, G., Haug, J.T., Maas, A., Waloszek, D., 2013. Functional aspects of the gammaridean mandibles with special reference to the lacinia mobilis (Crustacea, Amphipoda). Zoologischer Anzeiger 252, 536-547.

McIver, S.B., 1975. Structure of Cuticular Mechanoreceptors of Arthropods. Annual Review of Entomology 20, 381-397.

McKinney, M.L., McNamara, K.J., 1991. Heterochrony: the evolution of ontogeny. Plenum Press.

McNamara, K.J., 1986. A Guide to the Nomenclature of Heterochrony. Journal of Paleontology 60, 4-13.

McNamara, K.J., McKinney, M.L., 2005. Heterochrony, disparity, and macroevolution. Paleobiology 31, 17-26.

Mekhanikova, I.V., 2010. Morphology of mandible and lateralia in six endemic amphipods (Amphipoda, Gammaridea) from Lake Baikal, in relation to feeding. Crustaceana 83, 865.

Meyer, R., Lehmann, T., Melzer, R.R., Geiselbrecht, H., 2014. Morphology of the first zoeal stage of the Mediterranean bumblebee shrimp *Gnathophyllum elegans* studied with both light microscopy and scanning EM. Journal of the Marine Biological Association of the United Kingdom 94, 151-158.

Meyer, R., Wehrtmann, I.S., Melzer, R.R., 2006. Morphology of the first zoeal stage of *Portunus acuminatus* Stimpson, 1871 (Decapoda : Portunidae : Portuninae) reared in the laboratory. Scientia Marina 70, 261-270.

Milne-Edwards, H., 1835. Observations sur les changements de forme que les Crustcés éprouvent dans le jeune age., Paris.

Ng, P.K., Guinot, D., Davie, P.J., 2008. Systema Brachyurorum: Part I. An annotated checklist of extant brachyuran crabs of the world. The Raffles Bulletin of Zoology 17.

Nicastro, D., Melzer, R.R., Hruschka, H., Smola, U., 1998. Evolution of small sense organs: sensilla on the larval antennae traced back to the origin of the Diptera. Naturwissenschaften 85, 501-505.

Ong, J., 1969. The fine structure of the mandibular sensory receptors in the brackish water calanoid copepod *Gladioferens pectinatus* (Brady). Zeitschrift für Zellforschung und Mikroskopische Anatomie 97, 178-195.

Pires, A.M.S., 1987. *Potiicoara brasiliensis*: a new genus and species of Spelaeogriphacea (Crustacea: Peracarida) from Brazil with a phylogenetic analysis of the Peracarida. Journal of Natural History 21, 225-238.

Poore, G., 2005. Peracarida: monophyly, relationships and evolutionary success. Nauplius 13, 1-27.

Porter, M.L., Perez-Losada, M., Crandall, K.A., 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. Molecular Phylogenetics and Evolution 37, 355-369.

Remerie, T., Calderon, J., Deprez, T., Mees, J., Vanfleteren, J., Vanreusel, A., Vierstraete, A., Vincx, M., Wittmann, K., Wooldridge, T., 2004. Phylogenetic relationships within the Mysidae (Crustacea, Peracarida, Mysida) based on nuclear 18S ribosomal RNA sequences. Molecular Phylogenetics and Evolution 32, 770-777.

Rice, A., 1979. A plea for improved standards in descriptions of crab zoeae. Crustaceana 37, 214-218.

Rice, A.L., 1964. The metamorphosis of a species of Homola (Crustacea Decapoda: Dromiacea). Bull Mar Sci Gulf Caribbean 14, 221-238.

Rice, A.L., 1975. The 1st zoeal stages of *Cancer Pagurus*, *Pinnotheres pisum* and *Macrophthalmus depressus* (Crustacea, Decapoda, Brachyura). Bulletin of the British Museum (Natural History) Zoology 28, 237-247.

Rice, A.L., 1980a. The 1st zoeal stage of *Ebalia nux* A. Milne edwards 1883, with a discussion of the zoeal characters of Leucosiidae. Journal of Natural History 14, 331-337.

Rice, A.L., 1980b. Crab zoeal morphology and its bearing on the classification of the Brachyura. The Transactions of the Zoological Society of London 35, 271-372.

Rice, A.L., Ingle, R.W., Allen, E., 1970. Larval development of the sponge crab, *Dromia personata* (L) (Crustacea, Decapoda, Dromiidae), reared in laboratory. Vie Et Milieu Serie a-Biologie Marine 21, 223-&.

Richter, S., Edgecombe, G.D., Wilson, G.D.F., 2002. The lacinia mobilis and Similar Structures – a Valuable Character in Arthropod Phylogenetics? Zoologischer Anzeiger - A Journal of Comparative Zoology 241, 339-361.

Richter, S., Kornicker, L.S., 2006. The mandibles of a halocyprid ostracode (Halocypridina : Halocypridae) - A new record of mandibular gnathal edges with a "laconia mobilis". Journal of Crustacean Biology 26, 113-118.

Richter, S., Scholtz, G., 2001. Phylogenetic analysis of the Malacostraca (Crustacea). Journal of Zoological Systematics and Evolutionary Research 39, 113-136.

Robles, R., Tudge, C.C., Dworschak, P.C., Poore, G.C., Felder, D., 2009. Molecular phylogeny of the Thalassinidea based on nuclear and mitochondrial genes. Decapod crustacean phylogenetics, 309-326.

Rota-Stabelli, O., Campbell, L., Brinkmann, H., Edgecombe, G.D., Longhorn, S.J., Peterson, K.J., Pisani, D., Philippe, H., Telford, M.J., 2011. A congruent solution to arthropod phylogeny: phylogenomics, microRNAs and morphology support monophyletic Mandibulata. Proceedings of the Royal Society B: Biological Sciences 278, 298-306.

Rotllant, G., Charmantier-Daures, M., De Kleijn, D., Charmantier, G., Van Herp, F., 1995. Ontogeny of neuroendocrine centers in the eyestalk of *Homarus gammarus* embryos: an anatomical and hormonal approach. Invertebrate Reproduction & Development 27, 233-245.

Rotllant, G., Charmantier-Daures, M., Trilles, J., Charmantier, G., 1994. Ontogeny of the sinus gland and of the organ of Bellonci in larvae and postlarvae of the European lobster *Homarus gammarus*. Invertebrate Reproduction & Development 26, 13-22.

Rotllant, G., De Kleijn, D., Charmantier-Daures, M., Charmantier, G., Van Herp, F., 1993. Localization of crustacean hyperglycemic hormone (CHH) and gonad-inhibiting hormone (GIH) in the eyestalk of *Homarus gammarus* larvae by immunocytochemistry and in situ hybridization. Cell and Tissue Research 271, 507-512.

Russell, E.S., 1917. Form and function: A contribution to the history of animal morphology. John Murray Ltd., London.

Sandeman, D., Sandeman, R., Derby, C., Schmidt, M., 1992. Morphology of the Brain of Crayfish, Crabs, and Spiny Lobsters: A Common Nomenclature for Homologous Structures. Biological Bulletin 183, 304-326.

Sandeman, D., Scholtz, G., Sandeman, R., 1993. Brain Evolution in Decapod Crustacea. Journal of Experimental Zoology 265, 112 - 133.

Schminke, H.K., 2007. Crustacea: Decapoda, In: Westheide, W., Rieger, R. (Eds.), Spezielle Zoologie. Teil 1: einzeller und Wirbellose Tiere, 2 ed. Spektrum Akademischer Verlag, Berlin, pp. 611-619.

Schram, F.R., 1969. Polyphyly in the Eumalacostraca? Crustaceana, 243-250.

Schram, F.R., 1981. On the Classification of Eumalacostraca. Journal of Crustacean Biology 1, 1-10.

Selverston, A.I., Moulins, M., 1987. The crustacean stomatogastric system: a model for the study of central nervous systems. Springer, Berlin.

Slabber, M., 1778. Natuurkundige verlustingen, behelzende microscopiese waarnemingen van in- en uitlandse water en land-dieren. Bosch, Haarlem.

Smith, K.K., 2001. Heterochrony revisited: the evolution of developmental sequences. Biological Journal of the Linnean Society 73, 169-186.

Števčić, Z., 2005. The reclassification of brachyuran crabs (Crustacea: Decapoda: Brachyura). Natura Croatica 14, 1-159.

Stoffolano, J.G., Yin, L.R.S., 1983. Comparative study of the mouthparts and associated sensilla of adult male and female *Tabanus nigrovittatus* (DIPTERA: TABANIDAE). Journal of Medical Entomology 20, 11-32.

Strausfeld, N.J., 2012. Arthropod Brains: Evolution, Functional Elegance, And Historical Significance. Belknap Press Of Harvard University Press, p. 848.

Sudhaus, W., 2007. Die Notwendigkeit morphologischer Analysen zur Rekonstruktion der Stammesgeschichte. Species, Phylogeny and Evolution 1, 17-32.

Thompson, J., 1828. On the Metamorphoses of the Crustacea, and on Zoea, exposing their singular structure and demonstrating that they are not, as has been supposed, a peculiar Genus, but the Larva of Crustacea!!, Thompson, J.V. (1828-1834) Zoological Researches, and illustrations; or, natural history of nondescript or imperfectly known animals, in a series of memoirs, illustrated by numerous figures., pp. pp. 1-11, plates I-II.

Tsang, L.M., Ma, K.Y., Ahyong, S.T., Chan, T.Y., Chu, K.H., 2008. Phylogeny of Decapoda using two nuclear protein-coding genes: Origin and evolution of the Reptantia. Molecular Phylogenetics and Evolution 48, 359-368.

Tyson, G.E., Sullivan, M.L., 1981. A scanning electron microscopic study of the molar surfaces of the mandibles of the brine shrimp (Cl. Branchiopoda: O. Anostraca). Journal of Morphology 170, 239-251.

Valdecasas, A.G., Williams, D., Wheeler, Q.D., 2008. 'Integrative taxonomy'then and now: a response to Dayrat (2005). Biological Journal of the Linnean Society 93, 211-216.

Wagner, H., 1994. A monographic review of the Thermosbaenacea (Crustacea: Peracarida). Nationaal Natuurhistorisch Museum, Leiden.

Watling, L., 1981. An Alternative Phylogeny of Peracarid Crustaceans. Journal of Crustacean Biology 1, 201-210.

Westwood, J.O., 1835. On the Supposed Existence of Metamorphoses in the Crustacea. Philosophical Transactions of the Royal Society of London 125, 311-328.

Wetzer, R., 2002. Mitochondrial genes and isopod phylogeny (Peracarida: Isopoda). Journal of Crustacean Biology 22, 1-14.

Wheeler, Q.D., 2008. Undisciplined thinking: morphology and Hennig's unfinished revolution. Systematic Entomology 33, 2-7.

Whitehead, A.T., Larsen, J.R., 1976. Ultrastructure of the contact chemoreceptors of *Apis mellifera* L. (Hymenoptera: Apidae). International Journal of Insect Morphology and Embryology 5, 301-315.

Will, K.W., Mishler, B.D., Wheeler, Q.D., 2005. The perils of DNA barcoding and the need for integrative taxonomy. Systematic Biology 54, 844-851.

Williamson, D.I., 1957. Crustacea Decapoda: Larvae. V. Caridea: Family Hippolytidae. Conseil international pour l'exploration de la mer.

Williamson, D.I., 1969. Names of Larvae in the Decapoda and Euphausiacea. Crustaceana 16, 210-213.

Williamson, D.I., 1976. Larvae of Stenopodidea (CRUSTACEA, DECAPODA) from indian ocean. Journal of Natural History 10, 497-509.

Williamson, D.I., 1982. Larval morphology and diversity, In: Abele, L.G. (Ed.), The biology of Crustacea: 2. Embryology, morphology and genetics. The biology of Crustacea. Academic Press, New York, pp. 43-110.

Wills, M.A., Jenner, R.A., Dhubhghaill, C.N., 2009. Eumalacostracan Evolution: Conflict between Three Sources of Data. Arthropod Systematics & Phylogeny 67, 71-90.

Wilson, G.D., 2009. The phylogenetic position of the Isopoda in the Peracarida (Crustacea: Malacostraca). Arthropod Systematics and Phylogeny 67, 159-198.

Zacharuk, R.Y., Albert, P.J., 1978. Ultrastructure and function of scolopophorous sensilla in the mandible of an elaterid larva (Coleoptera). Canadian Journal of Zoology 56, 246-259.

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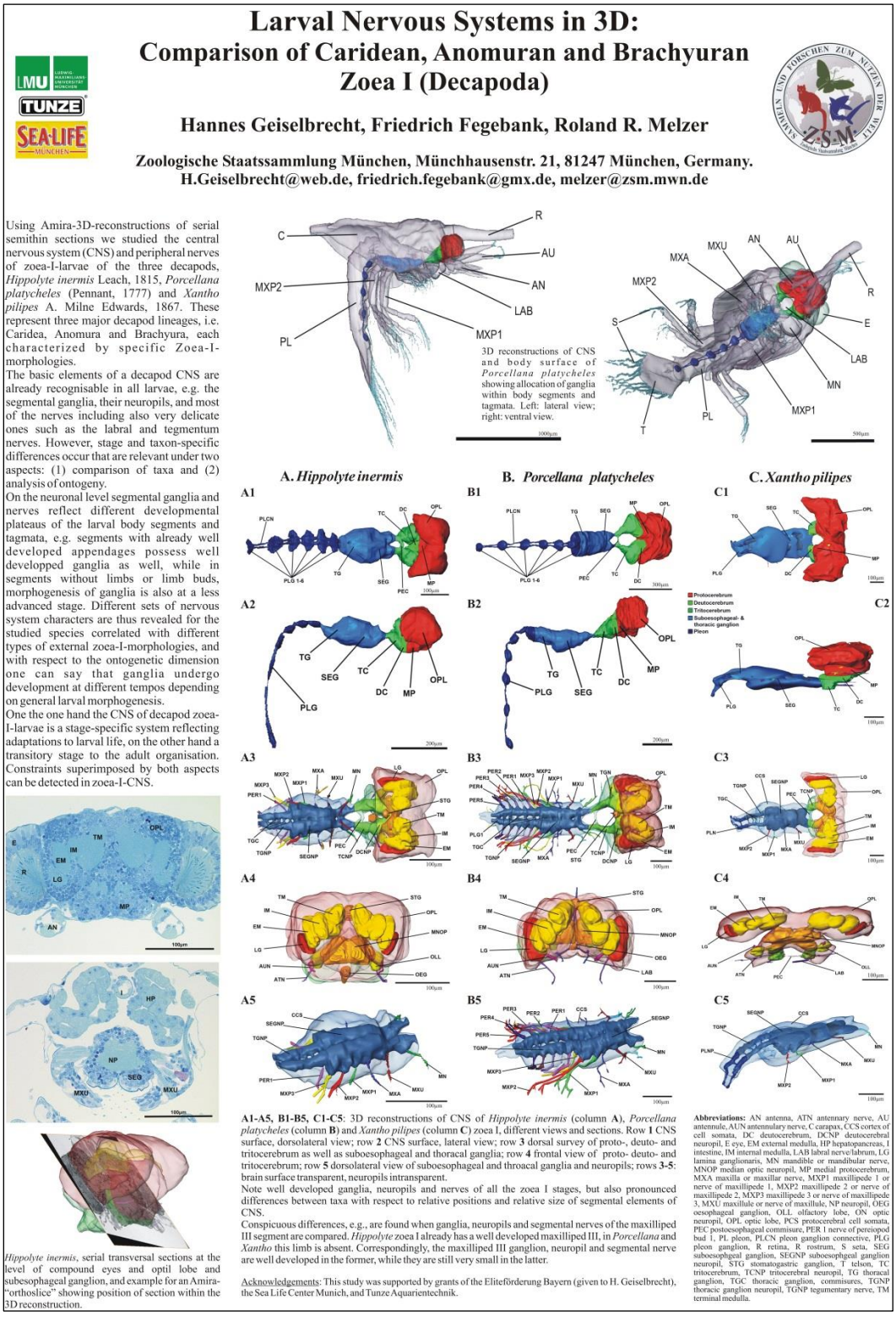
Zuletzt möchte ich mich ganz besonders bei meinen Eltern für die verständnisvolle und geduldige Unterstützung bedanken und bei meiner Frau Susi für ein immer offenes Ohr und viel Verständnis, für die Geduld, die Motivation und für den Rückhalt den Du mir gegeben hast.

12. Appendix: Relevant posters

Geiselbrecht, H., Fegebank, F. & Melzer, R.R. (2011): Larval nervous system in 3D: Comparison of Caridean, Anomuran and Brachyuran Zoea I (DECAPODA). 2nd International Congress on Invertebrate Morphology (ICIM),19. – 22. Juni 2011, Boston



Larval Nervous Systems in 3D: Comparison of Caridean, Anomuran and Brachyuran Zoea I (Decapoda)

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Using Amira-3D-reconstructions of serial semithin sections we studied the central nervous system (CNS) and peripheral nerves of zoea-I-larvae of the three decapods, *Hippolyte inermis* Leach, 1815, *Porcellana platycheles* (Pennant, 1777) and *Xantho pilipes* A. Milne Edwards, 1867. These represent three major decapod lineages, i.e. Caridea, Anomura and Brachyura, each characterized by specific Zoea-I-morphologies.

The basic elements of a decapod CNS are already recognisable in all larvae, e.g. the segmental ganglia, their neuropils, and most of the nerves including also very delicate ones such as the labral and tegmentum nerves. However, stage and taxon-specific differences occur that are relevant under two aspects: (1) comparison of taxa and (2) analysis of ontogeny.

On the neuronal level segmental ganglia and nerves reflect different developmental plateaus of the larval body segments and tagmata, e.g. segments with already well developed appendages possess well developped ganglia as well, while in segments without limbs or limb buds, morphogenesis of ganglia is also at a less advanced stage. Different sets of nervous system characters are thus revealed for the studied species correlated with different types of external zoea-I-morphologies, and with respect to the ontogenetic dimension one can say that ganglia undergo development at different tempos depending on general larval morphogenesis.

One the one hand the CNS of decapod zoea-I-larvae is a stage-specific system reflecting adaptations to larval life, on the other hand a transitory stage to the adult organisation. Constraints superimposed by both aspects can be detected in zoea-I-CNS.

Hippolyte inermis, serial transversal sections at the level of compound eyes and optil lobe and subesophageal ganglion, and example for an Amira-"orthoslice" showing position of section within the 3D reconstruction.

3D reconstructions of CNS and body surface of *Porcellana platycheles* showing allocation of ganglia within body segments and tagmata. Left: lateral view; right: ventral view.

A1-A5, B1-B5, C1-C5: 3D reconstructions of CNS of *Hippolyte inermis* (column **A**), *Porcellana platycheles* (column **B**) and *Xantho pilipes* (column **C**) zoea I, different views and sections. Row **1** CNS surface, dorsolateral view; row **2** CNS surface, lateral view; row **3** dorsal survey of proto-, deuto- and tritocerebrum as well as suboesophageal and thoracal ganglia; row **4** frontal view of proto- deuto- and tritocerebrum; row **5** dorsolateral view of suboesophageal and throacal ganglia and neuropils; rows **3-5**: brain surface transparent, neuropils intransparent.

Note well developed ganglia, neuropils and nerves of all the zoea I stages, but also pronounced differences between taxa with respect to relative positions and relative size of segmental elements of CNS.

Conspicuous differences, e.g., are found when ganglia, neuropils and segmental nerves of the maxilliped III segment are compared. *Hippolyte* zoea I already has a well developed maxilliped III, in *Porcellana* and *Xantho* this limb is absent. Correspondingly, the maxilliped III ganglion, neuropil and segmental nerve are well developed in the former, while they are still very small in the latter.

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Abbreviations: AN antenna, ATN antennary nerve, AU antennule, AUN antennulary nerve, C carapax, CCS cortex of cell somata, DC deutocerebrum, DCNP deutocerebral neuropil, E eye, EM external medulla, HP hepatopancreas, I intestine, IM internal medulla, LAB labral nerve/labrum, LG lamina ganglionaris, MN mandible or mandibular nerve, MNOP median optic neuropil, MP medial protocerebrum, MXA maxilla or maxillar nerve, MXP1 maxillipede 1 or nerve of maxillipede 1, MXP2 maxillipede 2 or nerve of maxillipede 2, MXP3 maxillipede 3 or nerve of maxillipede 3, MXU maxillule or nerve of maxillule, NP neuropil, OEG oesophageal ganglion, OLL olfactory lobe, ON optic neuropil, OPL optic lobe, PCS protocerebral cell somata, PEC postoesophageal commisure, PER 1 nerve of pereiopod bud 1, PL pleon, PLCN pleon ganglion connective, PLG pleon ganglion, R retina, R rostrum, S seta, SEG subesophgeal ganglion, SEGNP subesophgeal ganglion neuropil, STG stomatogastric ganglion, T telson, TC tritocerebrum, TCNP tritocerebral neuropil, TG thoracal ganglion, TGC thoracic ganglion, commisures, TGNP thoracic ganglion neuropil, TGNP tegumentary nerve, TM terminal medulla.

Batel, A., Melzer, R.R. & Geiselbrecht, H. (2012): Heterochrony in mandible development. SEM analysis of five zoea stages in two carideans (DECAPODA, PALAEMONIDAE). TCS-Summer Meeting, 03. – 07. Juni 2012, Athen

Heterochrony in mandible development
SEM analysis of five zoea stages of two carideans (DECAPODA, PALAEMONIDAE)

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INTRODUCTION

This study analyzed the mandible morphology of two different caridean zoea larvae, *Palaemon elegans* (Rathke, 1837) and *Macrobrachium amazonicum* (Heller, 1862). Focus was set on taxon specific features and the development of mandibles especially comparing *P. elegans* and *M. amazonicum*. Larvae of *P. elegans* feed in stage I in contrast to *M. amazonicum*, where larvae feed from stage II on (Anger & Hayd 2010).

Thus, a taxon and development analysis with different feeding habits was conducted.

RESULTS

Comparing the mandible development of *P. elegans* and *M. amazonicum*, a retarded morphogenesis was observed for *M. amazonicum*. Main development of both species was an increase in sizes of gnathal edges, pars incisivus, lacinia mobilis and the sizes of the small spines at pars molaris as well as appearance of additional submarginal spines. The mandibles of *M. amazonicum* zoea I had only small appendages, but due to an increased growth rate the mandibles of zoea stage V reached similar dimensions as mandibles of *P. elegans* zoea stage V.

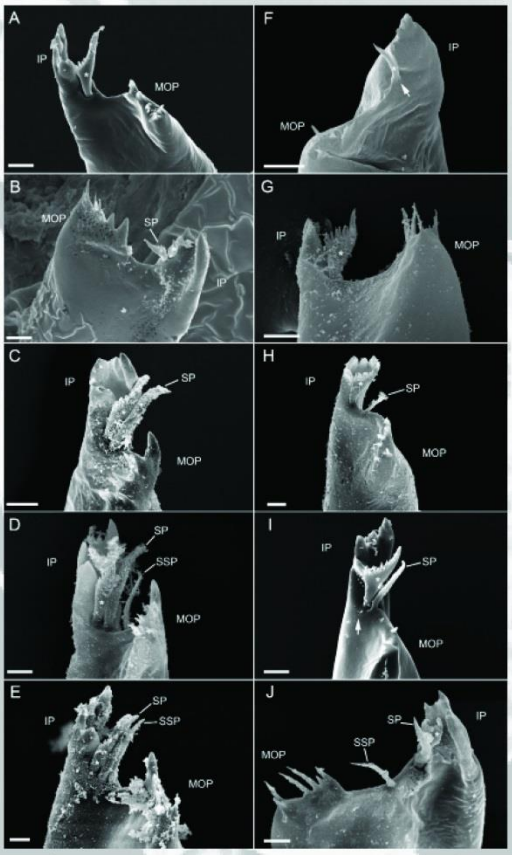

FIG. 1 (left): SEM pictures of dissected left mandibles, comparing morphological development of *P. elegans* and *M. amzonicum*. **A-E**: *P. elegans*, left mandible, stage I-V. **F-J**: *M. amazonicum*, left mandible, stage I-V. Note the submarginal spine development and increase in length of appendages. IP, incisor process; MOP, molar process; SP, submarginal spine; SSP, second submarginal spine; asterisk, lacinia mobilis; arrows, pores. Bars 10 µm

DISCUSSION

The retarded morphogenesis of *M. amazonicum* is interpreted as heterochrony, an altered timing in phenotypic development compared to close relatives or ancestors (Haeckel 1866). Due to the non-utilization of mandibles in zoea stage I, the development of *M. amazonicum* is compared to *P. elegans* retarded. Still, in later stages *M. amazonicum* showed the same developmental pattern and also the taxon specific features as *P. elegans*.

This supports the hypothesis mentioned by Geiselbrecht & Melzer (2010), that there exists a phylogenetically relevant signal on early larval mandibles. The evolutionary ground pattern is present even in species with highly aberrant feeding patterns such as in *M. amazonicum*.

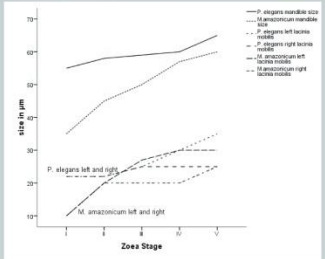

FIG. 2: Development of length of gnathal edges of mandibles and lacinia mobilis of *P. elegans* and *M. amazonicum*, stage I-V. Note the greater differences in stage I compared to stage V.

CONCLUSION

This study is an example of the importance of holomorphology, a morphological analysis including many sources of information, e.g. ontogeny. Without the identification of unequivocal developmental stages and morphogenetic hallmarks, the specific ground pattern in mandible development of Palaemonidae could be opposed. In a broader view, phylogenetic studies should, besides molecular analyses, include morphology and ontogeny.

REFERENCES

Anger, K. & Hayd, L. (2010): Feeding and growth in early larval shrimp *Macrobrachium amazonicum* from the Pantanal, southwestern Brazil. Aquat Biol 9:251-261

Geiselbrecht, H. & Melzer, R. R. (2010): Mandibles of zoea I larvae of nine decapod species: a scanning EM analysis (Crustacea, Decapoda). Spixiana 33: 27-47.

Haeckel, E. (1866): Generelle Morphologie der Organismen: Allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformierte Deszendenz-Theorie, Vol. 2. Berlin: George Reimer.

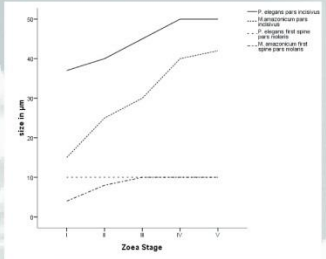

FIG. 3: Development of length of pars incisivus and first small spine at pars molaris of *P. elegans* and *M. amazonicum*, stage I-V. Note the greater difference in stage I compared to stage V.

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Geiselbrecht, H. & Melzer, R.R. (2012): The sensory apparatus of larval mandibles in *Palaemon elegans* Rathke, 1837 (DECAPODA, PALAEMONIDAE). TCS-Summer Meeting, 03. – 07. Juni 2012, Athen

The sensory apparatus of larval mandibles of *Palaemon elegans* (DECAPODA, PALAEMONIDAE)

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INTRODUCTION

The mandibles of decapod zoea-I-larvae are robustly built masticating mouthparts, equipped with several processes and spines. An external observation of these structures might suggest the absence of sensory receptors because they mostly appear inflexible and robust.

Conversely, a detailed TEM analysis of the ultrastructure now showed up to 11 sensillar cell clusters on the gnathal edges of the mandibles of the zoea-I of *Palaemon elegans*.

This study is part of a project dealing with questions about the sensory system and the homology of certain appendages, like the ‚lacinia mobilis', of decapod zoea mandibles (Geiselbrecht & Melzer 2010).

Figure 1: *Palaemon elegans* (Rathke, 1837)Zoea-I.Lateralview,(bar=0,25mm)

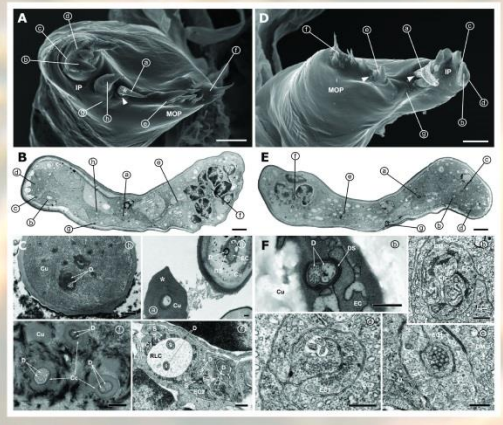
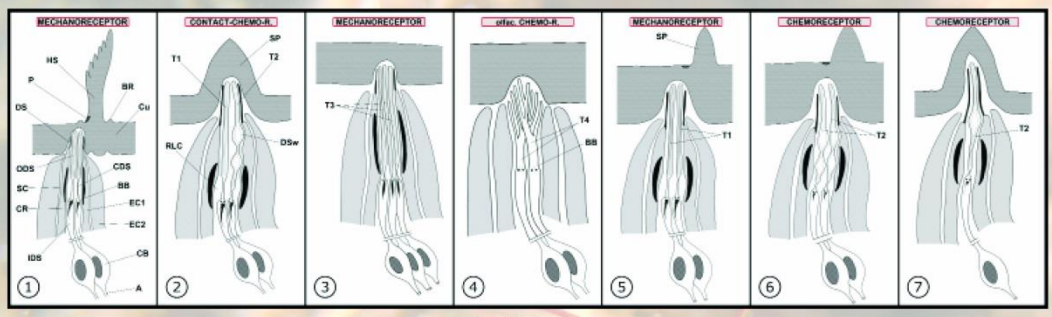


Figure 2 (right): Scanning (A, B) and transmission (B, C, E, F) electron micrographs showing morphology of right (A, B, C) and left (D, E, F) mandible, sensillar arrangement and ultrastructure. **A, D**: Inner views of mandibles showing sensillar spines, pores (arrowheads) and ‚lacinia mobilis' (asterisks) (bars=10µm). **B, E**: Sagittal sections of mandibles showing position of sensilla (bars=4µm). **C, F**: Dendritic outer segments of sensilla in different sections (bars=0,5µm). Small encircled letters mark congruent sensilla or groups of sensilla for right and left mandible. Cc, cuticular canal; CR, ciliary rootlet; Cu, cuticle; D, dendrite; DS, dentritic sheath; EC, epithelial cell; EC1, inner enveloping cell; EC2, second enveloping cell; IP, incisor process; MOP, molar process; RLC, receptor lymph cavity; S, scolopale.

Figure 3 (below): Schematic drawings showing the 7 different types of sensilla (1-7). A, Axon; BB, basal body; BR, basal ring; CB, cell body; CDS, ciliary dendritic segment; CR, ciliary rootlet; Cu, cuticle; DS, dendritic sheath; DSw, dendritic swelling; EC1, inner enveloping cell; EC2, second enveloping cell; HS, hair shaft; IDS, inner dendritic segment; ODS, outer dendritic segment; P, pore; RLC, receptor lymph cavity; SC, scolopale; SP, spine; T1-T4, dendrite type 1-4



RESULTS

- **11** sensillar cell clusters on the left and **10** on the right mandible
- Sensilla are **(1)** either of articulated seta-like structure, i.e the 'lacinia mobilis' (Fig.4), or **(2)** are found in form of non-articulated solid spines or **(3)** do not show special external cuticular structures
- All sensory units show the typical features of arthropod sensilla (McIver 1975)
- Innervating dendrites can be assigned to four different types (Figure 3, T1-T4)
- On ultrastructural level there are **7** different types of sensilla. (Figure 2, 1-7)

DISCUSSION

- Larval mandibles are equipped with a relatively high number of sensory structures
- Different structural features of the 7 sensilla types reflect a diversity in the mechanisms of adequate stimulation.
- Using the concept of modality specific structures (e.g. Schmidt and Gnatzy 1984; Altner, Hatt et al. 1986) mandibular sensilla can be assigned to their potential function: Mechanoreception, Bimodal Contact-chemoreception and Chemoreception.
- Mandibles as the main masticating organs of decapod larvae (Factor 1989) need sensory equipment to check food quality and mechanical forces. Their function also provoques the robustness of the mandibles and brings about uncommon external cuticular structures of sensilla.

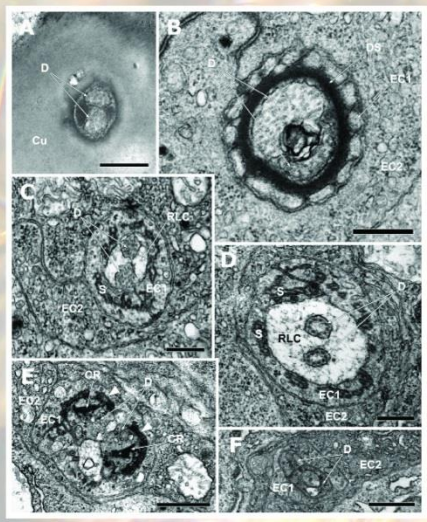


Figure 4 (left): Transmission electron micrographs showing ultrastructural features of dendrites innervating the ‚lacinia mobilis' of the right mandible. **A**: Termination region of the dendrites near the hair base. **B**: Two ODS proximal to hair base. **C**: ODS distal to transitional region. **D**: Ciliary dendritic segments. **E**: Transitional region with ciliary rootlets of dendrites and desmosomal junctions (arrowheads) with inner enveloping cell. **F**: Inner dendritic segments proximal to transitional region. Abbreviations as in Figure 2.

REFERENCES

Altner, H., H. Hatt, et al. (1986). Structural and functional properties of the mechanoreceptors and chemoreceptors in the anterior oesophageal sensilla of the crayfish, *Astacus astacus*. Cell and Tissue Research 244 (3): 537-547.

Factor, J.R. (1989). Development of the feeding apparatus in decapod crustaceans; published in: Functional morphology of feeding and grooming in crustacean; Crustacean Issues 6, ed. By B.E. Felgenhauer - Rotterdam A.A. Balkema. 175-184.

Geiselbrecht, H. and R. R. Melzer (2010). Mandibles of zoea-I-larvae of nine decapod species. A scanningEM analysis. Spixiana 33 (1): 27-47.

McIver, S. B. (1975). Structure of Cuticular Mechanoreceptors of Arthropods. Annual Review of Entomology 20 (1): 381-397.

Schmidt, M. and W. Gnatzy (1984). Are the funnel-canal organs the 'campaniform sensilla' of the shore crab, *Carcinus maenas* (Decapoda, Crustacea)? II. Ultrastructure. Cell and Tissue Research 237 (1): 81-93.

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