

Approaching the evolution of parasitism in a historical context: isopod crustaceans as an example

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EIDESSTATTLICHE ERKLÄRUNG

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

Serita van der Wal

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ERKLÄRUNG

Hiermit erkläre ich, dass diese Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist. Ich erkläre, dass ich mich nicht anderweitig (mit oder ohne Erfolg) einer Doktorprüfung unterzogen habe.

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LIST OF PUBLICATIONS INCORPORATED IN THIS DISSERTATION

- I. Van der Wal S & Haug JT. 2019. Letter to the editor referencing “The apparent kleptoparasitism in fish-parasitic gnathiid isopods” 10.1007/s00436-018-6152-8. *Parasitology Research*, 118(5), 1679–1682. <https://doi.org/10.1007/s00436-019-06281-2>
- II. Schädel M, Pazinato, PG, Van der Wal, S & Haug JT. 2019. A fossil tanaidacean crustacean from the Middle Jurassic of southern Germany. *Palaeodiversity*, 12(1), 13–30. <https://doi.org/10.18476/pale.v12.a2>
- III. Van der Wal S & Haug JT. 2020. Shape of attachment structures in parasitic isopodan crustaceans: the influence of attachment site and ontogeny. *PeerJ*, 8:e9181. <https://doi.org/10.7717/peerj.9181>
- IV. Van der Wal S, Schädel M, Ekrt B & Haug JT. 2021. Description and ontogeny of a 40-million-year-old parasitic isopodan crustacean: *Parvucymoides dvorakorum* gen. et sp. nov. *PeerJ*, 9:e12317. <https://doi.org/10.7717/peerj.12317>
- V. Van der Wal S, Haug JT. 2023. Reconstructing the life cycle of the isopodan group Aegidae with morphological descriptions and the importance of immature stages. *Nauplius*, 31. <https://doi.org/10.1590/2358-2936e2023007>

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- I. Haug GT, Haug C, Van der Wal S, Müller P, Haug JT. 2021. Split-footed lacewings declined over time: indications from the morphological diversity of their antlion-like larvae. *Paläontologische Zeitschrift*, 1–22. <https://doi.org/10.1007/s12542-021-00550-1>
- II. Haug JT, Haug GT, Zippel A, Van der Wal S, Müller P, Gröhn C, Wunderlich J, Hoffeins C, Hoffeins H-W, Haug, C. 2021. Changes in the morphological diversity of larvae of lance lacewings, mantis lacewings and their closer relatives over 100 million years. *Insects*, 12(10), 860. <https://doi.org/10.3390/insects12100860>
- III. Haug C, Haug GT, Zippel A, Van Der Wal S, Haug JT. 2021. The earliest record of fossil solid-wood-borer larvae—immature beetles in 99-million-year-old Myanmar amber. *Palaeoentomology*, 4(4), 390–404. <https://doi.org/10.11646/palaeoentomology.4.4.14>
- IV. Haug JT, Van der Wal S, Groehn C, Hoffeins C, Hoffeins H, Haug C. 2022. Diversity and fossil record of larvae of three groups of lacewings with unusual ecology and functional morphology: Ithonidae, Coniopterygidae and Sisyridae. *Palaeontologia Electronica*, 25(2):a14. <https://doi.org/10.26879/1212>

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STATEMENT OF AUTHOR CONTRIBUTIONS

This thesis presents the results from research conducted between 2018 to 2022, carried out under the supervision of Prof. Dr. Joachim T. Haug, Prof. Dr. Matthias J. Starck and Prof. Dr. Roland Melzer associated with the Ludwig Maximilian University of Munich. The author's contribution is written according to the CRediT taxonomy.

- I. Van der Wal & Haug 2019. Letter to the editor referencing “The apparent kleptoparasitism in fish-parasitic gnathiid isopods” 10.1007/s00436-018-6152-8. *Parasitology Research*, 118(5), 1679-1682.
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SUMMARY

Parasites are often depicted as harmful antagonists in an ecosystem, hindering the fitness of those they rely on for survival, reproduction and dispersal. Even though this may be the case for a number of cases, parasites and parasitic associations are, and have been crucial in the evolutionary diversification and survival of species. In fact, parasitism is such a highly successful life strategy, that almost half of all multicellular species exhibit some degree of parasitism throughout its lifecycle. In addition, parasites play an essential evolutionary role in the health and sustainability of an ecosystem. By understanding their effects, ecological roles and development, we are able to better manage and describe parasitic interactions and their evolutionary trends. In this dissertation, isopod crustaceans are used as an example group to study the evolution of parasitism in a historical context. This group serves as the ideal ‘model’ group as they are quite well-known; abundant in almost all habitats on earth; have complex and varying ontogenetic developmental patterns; and exhibit extraordinary parasitic life and feeding strategies.

Comparative morphometric analyses of attachment structures of groups exhibiting various parasitic life strategies and ecological roles through development, can provide insight into the evolutionary diversification and specialisation of structures for a specific parasitic lifestyle. Including both extant and fossil specimen material from various ingroups and ontogenetic stages, allows for a more comprehensive analysis of differentiation in these structures over time and through development. As adult and immature stages often have varying ecological functions (usually correlated with feeding behaviour and level of maturity) the inclusion of the often overlooked and highly underrepresented immature stages are essential in this study.

This study aimed to provide data that can substantiate if and how attachment structure morphologies and changes in these morphologies, are derived from the specific parasitic strategies and ecological functions among ingroups of Cymothoidea. The results present and discuss in particular the morphological variation and differentiation in morphology of the distalmost part of the thoracopod, the dactylus, used to attach to a host. The variation in shape is compared between representatives of non-parasitic- temporary parasitic and permanent parasitic ingroups morphological change resulting from ecological function and feeding habit.

The results have shown that parasitic strategy and ontogeny play a role in the shape of attachment structures, with most variation seen in the curvature and thickness of the dactylus. The evolution of dactylus shape through parasitic strategies is proposed as a stepwise process where: 1. the posterior dactyli of representatives of temporary parasitic ingroups retain its resemblance to the plesiomorphic condition as seen in the non-parasitic representatives, while the anterior dactyli shapes become specialised for temporary attachment to a host. Finally, 2. the posterior dactyli of representatives of permanent parasitic groups deviate from the plesiomorphic condition and diversify along with the anterior dactyli, specialised for permanent association with a host using all attachment structures. The results additionally suggest that the attachment structures of representatives of the permanent parasitic ingroup, Epicaridea follow a similar pattern to representatives of the temporary parasitic ingroup, Aegidae, rather than that of the remaining permanent parasitic representatives of Cymothoidea.

1. INTRODUCTION

1.1. Types of parasitic associations

Parasitism is a specific association between organisms, but like most terms for ecological concepts, the use of the term parasitism and the various terms for types of parasitism, are not completely unambiguous. Leung & Poulin (2008) also mentioned that the ‘labels’ we assign to symbionts may not necessarily be accurate descriptions of the association. Descriptions of parasitism are often based on informed, but subjective, view of a scientist or researcher, which is in turn is based on their field of study; the phylogenetic group of organisms of their interest; and the circumstance in which the association was observed. Associations among organisms are ever changing and evolving, rarely exhibiting textbook behaviour that can be defined and categorised based on established concepts and terms (Leung & Poulin 2008). The terminology we have to our disposal need to be challenged and/or refined as we discover more intricate and complex organism interactions and expand our understanding of ecological, and more specifically to this study, parasitic interactions.

Terminology relating to types of parasites and parasitism, are given throughout a myriad of publications and textbooks, with most providing simple, but often ambiguous examples and characteristics. Most of these terms have been proposed by early ecologists and biologists in an attempt to group and/ or classify observations and organisms, a human-based strategy to better understand and comprehend our environment and nature. Most of these established terms for types of parasitism and types of parasites are well-understood and can be used to describe basic, well-known examples. However, the challenge of categorising these concepts, lie in the use of specialised terms of more complex, more recently discovered or evolved associations that do not quite fall into the currently established categories. Other challenges lie in the accurate interpretation of terms and/ or interactions; accurately describing observations; and accurate use of terminology with clearly outlined criteria regarding parasitic associations and types of parasites.

Perhaps the most basic and broad conceptualisation of ‘parasitism’ is the association between organisms where one organism benefits from the association (the ‘parasite’), at the expense of the other (the ‘host’) (Paracer & Ahmadjian 2000, Leung & Poulin 2008, Roberts & Janovy 2009). This association can either be between individuals of different species (interspecific) or between individuals of the same species (intraspecific). As with many other inter- or intraspecies associations, parasitism is primarily driven, but not limited to, nutrition and reproduction. The benefitting organism (the parasite) usually relies on its host’s body as a nutritional source, but can additionally depend on the host for an array of other biological or physiological needs such as optimal environmental conditions; shelter and safety from the environment and threats; transfer to a final host via an intermediate host; as well as reproduction and distribution. The degree of dependency on the host varies, as does the degree of harm done to the host. In many cases, the cost of the association to the host extends beyond pathological effects or disease (*1.1.5. The cost to the host*).

A host organism can be categorised by its role in the development and life cycle of its parasites (Toft & Karter 1990). Some parasites depend on two or more host species for the completion of their life cycles. Intermediate hosts are those that are the initial hosts for the parasite before sexual maturity is reached (Bush *et al.* 2001, Roberts & Janovy 2009, Cheng 2012). These hosts aid in the development of the parasite to the life stage where it can be released to reach the final host. Definitive (final) hosts are those in or on which the parasitic organism reaches sexual maturity (Bush *et al.* 2001, Roberts & Janovy 2009, Cheng 2012) and would spend the remainder of its life cycle, unless disturbed or removed by external factors.

In some cases, an intermediate host is not affected by the parasite(s) or shows no signs of damage or illness due to the presence of the parasite. These hosts merely serve as a transport and transfer mechanism for the parasite to reach the next host and are referred to as reservoir hosts, transport hosts or vectors (Bush *et al.* 2001, Roberts & Janovy 2009, Cheng 2012) depending on the type of pathogenic organism it carries. Various publications have proposed and revised the terminology for the categorisation of parasites. A common strategy persists, whereby parasites are grouped based on the following aspects:

1.1.1. The location of the parasite relative to the hosts' body

The location of the parasite relative to the host is perhaps the simplest and most widely used for categorising parasitic organisms. Bush *et al.* (2001) and Cheng (2012) defines an ectoparasite as a parasite that resides on the outside of a host's body and an endoparasite is a parasite that resides inside the body of a host. The ambiguity with these definitions is that they lack criteria that specify what constitutes the outside and inside of a host organism. While it might seem obvious, it is important to distinguish that ectoparasites reside on, and are most likely attached to, either the epidermis of a host, or any other outer layer of tissue i.e., hair, fur, scales etc. These include parasites that reside in a body cavity that is lined with epidermis, or is superficially embedded in the body i.e., inside the mouth, inside the gills, superficially embedded in the body surface. Endoparasites are therefore those parasites that reside within the body tissue of a host i.e., intestinal and inter-cellular inhabiting organisms or inside the organs of a host.

1.1.2. The duration of the association

Due to their location within a final host, endoparasites are usually the most persistent (Paracer & Ahmadjian 2000), spending the entire duration of its life cycle in association with the same host organism. Although not exclusive to endoparasites, these are categorised as permanent parasites (Roberts & Janovy 2009), as they are 'permanently' associated with, and dependant on the host, without the need to be transferred to another. Ectoparasites are usually more mobile and able to move more freely between hosts or between hosts and the environment. Some ectoparasites persist in their association with a single host, until the host dies, or external conditions causes the parasitic organism to release/ detach from the host, in which case the parasite would immediately search for another host. Other ectoparasites are intermittent in their association with a host, only attaching to a host when nutrition is needed and release into the environment once fed. Such parasites that only attach or interact with a host in intervals and are able to survive for some periods of time away from a host, are referred to as temporary parasites.

Temporary parasites are sometimes referred to as ‘micropredators’ and these terms are often used interchangeably. In fact, ‘micro’ in ‘micropredators’ are often viewed with different criteria depending on the author’s point of reference. Roberts & Janovy (2009) defines a micropredator as a predator that is small in body size, typically requiring a microscope to be visible to the human eye. In this definition, the prefix ‘micro’ is used in the same manner as ‘micro’ in for example, ‘microplankton’, referring to the small size of organisms.

In contrast, Bush *et al.* (2001) distinguish between a predator and an micropredator on the bases of whether or not the prey is always killed by the predator. In the latter publication, authors define a micropredator as an aggressor that attacks prey, but typically does not kill it, while a predator is an aggressor that attacks prey and always kills it. Thus, in this instance, the prefix ‘micro’ refers to the ‘less fatal’ nature of the interaction between the predator and the prey. The term micropredator has caused much confusion and disagreement as its definition is rather ambiguous and unspecific, hindering the accurate description of the lifestyle of the organism observed, leading to larger ecological implications. Roberts & Janovy (2009) provides a distinction between these concepts, stating that parasites are typically not directly fatal to a host; has only a single host at a time (at least during each stage of its life cycle); and is dependent on the association with a compatible host. A predator is typically the direct killer of its prey; has numerous sources and instances of prey; and is not dependant on a ‘compatible’ species of prey.

1.1.3. Specificity

Parasites have evolved diverse strategies to ensure a successful parasitic association and reproduction, to compensate for the availability and abundance of host species. Some parasites only infest a small range or specific groups of species, while others are only found to infest a single host species. The latter are referred to as specialists that have the advantage of highly specialised physiology, pathology and morphology that enable a consistently successful association with a specific host species. Generalists are less picky and would feed on a wide range of species or groups of species. Parasitic associations in which the host is a parasite of another organism, is referred to as hyperparasitism (Cheng 2012).

1.1.4. The degree of dependence on the association

Most parasitic organisms can be categorised as obligate parasites – highly specialised organisms that are completely dependent on the association with a host species for its survival and/ or reproduction (Paracer & Ahmadjian 2000, Cheng 2012). Without the association, at least for some duration of time, the species cannot survive. Obligate parasites can include all types of parasites mentioned above, even temporary parasites – as these still need a temporary association to a host for the benefit of nutrition for some duration of time.

Facultative parasites are individuals or populations of species that may, or may not, exhibit parasitic behaviour depending on the circumstantial contact with a host. In other words, some individuals or populations depend on another species for survival or reproduction, while other individuals or populations do not form any such association throughout its life cycle (Paracer & Ahmadjian 2000, Roberts & Janovy 2009, Cheng 2012). For example, some amoebas and

nematodes are non-parasitic, but can form a highly pathogenic parasitic association with humans if ingested or entered through a wound (Roberts & Janovy 2009). Similarly, some species of algae form a parasitic association with lichens, even though some colonies have been found to live separate, without such an association (Paracer & Ahmadjian 2000).

1.1.5. The cost to the host

In most parasitic associations, species have been able to co-evolve in a way that the host is not severely damaged by the presence of the parasite and will survive with minimal damage, for a long period of time (Ebert & Herre 1996, Paracer & Ahmadjian 2000). Such an association is beneficial to both the host and the parasite in the evolutionary sense, and is favoured by selection. This hypothesis of evolution through competitive interactions between species was coined by Van Valen (1973) as the Red Queen hypothesis – proposing that interacting species will individually evolve to minimise their ‘disadvantage’, but that the overall fitness of the interacting species will not change relative to each other (Pearson 2001). However, the Red Queen hypothesis is in essence nothing more than Darwin’s theory of evolution by competition (Pearson 2001).

Pathogenic parasites cause serious disease and illness to their hosts shortly after infestation (Roberts & Janovy 2009, Cheng 2012), effecting the overall fitness and longevity of the host. The duration of longevity of the host depends on various factors such as the host health; the degree of parasitic infestation; the severity of disease caused by the parasite etc. (Sikkel & Welicky 2019). The serious disease and illness caused by pathogenic parasites are usually fatal to their hosts, as the host in the association has not developed mechanisms to overcome these effects through time. Such parasites that directly cause the death of their hosts are referred to as obligate parasitoids.

As mentioned, parasites can have numerous negative effects on a host, which are not limited to pathogenetic effects and disease. Other commonly observed and studied fitness decreasing effects of parasites include: wounds and damage to host tissue (Baker 1976, Avenant-Oldewage 1994, Cheng 2012); impaired mobility, dispersal and ability to avoid predators (Östlund-Nilsson *et al.* 2005, Fellous *et al.* 2011); an increase or limitation in growth and development (Poulin 1995, Ebert *et al.* 2004, Cheng 2012); metabolic and immunologic effects (Li *et al.* 2018, Nadler *et al.* 2021); and effects on reproductive success, including host castration and sex reversals (Callan 1940, Hamilton & Zuk 1982, Worden *et al.* 2000, Marzal *et al.* 2005, Lafferty & Kuris 2009, Cheng 2012). Parasites that cause host castration are referred to as parasitic castrators. These parasites have the ability to consequently inhibit the continued existence of a species in an evolutionary sense.

1.2. The loss and evolution of parasitism

Inter- and intraspecies associations and types of lifestyles may change and evolve over time, either as a result of environmental changes or changes in the physiology or development of either the parasite or host organism (Paracer & Ahmadjian 2000, Leung & Poulin 2008). To be able to discuss the loss or evolution of parasitic associations and the loss or evolution of parasitic lifestyles, it is important to distinguish between the two concepts. A parasitic association involves both species,

the parasite and the host, and refers to the essential dependency of the parasite on the host. A parasitic lifestyle involves only the organism that exhibits parasitic feeding behaviour, the parasite.

Parasitic associations can (and have) evolve into neutral associations (Paracer & Ahmadjian 2000, Miller *et al.* 2006, Canestrari *et al.* 2014), where one or both species benefit from the association ('mutualism'), or no harm is done to either species ('commensalism'). In these cases, the host becomes less effected by the presence of the parasitic organism, by developing mechanisms or strategies for overcoming the damage done by the parasite, or by developing the ability to terminate the association in response to damage or exploitation. Eventually the host becomes unaffected by the association (Paracer & Ahmadjian 2000, Johnstone & Bshary 2002, Miller *et al.* 2006). Thus, changes in the host's tolerance for the parasite, and/ or the parasites response to the host changes, brings about the change in the association. For example, Jansen *et al.* (2015) demonstrated how a 'commensalistic' association between a bacterium and a metazoan host can evolve from an initial pathogenic association. Similarly, Shapiro & Turner (2018) empirically observed the conditions under which a parasitic association between selected viruses and bacteria evolved into a 'mutualistic' (mutually beneficial) association over time.

Organisms that have a parasitic lifestyle at least during some stage of its life cycle, rarely evolve to become less parasitic or adopt a new lifestyle. This might be because only a few examples of such cases exist and are known, or more likely, because the specialisation and complexity of a parasitic lifestyle enable organisms to be more successful through evolution. Even so, some examples exist where a parasitic, 'exploitive' lifestyle is lost through evolution. In an attempt to test the 'reversibility' of parasitism, Klimov & O'Connor (2013) applied a multigene topology; an ancestral character state reconstruction; and a test for irreversible evolution that showed that the common, non-parasitic house dust mites (Acari) evolved from permanent parasitic ancestral forms, that parasitised vertebrate hosts. In another example, some Hymenopterans, notably species of Aculeata, are suggested to have evolved from being parasitoids (killing the host), to having a less fatal parasitic association with a host/prey, including resorting to a kleptoparasitic lifestyle; egg predation; provisioning predation and omnivory, or phytophagy (herbivory) (see Eggleton & Belshaw 1992) Alternative strategies in the loss of a parasitic lifestyle is, are either through a heterogonic lifecycle –alternating between parasitic and non-parasitic generations within a single species, for example the parasitic nematode group *Strongyloides* (Dorris *et al.* 2002) – or through a heterogonic lifecycle. Weinstein & Kuris (2016) explain that the loss in parasitic lifestyle is most often due to the loss of a specific life stage in a complex life cycle, rather than a morphological or physiological change to a non-parasitic lifestyle.

The more likely theory is that parasitic lifestyles have evolved from an ancestral, non-parasitic lifestyle. This theory is widely accepted, studied and well-documented for various groups (Littlewood 1999, Paracer & Ahmadjian 2000, Ebert 2005, Cheng 2012, Poulin & Randhawa 2013, Blaxter & Koutsovoulos 2015, Lukeš *et al.* 2014, Janouskovec & Keeling 2016, Nagler *et al.* 2017), especially those that contain the overwhelming majority of groups with at least one parasitic life stage as well as those that are of commercial and medical importance.

1.3. The evolutionary role and importance of parasitism

Parasites are commonly perceived and presented as antagonists, likely due to a selected few, well-known organisms that cause severe disease or death to humans and livestock, such as intestinal worms, malaria, ticks and lice. A parasitic lifestyle has proven to be highly successful, with approximately half of all metazoans exhibiting a parasitic lifestyle during some stage of development (Price 1980, Windsor 1998, De Meeûs & Renaud 2002, Roberts & Janovy 2009, Weinstein & Kuris 2016).

The study of parasites, including their life cycles and morphologies, are usually done with the aim to understand and mitigate their distribution, pathogenic effects, and to develop preventative measures against them. One important, but often overlooked or limited topic is the ecological, and eventual evolutionary role and impact of parasites and parasitism:

1.3.1. Speciation and biodiversity

A loss or evolution of parasitic associations, or the loss or evolution of a parasitic lifestyles within individual groups or species, all contribute to speciation and diversity (Poulin & Morand 2000, Marcogliese 2004, Hudson *et al.* 2006, Weinstein & Kuris 2016). Through co-evolution, inter-species interactions can result in genetic changes (mutations and specialisations) in either or both associates, or create a genetic separation and reproductive isolation between populations (Tripathy & Pradhan 2018, Groussin *et al.* 2020). Parasites and parasitic associations can therefore be a valuable tool or model for studying the origins, evolution and other key aspects of biodiversity (Meeûs *et al.* 1998, Poulin & Morand 2000, Hoberg & Klassen 2002). Understanding host transfers and switches between hosts, contribute to our understanding and management of parasites that are of commercial and medical importance (Poulin & Morand 2000, Gandon 2004).

1.3.2. Animal personalities

Only very recently, have evolutionary ecologists begun to study the effects and role of parasitism in the evolution of animal personalities. Barber & Dingemanse (2010) suggested that parasitism likely plays an important role in the evolution of animal personalities. Curious, risk-taking potential host individuals are more likely to be exposed to previously un-encountered parasites and pathogens that may come as a cost to their explorative behaviour and risk-taking (also reported by Wilson *et al.* 1993). Similarly, social behaviour of potential host individuals may influence the likelihood of them being infested through contact to parasites present in/ on other individuals of the population. Parasite strategies may favour these types of 'outgoing', risk-taking individuals for better success of transmission and infestation. The internal state or wellbeing of a host individual influences the behaviour and personality traits of that individual. As parasites may alter the wellbeing of hosts, evolution will likely favour infestation of individuals that can and do take risks and that can withstand the negative effects parasites and ensure compatible hosts (Barber & Dingemanse 2010, Kortet *et al.* 2010).

1.3.3. Behavioural changes

As the term suggests, behaviour-altering parasites have the ability to directly alter the behaviour or behavioural patterns of its host (Huebner & Chadwick 2012, Sato *et al.* 2012). These are usually, but not limited to, parasitic species or groups or species that depend on an intermediate host in order to complete its life cycle. This intermediate host is subject to behavioural change directly induced by the parasite to ‘force’ the host into a specific physical or physiological state that is favourable for the parasite to be dispersed or transferred to the next host (Poulin 1994, Schwanz 2006, Heil 2016). Well-known examples include the “Zombie ants” (Poulin 2010), “lighthouse snails” (Hughes 2014) and “fatal attraction” (Poulin 2010, Poirotte *et al.* 2016). The latter example includes the protozoan parasite *Toxoplasma gondii*, that alters the cognitive perception of its intermediate host (primarily rodents). An increase in activity and a decrease its fear of predation by the definitive host (felines), increases the rodent’s exposure and accessibility to the feline to ensure the transmission of *T. gondii* (Webster & McConkey 2010, Heil 2016). This phenomenon where parasitic organisms induce a behavioural change in its host to increase its own success and fitness, is referred to as the adaptive manipulation hypothesis (Poulin 1995, Lagrue *et al.* 2007) and is suggested to have evolved as a result of natural selection.

1.3.4. Indicators of ecosystem health

The study of parasite populations and communities have received increasing interest as potential bioindicators of ecosystem health with regards to community structure and environmental stress degradation (Lafferty 1997, Jameson *et al.* 1998, Marcogliese 2004, 2005. Howells *et al.* 2011). Organisms from virtually all animal groups and from all trophic levels, have parasites. As many parasites depend on an intermediate host for the completion of its life cycle, they are transferred through trophic levels. This interaction of a single organism with species from one or multiple ecosystems, have the potential to provide important ecological information about food webs and about the host and the interactions of the host with its environment (Marcogliese 2005, Hudson 2006, Amundsen *et al.* 2009). Additional information about host feeding behaviour and diet can also be acquired through the study of its parasites. Parasites can potentially also provide useful information regarding climate change, as the latter is predicted to affect entire ecosystems and species compositions (Marcogliese 2005, Polley & Thompson 2009). Pollution and anthropogenically induced environmental stress may affect the non-parasitic life stages of temporary parasites through the change in abundance or availability of compatible intermediate hosts (Lee 1977, Overstreet 1993, Sures 2004, Marcogliese 2005, Mehana 2020). Additionally, these non-parasitic stages can, and have been used in standardised toxicity tests to provide insight into ecosystem health (Morley *et al.* 2003).

1.4. Isopoda

1.4.1. Morphology & distribution

Isopoda is a species rich ingroup of Eucrustacea, and arguably one of the better-known ingroups of Peracarida (WoRMS database available at <https://www.marinespecies.org>, Del Carmen Espinosa-Pérez & Hendrickx 2001, Wilson 2009, Kazmi & Yousuf 2013). They are widely recognised by researchers and the public as pill bugs, wood lice, slaters, snow bugs, potato bugs,

woodlice and roly-polies (Hampton & Hampton 1975, Jass & Klausineier 1987, Bruce 2001, Snyder & Hendrix 2008, Hornung *et al.* 2018).

Isopods are diverse in their morphology, body shapes and sizes (Dreyer & Wägele 2002, Hickman *et al.* 2006, Kazmi & Yousuf 2013, Boyko & Wolff 2014), ranging from wide, rounded, dorso-ventrally flattened bodies (e.g., Bathynataliidae, Serolidae); disc-shaped forms (e.g., Sphaeromatidae); to long, slender, almost cylindrical body shapes (e.g., Anthuroidea and Phreatoicoidea) (see Wägele 1981, Brusca & Wilson 1991, Brandt & Poore 2003, Schädel *et al.* 2021). Eggs and immatures of representatives of Isopoda can be smaller than 1 mm (Holdich 1968, Johnson, 2001, Wolff 2009, Milatovic 2010). Adult body sizes range between 1–7 mm in total length for small species, such as *Mothocya powelli* (see Van der Wal *et al.* 2021b) and many species of Cirolanidae (see Bruce 1986); with the largest species, the giant isopod *Bathynomus giganteus* (Cirolanidae) reaching total body lengths of nearly 40 cm, (see Briones-Fourzán & Lozano-Alvarez 1991), with an adult male specimen holding the record at 42 cm (Soto & Mincarone 2001).

It is generally accepted that Isopoda forms a monophyletic group (Wägele 1989, Brusca & Wilson 1991, Wilson 2009), most often distinguished from other groups by the conjoined posterior trunk (pleon) segment 5 with the telson, forming the ‘pleotelson’ (Wägele 1989, Brusca & Wilson 1991, Poore 2001); and maxillulae without a palp, although these features are not exclusive to Isopoda. The only undisputed feature unique to Isopoda (autapomorphy) is the biphasic moulting cycle (see 1.4.2 *Life Cycles and Development*) where the posterior half of the exoskeleton is moulted some time before the anterior part of the exoskeleton (Whiteley & El Haj 1997, Wilson 2009). Brusca & Wilson (1991) mentioned further autapomorphies for Isopoda, which are largely internal and not easily accessible in all specimen material, especially fossil material. These features include striated muscles with unique myofibril ultrastructure; a gut tube without a midgut region; and respiration via respiratory structures in the posterior trunk appendages (pleopods).

Isopods are also hugely diverse in terms of distribution and ecological role (Brusca & Wilson 1991, Dreyer & Wägele 2002, Bruce, 2001, Hadfield, 2012; Kazmi and Yousuf 2013, Smit *et al.* 2014). Isopods are found in virtually all ecological niches on earth, ranging from moist to arid terrestrial habitats, including caves (predominantly Oniscidea, see Schmidt 2008, Schmalfuss 2003) and high altitudes reaching just below 4800 m (Beron 1997); freshwater (e.g., some species of Phreatoicoidea and Asellota see Wilson 2008); and brackish water sources (e.g., some species of Valvifera and Sphaeromatidae, see Jansen 1971). Most species are however, live in marine environments ranging from the seashore (Kensley 1978, Keskinen *et al.* 2002) to the deepest depths of the ocean (e.g., some species of Asellota and Cymothoidea, see Svavarsson *et al.* 1993, Lowry & Dempsey 2006) and even the icy water of Antarctica (Brandt 1992, Held 2003).

1.4.2. Life cycles and Development

Ingroups of Isopoda are equally diverse in their life cycles and developmental patterns, but as an ingroup of Peracarida, they all share the feature of bearing their eggs in a brood pouch, a specialised ventral cavity formed by the ‘oostegites’ (thoracopod epipods) of a mature female, referred to as an ovigerous female (Kensley 2001). Spermatophores are transferred during copulation from a male individual, with the aid of specialised copulatory structures such as the pleon appendage 2 epipod or ‘stylet’, referred to as the appendix masculina.

The life cycles of many ingroups of Isopoda are well-described. Most are however, based on a single species or ingroups of closely related species, making individual species' life cycle descriptions quite scarce. In many instances, a life cycle is described without morphological descriptions of immature stages and without inter-species and developmental stage comparisons and differentiations. Perhaps the most restrictive factor in the study of life cycles of representative ingroups of Isopoda, is the lack of descriptions, illustrations or photographs of immature stages in literature. Even more, many species only have a single ontogenetic stage described, usually an adult female or adult male. Immature stages and additional developmental stages are usually excluded from works due to the widely accepted notion that Isopoda are 'direct developers' (Kensley 2001, see Haug, 2019 for challenges of the concept), which means that the eggs develop into an immature stage which resembles the adult in most aspects. Yet, many ingroups have a distinct differentiation between immature stages and adult stages, both in terms of morphology and ecological role. The immatures of these ingroups are referred to as larvae (Boyko and Wolff 2014, Haug 2020).

The modes of reproduction within Isopoda are just as diverse and can possibly provide insight into the evolution of sexual systems (Juchault 1999, Allsop & West 2004) and possible correlation to specific lifestyles. Gonochorism (dioecy), is the sexual system where individuals develop as separate sexes (i.e., functional females and functional males). This is a common sexual system within Isopoda, but many species, especially parasitic isopods, have evolved more specialised systems, such as Sequential hermaphroditism. The transition between sexes is regulated by a hormone produced by the androgenic gland (Chang & Sagi 2008, Benvenuto & Weeks 2020) and depends on a variety of factors including sex ratios, abundance and presence of the opposite sex etc. (Brook et al. 1994, Allsop & West 2004). Two types of sequential hermaphroditism exist, both of which are found within ingroups of Isopoda:

Protogyny or protogynic hermaphroditism refers to species in which all offspring initially develop as females and are able to transition into males under specific conditions (Brook *et al.* 1994, Benvenuto & Weeks 2020). The transition is not necessarily required, and some females may never transition into males. Examples of protogyny are usually found in non-parasitic, aquatic species and has been described for species of Anthuroidea (Bamber 1985, Brook *et al.* 1994, Tsai *et al.* 1999). The more commonly observed sexual system in isopods, protandry or protandric hermaphroditism, refers to species in which all offspring initially develop as males and are able to transition into females under specific conditions (Bullar 1876, Mayer 1879, Benvenuto & Weeks 2020). This transition is also not necessarily required, and some males never transition into females. Examples of protandry are found in some species of Epicaridea and all species of Cymothoidea (Brook *et al.* 1994, Jacobsen & Collins 2008, Kottarathil & Kappalli 2019).

Sexual dimorphism well-known within Isopoda, where separate sexes (i.e., functional females and functional males), are phenotypically, morphologically and physiologically distinct (Bertin *et al.* 2002). Sexual dimorphism is usually most obvious by comparing the body sizes of adult male and adult female specimens, where one sex is notably larger than the other, with distinct morphological features (usually external sexual structures). In some cases, the male is larger than the female (as in the freshwater detritivores Asellota, see Bertin *et al.* 2002), while in other cases the female is larger than the male (as in the marine temporary ectoparasites, Gnathiidae, see Hispano *et al.* 2014).

The duration of the lifespan of isopods are not well recorded due to the difficulties of accessing rare species and keeping these animals in laboratory conditions. Even so, the general lifespan is estimated between one to two years (Brusca 1981), while some survive for up to nine years (Maxwell 1982).

1.4.3. Lifestyles & feeding habits

The lifestyle and feeding habits of ingroups of Isopoda are as diverse. Most groups are predominantly terrestrial and aquatic detritivores, feeding on and breaking down dead plant or animal matter, such as most representatives of Sphaeromatidae (Constantini & Rossi 1998, Lester 2005, Snyder & Hendrix 2008, Poore & Bruce 2012). Others are strict herbivores, grazing on seaweed and other plant material (Paris & Sikora 1965, Salemaa 1987). Many species are carnivorous predators, actively hunting prey, such as representatives of Cirolanidae (Holdich 1981), including the well-known giant marine isopod *Bathynomus giganteus*. Scavenging individuals usually feed on debris and dead organic material. Various species of Cirolanidae scavenge on and clean shark carcass (Wong & Moore 1996, Poore & Bruce 2012). A few ingroups of Isopoda have evolved to form parasitic associations with other marine animals during a specific life stage or for a specific duration of time. These ingroups serve as example groups and are main focus groups within this thesis.

1.5. The focus groups: parasitic representatives of Cymothoida sensu Wägele (1989)

In Isopoda, parasitism only evolved in the predominantly marine inhabiting group, Cymothoida sensu Wägele (1989) (Brandt & Poore 2003, Nagler *et al.* 2017). An apomorphy of Cymothoida sensu Wägele (1989) is a triangular uropod basipod with the median angle of the uropod basipod ventral to the pleotelson. Parasitic representatives of Cymothoida are grouped within Aegidae, Cymothoidae, Gnathiidae, Epicaridea and the fossil group Urda (see *1.5.1 Parasitic strategies*). All representatives from these ingroups are characterised by a hook-like dactylus (distal-most article) on the anterior trunk appendage 2 (thoracopod 3/ leg 2), a specialisation (autapomorphy) for attaching to a host (Nagler *et al.* 2017). A few questionable ‘parasitic’ association instances have been reported for species of Corallanidae and Tridentellidae, but these associations are likely only accidental interactions of species from these ingroups with other marine animals, or due to misidentifications of the species. Due to the lack of conclusive indication of parasitism and the lack of a hook-like dactylus on trunk appendage 2, these groups are herein treated as non-parasitic ingroups of Cymothoida.

Even before the phylogenetic debates regarding Cymothoida and its ingroups, the relatedness of parasitic groups has been widely accepted and they are often treated as an “ecological and phylogenetic lineage” (Delaney 1989), based on the theory that parasitic forms evolved from non-parasitic forms (*1.2 The loss and evolution of parasitism*). The evolution of parasitic forms from non-parasitic forms suggests that scavenging and predatory individuals (such as from Cirolanidae and Corallanidae) are ancestral to the groups containing parasitic individuals (such as from Aegidae, Cymothoidae, Gnathiidae, Epicaridea, Urda).

1.5.1. Parasitic strategies

Representatives of Aegidae form a temporary ectoparasitic association typically with fish species, comparable to that of a mosquito, where an individual would attach to, and feed on host tissue until satiated (Bruce 2009, Van der Wal & Haug 2022). Non-feeding individuals (usually adult females and brooding females) are usually found inactive and hiding on the seabed (Wägele 1989, Wägele 1990, Wing & Moles 1995) as discovered with in vivo laboratory studies. A few species are known to inhabit and feed on sponges (Stebbing 1893, Nunomura 1988), while some have been recorded in association with cartilaginous fishes, sea squirts (Wetzer 1990) and squids (Bruce 1996). Some records also suggest that representatives of Aegidae may scavenge and feed on debris and carcasses on the benthos (Bruce 1983, Brusca & Iverson 1985). Once fed, the individual detached from the host and continue to live un-associated until the next feeding. All ontogenetic and developmental studies suggest that representatives of Aegidae develop as separate sexes (gonochoric) and are not hermaphroditic (Wägele 1990, Ramdane & Trilles 2008, Van der Wal & Haug 2022). This type of sexual system is common for non-parasitic marine representatives of Isopoda (Johnson 2001). Furthermore, the sexual dimorphism between adult male and female aegids are quite subtle (Brusca & Iverson 1985, Van der Wal & Haug 2022).

Due to their feeding strategy, these animals are often described as predators (Ramdane & Trilles 2008, Öktener *et al.* 2020), ‘micro-predators’ (Lafferty & Kuris 2002, Bruce 2009) and scavengers (Stebbing 1893, Brusca & Iverson 1985). The temporary association of representatives of Aegidae with hosts are not fatal, and only lasts for the time of feeding (Haug *et al.* 2021) and are therefore more accurately described as temporary parasites. The complete life cycle and most immature ontogenetic stages of Aegidae were unknown prior to a publication incorporated in this thesis (Publication V: Van der Wal & Haug 2023).

All representatives of Cymothoidae are permanent, obligate ectoparasites of various (predominantly marine teleost) fish species. Once immatures are released from the brood pouch of the female, they immediately swim and search for a compatible host to attach to. Once attached, the association is permanent for the completion of the individual’s life cycle, unless successfully removed by the host or the death of the host (Adlard & Lester 1995, Bakenhaster *et al.* 2006, Aneesh *et al.* 2018). Cymothoiids exhibit clear protandric hermaphroditism which is well-described for many species (for example in Bakenhaster *et al.* 2006, Kottarathil & Kappalli 2019, Aneesh *et al.* 2020) as well as strong sexual dimorphism, which affects body size, appendage dimorphism and primary sexual characters (Bunkley-Williams & Williams 1998, Bruce 2002, Poore & Bruce 2012).

Four major site attachment strategies have evolved in ingroups of Cymthoidae, whereby representatives of some ingroups attach to the external surface of a fish host (Nagler *et al.* 2016, Welicky *et al.* 2017); some infest the host through the gill cavities and attach to the inside the mouth (González *et al.* 2019, Vigneshwaran *et al.* 2019); others attach to the inside the gill chamber (De Souza *et al.* 2019, Van der Wal *et al.* 2019); and a small number of species burrow superficially into the muscle tissue of the host (Tsai *et al.* 1999, Azevedo *et al.* 2006). The site of attachment, positioning, and orientation after attachment to a host is usually species specific and often used as indication to characterise genera. Some species (or groups of species) of Cymothoidae are highly specific, attaching only to a single host species, while others are generalists, attaching to an array

of fish hosts (Smit *et al.* 2014). Some instances have been reported where single individuals of Cymothoidae have been found attached to unusual hosts such as cephalopods, sponges, jellyfish, other crustaceans, chondrichthyans, amphibians and even humans (Trilles & Öktener 2004 and references therein). These instances are uncommon and most likely accidental (Williams *et al.* 2010).

Representatives of Gnathiidae are temporary ectoparasites of various fish species, but only during their larval stages, with non-feeding, non-parasitic adults (Sikkel *et al.* 2006, Artim *et al.* 2015), thus having a similar feeding strategy to that of Aegidae and immature stages of Cymothoidae (Haug *et al.* 2021). The life cycle of various species is well-recorded (Upton 1987, Smit *et al.* 2003, Chong *et al.* 2015), including the morphology and development of larval stages (Smit *et al.* 2003, Smit & Davies 2004, Boyko & Wolff 2014). Representatives of Gnathiidae are not protandric hermaphrodites and separate sexes can be distinguished as early as within the Zuphea 3 (Z3) larval stage (Smit *et al.* 2003).

Sexual dimorphism between adult males and adult females of Gnathiidae are quite striking, not necessarily in terms of body size as seen in representatives of Cymothoidae, but notably in terms of morphology – males have large, protruding mandibles, absent in females (Upton 1987). Some representatives of Gnathiidae are known to form ‘harems’ of females, collected and guarded by males during breeding season (Upton 1987, Wägele 1988). In an interesting case reported by Shodipo *et al.* (2018), an unfed gnathiid was found in association with a fed gnathiid, feeding on the ingested blood of the fed gnathiid. The type of association was described as kleptoparasitism, but is more accurately described as hyperparasitism in a publication incorporated in this thesis (Publication I: Van der Wal & Haug 2019).

Representatives of Epicaridea, including ingroups Bopyridae, Cryptoniscidae, Entoniscidae and Dajidae are somewhat unique among the parasitic cymothoidans in terms of their parasitic strategy and lifestyle. Representatives of Epicaridea are parasites of crustaceans – typically decapods – with an intermediate- and definitive crustacean host. Individuals of Bopyridae and Dajidae are (nearly all) ectoparasitic, while those of Cryptoniscidae and Entoniscidae are endoparasitic (Williams & Boyko 2012). Hyperparasitism has quite often reported for some individuals of Cryptoniscidae (Peresan & Roccatagliata 2005) and Bopyridae (Williams & Boyko 2004). Parasitic castration has been reported for a few individuals of Bopyridae and Dajidae (Blower & Roughgarden 1988).

With true larvae (epicaridium, microniscium and cryptoniscium larvae), these permanent parasitic individuals infest crustaceans during their larval and adult stages (Williams & Boyko 2012, Boyko & Wolff 2014, Haug *et al.* 2021). Hatched immatures, the planktic epicaridium larva, infest an intermediate crustacean host, typically a copepod, and develop into the least known of the larval stages, the microniscium (Schädel *et al.* 2019). The microniscium larva grows and develops into a planktic cryptoniscium larva, which infest the final decapod host where it reaches sexual maturity (Anderson & Dale 1981). Representatives of Epicaridea are protandric hermaphrodites (Jacobsen & Collins 2008) and clear sexual dimorphism (Harnoll 1966) with adult females larger than males (Williams & Boyko 2012).

Urda is an ingroup comprised exclusively of fossils and are closely related to modern ingroups of Cymothoidea, especially to Gnathiidae and Cymothoidea (see Nagler *et al.* 2017, Schädel *et al.* 2021). This phylogenetic position inference, along with extensive functional morphological examination of mouthparts and appendages, suggest that individuals of Urda were parasitic, with the oldest known case of parasitism within Cymothoidea reported at 168 mya (Nagler *et al.* 2017).

1.5.2. The fossil record

The large number of species and the morphological diversity of extant representatives of Cymothoidea is unfortunately not yet reflected in the fossil record (Hyžný *et al.* 2013, Smit *et al.* 2014). A large number of fossil specimens that have been interpreted as representatives of Cymothoidea, seem to be predatory or scavenging forms, most of which have been interpreted as representatives of Cirolanidae (Wieder & Feldmann 1992, Hyžný *et al.* 2013, Etter 2014, Robin *et al.* 2019). Even more scarce, is the fossil record of possible parasitic representatives. This sparsity is likely due to a number of factors including the limited preservation potential of individuals of these groups (Klompaker *et al.* 2017) or the fragmented preservation (usually only the dorsal sclerites) attributed to the characteristic biphasic moulting of ingroup representatives of Isopoda (Wieder & Feldmann 1992, Feldmann & Goolaerts 2005, Hansen & Hansen 2010, Hyžný *et al.* 2013, Etter 2014). Additionally in many cases, the position and orientation in which the specimen is preserved make appendages and other essential morphological structures inaccessible for further systematic interpretation and/ or comparative analyses (Hyžný *et al.* 2013, Smit *et al.* 2014, Maguire *et al.* 2018).

Direct indications of parasitic behaviour or associations (i.e., body fossils) are rare in the fossil records. In most cases of fossil specimens, a parasitic lifestyle or association can be indirectly inferred (Nagler *et al.* 2016, Haug *et al.* 2021). These inferences can be made from the resulting deformations of the host (such as body swellings) caused by representatives of Epicaridea; the reconstructed functional morphology as possible indication of a parasitic lifestyle; the preservation of a unique or distinct life stage that is only known in parasitic species of the modern fauna; and an interpreted phylogenetic position of exclusively parasitic representatives (supported by accessible morphological characters). Nagler *et al.* (2016) described and presented a direct parasite-host interaction from 150 million years old fossils, containing both the host and the interpreted parasitic representatives of Cymothoidea attached to it.

The current fossil record of parasitic representatives of Cymothoidea consist of: 1. A specimen interpreted as Urda from the Early Jurassic (168 mya), the oldest fossil parasitic representatives of Cymothoidea (Nagler *et al.* 2017); 2. A collection of specimens preserved in association with fishes from the Jurassic (150 mya), that have been interpreted as closely related to Cymothoidea (Nagler *et al.* 2016, Haug *et al.* 2021); 3. A collection of specimens from the late Eocene (40 mya) interpreted as representatives of Cymothoidea or at least closely related (Publication IV: Van der Wal *et al.* 2021a); 4. A single specimen interpreted as representative of Aegidae from the Late Miocene (20mya, see Hansen & Hansen 2010); 5. Body fossils of infective larval stages of representatives of Epicaridea from the Cretaceous (100 mya) as well as indirect, questionable interpretations of representatives of Epicaridea from Early to Late Jurassic (Haug *et al.* 2021 and references therein).

2. AIMS OF THE STUDY

The role of parasites and parasitic interactions are of economic, ecological and evolutionary importance (1.3 *The evolutionary role and importance of parasitism*). Representatives of Cymothoidea is an interesting group for studying the evolution of parasitism, parasitic strategies and specialisations for a parasitic lifestyle (1.5. *The focus groups: parasitic representatives of Cymothoidea sensu Wägele (1989)*). Representatives of Tanaidacea are closely related to those of Isopoda and are therefore herein used as an outgroup taxon to Isopoda. Unlike some representatives of Isopoda, those of Tanaidacea are primarily tube-dwelling and exclusively non-parasitic, and are morphologically differentiated from representatives of Isopoda. Parasitic representatives of Cymothoidea are used as ‘model parasites’ and they are especially ideal candidates to study deep-time aspects of parasitic strategies and specialisations as:

- Quite a few fossil collections are available to study these deep-time aspects and be able to compare parasitic strategies and specialisations through time.
- They are abundant in marine habitats and quite abundant in museum collections as preserved material
- They are highly diverse in morphology, ecological roles and ontogeny
- They are relatively large and therefore more easily observed and studied

Adult and immature stages of parasitic forms have different ecological functions, with mobile immature stages being essential for dispersal and host infestation, with reproducing adults (especially females) often becoming less mobile and/ or non-feeding. The aim of this dissertation is to study if and how morphological states and changes in morphological states are derived from ecological functions and parasitic strategies, as it relates to the evolutionary history of parasitism within Cymothoidea. With the set aim, the following hypotheses are tested:

1. There is an increase in morphological differentiation of thoracopod attachment structures, from non-parasitic forms to more parasitic and permanent parasitic forms.
2. Immature stages among more parasitic forms retain plesiomorphic morphologies in comparison to their adults, while the differentiation is more subtle between immatures and adults of non-parasitic or less parasitic forms.

In order to test the hypotheses, the objectives are to examine, document and describe the morphological differentiation, especially regarding the anterior and posterior thoracopod attachment structures, between adult and immature stages and among parasitic forms from non-parasitic forms to temporary parasitic forms and permanent parasitic forms. Morphospace analyses will be done to compare morphologies between parasitic forms and between adult and immature stage individuals.

3. RESULTS

3.1. Publication I

Van der Wal S & Haug JT. 2019. Letter to the editor referencing “The apparent kleptoparasitism in fish-parasitic gnathiid isopods” 10.1007/s00436-018-6152-8. *Parasitology Research*, 118(5), 1679–1682. <https://doi.org/10.1007/s00436-019-06281-2>

3.2. Publication II

Schädel M, Pazinato, PG, Van der Wal, S & Haug JT. 2019. A fossil tanaidacean crustacean from the Middle Jurassic of southern Germany. *Palaeodiversity*, 12(1), 13–30. <https://doi.org/10.18476/pale.v12.a2>

3.3. Publication III

Van der Wal S & Haug JT. 2020. Shape of attachment structures in parasitic isopodan crustaceans: the influence of attachment site and ontogeny. *PeerJ*, 8:e9181. <https://doi.org/10.7717/peerj.9181>

3.4. Publication IV

Van der Wal S, Schädel M, Ekt B & Haug JT. 2021a. Description and ontogeny of a 40-million-year-old parasitic isopodan crustacean: *Parvucymoides dvorakorum* gen. et sp. nov. *PeerJ*, 9:e12317. <https://doi.org/10.7717/peerj.12317>

3.5. Publication V

Van der Wal S, Haug JT. 2023. Reconstructing the life cycle of the isopodan group Aegidae with morphological descriptions and the importance of immature stages. *Nauplius*, 31. <https://doi.org/10.1590/2358-2936e2023007>

4. DISCUSSION

4.1. Importance and ecological function of immature stages

Some ingroups of Cymothoida have well-defined and described immature stages, specifically those that are morphologically well-distinguished from adult stages, i.e., those that have true larvae, such as Epicaridea and Gnathiidae. The larval stages of the latter groups are not only morphologically distinguished from adults, but their morphological differences also support their differences in ecological function. With the inclusion of immature (larval) stages in studies of these groups, complete life cycles, developmental patterns and behavioural studies have been presented, which would not have been possible without the inclusion of these larval stages.

This is unfortunately not the case for many of the other ingroups of Cymothoida, especially those that are considered to be ‘direct developers’ that do not have true larval stages. The immatures of these ingroups, including the non-parasitic representatives of Cirolanidae, Corallanidae; the temporary parasitic representatives of Aegidae; and the permanent parasitic representatives of Cymothoidae, are typically considered as ‘young adults’ upon hatching and that they are morphologically indistinguishable among species and from their adult stages. The results from morphological comparative analyses in Publication III: Van der Wal & Haug (2020) suggest that with proper data available, immature stages of species of Cymothoidae can be well distinguished from each other and that developmental morphological variation can provide a basis for species identification. The quantitative analysis for the latter publication further indicates that ontogenetic development in Cymothoidae plays a role in the shape of the dactyli as attaching structures.

The lack in availability of immature stage individuals in literature has become evident, with immature stages often mentioned without morphological descriptions, or with only a few noted regarding a single immature stage. Other publications, typically the early pioneering works, have inclusions of immature stage morphological structures. More recent works tend to exclude the descriptions and presentations of these immature stages altogether unless the species is only known from a single immature stage specimen as holotype. Even though the immature stages of these ingroups might seem morphologically similar to adult stages, the inclusion of these stages in especially taxonomic and phylogenetic works are essential for further research and studies such as comparative morphological and developmental differentiation analyses as conducted here. The lack of descriptions and morphological illustrations of immature stage individuals in literature also hinder evolutionary studies, as fossil material can often only be compared to extant material of adult representatives in the case of Cymothoida, making it even more challenging to compare and interpret fossil finds.

Without the availability of immature stages in literature, any further research on the ecology, development and ecological function of species of these groups are hindered and limited. This has been made evident with the study of the ontogenetic development of Aegidae, with the first representation of a life cycle for individuals of this group being presented in Publication V: Van der Wal & Haug (2023). The latter study included the morphological comparison between the

scarcely represented immature stages of Aegidae through ontogenetic development. The results from this study would not have been possible without the inclusion of the examined immature and embryonic stages of Aegidae, as these are almost completely lacking in literature. This study also presents the first substantiation that representatives of Aegidae are not hermaphroditic, but develop as separate sexes. A major challenge in improving the understanding of ecology and evolution of the group Cymothoidae, is the very imperfect knowledge about the ontogeny. Approximately 22% of all species of Cymothoidae have at least some information on immature stages. Even so, for most of these records, there is only single illustrations and a short description. Regardless of parasitic strategy, the results suggest that immature stages, including larvae of all parasitic forms within Cymothoida are the dispersal and infective stages, ensuring an association with a host, whether temporary or permanent. The ecological role of these immature and larval stages is to find a suitable host, ensure attachment to the host, to feed and to grow. Adult females are almost exclusively sedentary, non-feeding and breeding.

Representatives of Gnathiidae and Epicaridea have well-defined and described true larval stages, in contrast to representatives of Cymothoidae, Aegidae and non-parasitic ingroups of Cymothoida. Non-adult individuals of the former and latter groups are in literature usually collectively (and interchangeably) referred to under the umbrella terms of ‘manca’, ‘juvenile’ or ‘larva’ (Pillai 1964, Williams & Bunkley-Williams 1980, Bakenhaster 2004, Trilles & Justine 2006, Čolak *et al.* 2019). The term larva refers to non-adult individuals with specific morphological characters and ecological functions that cannot be applied to any non-adult individual and have proven to be highly problematic in many groups (Haug 2020). Within Isopoda, the use of the term ‘larva’ has also caused some discussion (Boyko & Wolff 2014). To address these inconsistencies in terminology, the term ‘immature’ is used herein and proposed to be used in future studies, to neutrally refer to the non-adult life stages of representatives of Aegidae and Cymothoidae, thus those in the ‘post-hatched’ developmental stages before maturation is reached (Publication III: Van der Wal & Haug 2020).

For groups that do not have true larvae (i.e., the ‘direct developers’), the proposed terminology for immature stage individuals is based on the presence or absence of morphological structures, in particular the development of the anterior trunk appendage 7 (thoracopod 7), which seems to be consistent among ingroup representatives of Aegidae, Cymothoidae and the newly described fossil species *Parvucymoides dvorakorum* (Publications III – V). To date, there is no record of a possible immature stage individual of Urda. Additional stage specific characters can show variation among ingroups and should be recorded in ontogenetic stage descriptions and life cycle descriptions. The following terminology is proposed and contributed to the works of this dissertation. Immature stage 1 individuals are those often referred to in literature as ‘pre-manca’, ‘pre-hatch II’, ‘first manca stage’, manca-I or ‘pullus I’. This stage is identifiable as the first stage after the individual has hatched from the egg membranes inside the brood pouch of the female, which will be release from the brood pouch and start further post-hatch development. These individuals completely lack the pair of thoracopods 7. Immature stage 2 individuals are those often referred to in literature as ‘manca’, manca-II or ‘pullus II’. Individuals during this stage of development start to develop the posterior most pair of thoracopods, and may be at an early stage (without visible indication of thoracopods 7) or during a later stage (with visible underdeveloped thoracopods 7). Immature stage 3 individuals are those often referred to in literature as ‘natatory-stage’, ‘juvenile’ or ‘aegathoid’.

These individuals have a fully developed pair of thoracopods 7, but still lack adult sexual structures or have underdeveloped adult sexual structures. Additional developmental stage and species or group specific characters may be observed, such as the presence or absence of yolk, eyes, setae, body shape and/ or swimming ability. These characters are variable among groups and should rather serve as additional stage specific characters in specimen descriptions and comparative analyses.

Adult stages are usually more clearly defined and categorised based on internal or external sexual structures as male or female individual. As an ingroup of Peracarida, adult female individuals develop a brood pouch by forming oostegites, originating from the coxae, which are epipod-like structures arising from anterior trunk appendages. The presence or absence of a brood pouch and/ or eggs in the brood pouch is indicative of developmental stages of adult female individuals. The formation of oostegites (oostegital moult) seem to occur after copulation, as a facilitation before the brood pouch is completely formed and hardened (Holdich, 1968; Johnson, 2001). Females with such developed oostegites are referred to as ovigerous females. Non-ovigerous females are adults that do not have developed external male structures and also no developed brood pouch. Once fertilisation has occurred and the brood pouch has developed, the eggs are deposited in the pouch and the individual is now referred to as a gravid female. The transfer of sperm to the female seems to be facilitated by the appendix masculina, a type of modified endite arising from the base of the endopod of the 2nd pleon appendage (Wilson 1991, Johnson 2001, Messana 2004).

4.2. Character evolution: dactylus differentiation among parasitic strategies

The complete dataset of analysed dactyli, including the data imported to R, species names, Principal Component values and references of digital illustrations, is provided in Table S2. The RStudio script used to run the analysis and generate the plots, is available in Doc S3. A total of 150 dactyli were analysed together, which consisted of: 7 species of Aegidae (6 adult and 7 immature stage individuals); 5 species of Cirolanidae (4 adult and 1 immature stage individuals); 1 species of Corallanidae (1 immature stage individual); 15 species of Cymothoidae (15 adult and 15 immature stage individuals); 1 species of the fossil group *Parvucymoides* (1 adult and 1 immature stage individuals); 5 species of Gnathiidae (4 adult and 5 immature stage individuals); 14 species of Epicaridea (6 adult and 9 immature stage individuals). Aligned and scaled digital illustrations of all dactyli outlines used in the PCA, are provided in Figure S4, colour coded by ingroup.

The variation in the first 5 Principal Components (PC1–PC5) are visualised in a boxplot in Figure S5. The dactylus shape along PC axes (PC1–PC5) are visualised in Figure S6. Examining the eigenvalues from the scree plot (Figure S7), PC1 and PC2 account almost equally for most of the total variation in shape and will be used to present the results. Principal Component 1 accounts for 45.4% of the total variation and Principal Component 2 accounts for 40.0% of the total variation. Cumulatively, PC1–PC2 accounts for 85.5% of the total variation and share the proportion of variation almost equally (Figure S7). PC3–PC5 accounts for a further approximate 10% of the total variation, resulting in PC1–PC5 cumulatively accounting for 95.9% of the total variation.

The variation in shape of the analysed dactyli, is presented as a multidimensional morphospace, where each included dactylus is represented by a point (Figs. 1–4). The variation among all 150

shapes included in the analysis, grouped by ingroup, is provided in Figure 1A–B. The same results are visualised based on variation in anterior and posterior dactyli of all ingroups, in Figure 2A and based on the parasitic strategy of all ingroups, in Figure 2B. The variation in dactyli shapes is additionally visualised for adult specimens (Fig. 3) and for immature specimens (Fig. 4). The first dimension in these plots, (PC1, represented on the x-axis), is strongly influenced by the degree of curvature of the dactylus. Dactyli are more curved toward negative values and less curved (straighter) towards the positive values. The second dimension, (PC2, represented on the y-axis), is influenced by the thickness of the dactylus. Dactyli curves are thicker toward negative values and thinner towards positive values.

The differentiation in dactyli shapes among adult and immature specimens, as well as between anterior and posterior dactyli, based on the values of the Principal Components that describe the most variation (PC1–PC2), are visualised in Figure 5. Mean shapes, standard deviations and mean plesiomorphic conditions indicate how dactyli shapes among the groups are differentiated and how these resemble or deviate from the general plesiomorphic condition. Due to the lack of available data for the second non-parasitic ingroup, Corallanidae, this group was not included in Figure 5.

The results from the combined morphometric analysis substantiate and visualise the variation in dactylus morphology among the selected ingroups of Cymothoida. Some overall trends and clustering of dactyli morphologies between ingroups are notable (Figs. 1–5). It is worth noting that some of these trends may be attributed to the uneven (or not especially high) number of species per group included in the analysis, which was due to the lack of availability of dactyli illustrations in literature. For the non-parasitic ingroups Cirolanidae and Corallanidae, immature stage depictions in literature are almost non-existent. The same is true for Aegidae, of which all dactyli shapes for the analysis were generated from this study (Publication V: Van der Wal & Haug 2023). In the case of species from the remaining ingroups included in the analysis, literature often only contains only a single thoracopod depiction, or an illustration/ photograph at an angle which influences the perspective of the morphology of the structure, making it unusable in a comparative morphometric analysis. Therefore, these discussions are solely based on the available data and the trends observed from the data generated in this study.

When considering the three overarching parasitic strategies (non-parasitic, temporary parasitic and permanent parasitic), the morphometric results (Fig. 2) show that representatives with permanent parasitic strategies occupy the majority of the total morphospace, indicating that dactyli from these ingroups (Cymothoidae and Epicaridea) are the most diverse and can range from slender, straight outlines, to thick, strongly curved outlines, followed by representatives of Aegidae, the ingroup with temporary parasitic strategies. Epicaridea and Cymothoidae show the most variation in dactylus curvature (PC1), but this variation extends in different directions (Figs. 1–5). The dactyli seen in Cymothoidae range from moderately curved, resembling the plesiomorphic condition in some cases, to some of the most strongly curved dactyli shapes included in the analysis. On the other hand, the dactyli seen in Epicaridea range from a curved and moderately curved, to straight, uncurved dactyli shapes. Epicaridea show the largest variation in dactyli thickness (PC2), ranging from slender and straight (needle-like) shapes to thick (balloon-like) shapes.

Representatives of non-parasitic ingroups (Cirolanidae and Corallanidae) occupy the smallest total morphospace with no, to only slight variation dactyli shapes. A larger availability of dactyli illustrations in future publications may provide more accuracy in the trends of variation in ingroups with non-parasitic strategies. The morphometric results (specifically Fig. 1) further substantiate the interpretation of the fossil species *Parvucymoides dvorakorum* (Van der Wal, Schädel, Ekrt & Haug (2021)), as ingroup representative of Cymothoidae, as all the dactyli shapes of *P. dvorakorum* fall well within the morphospace of extant representatives of Cymothoidae.

Considering the anterior dactyli shapes of the *adults* only (Fig. 5A), a complete separation is seen between: Cymothoidae and all other groups except Aegidae; Aegidae and all other groups except Cymothoidae; Epicaridea and all other groups; and between Gnathiidae and Cirolanidae (combined) with all other groups. The latter two groups overlap only very slightly in anterior dactyli curvature. No data points for adult stage Corallanidae representatives were available. Notably, the anterior dactyli of representatives of Aegidae and *P. dvorakorum* are similar in thickness and curvature to those of Cymothoidae. This is indicated by these dactyli shapes occupying the same morphospace area – strongly curved, hook-like dactyli shapes. Posterior dactyli shapes of *adult* specimens show separation to a lesser extent, with all extant groups overlapping at a moderately curved (PC1 values between 0 to 0.1), moderately thick (PC2 values between -0 to -0.05) dactylus shape.

Similarly, more separation is seen in the anterior dactyli shapes of the *immatures* only (Fig. 5B), compared to those of posterior dactyli shapes. A complete separation is seen between the anterior dactyli shapes of the *immatures* of: Cymothoidae and Epicaridea; Cymothoidae and Gnathiidae; Aegidae and Epicaridea; and Aegidae and Gnathiidae. As with the adult comparison, the anterior dactyli of immature representatives of Aegidae and *P. dvorakorum* are similar in thickness and curvature to those of Cymothoidae. The anterior dactyli shapes of Epicaridea encompass the entire morphospace of anterior dactyli shapes of Gnathiidae. The posterior dactyli shapes of *immature* specimens show separation to a lesser extent than the anterior counterparts, with all posterior dactyli of extant groups at moderately curved (PC1 values between 0 to 0.05) and moderately thick (PC2 values between -0 to -0.075). A clear separation is seen in the posterior dactyli shapes between: Cymothoidae and Epicaridea; and Cymothoidae and Gnathiidae.

Based on the parasitic strategy of adult and immature stages (Fig. 4), the following trends become visible. As expected, adult representatives of ingroups with permanent parasitic strategies have anterior dactyli that are distinct from those of non-parasitic lifestyle adults (Fig. 4A). Adult representatives of Cymothoidae and Epicaridea show significantly more variation in the thickness of dactyli, than adults of Cirolanidae and are always more strongly curved. The posterior dactyli of Aegidae and Cirolanidae are similar (straight to slightly curved). From Fig. 4B, no clear separation can be seen in the anterior and posterior dactyli shapes of immature representatives.

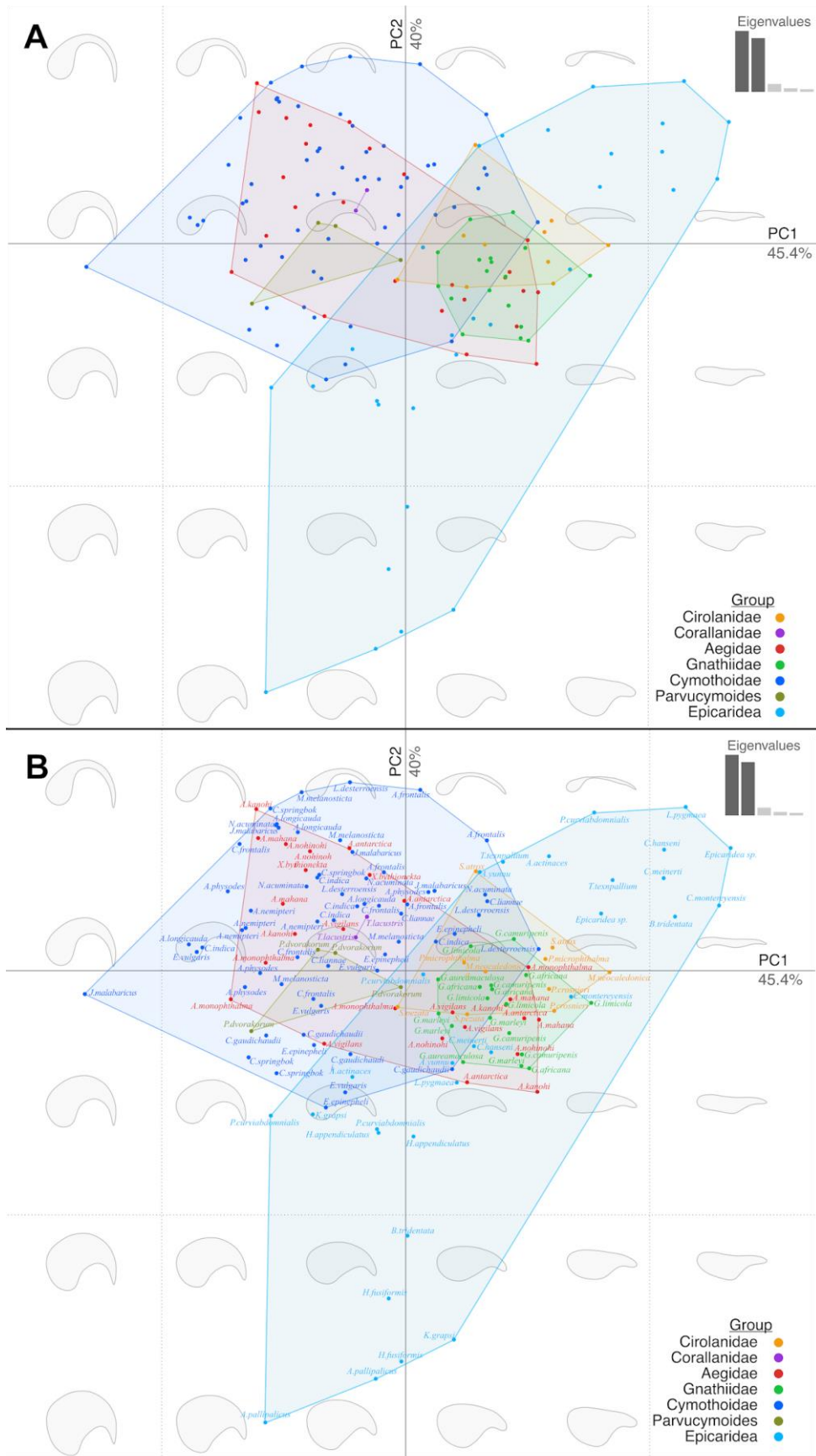


Figure 1. Principal Component Analysis representation of the dactylus outline variation of all 150 analysed specimens of various ingroups of Cymothoidea included in the elliptical Fourier analysis. **A.** PCA plot of all specimens. **B.** PCA plot of all specimens, with species names.

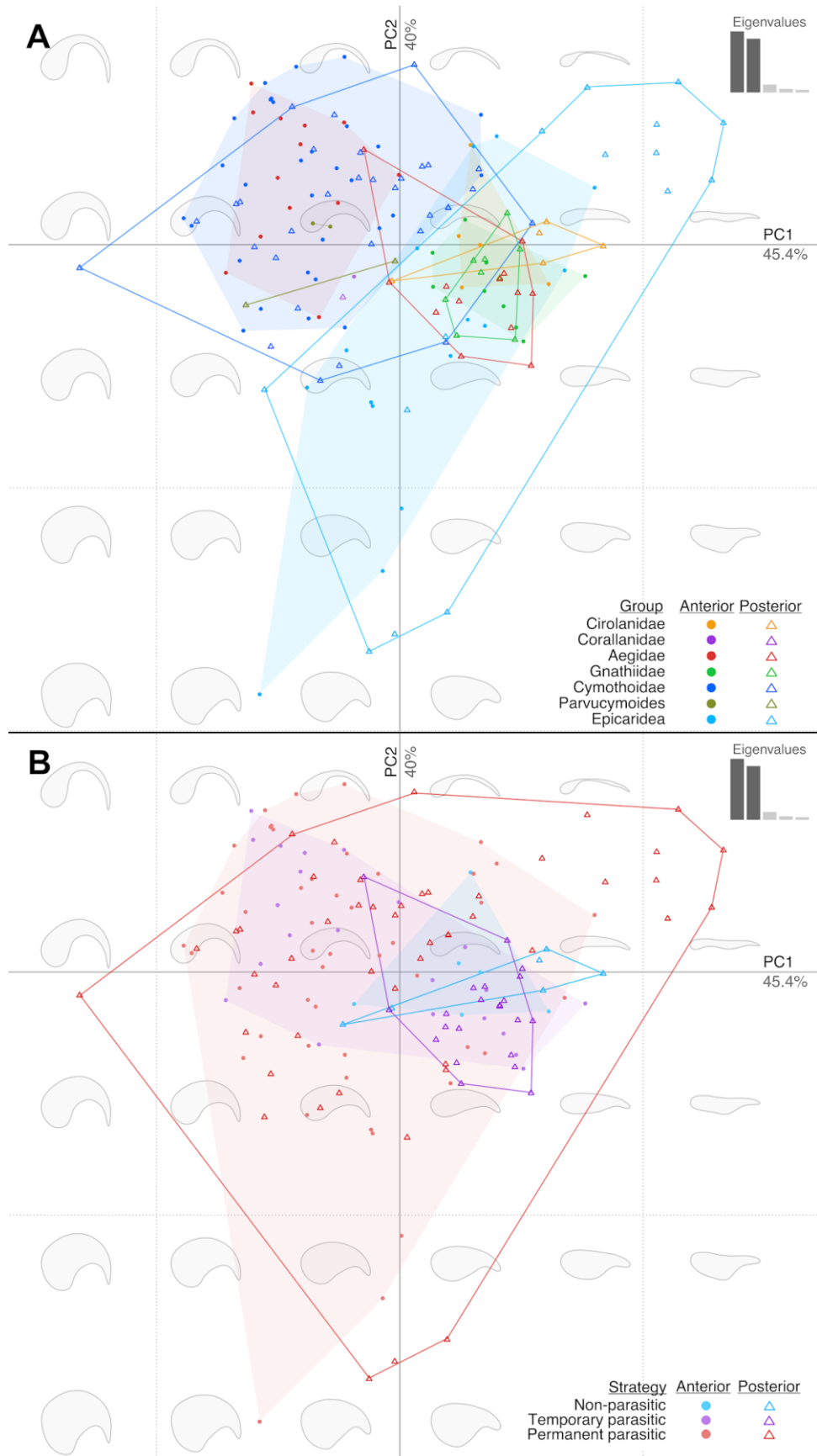


Figure 2. Principal Component Analysis representation of the dactylus outline variation of all 150 analysed specimens of various ingroups of Cymothoidea included in the elliptical Fourier analysis. **A.** PCA plot of all specimens, grouped by dactylus location. **B.** PCA plot of all specimens, grouped by parasitic strategy.

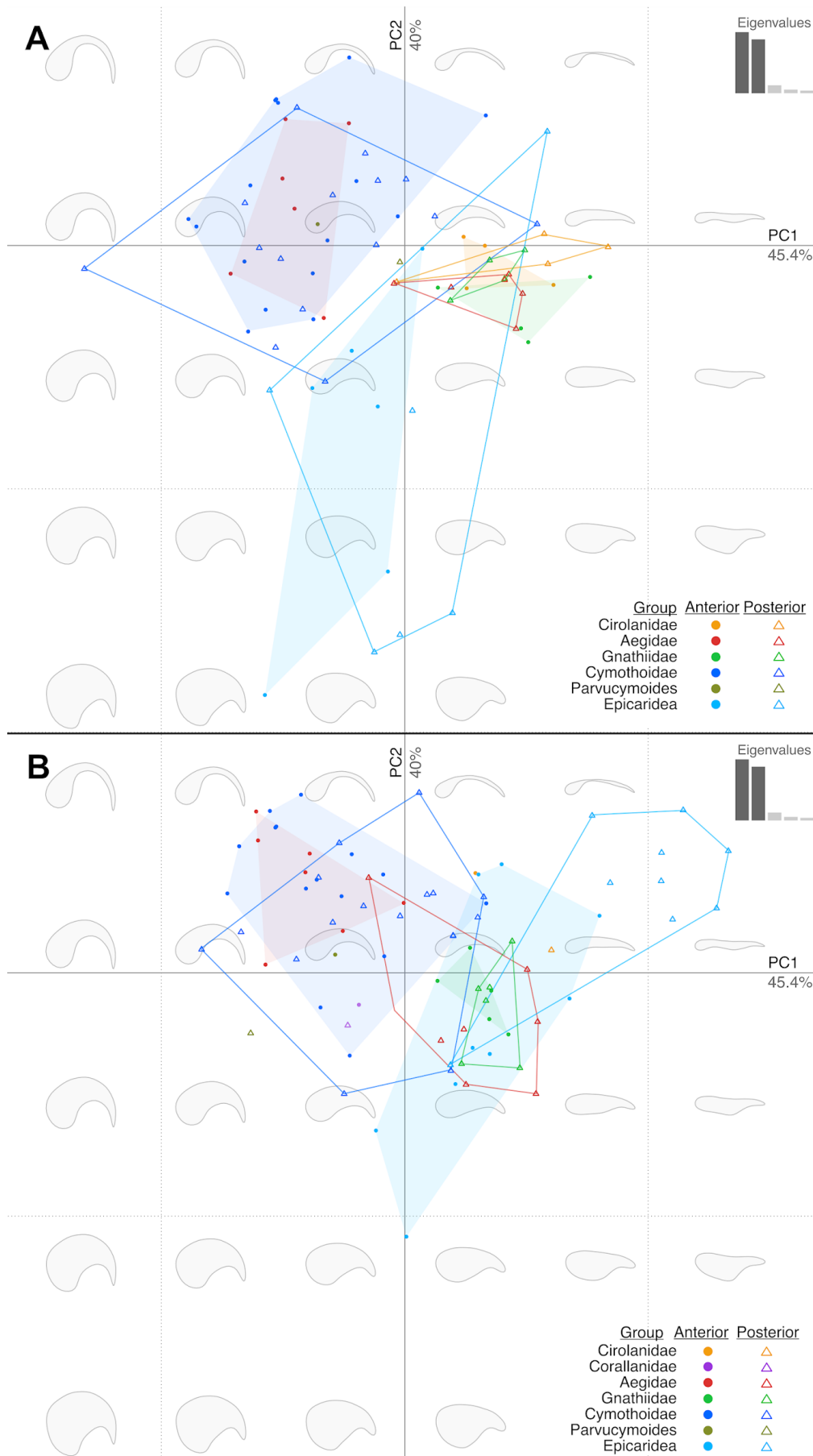


Figure 3. Principal Component Analysis representation of the anterior and posterior dactyli outline variation **A.** Adult stage specimens only. **B.** Immature stage specimens only.

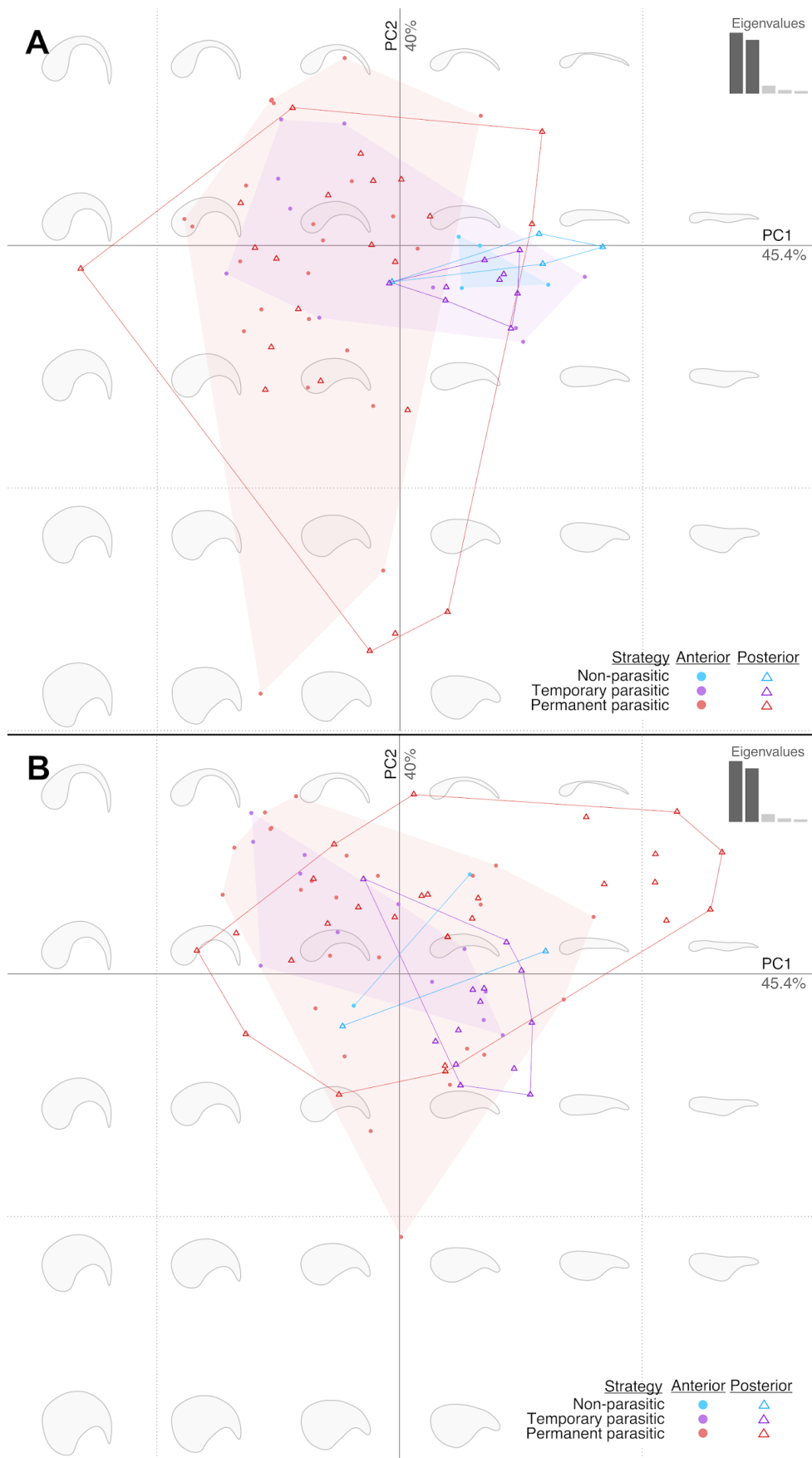


Figure 4. Principal Component Analysis representation of the anterior and posterior dactyli outline variation, grouped by parasitic strategy **A.** Adult stage specimens only. **B.** Immature stage specimens only.

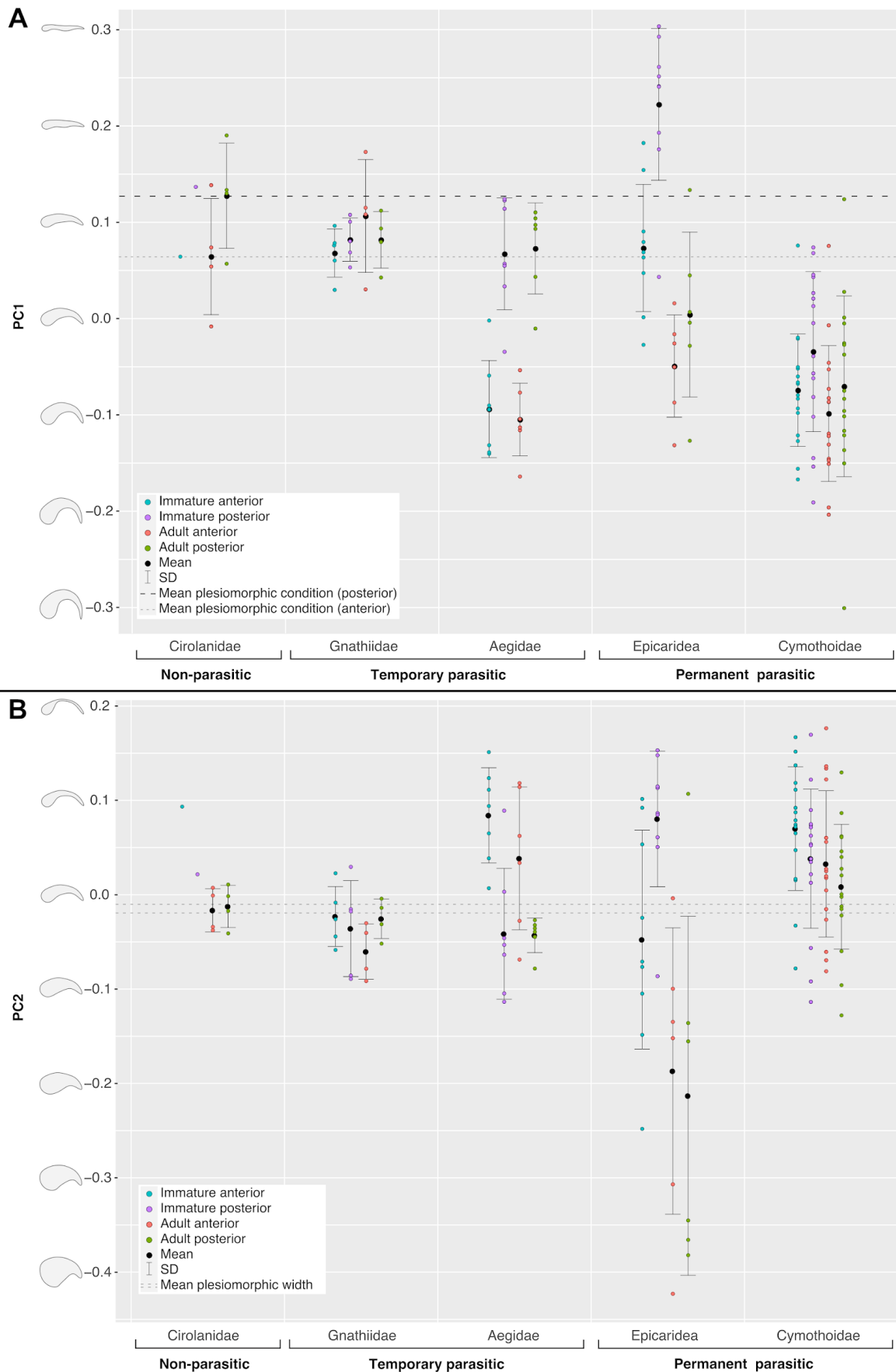


Figure 5. Visual representation of dactyli shape variation and differentiation based on **A.** Principal Component 1 (PC1) values. **B.** Principal Component 2 (PC2) values.

The differentiation of anterior and posterior dactylus shapes among adult and immature stages of the ingroups are best visualised by comparing the values of Principal Components 1–2 (Fig. 5). These results show a few interesting signals regarding character evolution of dactylus shape, which can be discussed in correlation with the feeding habits and ontogenetic development of the groups included in the analysis.

4.2.1. Representatives of non-parasitic ingroups

It is known that representatives of Cirolanidae and Corallanidae are non-parasitic, carnivorous, active swimming and hunting predators, while others scavenge on debris and dead organic material on the benthos. The dactyli of representatives of Cirolanidae represent the plesiomorphic condition of dactyli shapes for ingroups of Cymothoidea as rather straight (Fig. 5A) and relatively uniform in thickness (Fig. 5B), with a low anterior-posterior differentiation. The anterior and posterior dactyli curves of the only immature representative of Cirolanidae (*S. atrox*) strongly resemble the mean shapes of those of the adult specimens (Fig. 5A) and are slightly thinner than those of the adults (Fig. 5B). Likely due to the underdevelopment of these structures in immature specimens. The relative similarity in dactylus morphology through ontogenetic stages correspond to the specialisation of these structures for the life habit of non-parasitic representatives of Cymothoidea, including swimming, grasping and scavenging, rather than attachment to, or tearing through, the tissue of a host.

Even though species of Cirolanidae and Corallanidae are well-known and documented, specimen and species descriptions and illustrations are typically exclusively provided for adult stage specimens, with the motivation that immature stage specimens are identical to adult stages in morphology and are therefore not included. The future inclusion of immature stage individuals in species descriptions, illustrations and photographs can have far-reaching positive implications, not only for taxonomy but also for additional studies such as this morphometric analyses.

4.2.2. Representatives of temporary parasitic ingroups

Representatives of Gnathiidae are ectoparasites of fish during their immature/larval stages. The results (Fig. 5) for representatives of Gnathiidae are overall not especially informative, but show that the dactyli of these specimens are similarly rather straight (all PC1 values above 0) and similar in thickness. There does not seem to be a differentiation between the dactylus morphologies of the non-feeding adults versus the temporary parasitic immature individuals' dactyli, or between the morphology of anterior versus posterior dactyli. The mean shapes for both anterior and posterior dactyli of adult and immature stages of Gnathiidae closely resemble the plesiomorphic states. The results suggest that the immature dactylus morphology of Gnathiidae is kept though development into adulthood, even though the ecological role of adults differ from that of the larval stages.

In a study published by Shodipo *et al.* (2018), authors describe on an interesting interspecific interaction between representatives of Gnathiidae, in which some larval individuals feed on the blood meal of other larval individuals. This interaction was verified and monitored in vivo and is unique as larval representatives of Gnathiidae typically feed on blood from various fish species, while adults are non-feeding. The interaction is described as kleptoparasitism; the act of one organism stealing food from another. Even though the concept is accurately described, the

interaction can also be characterised as hyperparasitism, which is the parasitic associations in which the host (unfed gnathiid larva) is a parasite of another organism (fed gnathiid larva). In essence, both concepts apply to the observed interaction, which substantiates the importance of considering a wide variety of descriptions and characteristics of specific animal associations before categorising an interaction.

The dactyli of the other temporary parasitic representatives, Aegidae, have an overall wide range of morphologies due to the prominent differentiation between anterior and posterior dactylus shapes. Dactyli of anterior thoracopods (thoracopods 2–4) are strongly curved and hook-like, resembling those of Cymothoidae, and vary in thickness. Dactyli of the posterior thoracopods (thoracopods 5–8) are rather straight and resemble the plesiomorphic condition (and significantly smaller overall; not visible from these results, see Publication V: Van der Wal & Haug 2023). This morphology corresponds to the temporary parasitic feeding habit of representatives of Aegidae, using the anterior thoracopods for attaching and maintaining attachment to a host (as immature and adult male stages) for the duration of a feeding, while being able to swim and/ or walk on the benthos using posterior thoracopods after detaching from a host after a feeding.

The gap in knowledge and understanding of Aegidae life cycles and development has caused some variation in the description of lifestyle and feeding habit for representatives of Aegidae. Many specimens are collected from the surfaces of sponges, cartilaginous fishes, sea squirts and squids, leading to the assumption that individuals of Aegidae feed on these animals while in association/ attached to them. Consequently, literature often describes representatives of Aegidae as ‘micro-predators’, predators, scavengers and/ or temporary parasites. In fact, no study has provided sufficient indication of consumption of these marine organism by representatives of Aegidae. Observations of Aegidae in association with these marine organisms can only definitively indicate a possible habitat, hiding place or mode of transport for non-feeding individuals rather than being an actual food source. Such individuals have likely detached from the host after a feeding, and continues to live un-associated to a host until the next feeding.

Additionally, representatives of Aegidae are often collected by means of trawl and sledging of the ocean benthos by a Research Vessel. The occurrences often lead to the conclusion that representatives of Aegidae feed on debris and carcasses on the ocean benthos. Such feeding behaviour is unconfirmed and merely speculative without in vivo study or the examination of the gut contents of these individuals. It is, however, not surprising that representatives of Aegidae collected in this manner from the ocean benthos, are exclusively female individuals, which correlate with the life cycle of Aegidae, as described as part of this dissertation (Publication V: Van der Wal & Haug 2023). The collection of only female individuals substantiates the non-feeding, bottom dwelling habit of adult females and brooding female individuals of Aegidae. Previous in vivo studies recorded the non-feeding, inactive behaviour of females, usually found hiding on the seabed (Wägele 1989, Wägele 1990, Wing & Moles 1995). Furthermore, the temporary association of representatives of Aegidae with confirmed fish hosts are not fatal, and only lasts for the time of feeding (Haug *et al.* 2021) and are therefore more accurately described as temporary parasites.

4.2.3. Representatives of permanent parasitic ingroups

The results for the group of representatives that permanently parasitises on crustaceans as adults and larvae, Epicaridea show the widest range in dactylus thickness (Fig. 7B), from straight, needle-like dactyli shapes to some of the thickest, curved dactyli of the specimens included in the analysis. Immature (larval) and adult dactyli shapes are well differentiated, both in curvature and in thickness. Adult dactyli tend to be thick with a short curved distal end, but surprisingly, larval stages tend to have thinner, straight dactyli. A striking differentiation is seen between anterior and posterior dactyli of especially the larval stages. Anterior dactyli tend to be more curved than posterior dactyli. The pattern of dactyli differentiation in Epicaridea is surprisingly similar the pattern shown by the temporary parasitic representatives of Aegidae. This high differentiation between anterior and posterior dactyli shapes in Epicaridea challenges the idea that Epicaridea are more closely related to Cymothoidae based on the character evolution of attachment structures proposed by Nagler *et al.* (2017), that all pairs of functional legs of Epicaridea and Cymothoidae are strongly curved and specialised for attaching to a host. The differentiation between anterior and posterior dactyli shapes may represent a plesiomorphic state at the node with groups containing Aegidae + (Cymothoidae + Epicaridea + Gnathiidae).

The dataset of dactyli shapes for Cymothoidae consists of those used in Publication III: Van der Wal & Haug (2020) and supplemented with dactyli shapes used in Publication IV: Van der Wal *et al.* (2021a) for *P. dvorakorum*. All dactyli of both adult and immature representatives are strongly curved and hook-like, similar in shape to the anterior dactyli of Aegidae. A low differentiation is seen between the anterior and posterior dactyli shapes. This corresponding to their parasitic strategy where immature stages attached to a fish host where it will remain in the gill cavity, buccal cavity or attached to the body surface, using all 7 pairs of anterior trunk appendages to dig into the tissue of the host to remain fixed.

5. CONCLUSIONS & OUTLOOK

Incorporating fossil material in comparative analyses of morphological character states of parasitic ingroups of Cymothoidea, provides a more informative overview of changes or consistencies in morphological and developmental specialisation over time. The examination and description of the fossil species, *Parvucymoides dvorakorum* gen. et sp. nov., revealed the group's close affinity to the permanent parasitic representatives of Cymothoidea, based on their similarities in morphological specialisations for a parasitic lifestyle. The fossil material examined in this study additionally provide insight into the early origin and shifts in habitat especially for species of Cymothoidea, from freshwater to marine.

One of aims of this dissertation was to examine the morphological variation in dactylus shape, between adult and immature stage specimens of groups exhibiting varying parasitic strategies and ultimately, distinct ecological roles. The comparative analyses indicate considerable thoracopod attachment structures (dactyli) shape variation among the groups examined, as well as an increase in variation of dactyli shapes, from non-parasitic forms to more parasitic and permanent parasitic forms. Furthermore, dactyli morphologies vary predominantly and almost equally, in curvature and width.

Another aim was to examine the differentiation in dactylus shape through ontogeny and between those of anterior and posterior thoracopods. The results herein indicate that ontogenetic stage, parasitic strategy and location of the attachment appendage (anterior/ posterior) play a role in the shape of the dactylus. All of the examined groups show a low differentiation in anterior- posterior dactyli shapes, except Aegidae. The latter group shows a significant and clear differentiation between anterior and posterior thoracopod dactyli, corresponding to their temporary parasitic life strategy. The plesiomorphic condition of the dactylus morphology is represented by the non-parasitic species of Cirolanidae. The analysis of the first Principal Component, the dactylus curvature, suggest a character evolution on two steps:

1. The posterior dactyli of representatives of temporary parasitic ingroups retain its resemblance to the plesiomorphic condition, while the anterior dactyli shapes diversify as specialisation for temporary attachment to a host. This first step is especially evident within Aegidae.
2. The posterior dactyli of representatives of permanent parasitic groups (Epicaridea and Cymothoidea) deviate from the plesiomorphic condition (diversify), but in the opposite directions. While the posterior dactyli of Cymothoidea have diversified with the anterior dactyli to strongly curved, the posterior dactyli of Epicaridea surprisingly diversified to straight, needle-like structures. The differentiation of posterior dactyli of representatives of permanent parasitic groups from the plesiomorphic condition is as a result of specialisation for permanently attaching to a host using all thoracopod appendages.

These results challenge the previously proposed character evolution and therefore the relatedness of Cymothoidea and Epicaridea. The similarity in the differentiation between anterior and posterior dactyli shapes of Aegidae and Epicaridea may suggest that this character state is a plesiomorphic state for at the node of Aegidae + (Cymothoidea + Epicaridea + Gnathiidae).

Examining and comparing individual specimens and species with different parasitic strategies to that available in literature, substantiated and emphasised the importance of accurate descriptions of parasitic associations and observations. The variation in uses of terms demonstrate that many obstacles can be overcome by providing clear criteria for terms used (also suggested by Haug 2018). This study provides new knowledge on the ontogenetic development and post-embryonic developmental stages of various groups and species examined. Well-documented immature stages and comparative notes amend a previously scarce dataset on immature stages, and improve our understanding of the ecological roles and importance of the lesser-known immature and larval stages of ingroups of Cymothoidea.

6. REFERENCES

- Adlard RD & Lester RJG. 1995. The life-cycle and biology of *Anilocra pomacentri* (Isopoda, Cymothoidae), an ectoparasitic isopod of the coral-reef fish, *Chromis nitida* (Perciformes, Pomacentridae). *Australian Journal of Zoology*, 43(3), 271–281. <https://doi.org/10.1071/ZO9950271>
- Allsop DJ & West SA. 2004. Sex-ratio evolution in sex changing animals. *Evolution*, 58(5), 1019–1027. Available at: <https://www.jstor.org/stable/3449196>
- Amundsen PA, Lafferty KD, Knudsen R, Primicerio R, Klemetsen A, Kuris AM .2009. Food web topology and parasites in the pelagic zone of a subarctic lake. *Journal of Animal Ecology*, 78:563–572. <https://doi.org/10.1111/j.1365-2656.2008.01518.x>
- Anderson G & Dale WE. 1981. *Probopyrus pandalicola* (Packard) (Isopoda, Epicaridea): morphology and development of larvae in culture. *Crustaceana*, 41(2), 143–161. <https://doi.org/10.1163/156854081X00192>
- Aneesh PT & Kappalli S. 2020. Protandrous hermaphroditic reproductive system in the adult phases of *Mothocya renardi* (Bleeker, 1857) (Cymothoidae: Isopoda: Crustacea)–Light and electron microscopy study. *Zoological Studies*, 59. <https://doi.org/10.6620/ZS.2020.59-61>
- Aneesh PT, Sudha K, Helna AK & Anilkumar G. 2018. *Agarna malayi* Tiwari 1952 (Crustacea: Isopoda: Cymothoidae) parasitising the marine fish, *Tenualosa toli* (Clupeidae) from India: re-description/description of parasite life cycle and patterns of occurrence. *Zoological Studies*, 57. <https://dx.doi.org/10.6620/ZS.2018.57-25>
- Artim JM, Sellers JC & Sikkil PC. 2015. Micropredation by gnathiid isopods on settlement-stage reef fish in the eastern Caribbean Sea. *Bulletin of Marine Science*, 91(4), 479–487. <http://dx.doi.org/10.5343/bms.2015.1023>
- Avenant-Oldewage A. 1994. Integumental damage caused by *Dolops ranarum* (Stuhlmann, 1891)(Crustacea: Branchiura) to *Clarias gariepinus* (Burchell), with reference to normal histology and wound-inflicting structures. *Journal of Fish Diseases*, 17(6), 641–647. <https://doi.org/10.1111/j.1365-2761.1994.tb00262.x>
- Azevedo JDS, Silva LGD, Bizzeri CRSF, Dansa-Petretski MA & Lima NRW. 2006. Infestation pattern and parasitic castration of the crustacean *Riggia paranensis* (Crustacea: Cymothoidae) on the freshwater fish *Cyphocharax gilbert* (Teleostei: Curimatidae). *Neotropical Ichthyology*, 4, 363–369. <https://doi.org/10.1590/S1679-62252006000300008>
- Bakenhaster MD. 2004. External morphological features of mancas of four parasitic isopod species (Cymothoidae) in the northern Gulf of Mexico (Doctoral dissertation, University of Southern Mississippi). Available at: https://www.researchgate.net/profile/Micah-Bakenhaster/publication/321642639_External_Morphological_Features_of_Mancas_of_Four_Parasitic_Isopod_Species_Cymothoidae_in_the_Northern_Gulf_of_Mexico/links/5a29b2c2aca2728e05dae49c/External-Morphological-Features-of-Mancas-of-Four-Parasitic-Isopod-Species-Cymothoidae-in-the-Northern-Gulf-of-Mexico.pdf
- Bakenhaster MD, McBride RS & Price WW. 2006. Life history of *Glossobius hemiramphi* (Isopoda: Cymothoidae): development, reproduction, and symbiosis with its host *Hemiramphus brasiliensis* (Pisces: Hemiramphidae). *Journal of Crustacean Biology*, 26(3), 283–294. <https://doi.org/10.1651/C-2573.1>

- Baker RA. 1976. Tissue damage and leukocytic infiltration following attachment of the mite *Unionicola intermedia* to the gills of the bivalve mollusc *Anodonta anatina*. *Journal of Invertebrate Pathology*, 27(3), 371–376. [https://doi.org/10.1016/0022-2011\(76\)90099-9](https://doi.org/10.1016/0022-2011(76)90099-9)
- Bamber RN .1985. The autecology of *Cyathura carinata* (Crustacea: Isopoda) in a cooling water discharge lagoon. *Journal of the Marine Biological Association of the United Kingdom* 65: 181–194. <https://doi.org/10.1017/S0025315400060896>
- Barber I & Dingemans NJ. 2010. Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1560), 4077–4088. <https://doi.org/10.1098/rstb.2010.0182>
- Benvenuto C & Weeks SC. 2020. Hermaphroditism and gonochorism. *The Natural History of the Crustacea: Reproductive Biology*, 6, 197–241. <http://dx.doi.org/10.1093/oso/9780190688554.003.0008>
- Beron P. 1997. On the high mountain Isopoda Oniscidea in the Old World. *Historia naturalis bulgarica*, 8, 85–100. Available at: <https://www.nmnh.com/historia-naturalis-bulgarica/pdfs/000100000081997.pdf>
- Bertin A, David B, Cézilly F & Alibert P. 2002. Quantification of sexual dimorphism in *Asellus aquaticus* (Crustacea: Isopoda) using outline approaches. *Biological Journal of the Linnean Society*, 77(4), 523–533. <https://doi.org/10.1046/J.1095-8312.2002.00125.X>
- Blaxter M & Koutsovoulos, G. 2015. The evolution of parasitism in Nematoda. *Parasitology*, 142(S1), S26–S39. <https://doi.org/10.1017/S0031182014000791>
- Blower SM, Roughgarden J. 1988. Parasitic castration: host species preferences, size-selectivity and spatial heterogeneity. *Oecologia*, (Berlin) 75: 512–515. <https://doi.org/10.1007/BF00776413>
- Bonhomme V, Picq S, Gaucherel C & Claude J. 2014. Momocs: outline analysis using R. *Journal of Statistical Software*, 56(13), 24. <https://doi.org/10.18637/jss.v056.i13>
- Boyko CB & Wolff C. 2014. Isopoda and Tanaidacea. *Atlas of crustacean Larvae*, 210–215. Available at: https://books.google.co.za/books?hl=en&lr=&id=61rCAwAAQBAJ&oi=fnd&pg=PA210&dq=Boyko+CB+%26+Wolff+C.+2014.+Isopoda+and+tanaidacea.+Atlas+of+crustacean+Larvae,+210%E2%80%93215.&ots=ctjYwaeCxb&sig=aFEVAY7TdZV7H_H5x0hNW2ocgH4#v=onepage&q&f=false
- Brandt A. 1992. Origin of Antarctic Isopoda (Crustacea, Malacostraca). *Marine Biology*, 113(3), 415–423. <https://doi.org/10.1007/BF00349167>
- Brandt A & Poore GC. 2003. Higher classification of the flabelliferan and related Isopoda based on a reappraisal of relationships. *Invertebrate Systematics*, 17(6), 893–923. <https://doi.org/10.1071/IS02032>
- Briones-Fourzán P & Lozano-Alvarez E. 1991. Aspects of the biology of the giant isopod *Bathynomus giganteus* A. Milne Edwards, 1879 (Flabellifera: Cirolanidae), off the Yucatan Peninsula. *Journal of Crustacean Biology*, 11(3), 375–385. <https://www.jstor.org/stable/1548464>
- Brook HJ, Rawlings TA & Davies RW. 1994. Protogynous sex change in the intertidal isopod *Gnorimosphaeroma oregonense* (Crustacea: Isopoda). *The Biological Bulletin*, 187(1), 99–111. <https://doi.org/10.2307/1542169>

- Bruce NL, Lew Ton HM, Poore GC & Poore GCB. 2002. Cymothoidae Leach, 1814. Crustacea: Malacostraca: Syncarida and Peracarida: Isopoda, Tanaidacea, Mictacea, Thermosbaenacea, Spelaeogriphacea. Zoological Catalogue of Australia. CSIRO Publishing, Melbourne, 168–183. ISBN 10:0643069011
- Bruce NL. 1983. Aegidae (Isopoda: Crustacea) from Australia with descriptions of three new species. *Journal of Natural History*, 17(5), 757–788.
<https://doi.org/10.1080/00222938300770591>
- Bruce NL. 1986. Cirolanidae (Crustacea: Isopoda) of Australia (Vol. 6). Australia. Records of the Australian Museum. Supplement 6, 1–239. <https://doi.org/10.3853/j.0812-7387.6.1986.98>
- Bruce NL. 1996. *Aega komai*, a new species of marine isopod crustacean (Aegidae: Flabellifera) from Japan. *Crustacean Research*, 25, 129–136. Available at:
https://www.jstage.jst.go.jp/article/crustacea/25/0/25_KJ00003243357/pdf
- Bruce NL. 2001. Marine isopod crustaceans in New Zealand. *Water and Atmosphere*, 9(3), 12–13. Available at: <https://isopods.nhm.org/pdfs/27640/27640.pdf>
- Bruce NL. 2009. The marine fauna of New Zealand: Isopoda, Aegidae (Crustacea). *NIWA Biodiversity Memoir*, 12, 21–252 pp. Available at:
<https://research.nhm.org/pdfs/31057/31057.pdf>
- Brusca RC. 1981. A monograph on the Isopoda Cymothoidae (Crustacea) of the eastern Pacific. *Zoological Journal of the Linnean Society*, 73(2), 117–199.
<https://doi.org/10.1111/j.1096-3642.1981.tb01592.x>
- Brusca RC & Iverson EW. 1985. A guide to the marine isopod Crustacea of Pacific Costa Rica. *Revista de Biología Tropical*, 33 (Supl. 1). Available at:
<https://research.nhm.org/pdfs/2242/2242.pdf>
- Brusca RC, Wilson GDF. 1991. A phylogenetic analysis of the Isopoda with some classificatory recommendations. *Memoirs of the Queensland Museum*, 31, 143–204. Available at:
<https://decapoda.nhm.org/pdfs/1011/1011.pdf>
- Bunkley-Williams L & Williams EH. 1998. Isopods associated with fishes: a synopsis and corrections. *The Journal of parasitology*, 84(5), 893–896.
<https://doi.org/10.2307/3284615>
- Bush AO, Fernandez JC, Esch GW, Seed JR & Ndez JCF. 2001. Parasitism: the diversity and ecology of animal parasites. Cambridge university press. 497pp. ISBN 0521664470.
- Bullar JF. 1876. The generative organs of the parasitic Isopoda. *Journal of Anatomy and Physiology*, 11(1), 118. Available at:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1309767/pdf/janatphys00171-0125.pdf>
- Callan HG. 1940. The effects of castration by parasites and X-rays on the secondary sex character of prawns (*Leander* spp.). *Journal of Experimental Biology*, 17, 168–179.
<https://doi.org/10.1242/jeb.17.2.168>
- Canestrari D, Bolopo D, Turlings TC, Röder G, Marcos JM & Baglione V. 2014. From parasitism to mutualism: unexpected interactions between a cuckoo and its host. *Science*, 343(6177), 1350–1352. <https://doi.org/10.1126/science.1249008>
- Chang ES & Sagi A. 2008. Male reproductive hormones. In: *Reproductive Biology of Crustaceans: Case Studies of Decapod Crustaceans* (ed. E Mente) Science Publishers, Enfield. 299–317pp. <https://doi.org/10.1201/9781439843345-7>

- Chong YT, Hatai K & Ransangan J. 2015. Life cycle of *Caecognathia coralliophila* (Crustacea, Isopoda, Gnathiidae) in hatchery reared tiger grouper, *Epinephelus fuscoguttatus*. *Bulletin of the European Association of Fish Pathologists*, 35(5), 177. Cheng TC. 2012. General parasitology. Elsevier. 827 pp. <https://doi.org/10.1016/C2009-0-02920-1>
- Čolak S, Barić R, Kolega M, Mejdandžić D, Mustać B, Petani B & Šarić, T. 2019. Effect of the pesticide deltamethrin as a treatment of *Ceratothoa oestroides* infestations of farmed sea bass *Dicentrarchus labrax*. *Aquaculture*, 500, 322–326. <https://doi.org/10.1016/j.aquaculture.2018.10.044>
- Delaney PM. 1989. Phylogeny and biogeography of the marine isopod family Corallanidae (Crustacea, Isopoda, Flabellifera). *Contributions in science*, 409,1–75. Available at: <https://archive.org/details/biostor-215007>
- Del Carmen Espinosa-Pérez M & Hendrickx ME. 2001. Checklist of isopods (Crustacea: Peracarida: Isopoda) from the eastern tropical Pacific. *Belgian Journal of Zoology*, 131(1), 43–56. Available at: <http://files.belgianjournalofzoology.eu/download/Volume%20131%281%29%2C%20pp.%2043-55.pdf>
- De Meeûs T, Michalakis Y & Renaud F. 1998. Santa Rosalia Revisited: or why are there so many kinds of parasites in the garden of earthly delights?. *Parasitology Today*, 14(1), 10–13. [https://doi.org/10.1016/s0169-4758\(97\)01163-0](https://doi.org/10.1016/s0169-4758(97)01163-0)
- De Meeûs T & Renaud, F. 2002. Parasites within the new phylogeny of eukaryotes. *Trends in parasitology*, 18(6), 247–251. [https://doi.org/10.1016/s1471-4922\(02\)02269-9](https://doi.org/10.1016/s1471-4922(02)02269-9)
- De Souza JS, da Cruz Canellas BG, Sakabe R, Dos Santos LN & Dos Santos AFGN. 2019. The parasitic isopod *Mothocya nana* drives dietary shifts and poorer condition of Brazilian silversides *Atherinella brasiliensis*. *Diseases of Aquatic Organisms*, 132(3), 229–239. <https://doi.org/10.3354/dao03307>
- Dorris M, Viney ME, Blaxter ML. 2002. Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. *International Journal for Parasitology*, 32, 1507–1517. [https://doi.org/10.1016/S0020-7519\(02\)00156-X](https://doi.org/10.1016/S0020-7519(02)00156-X)
- Dreyer H & Wägele JW. 2002. The Scutocoxifera tax. nov. and the information content of nuclear ssu rDNA sequences for reconstruction of isopod phylogeny (Peracarida: Isopoda). *Journal of Crustacean Biology*, 22(2), 217–234. <https://doi.org/10.1163/20021975-99990229>
- Ebert D. 2005. Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Library of Medicine (US), National Center for Biotechnology Information. Available at: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books>
- Ebert D, Carius JH, Little T & Decaestecker E. 2004. The evolution of virulence when parasites cause host castration and gigantism. *The American Naturalist*, 164(S5), S19–S32. <https://doi.org/10.1086/424606>
- Ebert D & Herre EA. 1996. The evolution of parasitic diseases. *Parasitology today*, 12(3), 96–101. [https://doi.org/10.1016/0169-4758\(96\)80668-5](https://doi.org/10.1016/0169-4758(96)80668-5)
- Eggleton P, Belshaw R. 1992. Insect parasitoids: an evolutionary overview. *Philosophical Transactions of the Royal Society of London*, 337, 1–20. <https://doi.org/10.1098/rstb.1992.0079>

- Etter W. 2014. A well-preserved isopod from the Middle Jurassic of southern Germany and implications for the isopod fossil record. *Palaeontology*, 57(5), 931–949. <https://doi.org/10.1111/pala.12095>
- Feldmann RM & Goolaerts S. 2005: *Palaeoga rugosa*, a new species of fossil isopod (Crustacea) from Maastrichtian rocks of Tunisia. *Journal of Paleontology*, vol. 79, p. 1031–1035. <https://www.jstor.org/stable/4095077>
- Fellous S, Quillery E, Duncan AB & Kaltz O. 2011. Parasitic infection reduces dispersal of ciliate host. *Biology letters*, 7(3), 327–329. <https://doi.org/10.1098%2Frsbl.2010.0862>
- Gandon S. 2004. Evolution of multihost parasites. *Evolution*, 58(3), 455–469. <https://www.jstor.org/stable/3449239>
- González L, Taylor RS, Bridle AR, Crosbie PB & Nowak BF. 2019. Parasitic isopods *Ceratothoa banksii* (Leach, 1818) and *Nerocila orbignyi* (Guérin-Méneville, 1832) of farmed Atlantic salmon and their potential as vectors of *Neoparamoeba perurans* (Young et al. 2007) in Tasmania. *Aquaculture*, 507, 28–34. <http://dx.doi.org/10.1016/j.aquaculture.2019.04.008>
- Groussin M, Mazel F & Alm EJ. 2020. Co-evolution and co-speciation of host-gut bacteria systems. *Cell Host & Microbe*, 28(1), 12–22. <https://doi.org/10.1016/j.chom.2020.06.013>
- Hadfield KA. 2012. The biodiversity and systematics of marine fish parasitic isopods of the family Cymothoidae from southern Africa. University of Johannesburg (South Africa). Available at: <https://hdl.handle.net/10210/8063>
- Hamilton WD & Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites?. *Science*, 218(4570), 384–387. <https://doi.org/10.1126/science.7123238>
- Hampton CH & Hampton CD. 1975. Living Organisms for the Elementary Classroom. East Carolina Univ., Greenville, N.C. 21pp. Available at: <https://files.eric.ed.gov/fulltext/ED115501.pdf>
- Hansen T & Hansen J. 2010. First fossils of the isopod genus *Aega* Leach, 1815. *Journal of Paleontology*, 84(1), 141–147. <https://doi.org/10.1666/08-083.1>
- Haug C, Mayer G, Kutschera V, Waloszek D, Maas A & Haug JT. 2011. Imaging and documenting gammarideans. *International Journal of Zoology*, 2011, 1–9. <https://doi.org/10.1155/2011/380829>
- Haug JT. 2019. Categories of developmental biology: Examples of ambiguities and how to deal with them. Essays for Alessandro Minelli, *Festschrift*, 2, 93–102. Available at: https://www.padovauniversitypress.it/system/files/attachments_field/9788869381409-0a.pdf
- Haug JT. 2020. Why the term “larva” is ambiguous, or what makes a larva?. *Acta Zoologica*, 101(2), 167–188. <https://onlinelibrary.wiley.com/doi/abs/10.1111/azo.12283>
- Haug JT, Haug C & Nagler C. 2021. Evolutionary History of Crustaceans as Parasites. In: *The Evolution and Fossil Record of Parasitism* (eds. K De Baets, JW Huntley) Topics in Geobiology, vol 49. Springer, Cham. https://doi.org/10.1007/978-3-030-42484-8_10
- Held C. 2003. Molecular evidence for cryptic speciation within the widespread Antarctic crustacean *Ceratoserolis trilobitoides* (Crustacea, Isopoda). *Antarctic biology in a global context*, 1–5. Available at: <https://epic.awi.de/id/eprint/14270/1/Hel2003j.pdf>
- Heil M. 2016. Host manipulation by parasites: cases, patterns, and remaining doubts. *Frontiers in Ecology and Evolution*, 4, 80. <https://doi.org/10.3389/fevo.2016.00080>

- Hickman CP, Roberts LS, Keen SL, Larson A, I'Anson H & Eisenhour D. 2008. Integrated Principles of Zoology. 14th edition. McGraw-Hill, Dubuque. 936 pp. ISBN 978-0-07-297004-3. Available at:
https://www.academia.edu/40498768/Hickman_Zoology_14th_ed
- Hispano C, Bultó P & Blanch AR. 2014. Life cycle of the fish parasite *Gnathia maxillaris* (Crustacea: Isopoda: Gnathiidae). *Folia Parasitologica*, 61(3), 277.
<https://doi.org/10.14411/fp.2014.026>
- Hoffman GL. 2019. Parasites of North American Freshwater Fishes, Ithaca, NY: Cornell University Press. <https://doi.org/10.7591/9781501735059>
- Holdich DM. 1968. Reproduction, growth and bionomics of *Dynamene bidentata* (Crustacea: Isopoda). *Journal of Zoology*, 156(2), 137–153. <https://doi.org/10.1111/j.1469-7998.1968.tb05925.x>
- Holdich DM. 1981. Opportunistic feeding behaviour in a predatory isopod. *Crustaceana*, 41(1), 101–103. <https://www.jstor.org/stable/20103640>
- Hoberg EP & Klassen GJ. 2002. Revealing the faunal tapestry: co-evolution and historical biogeography of hosts and parasites in marine systems. *Parasitology*, 124(07).
<http://dx.doi.org/10.1017/s0031182002001841>
- Hornung E, Taiti S & Szlavecz K. 2018. Preface – 10th ISTIB. In: *Isopods in a Changing World* (eds. E Hornung, S Taiti, K Szlavecz). *ZooKeys*, 801: 1–3.
<http://dx.doi.org/10.3897/zookeys.801.31568>
- Howells ME, Pruetz J & Gillespie TR. 2011. Patterns of gastro-intestinal parasites and commensals as an index of population and ecosystem health: the case of sympatric western chimpanzees (*Pan troglodytes verus*) and Guinea baboons (*Papio hamadryas papio*) at Fongoli, Senegal. *American Journal of Primatology*, 73(2), 173–179.
<https://doi.org/10.1002/ajp.20884>
- Hudson PJ, Dobson AP & Lafferty KD. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in ecology & evolution*, 21(7), 381–385.
<https://doi.org/10.1016/j.tree.2006.04.007>
- Huebner LK, Chadwick NE .2012. Patterns of cleaning behaviour on coral reef fish by the anemone shrimp *Ancylomenes pedersoni*. *Journal of the Marine Biological Association UK*, 92:1557–1562. <https://doi.org/10.1017/S0025315411001822>
- Hughes DP. 2014. On the origins of parasite-extended phenotypes. *Integrative and Comparative Biology*, 54, 210–217. <https://doi.org/10.1093/icb/icu079>
- Hyžný M, Bruce NL & Schloegl J. 2013. An appraisal of the fossil record for the Cirolanidae (Malacostraca: Peracarida: Isopoda: Cymothoida), with a description of a new cirolanid isopod crustacean from the Early Miocene of the Vienna Basin (Western Carpathians). *Palaeontology*, 56(3), 615–630. <https://doi.org/10.1111/pala.12006>
- Iwata H & Ukai Y. 2002. SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity*, 93: 384–385. <https://doi.org/10.1093/jhered/93.5.384>
- Jackson HG. 1926. The morphology of the isopod head. I. The head of *Ligia oceanica*. *Proceedings of the Zoological Society of London*, 1926, 885–911.
<https://doi.org/10.1111/J.1469-7998.1926.TB07133.X>

- Jameson SC, Erdmann MV, Gibson GR, Potts KW. 1998. Development of biological criteria for coral reef ecosystem assessment. *Atoll Research Bulletin*, 450: 108.
<https://doi.org/10.5479/si.00775630.450.1>
- Jansen G, Crummenerl LL, Gilbert F, Mohr T, Pfefferkorn R, Thänert R, Rosenstiel P & Schulenburg H. 2015. Evolutionary transition from pathogenicity to commensalism: global regulator mutations mediate fitness gains through virulence attenuation. *Molecular Biology and Evolution*, 32(11), 2883–2896.
<https://doi.org/10.1093/molbev/mst160>
- Jansen KP. 1971. Ecological studies on intertidal New Zealand Sphaeromatidae (Isopoda: Flabellifera). *Marine Biology*, 11(3), 262–285. <https://doi.org/10.1007/BF00401274>
- Janouskovec J & Keeling PJ. 2016. Evolution: causality and the origin of parasitism. *Current Biology*, 26(4), R174–R177. <https://doi.org/10.1016/j.cub.2015.12.057>
- Jass JP & Klauseneier B. 1987. Terrestrial isopods at the UWM Field Station. *Field Station Bulletin*, 20(2): 17–21. Available at:
https://dc.uwm.edu/cgi/viewcontent.cgi?article=1122&context=fieldstation_bulletins
- Johnson WS, Stevens M & Watling L. 2001. Reproduction and development of marine peracaridans. *Advances in Marine Biology*, 105–260. [https://doi.org/10.1016/S0065-2881\(01\)39009-0](https://doi.org/10.1016/S0065-2881(01)39009-0)
- Johnstone RA & Bshary R. 2002. From parasitism to mutualism: partner control in asymmetric interactions. *Ecology Letters*, 5(5), 634–639. <http://dx.doi.org/10.1046/j.1461-0248.2002.00358.x>
- Juchault P. 1999. Hermaphroditism and gonochorism. A new hypothesis on the evolution of sexuality in crustacea. *Comptes Rendus de l'Académie Des Sciences, Series III – Sciences de La Vie*, 322(5), 423–427. [https://doi.org/10.1016/s0764-4469\(99\)80078-x](https://doi.org/10.1016/s0764-4469(99)80078-x)
- Kazmi K & Yousuf F. 2013. Checklist of Peracarida of Pakistan a-marine and b-terrestrial Isopoda marine Tanaidacea. *FUUAST Journal of Biology*, 3(2), 129–139. Available at:
<https://fuuastjb.org/index.php/fuuastjb/article/view/250/231>
- Kensley BF. 2001. Isopoda. In: *Guides to the Freshwater Invertebrates of Southern Africa. Volume 4: Crustacea III, Bathynellacea, Amphipoda, Isopoda, Spelaeogriphacea, Tanaidacea and Decapoda* (eds. JA Day, BA Stewart, IJ de Moor & AE Louw). WRC Report No. TT 141/01. Available at: <https://www.wrc.org.za/wp-content/uploads/mdocs/TT-141-01.pdf>
- Kensley BF. 1978. A new marine isopod family from the south-western Indian Ocean. *Annals of the South African Museum*, 75: 41–50. Available at:
https://repository.si.edu/bitstream/handle/10088/11169/iz_1978_Kensley_A_new_marine_family_from_the_south-western_Indian_Ocean.pdf.pdf
- Keskinen E, Takaku Y, Meyer-Rochow VB & Hariyama T. 2002. Postembryonic eye growth in the seashore isopod *Ligia exotica* (Crustacea, Isopoda). *The Biological Bulletin*, 202(3), 223–231. <https://doi.org/10.2307/1543472>
- Klimov PB, O'Connor B. 2013. Is permanent parasitism reversible? Critical evidence from early evolution of house dust mites. *Systematic Biology*, 62, 411–423.
<https://doi.org/10.1093/sysbio/syt008>
- Klompmaker AA, Portell RW & Frick MG. 2017. Comparative experimental taphonomy of eight marine arthropods indicates distinct differences in preservation potential. *Palaeontology*, 60(6), 773–794. <https://doi.org/10.1111/pala.12314>

- Kortet R, Hedrick AV & Vainikka A. 2010. Parasitism, predation and the evolution of animal personalities. *Ecology letters*, 13(12), 1449–1458. <https://doi.org/10.1111/j.1461-0248.2010.01536.x>
- Kottarathil HA, Kappalli S. 2019. Reproductive system in the male phase of a parasitic isopod (Crustacea) – morphological, histological and ultrastructural evidence for sequential protandrous hermaphroditic changes. *Zoological Studies*, 58:4. <https://doi.org/10.6620/ZS.2019.58-04>.
- Lafferty D. 1997. Environmental parasitology: What can parasites tell us about human impacts on the environment?. *Parasitology today*, 13, 251–255. [https://doi.org/10.1016/s0169-4758\(97\)01072-7](https://doi.org/10.1016/s0169-4758(97)01072-7)
- Lafferty KD & Kuris AM. 2009. Parasitic castration: the evolution and ecology of body snatchers. *Trends in parasitology*, 25(12), 564–572. <https://doi.org/10.1016/j.pt.2009.09.003>
- Laguerre C, Kaldonski N, Perrot-Minnot MJ, Motreuil S & Bollache L. 2007. Modification of hosts behavior by a parasite: field evidence for adaptive manipulation. *Ecology*, 88(11), 2839–2847. Available at: <https://www.jstor.org/stable/27651442>
- Lee WY. 1977. Some laboratory cultured Crustaceans for marine pollution studies. *Marine Pollution Bulletin*, 8: 258–259. [https://doi.org/10.1016/0025-326X\(77\)90324-1](https://doi.org/10.1016/0025-326X(77)90324-1)
- Leung TL & Poulin R. 2008. Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie et Milieu/Life & Environment*, 107–115. Available at: <http://www.obs-banyuls.fr/Viemilieu/index.php/volume-58-2008/58-issue-2/582-article-2.html>
- Lester RJG. 2005. Isopoda. In: *Marine parasitology* (ed. K Rohde). 138–144 pp. ISBN0643 090258. Available at: <https://de.cdn-website.com/dcd39678c7e140728c4783df708ba9c9/files/uploaded/19.pdf>
- Leung TL & Poulin R. 2008. Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie et Milieu/Life & Environment*, 107–115. Available at: <https://hdl.handle.net/1959.11/8871>
- Littlewood DTJ, Rohde K, Bray RA & Herniou EA. 1999. Phylogeny of the Platyhelminthes and the evolution of parasitism. *Biological Journal of the Linnean Society*, 68(1–2), 257–287. <https://doi.org/10.1111/j.1095-8312.1999.tb01169.x>
- Lowry JK & Dempsey K. 2006. The giant deep-sea scavenger genus *Bathynomus* (Crustacea, Isopoda, Cirolanidae) in the Indo-West Pacific. *Tropical deep-sea benthos*, 24, 163–192. Available at: <https://isopods.nhm.org/pdfs/38866/38866.pdf>
- Lukeš J, Skalický T, Týč J, Votýpka J & Yurchenko V. 2014. Evolution of parasitism in kinetoplastid flagellates. *Molecular and biochemical parasitology*, 195(2), 115–122. <https://doi.org/10.1016/j.molbiopara.2014.05.007>
- Maguire EP, Feldmann RM, Jones WT, Schweitzer CE & Casadío S. 2018. The first fossil isopod from Argentina: a new species of Cirolanidae (Crustacea: Peracarida) from the Miocene of Patagonia. *Journal of Crustacean Biology*, 38(1), 34–44. , <https://doi.org/10.1093/jcobiol/rux100>
- Marcogliese DJ. 2004. Parasites: small players with crucial roles in the ecological theatre. *EcoHealth*, 1, 151–164. <https://doi.org/10.1007/s10393-004-0028-3>

- Marcogliese DJ. 2005. Parasites of the superorganism: are they indicators of ecosystem health?. *International journal for parasitology*, 35(7), 705–716.
<https://doi.org/10.1016/j.ijpara.2005.01.015>
- Marzal A, Lope FD, Navarro C & Møller AP. 2005. Malarial parasites decrease reproductive success: an experimental study in a passerine bird. *Oecologia*, 142(4), 541–545.
<https://doi.org/10.1007/s00442-004-1757-2>
- Maxwell JGH. 1982. Infestation of the jack mackerel, *Trachurus declivis* (Jenyns), with the cymothoid isopod, *Ceratothoa imbricatus* (Fabricus), in southeastern Australian waters. *Journal of Fish Biology*, 20(3), 341–349. <https://doi.org/10.1111/j.1095-8649.1982.tb04716.x>
- Mehana ES. E, Khafaga AF, Elblehi SS, Abd El-Hack ME, Naiel MA, Bin-Jumah M, Allam AA. 2020. Biomonitoring of heavy metal pollution using acanthocephalans parasite in ecosystem: An updated overview. *Animals*, 10(5), 811.
<https://doi.org/10.3390%2Fani10050811>
- Messana G. 2004. How can I mate without an appendix masculina? The case of *Sphaeroma terebrans* Bate, 1866 (Isopoda, Sphaeromatidae). *Crustaceana*, 77(4), 499–505.
<https://doi.org/10.1163/15685400416433346>
- Milatovič M, Kostanjšek R & Štrus J. 2010. Ontogenetic development of *Porcellio scaber*: staging based on microscopic anatomy. *Journal of Crustacean Biology*, 30(2), 225–235. Available at: <https://www.jstor.org/stable/40665213>
- Miller MR, White A & Boots M. 2006. The evolution of parasites in response to tolerance in their hosts: the good, the bad, and apparent commensalism. *Evolution*, 60(5), 945–956. Available at: <http://www.jstor.org/stable/4095397>
- Morley NJ, Irwin SWB & Lewis JW. 2003. Pollution toxicity to the transmission of larval digeneans through their molluscan hosts. *Parasitology*, 126(7), S5–S26.
<https://doi.org/10.1017/S0031182003003755>
- Nadler LE, Bengston E, Eliason EJ, Hassibi C, Helland-Riise SH, Johansen IB, & Hechinger RF. 2021. A brain-infecting parasite impacts host metabolism both during exposure and after infection is established. *Functional Ecology*, 35(1), 105–116.
<https://doi.org/10.1111/1365-2435.13695>
- Nagler C, Eiler SM & Haug JT. 2020. Examination of functional morphology of dajiid isopods using *Arthropryxus* sp. parasitising a mysid shrimp as an example. *Acta Zoologica*, 101(4), 339–352. <https://doi.org/10.1111/azo.12298>
- Nagler C & Haug JT. 2016. Functional morphology of parasitic isopods: understanding morphological adaptations of attachment and feeding structures in *Nerocila* as a prerequisite for reconstructing the evolution of Cymothoidae. *PeerJ*, 4, e2188.
<https://doi.org/10.7717%2Fpeerj.2188>
- Nagler C, Hyžný M & Haug, JT. 2017. 168 million years old “marine lice” and the evolution of parasitism within isopods. *BMC Evolutionary Biology*, 17(1), 1–14.
<https://doi.org/10.1186/s12862-017-0915-1>
- Nunomura N. 1988. A new aegid isopod (crustacea) collected from a glass sponge. *Bulletin of the Toyama science Museum*, 12: 23–26. Available at:
<http://repo.tsm.toyama.toyama.jp/?action=repos>
- Öktener A, Şirin M & Yurdigül E. 2020. Micropredator behavior of *Rocinela dumerilii* (Isopoda, Aegidae) on *Trachurus trachurus* in the Sea of Marmara (Turkey). *Transylvanian*

- Review of Systematical and Ecological Research*, 22(2), 57–72.
<https://doi.org/10.2478/trser-2020-0011>
- Östlund-Nilsson S, Curtis L, Nilsson GE & Grutter AS. 2005. Parasitic isopod *Anilocra apogonae*, a drag for the cardinal fish *Cheilodipterus quinquelineatus*. *Marine Ecology Progress Series*, 287, 209–216. <https://doi.org/10.3354/meps287209>
- Overstreet RM. 1993. Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. In: *Pathobiology of Marine and Estuarine Organisms* (eds. JA Couch, JW Fournie) CRC Press, Boca Raton, FL. 111–156pp.
<https://doi.org/10.1201/9781003069058>
- Paracer S & Ahmadjian V. 2000. Symbiosis: an introduction to biological associations. Oxford University Press. 306pp. Available at:
<https://books.google.com.jm/books?id=o5SDmAEACAAJ&printsec=copyright#v=onepage&q&f=false>
- Paris OH & Sikora A. 1965. Radio tracer demonstration of isopod herbivory. *Ecology*, 46(5), 729–734. <https://doi.org/10.2307/1935014>
- Pearson PN. 2001. Red Queen Hypothesis. eLS. <https://doi.org/10.1038/npg.els.0001667>
- Peresan L & Roccatagliata D. 2005. First record of the hyperparasite *Liriopsis pygmaea* (Cryptoniscidae, Isopoda) from a rhizocephalan parasite of the false king crab *Paralomis granulosa* from the Beagle Channel (Argentina), with a redescription. *Journal of Natural History*, 39(4), 311–324.
<https://doi.org/10.1080/0022293042000200103>
- Pillai NK. 1967. Littoral and parasitic isopods from Kerala: families Eurydicidae, Corallanidae and Aegidae. *Journal of the Bombay Natural History Society*, 64(2), 267–283.
 Available at: <https://biostor.org/reference/149153>
- Poirotte C, Kappeler PM, Ngoubangoye B, Bourgeois S, Moussodji M & Charpentier MJE. 2016. Morbid attraction to leopard urine in *Toxoplasma* infected chimpanzees. *Current Biology*, 26, R98–R99. <https://doi.org/10.1016/j.cub.2015.12.020>
- Poore GC. 2001. Isopoda Valvifera: diagnoses and relationships of the families. *Journal of Crustacean Biology*, 21(1), 205–230. [https://doi.org/10.1651/0278-0372\(2001\)021\[0205:IVDARO\]2.0.CO;2](https://doi.org/10.1651/0278-0372(2001)021[0205:IVDARO]2.0.CO;2)
- Polley L & Thompson, RA. 2009. Parasite zoonoses and climate change: molecular tools for tracking shifting boundaries. *Trends in Parasitology*, 25(6), 285–291.
<https://doi.org/10.1016/j.pt.2009.03.007>
- Poore GC & Bruce NL. 2012. Global diversity of marine isopods (except Asellota and crustacean symbionts). PLoS ONE 7(8): e43529. <https://doi.org/10.1371/journal.pone.0043529>
- Poulin R. 1994. The evolution of parasite manipulation of host behaviour: a theoretical analysis. *Parasitology*, 109(S1), S109–S118. Sures, B. 2004. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Parasitology today*, 20, 170–177. <https://doi.org/10.1017/s0031182000085127>
- Poulin R. 1995. “Adaptive” changes in the behaviour of parasitized animals: a critical review. *International journal for parasitology*, 25(12), 1371–1383.
[https://doi.org/10.1016/0020-7519\(95\)00100-x](https://doi.org/10.1016/0020-7519(95)00100-x)
- Poulin R. 2010. Parasite manipulation of host behavior: an update and frequently asked questions. In: *Advances in the Study of Behavior* (eds. HJ Brockmann, TJ Roper, M

- Naguib, KE Wynne-Edwards, JC Mitani, and WS Leigh). Burlington, Academic Press, 151–186. <https://doi.org/10.3389/fevo.2016.00080>
- Poulin R & Randhawa HS. 2015. Evolution of parasitism along convergent lines: from ecology to genomics. *Parasitology*, 142(S1), S6–S15. <https://doi.org/10.1017/S0031182013001674>
- Poulin R & Morand S. 2000. The diversity of parasites. *The Quarterly review of biology*, 75(3), 277–293. <https://doi.org/10.1086/393500>
- Price PW. 1980. Evolutionary biology of parasites. *Monographs in Population Biology*, 15:1–237. ISBN 9780691082578. Available at: <https://press.princeton.edu/books/paperback/9780691082578/evolutionary-biology-of-parasites-mpb-15-volume-15>
- Ramdane Z & Trilles JP. 2008. Cymothoidae and Aegidae (Crustacea, Isopoda) from Algeria. *Acta Parasitologica*, 53(2), 173–178. <https://doi.org/10.2478/s11686-008-0033-8>
- Roberts LS & Janovy Jr, J. 2009. Gerald D. Schmidt e Larry S. Roberts' foundations of parasitology. McGraw-Hill New York 8th edition. ISBN 978–0–07–302827–9.
- Robin N, Marramà G, Vonk R, Kriwet J & Carnevale G. 2019. Eocene isopods on electric rays: tracking ancient biological interactions from a complex fossil record. *Palaeontology*, 62(2), 287–303. <http://doi.org/10.1111/pala.12398>
- Rollmann W. 1853. Zwei neue stereoskopische Methoden. *Annalen der Physik (und Chemie)*, 166, 186–187. <https://doi.org/10.1002/andp.18531660914>
- Salemaa H. 1987. Herbivory and microhabitat preferences of *Idotea* spp. (Isopoda) in the northern Baltic Sea. *Ophelia*, 27(1), 1–15. <https://doi.org/10.1080/00785236.1987.10422007>
- Sato T, Egusa T, Fukushima K, Oda T, Ohte N, Tokuchi N, Watanabe K, Kanaiwa M, Murakami I & Lafferty KD. 2012. Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioral manipulation of their cricket hosts. *Ecology Letters*, 15:786–793. <https://doi.org/10.1111/j.1461-0248.2012.01798.x>
- Schädel M, Hyžný M & Haug JT. 2021. Ontogenetic development captured in amber—the first record of aquatic representatives of Isopoda in Cretaceous amber from Myanmar. *Nauplius*, 29. <https://doi.org/10.1590/2358-2936e2021003>
- Schädel M, Pazinato PG, Van der Wal S & Haug JT. A fossil tanaidacean crustacean from the Middle Jurassic of southern Germany. *Palaeodiversity* 12(1), 13–30. <https://doi.org/10.18476/pale.v12.a2>
- Schädel M, Perrichot V & Haug JT. 2019. Exceptionally preserved cryptoniscium larvae—morphological details of rare isopod crustaceans from French Cretaceous Vendean amber. *Palaeontologia Electronica*, 22(71), 1–46. <https://doi.org/10.26879/977>
- Schmalfuss H. 2003. World catalog of terrestrial isopods (Isopoda: Oniscidea). *Stuttgarter Beiträge zur Naturkunde*, A(654): 341. Available at: <https://decapoda.nhm.org/pdfs/27577/27577.pdf>
- Schmidt C. 2008. Phylogeny of the terrestrial Isopoda (Oniscidea): a review. *Arthropod systematics & phylogeny*, 66(2), 191–226. <https://doi.org/10.21203/rs.3.rs-25479/v1>
- Schwanz LE. 2006. Schistosome infection in deer mice (*Peromyscus maniculatus*): impacts on host physiology, behavior and energetics. *Journal of Experimental Biology*, 209(24), 5029–5037. <https://doi.org/10.1242/jeb.02601>
- Shapiro JW & Turner PE. 2018. Evolution of mutualism from parasitism in experimental virus populations. *Evolution*, 72(3), 707–712. <https://doi.org/10.1111/evo.13440>

- Shodipo MO, Gomez RDC, Welicky RL & Sikkel PC. 2019. Apparent kleptoparasitism in fish—parasitic gnathiid isopods. *Parasitology research*, 118(2), 653–655. <https://doi.org/10.1007/s00436-018-6152-8>
- Sikkel PC, Schaumburg C, Mathenia J. 2006. Diel infestation patterns of gnathiid isopod larvae on Caribbean reef fishes. *Coral Reefs*, 25:683–689. <https://doi.org/10.1007/s00338-006-0154-1>
- Sikkel PC & Welicky RL. 2019. The ecological significance of parasitic crustaceans. *Parasitic Crustacea*. Springer, Cham. 421–477 pp. https://doi.org/10.1007/978-3-030-17385-2_10
- Smit NJ, Bruce NL & Hadfield KA. 2014. Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae. *International Journal for Parasitology, Parasites and Wildlife*, 3(2), 188–197. <https://doi.org/10.1016/j.ijppaw.2014.03.004>
- Smit NJ, Basson L & Van As JG. 2003. Life cycle of the temporary fish parasite, *Gnathia africana* (Crustacea: Isopoda: Gnathiidae). *Folia Parasitologica*, 50(2), 135–142. <http://dx.doi.org/10.14411/fp.2003.024>
- Smit NJ, Davies AJ. 2004. The curious lifestyle of the parasitic stages of gnathiid isopods. *Advances in Parasitology*, 58:289–391. [https://doi.org/10.1016/s0065-308x\(04\)58005-3](https://doi.org/10.1016/s0065-308x(04)58005-3)
- Snyder BA & Hendrix PF. 2008. Current and potential roles of soil macroinvertebrates (earthworms, millipedes, and isopods) in ecological restoration. *Restoration Ecology*, 16(4), 629–636. <https://doi.org/10.1111/j.1526-100X.2008.00484.x>
- Soto JM & Mincarone MM. 2001. Distribution and morphology of the giant isopods *Bathynomus giganteus* and *Bathynomus miyarei* (Flabellifera, Cirolanidae) off southern Brazil. *Mare Magnum*, 1(2), 141–145. Available at: <https://www.univali.br/institucional/museu-oceanografico-univali/mare-magnum/volume-1-numero-2/Documents/maremagnum13.pdf>
- Stebbing TRR. 1893. A History of Crustacea. Recent Malacostraca. D. Appleton and Co. New York. 466 pp. Available at: <https://www.biodiversitylibrary.org/item/43034#page/13/mode/1up>
- Svavarsson J, Stromberg JO & Brattegard T. 1993. The deep-sea asellote (Isopoda, Crustacea) fauna of the Northern Seas: species composition, distributional patterns and origin. *Journal of Biogeography*, 537–555. <https://doi.org/10.2307/2845725>
- Toft CA & Karter AJ. 1990. Parasite-host coevolution. *Trends in Ecology & Evolution*, 5(10), 326–329. [https://doi.org/10.1016/0169-5347\(90\)90179-H](https://doi.org/10.1016/0169-5347(90)90179-H)
- Trilles JP & Justine JL. 2006. *Elthusa arnoglossi* sp. nov. (Crustacea: Isopoda: Cymothoidae), a branchial parasite of flatfishes (Bothidae) from the Chesterfield Islands, New Caledonia. *Zootaxa*, 1338(1), 57–68. <https://doi.org/10.11646/ZOOTAXA.1338.1.4>
- Trilles JP & Öktener A. 2004. *Livoneca sinuata* (Crustacea; Isopoda; Cymothoidae) on *Loligo vulgaris* from Turkey, and unusual cymothoid associations. *Diseases of Aquatic organisms*, 61(3), 235–240. Available at: <https://www.int-res.com/articles/dao2004/61/d061p235.pdf>
- Tripathy A & Pradhan RK. 2018. Symbiotic Interactions, Law of Purposive Association and the+/+ Nature of all Co-evolution. *Indian Journal of Science and Technology*, 11, 43. <http://dx.doi.org/10.17485/ijst/2018/v11i43/132577>

- Tsai ML, Li JJ & Dai CF. 1999. Why selection favors protandrous sex change for the parasitic isopod, *Ichthyoxenus fushanensis* (Isopoda: Cymothoidae). *Evolutionary Ecology*, 13(4), 327–338. <https://doi.org/10.1023/A:1006784330895>
- Upton NPD. 1987. Asynchronous male and female life cycles in the sexually dimorphic, harem-forming isopod *Paragnathia formica* (Crustacea: Isopoda). *Journal of Zoology*, 212(4), 677–690. <https://doi.org/10.1111/j.1469-7998.1987.tb05964.x>
- Van der Wal S & Haug JT. 2019. Letter to the editor referencing “The apparent kleptoparasitism in fish-parasitic gnathiid isopods” 10.1007/s00436-018-6152-8. *Parasitology Research*, 118(5), 1679–1682. <https://doi.org/10.1007/s00436-019-06281-2>
- Van der Wal S & Haug JT. 2020. Shape of attachment structures in parasitic isopodan crustaceans: the influence of attachment site and ontogeny. *PeerJ*, 8, e9181. <https://doi.org/10.7717/peerj.9181>
- Van der Wal S, Haug JT. 2022. Reconstructing the life cycle of the isopodan group Aegidae with morphological descriptions and the importance of immature stages. *Nauplius*, 31. <https://doi.org/10.1590/2358-2936e2023007>
- Van der Wal S, Schädel M, Ekrt B & Haug JT. 2021a. Description and ontogeny of a 40-million-year-old parasitic isopodan crustacean: *Parvucymoides davorakorum* gen. et sp. nov. *PeerJ*, 9, e12317. <https://doi.org/10.7717/peerj.12317>
- Van der Wal S, Smit NJ, Bruce NL, Olaosebikan B & Hadfield KA. 2021b. Two new species of branchial fish parasitic isopod of the genus *Mothocya* Costa, in Hope, 1851 (Isopoda, Cymothoidae) from Nigeria. *International Journal for Parasitology: Parasites and Wildlife*, 15, 1–11. <https://doi.org/10.1016/j.ijppaw.2021.03.001>
- Van Valen L. 1973. A new evolutionary law. *Evolutionary Theory*, 1:1–30. Available at: <https://www.mn.uio.no/cees/english/services/van-valen/evolutionary-theory/volume-1/vol-1-no-1-pages-1-30-1-van-valen-a-new-evolutionary-law.pdf>
- Vigneshwaran P, Ravichandran S & Prema M. 2019. Parasitic isopod *Cymothoa eremita* (Brünnich 1783) (Isopoda: Cymothoidae) affects the growth of black pomfret *Parastromateus niger* (Bloch 1795) in the southeast coast of India. *Thalassas: An International Journal of Marine Sciences*, 35(1), 109–115. <https://link.springer.com/article/10.1007/s41208-018-0097-7>
- Wägele JW. 1981. Study of the Anthuridae (Crustacea: Isopoda: Anthuridea) from the Mediterranean and the Red Sea. *Israel Journal of Ecology and Evolution*, 30(3), 113–159. <https://doi.org/10.1080/00212210.1981.10688509>
- Wägele JW. 1988. Aspects of the life-cycle of the Antarctic fish parasite *Gnathia calva* Vanhöffen (Crustacea: Isopoda). *Polar Biology*, 8(4), 287–291. <https://doi.org/10.1007/BF00263177>
- Wägele JW. 1989. Evolution und phylogenetisches System der Isopoda: Stand der Forschung und neue Erkenntnisse. *Zoologica*, 140:1–262. Available at: <https://isopods.nhm.org/pdfs/2393/2393.pdf>
- Wägele JW. 1990. Growth in captivity and aspects of reproductive biology of the Antarctic fish parasite *Aega antarctica* (Crustacea, Isopoda). *Polar Biology*, 10(7), 521–527.
- Walossek D. 1999. On the Cambrian diversity of Crustacea. In: *Crustaceans and the biodiversity crisis* (eds. FR Schram, JC von Vaupel & JC Klein). Proceedings of the Fourth International Crustacean Congress, vol 1. Brill Academic Publishers, Leiden. 3–27 pp. <https://doi.org/10.1007/BF00233701>

- Webster JP & McConkey GA. 2010. Toxoplasma gondii-altered host behaviour: clues as to mechanism of action. *Folia parasitologica*, 57(2), 95.
<https://doi.org/10.14411/fp.2010.012>
- Weinstein SB & Kuris AM. 2016. Independent origins of parasitism in Animalia. *Biology Letters*, 12(7), 20160324. <https://doi.org/10.1098/rsbl.2016.0324>
- Welicky RL, Hadfield KA, Sikkell PC & Smit NJ. 2017. Molecular assessment of three species of *Anilocra* (Isopoda, Cymothoidae) ectoparasites from Caribbean coral reef fishes, with the description of *Anilocra brillae* sp. n. *ZooKeys*, (663), 21.
<http://dx.doi.org/10.3897/zookeys.663.11415>
- Wetzer R. 1990. The cloaca of an ascidian from the Galapagos Islands. *Proceedings of the Biological Society of Washington*, 103(3), 655–662.
<https://ia600209.us.archive.org/12/items/biostor-74508/biostor-74508.pdf>
- Whiteley NM & El Haj AJ. 1997. Regulation of muscle gene expression over the moult in crustacea. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 117(3), 323–331. [https://doi.org/10.1016/S0305-0491\(97\)00130-2](https://doi.org/10.1016/S0305-0491(97)00130-2)
- Wieder RW & Feldmann RM. 1992. Mesozoic and Cenozoic fossil isopods of North America. *Journal of Paleontology*, 66, 958–972. <https://www.jstor.org/stable/1305950>
- Williams EH & Williams LB. 1980. Four new species of *Renocila* (Isopoda: Cymothoidae), the first reported from the New World. *Proceedings of the Biological Society of Washington*, 93, 573–592. Available at:
http://biologia.uprm.edu/facultad/publications/Lucy_Bunkley_19800101_3.pdf
- Williams EH, Bunkley-Williams L & Ebert D. 2010. An accidental attachment of *Elthusa raynaudii* (Isopoda, Cymothoidae) in *Etmopterus* sp. (Squaliformes, Etmopteridae). *Acta Parasitologica*, 55(1), 99–101. <https://doi.org/10.2478/s11686-010-0006-6>
- Williams JD, Boyko CB. 2004. A new species of *Schizobopyrina* Markham, 1985 (Crustacea: Isopoda: Bopyridae: Bopyrinae) parasitic on a *Gnathophyllum* shrimp from Polynesia, with description of an associated hyperparasitic isopod (Crustacea: Isopoda: Cabiropidae). *Proceedings of the California Academy of Sciences*, 55, 435–450. Available at: <https://eurekamag.com/research/004/020/004020106.php>
- Williams JD & Boyko CB. 2012. The global diversity of parasitic isopods associated with crustacean hosts (Isopoda: Bopyroidea and Cryptoniscoidea). *PLoS One*, 7(4), e35350. <https://doi.org/10.1371/journal.pone.0035350>
- Wilson GD. 1991. Functional Morphology and Evolution of Isopod Genitalia. In: *Crustacean sexual biology*. Columbia University Press. 228–245 pp. Available at:
<https://research.nhm.org/pdfs/24338/24338.pdf>
- Wilson GD. 2008. Global diversity of Isopod crustaceans (Crustacea; Isopoda) in freshwater. *Hydrobiologia*, 595:231–240. <http://dx.doi.org/10.1007/s10750-007-9019-z>
- Wilson GD. 2009. The phylogenetic position of the Isopoda in the Peracarida (Crustacea: Malacostraca). *Arthropod Systematics & Phylogeny*, 67(2), 159–198. Available at:
<http://www.arthropod-systematics.de/>
- Wilson DS, Coleman K, Clark AB & Biederman L. 1993. Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. *Journal of Comparative Psychology*, 107, 250–260. <https://doi.org/10.1037/0735-7036.107.3.250>

- Windsor DA. 1998. Controversies in parasitology, most of the species on Earth are parasites. *International journal for parasitology*, 12(28), 1939–1941.
[https://doi.org/10.1016/S0020-7519\(98\)00153-2](https://doi.org/10.1016/S0020-7519(98)00153-2)
- Wing BL & Moles DA. 1995. Behavior of *Rocinela angustata* (Isopoda, Aegidae), an ectoparasite of Alaskan marine fishes. *Journal of Aquatic Animal Health*, 7(1), 34–37.
[https://doi.org/10.1577/1548-8667\(1995\)007%3C0034:BORAIA%3E2.3.CO;2](https://doi.org/10.1577/1548-8667(1995)007%3C0034:BORAIA%3E2.3.CO;2)
- Wolff C. 2009. The embryonic development of the malacostracan crustacean *Porcellio scaber* (Isopoda, Oniscidea). *Development genes and evolution*, 219(11), 545–564.
<https://doi.org/10.1007/s00427-010-0316-6>
- Wong YM, Moore PG .1996. Observations on the activity and life history of the scavenging isopod *Natatolana borealis* Lilljeborg (Isopoda: Cirolanidae) from Loch Fyne, Scotland. *Estuarine, Coastal and Shelf Science* 42: 247–262.
<https://doi.org/10.1006/ECSS.1996.0018>
- Worden BD Parker PG & Pappas PW. 2000. Parasites reduce attractiveness and reproductive success in male grain beetles. *Animal Behaviour*, 59(3), 543–550.
<https://doi.org/10.1006/anbe.1999.1368>

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