Nauplius
THE JOURNAL OF THE BRAZILIAN CRUSTACEAN SOCIETY

e-ISSN 2358-2936
www.scielo.br/nau
www.crustacea.org.br

ORIGINAL ARTICLE

Diversity of hippoidean crabs – considering ontogeny, quantifiable morphology, and phenotypic plasticity

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ABSTRACT

Representatives of Hippoidea, often called sand crabs or mole crabs, are an ingroup of Anomala. These marine crustaceans inhabit the tropical and subtropical coasts of the world, yet some also appear in temperate climates. Their adults are specialized for digging and living in sandy substrates. Hippoidean zoea-type larvae are planktic and reach large sizes up to a few centimetres. These larvae transform into megalopa larvae, strongly resembling the adult, mediating the transition to the benthic lifestyle of the adult. We reconstructed outlines in dorsal view of over 80 shields of hippoideans, including representatives of Blepharipodidae (sister group to all others), Albuneidae, and Hippidae and including adults, megalopa-type, and zoea-type larvae from all three ingroups. We conducted a morphological analysis on this data using an elliptic Fourier transformation and principal component analysis. We used the results of the analysis to discuss the life history of hippoideans and the special function of megalopae, which often lack emphasis in current research. Early stage zoea larvae, megalopae, and adults show a linear gradient in their morphological development according to our analysis. However, late stage zoea larvae deviate from this pattern, possibly due to their specialization to a long-lasting planktic life. Lastly, we discuss the influence of phenotypic plasticity in hippoidean zoea larvae.

KEYWORDS

Eucrustacea, Fourier analysis, Hippoidea, life phases, morphometrics

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SUBMITTED 03 August 2020
ACCEPTED 12 February 2021
PUBLISHED 11 June 2021

DOI 10.1590/2358-2936e2021027

Introduction

Assessment of biodiversity is a task with increasing importance for zoologists as global species numbers are plummeting (Díaz *et al.*, 2019). When we do so, it is often taxonomic diversity, mostly of adults, that is recorded, as adult forms are easier to treat from a taxonomic point of view. Organisms, however, grow and develop throughout their ontogeny, and during this process they can change their overall morphology and ecological function. This has led to the evolution of several discrete phases during the ontogeny of organisms. One such phase (which can often be further differentiated into several sub-phases) is the 'larval phase' (although there is still no uniform concept of what a larva is; Haug, 2020b). The larva can have different ecological functions than the corresponding adult, which means that one organism can contribute in more than one way to the biodiversity of an ecosystem. This can make taxonomic diversity an imprecise proxy for the ecological diversity of a biota.

It is not only common to categorize certain phases (larva, adult) during ontogeny, but also entire life histories; those which include phases that are considered larval are often categorized as 'indirect development', but using such categories is an oversimplification (Haug, 2019). One group of crab-like eucrustaceans with highly specialised larval stages differing strongly in morphology and ecology from their adults is Hippoidea (*e.g.*, Harvey *et al.*, 2014). Representatives of the group are usually known as 'sand crabs' or 'mole crabs'. Two major ingroups of Hippoidea are usually differentiated: the first one has not been named, with two major ingroups (Boyko, 2002) Hippidae (28 species) and Albuneidae (53 species), the second one is Blepharipodidae (6 species; WoRMS Editorial Board, 2020). Adult hippoideans have a body that is specialised for digging, hence the common names 'sand crab' and 'mole crab' (*e.g.*, Borradaile, 1904; Faulkes and Paul, 1997; Lastra *et al.*, 2002). Hippoidea is not an ingroup of Brachyura therefore its representatives are not "true" eubrachyuran crabs, however these common names are established and therefore used for convenience herein.

Unlike in the adult phase, individuals in the long-lasting larval phase are planktic. The larval phase includes several stages of so-called zoea-type larvae, which can grow to large sizes (*e.g.*, Harvey *et al.*, 2014; Rudolf *et al.*, 2016; Fig. 1). Zoea is the term

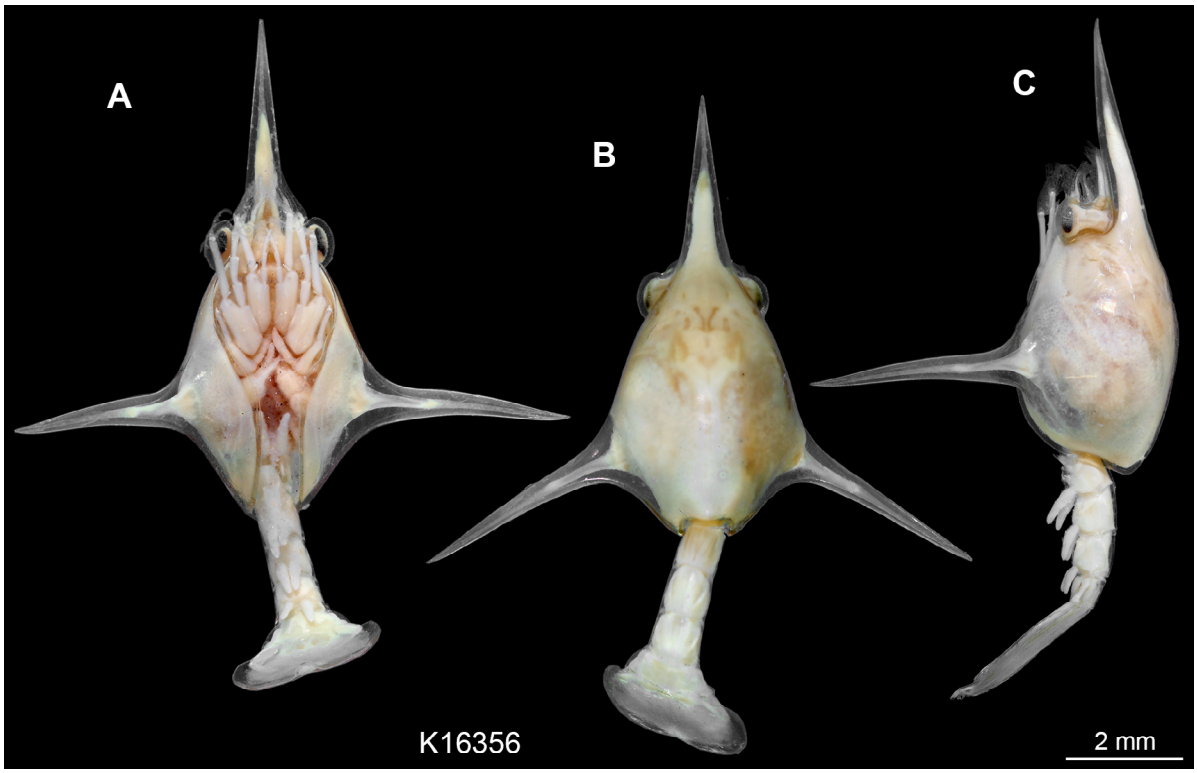

Figure 1. Larva of the group Hippidae, museum specimen ZMH-K16356 under cross-polarized light. **A**: Ventral view. **B**: Dorsal view. **C**: Lateral view.

for a morphologically distinct type of larva of many decapodan crustacean species and is characterised by having functional thorax appendages used for swimming (Fornshell, 2012; Harvey *et al.*, 2014). The last of several zoea stages moults into another type of larva, the megalopa. This larva typically performs the transition from the plankton to the benthos and is characterised by having functional swimming appendages on the pleon (Fornshell, 2012). The megalopa already resembles the adult more closely in hippoideans and moults into the first juvenile ("crab 1"). All major ingroups of Hippoidea can not only be well differentiated by their adult morphology, but also by that of their larval forms.

Here, we aim at providing background for assessing biodiversity based on all life phases of an organism in an ecosystem. We first investigate morphological differences between larval stages and adults of the group Hippoidea, not by qualitative methods but by larger scale quantitative methods, which enables us to analyse morphology quantitatively and compare larger data sets. We use outline morphometrics on the shield, the most prominent structure in larvae and adults. Based on this analysis, we evaluate the categorization of the different life phases. Lastly, we highlight the phenotypic plasticity found in larval morphology and discuss our findings.

Material and Methods

Material

Most of our data was based on specimens cited in literature that were presented in dorsal view. A complete list with figure references is provided in the appendix (App. 1). Additionally, different stages of larval specimens from plankton samples stored in the collections of the Muséum national d´Histoire naturelle in Paris (repository numbers MNHN-IU; Figs. 2–4) and of the Center of Natural History (CeNak), Universität Hamburg (repository numbers ZMH-K; Figs. 1, 3, 4) were examined (App. 1). The final number of analyzed specimens was 84 (including 34 adults, 7 megalopae, and 43 zoeae), of which 50 were representatives of Hippidae (16 adults, 4 megalopae, and 30 zoeae), 24 of Albuneidae (12 adults, 1 megalopa, and 11 zoeae) and 10 of Blepharipodidae (6 adults, 2 megalopae, and 2 zoeae).

Additionally, we further separated the zoea larvae into sub-groups. First, we differentiated them into specimens reared in the lab (exclusively specimens reported in the literature, 16) and those caught in the wild (27). Furthermore, we differentiated stage 1 zoea larvae (early stage zoea larvae) from later zoea larvae among lab-reared ones. We cannot exclude that some very small specimens among the wild-caught larvae were also stage 1 zoea larvae. Besides this uncertainty, we considered the wild-caught larvae as later zoeal stages.

Documentation methods

All specimens from museum collections were documented by the authors utilizing a macro-photography setup. The specimens were photographed using a Canon Rebel R3i digital camera with a MP-E 65mm macro lens. In order to reduce light-reflection induced artefacts, cross-polarized light was used; this was provided by a Canon Macro Twin Flash MT 24 or a Meike FC 100 LED ring light equipped with polarization filters and a cross-polarized filter in front of the camera lens (for a detailed description see Haug and Haug, 2014; Eiler *et al.*, 2016).

A prerequisite for scientific repeatability is making the basic data available. Therefore, all specimens that do not derive from the literature, that were documented by the authors, are depicted here.

Drawings

The images of the specimens gathered from the different sources were used to create reconstruction drawings of the shields. Source images of specimens in dorsal view were loaded into Inkscape version 0.92.4 (https://www.inkscape.org). Shield outlines were then redrawn (Fig. 5) using a mirroring method (only half of the specimen was drawn), focusing only on the outermost characteristics of the shield and ignoring additional details coming from other parts of the specimen (eyes, overlapping junctions, etc.). The use of scales was not necessary for the execution of the drawings due to the vector nature of the implemented analysis methods and the irrelevance of the factor of size using this method. Additionally, scales were often not available for specimens from the literature.

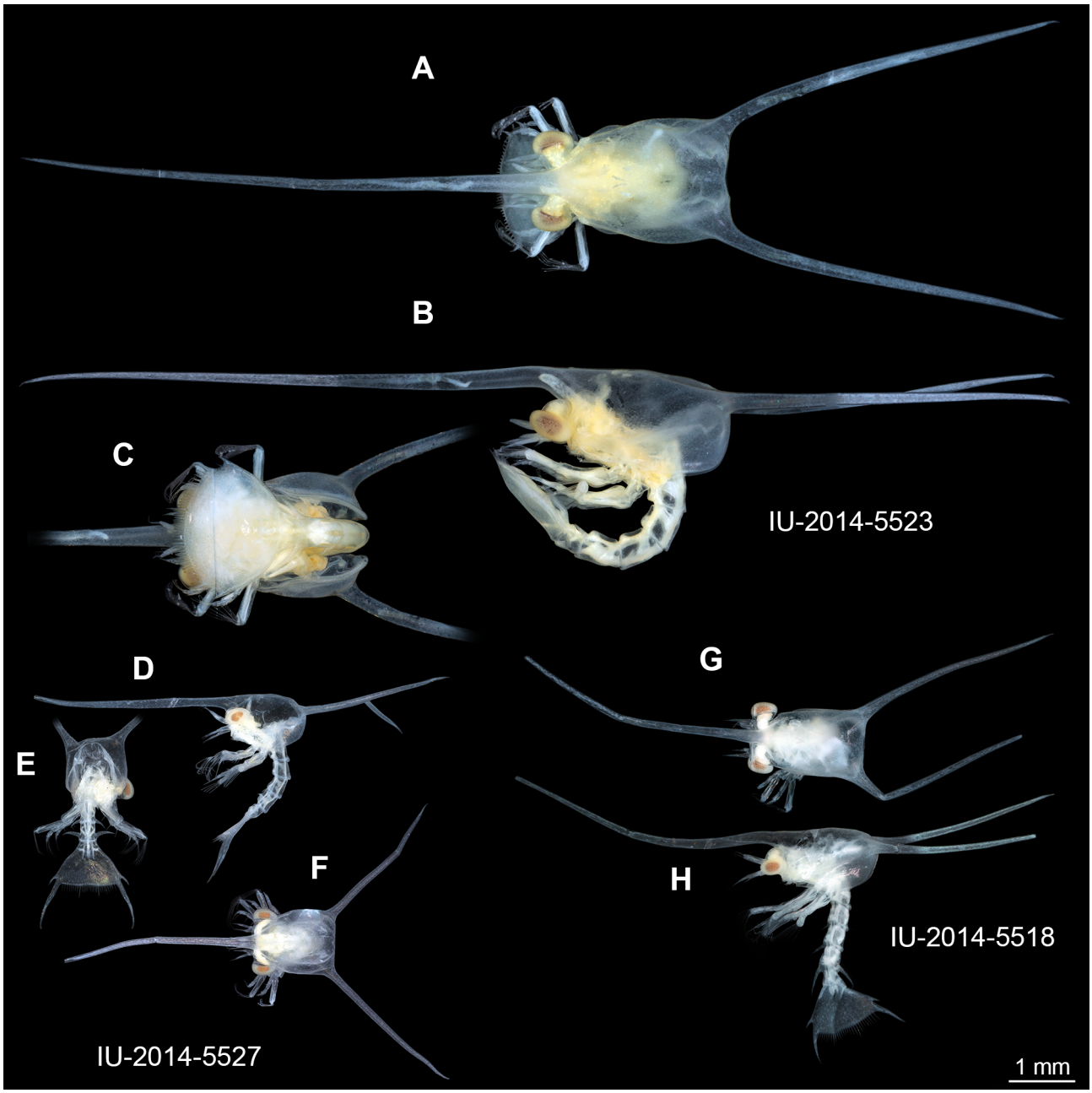

Figure 2. Larvae of the group Albuneidae, museum specimens under cross-polarized light. **A–C**: Specimen MNHN-IU-2014-5523. **A**. Dorsal view. **B**. Lateral view. **C**. Ventral view. **D–F**: Specimen MNHN-IU-2014-5527. **D**. Lateral view. **E**. Posterior view. **F**. Dorsal view. **G, H**: Specimen MNHN-IU-2014-5518. **G**. Dorsal view. **H**. Lateral view.

Shape analysis

For the statistical evaluation of shield outlines an elliptic Fourier analysis was performed using the SHAPE software package (Iwata and Ukai, 2002). The outlines of the (reconstructed) shield drawings were transformed into a vectorised object (represented by a chain code). This requires a vector-based stepwise approximation of an ellipse to the outline of the shield. The vectorised shapes (chain codes) are represented by numeric values, which are then transformed into normalised elliptic Fourier descriptors (EFDs). This technique represents a variation of the well-known Fourier transformation, practically applied on shapes of natural objects rather than (other) mathematical functions. The EFDs were analysed with a principal component analysis (PCA).

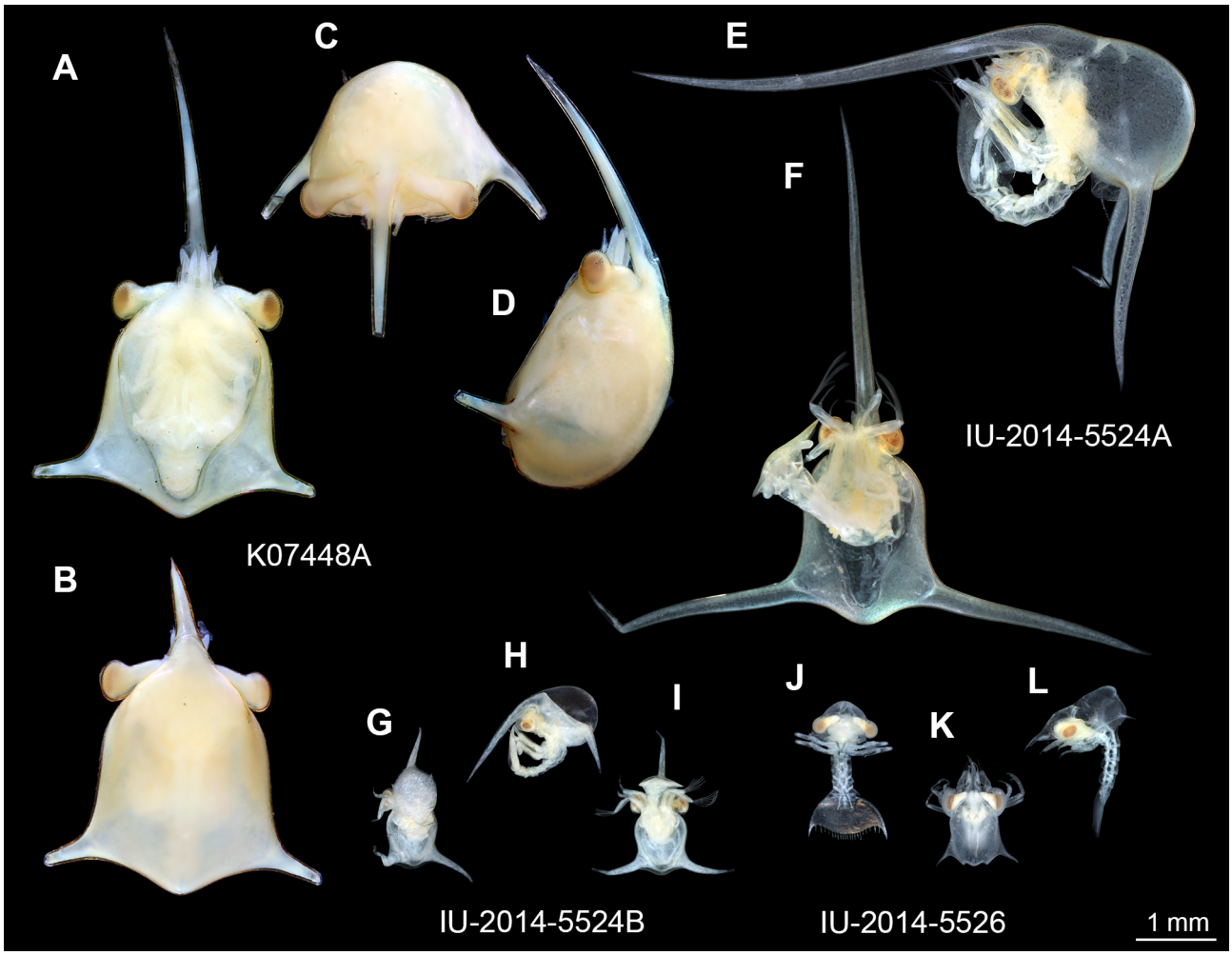

Figure 3. Larvae of the group Hippidae, museum specimens under cross-polarized light. **A–D**: Specimen ZMH-K07448A. **A**. Ventral view. **B**. Dorsal view. **C**. Anterior view. **D**. Lateral view. **E, F**: Specimen MNHN-IU-2014-5524A. **E**. Lateral view. **F**. Ventral view. **G–I**: Specimen MNHN-IU-2014-5524B. **G**. Ventral view. **H**. Lateral view. **I**. Posterior view. **J–L**: Specimen MNHN-IU-2014-5526. **J**. Anterior view. **K**. Dorsal view. **L**. Lateral view.

PCA is a multivariate ordination analysis method used to reduce a multi-dimensional data set down to a few dimensions describing the largest part of variation of said data set. In our case, 99 dimensions were analyzed which were reduced to ten dimensions. The entire procedure including the PCA was applied following Iwata and Ukai (2002), as applied in Braig *et al.* (2019). The results of the PCA were visualized using the R-statistics environment 3.4.3 (R Core Team, 2018), utilizing the interface R-studio. Packages used were ggplot2 (Wickham, 2016) and readxl (Wickham and Bryan, 2018).

Results

Dimensions of the shape analysis

The analysis resulted in a PCA with ten effective principal components showing most of the morphological diversity of shield shapes apparent in the data set (Apps. 2, 3). "Effective" in this case means that the proportion of total diversity depicted by each of these first ten created dimensions had a value larger than 1/(number of total analyzed components), in our case 1/99. Diversity here refers to the diversity in shield morphology apparent in the data set.

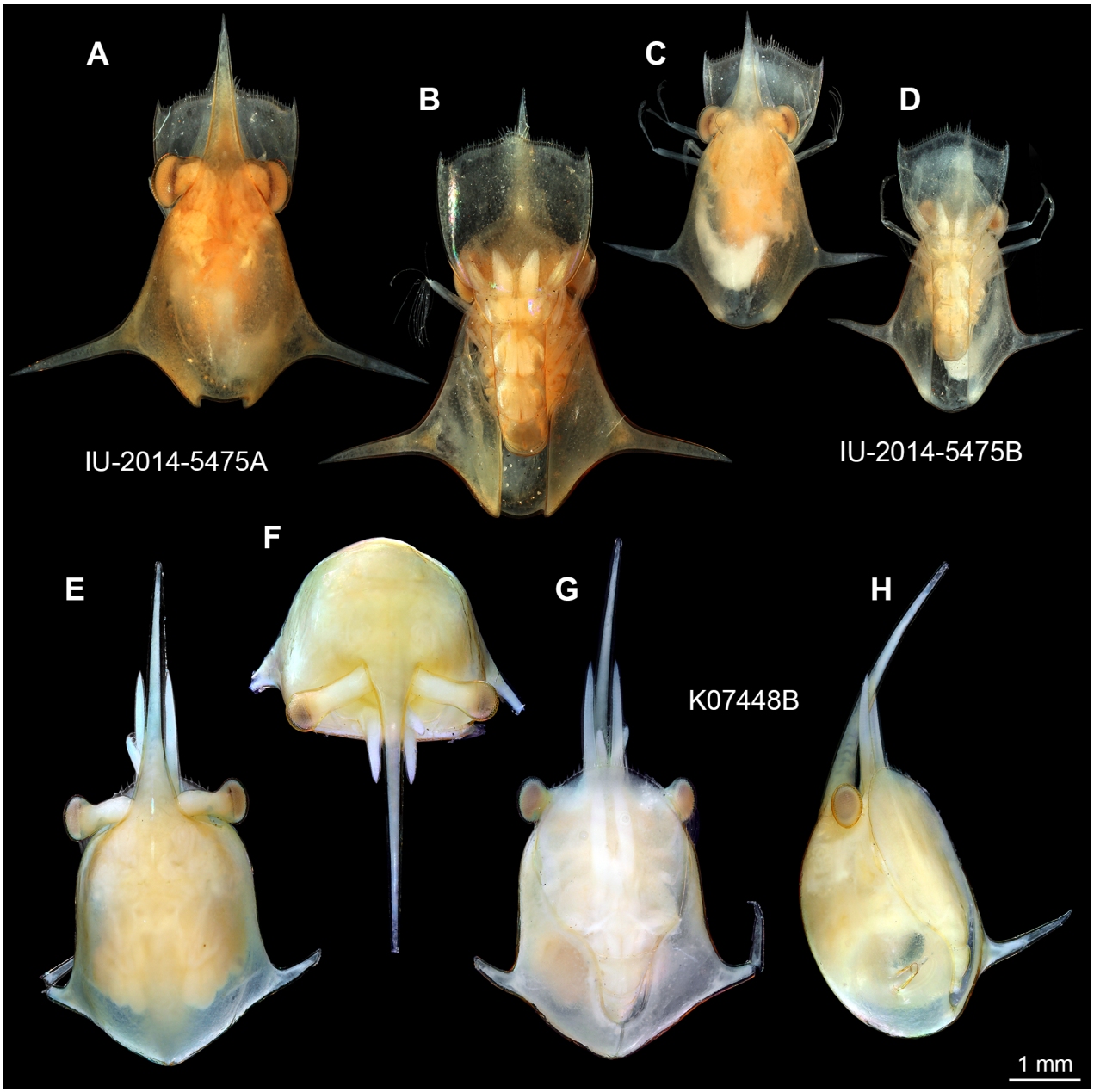

Figure 4. Larvae of the group Hippidae, museum specimens under cross-polarized light. **A, B**: Specimen MNHN-IU-2014-5475A. **A**. Dorsal view. **B**. Ventral view. **C, D**: Specimen MNHN-IU-2014-5475B. **C**. Dorsal view. **D**. Ventral view. **E–H**: Specimen ZMH-K07448B. **E**. Dorsal view. **F**. Anterior view. **G**. Ventral view. **H**. Lateral view.

The first dimension of the principal component analysis explains 86.1 % of the overall variation. It mainly describes the width or lateral extent of the shield and whether the outline is more square or more elliptic in shape (App. 2). Positive values suggest a slimmer and more elliptic shape, negative values a broader and more square shape of the shield. The median resembles a triangle with the tip being anterior, the posterior corners being rounded, and the posterior end being notched; here referred to as a "notched almond" shape.

The second dimension of the principal component analysis explains 6.6 % of the overall variation. It describes mostly whether the shield is triangular (for negative values) or elongate with an elliptic anterior and a notched posterior (for positive values). The median is weakly triangular with rounded posterior corners and a slight posterior notch, and therefore similar to the median of PC1 (App. 2).

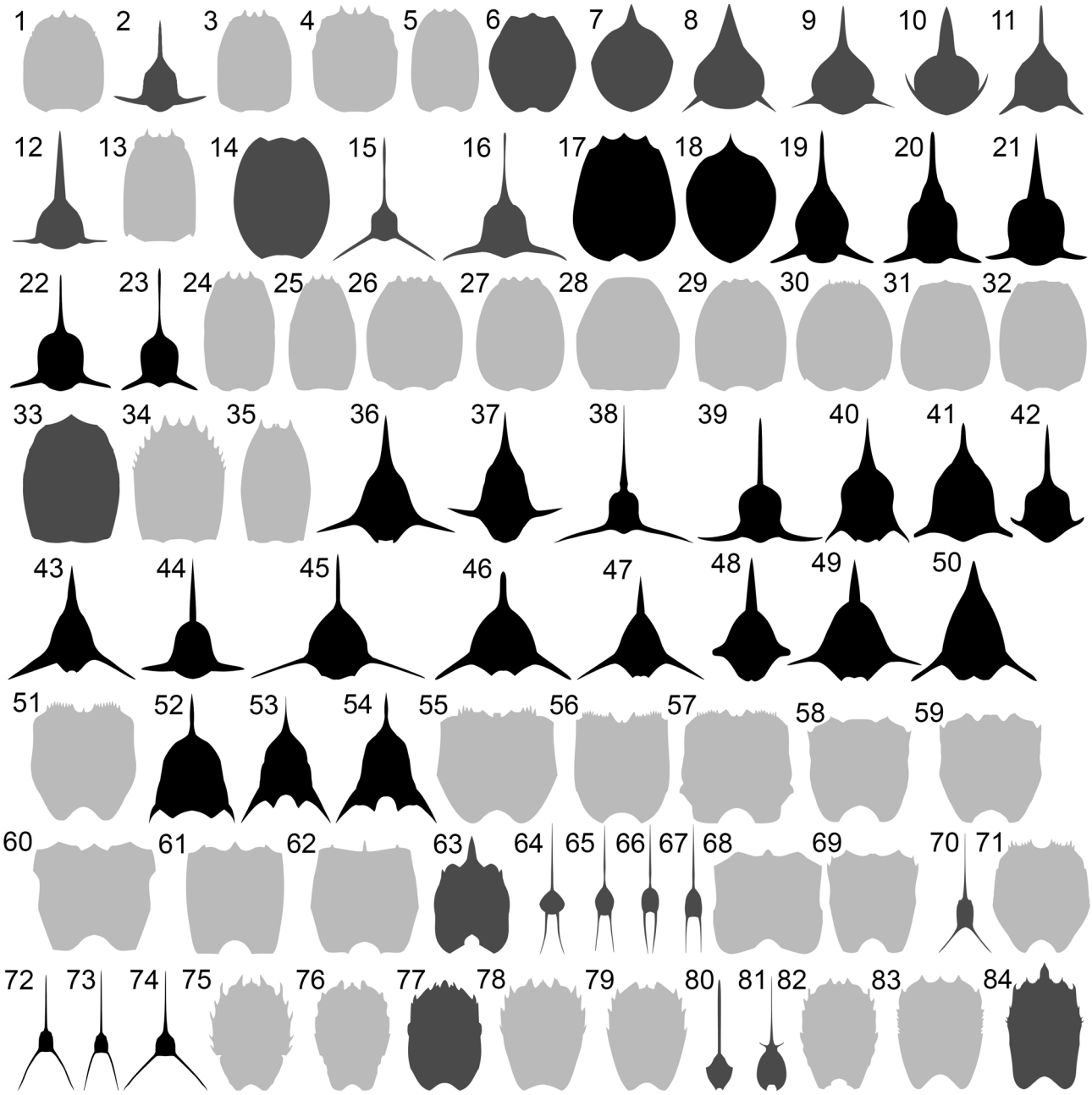

Figure 5. Overview of all reconstructed drawings of shields used for this study. List of specimens and sources given in the appendix (App. 1). Drawings not to scale. Color coding: Light grey: shields of adults; dark grey: shields of lab-reared larvae; black: shields of wild-caught larvae.

These first two principal components already explain over 90 % of variation apparent in the data set. The remaining eight principal components only explain 7.3 % (PC3 = 3.3 %; PC4 = 1.8 %; PC5 = 0.5 %; PC6 = 0.4 %; PC7 = 0.2 %; PC8 = 0.2 %; PC9 = 0.2 %; PC10 = 0.1 %) of the missing variation in the data set and are therefore not further considered here in detail.

Separation of life stages

Morphologically, the four life phases we have selected to use as distinctions in our analysis can be distinguished roughly into two groups concerning the shield. Early and late stage zoea larvae look rather similar with their elliptical to triangular long-spined shields, while megalopae look more like adults with their round to elliptical shields without long spines (Fig. 5).

When plotting the first two dimensions resulting from the principal component analysis (PC1 and PC2) in a two-dimensional plot, this qualitative separation is also represented quantitatively. The plot shows a separation of zoea larvae from both adults and megalopa larvae, the two latter overlapping strongly, excepting some very small zoea larvae which plot close to megalopae and adults (Fig. 6A). Zoea larvae plot on the right side of the plot, which indicates slim shields, elliptical to triangular in shape. Adults and megalopae plot more on the left side of the plot, which indicates broad, round to quadratic shields (Fig. 6; App. 3).

When only plotting PC1, another pattern becomes apparent (Fig. 7). The first ontogenetic stage, early zoea larvae, have mostly neutral values for PC1, which indicates "notched almond"-shaped shields (Fig. 7A; App. 3). Both megalopae and adults show more negative values for PC1, indicating the more broad and quadratic shield shapes (Fig. 7A; App. 3). Looking only at these three developmental stages one could envisage a simple gradient or linear line from early zoea stages through megalopae to adults representing a linear morphological gradient in development. Late stage zoeas however break with this pattern as they show mostly positive values for PC1 indicating slim and triangular shield forms (Fig. 7A; App. 3). Also, the variation within the group of late zoea stages seems to be lower compared to the other developmental groups, although there is a larger sample size. This morphological pattern in development is even more pronounced when discarding intra-group variation, *e.g.*, only looking at the group Hippidae (Fig. 7B).

Separation of three major systematic groups

The groups do not only show a discrete clustering according to their developmental stages, but within these ontogenetic groups there is also some further differentiation apparent. Adults and megalopae of Albuneidae plot mostly on the far-left side of the morphospace, again indicating broad, round to quadratic shields. One representative (a megalopa) is an exception; it plots slightly more to the right of the

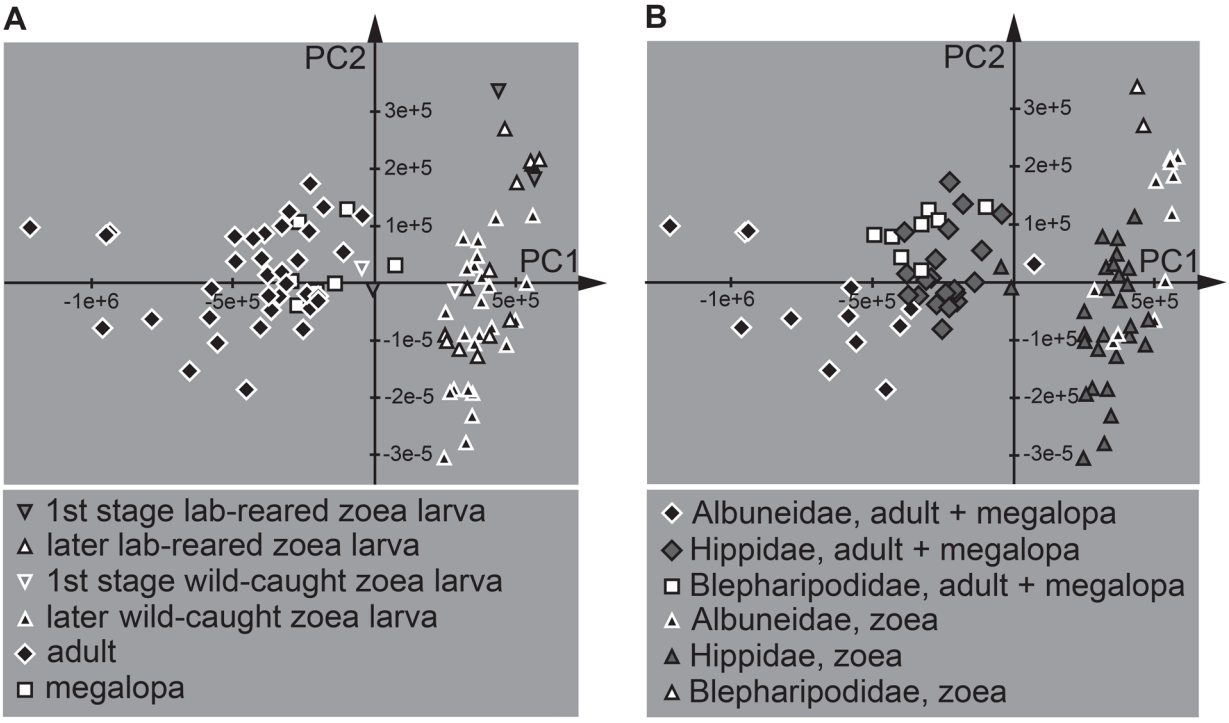

Figure 6. Plot of the first two principal components resulting from the principal component analysis (PCA) performed on the data generated by an elliptic Fourier transformation of shield shape within Hippoidea. Principal component one (PC1) explains 86.1 % of apparent diversity in the data set, principal component 2 (PC2) explains 6.6 % of the apparent diversity in the data set. Factor loadings given in the Appendix (App. 2). **A**: Grouping of specimens according to developmental stage and in case of the zoea stages also whether the material originated from the wild or from a lab-rearing. **B**: Grouping of specimens according to three major ingroups of Hippoidea.

morphospace (Fig. 6B). The individuals also mostly plot on the bottom side of the morphospace, indicating that they are wider on the posterior end than on the anterior end of the shield (App. 3). In total, Albuneidae occupy the largest area of the morphospace of all three ingroups indicating the largest morphological diversity.

The area of the morphospace occupied by adults and megalopae of Hippidae is much denser and smaller compared to that of Albuneidae, therefore covering less morphological diversity. The position of the cluster within the morphospace indicates a quadratic- to "notched almond"-shape of the shields (Fig. 6B; App. 3).

Adults and megalopae of Blepharipodidae plot also on the left side of the morphospace (Fig. 6B). This group has the smallest number of representatives and (not surprisingly) shows the smallest occupied area in the morphospace indicating smaller morphological diversity. Their position in the plot indicates again a quadratic to "notched almond" shield form (Fig. 6B; App. 3).

The zoea larvae also cluster according to their systematic affiliation. The two zoeae of Blepharipodidae plot at the top of the morphospace, indicating a very elliptic shape. Beneath them and slightly to the right plot most zoeae of Albuneidae in a dense small group indicating again the median "notched almond"-shape of the shield. Zoeae of Hippidae plot just below the denser sub-group of zoeae of Albuneidae indicating a more triangular form of the shield (Fig. 6B; App. 3).

Differences in larvae depending on their environment

When plotting the first two dimensions resulting from the principal component analysis in a two-dimensional plot, only including zoea stages, there is a difference apparent between zoea larvae obtained from the wild and zoea larvae reared in the lab (Fig. 8). Wild-caught zoeae plot more on the left side of the morphospace due to negative values for PC2. These values indicate a more triangular shield shape (App. 3). Lab-reared zoea larvae plot on the middle and right side of the morphospace due to their more positive values. The positive values indicate more elliptic shield shapes, while the neutral values indicate "notched almond"-shaped shields.

The cluster of lab-reared larvae has two outliers, both plotting in the center of the morphospace. Their position can be explained by being first stage larvae. Upon inspection, these two specimens were spineless except for a rostrum, explaining their position in the morphospace.

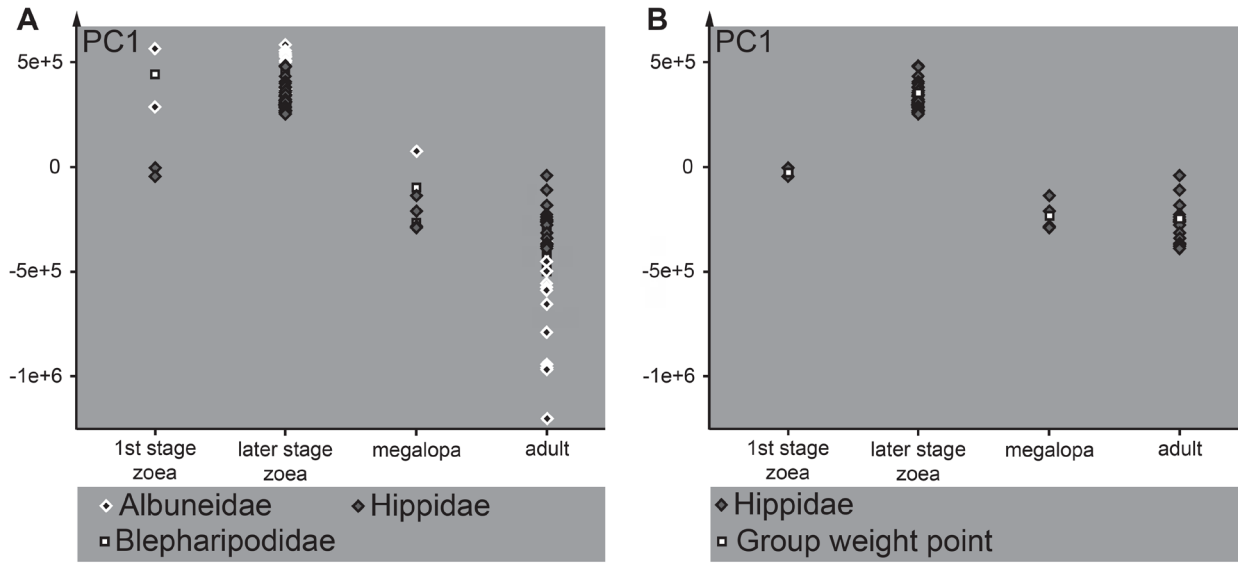

Figure 7. Plot of the first principal component resulting from the principal component analysis (PCA) performed on the data generated by an elliptic Fourier transformation of shield reconstruction drawings of the group Hippoidea. Principal component one (PC1) explains 86.1 % of apparent diversity in the data set. Factor loadings given in the Appendix (App. 2). **A**: All specimens included in the analysis. **B**: Representatives of Hippidae, with the addition of weight points to each developmental group for a clearer illustration of developmental pattern.

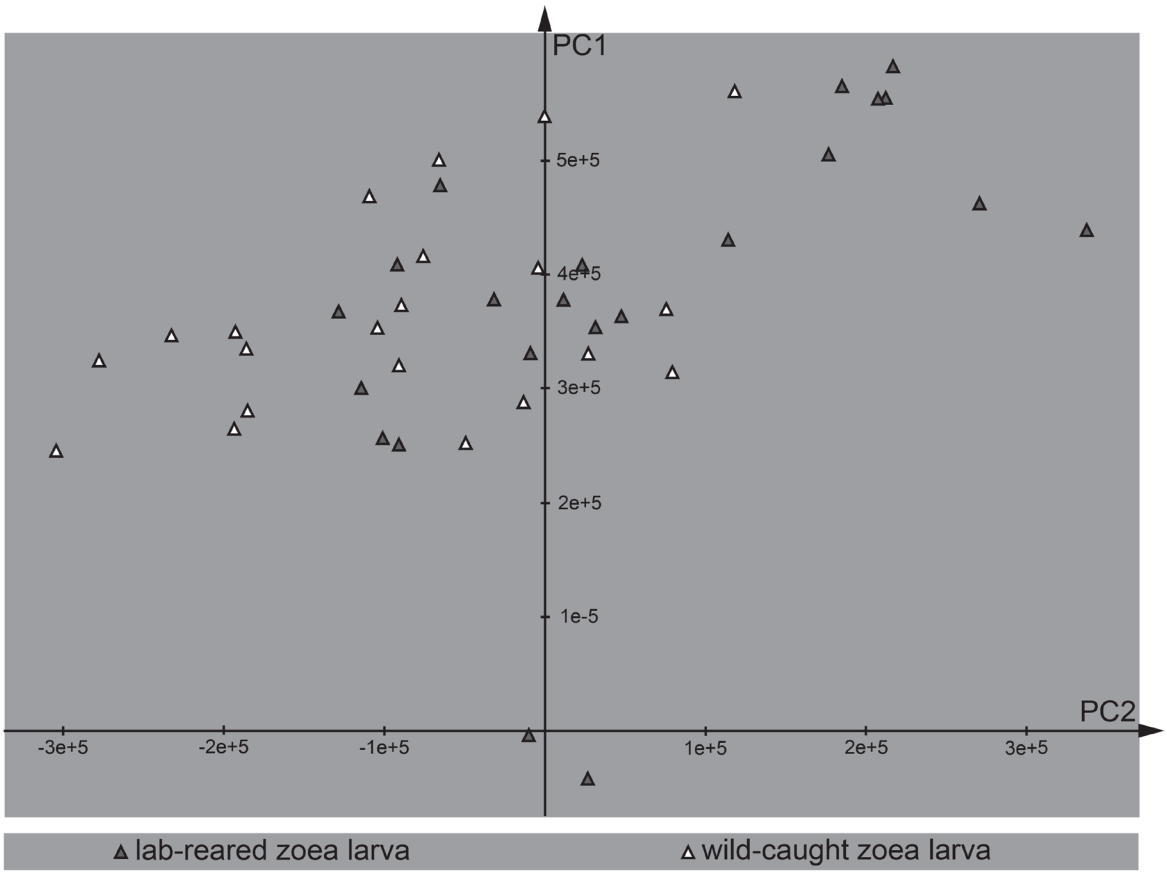

Figure 8. Plot of the first two principal components resulting from the principal component analysis (PCA) performed on the data generated by an elliptic Fourier transformation of shield shape in the group Hippoidea. Only zoeae larvae are considered. Principal component one (PC1) explains 86.1 % of apparent diversity in the data set, principal component 2 (PC2) explains 6.6 % of the apparent diversity in the data set. Factor loadings given in the Appendix (App. 2). Grouping of specimens according to origin of the material: from the wild or from a lab rearing.

Discussion

Limitations of the approach

The approach presented herein faces several challenges in practice:

1) The sample size for many sub-groups is quite limited. Faulkes (2017) mentions that field sampling more than a thousand specimens over several years yielded fewer than 10 gravid females, making laboratory-breeding challenging in itself. The tradition of rearing larvae from eggs carried by gravid females (*e.g.*, Knight, 1967; Siddiqi and Ghory, 2006) is useful, but also has pitfalls. Using this method, there are relatively large numbers of individuals available for studying early stages, but the number of available specimens drops, as fewer and fewer individuals survive (*e.g.*, Knight, 1967). Juveniles, immatures after the megalopa stage, which might still differ from adults ("crab 1" stages), are nearly absent in the literature and even megalopa stage specimens are very rare.

Although zoea stages should supposedly be more common with this approach to rearing, we again face limitations of availability. While there are quite a number of zoea larvae of the groups Albuneidae and Hippidae reported in the literature, there are only two zoea specimens for the group Blepharipodidae (Johnson and Lewis, 1942). This is due to several reasons. First, Blepharipodidae is rather species poor (<10) compared to the other two groups of Hippoidea, leading to fewer larvae being described. Furthermore, the larvae that were described are not necessarily depicted in dorsal view for species of Blepharipodidae, as the shape of these larvae differs from that of the other two ingroups (*e.g.*, Konishi, 1987; Báez, 1997).

However, lateral views are useless for our analysis. Finally, the larvae of species of Blepharipodidae are less recognizable, as such, in plankton samples, as they resemble larvae of other ingroups of Anomala in a number of aspects. Larvae of Albuneidae and Hippidae are very distinctive in appearance with their somewhat inflated shields, long spines and the large telson. Additionally, they can reach very large sizes for zoea larvae (Martin and Ormsby, 1991; Rudolf *et al.*, 2016) and therefore can be recognized more easily in plankton samples.

Uneven sample sizes are a general challenge when dealing with comparison of larval forms. We therefore hope that studies such as the present one will raise awareness that descriptions of larvae can be positively and effectively integrated into larger-framed research questions (such as quantitative morphometrics, ecosystem-function assessment, food web analysis, and biodiversity studies).

2) In the present study, we only gathered data from a single structure, the shield, and used it as a proxy in a more complex context. When comparing larvae with their adult counterparts it is a general challenge to find structures that can be used for comparison. Larval forms lack some of the structures that are later present in adults (zoea larvae lack, for example, functional chelipeds). Also, structures prominent in larvae might be rather small and inconspicuous in adults. Therefore, it is necessary that any structure that is to be investigated is available for many specimens (commonly depicted in the literature), to create a broad data set. Finally, even if a structure is illustrated it still needs to be depicted in the same orientation in all sources (see above for larvae of Blepharipodidae). In our case, the shield of hippoideans has the advantage that it is available for many specimens. In addition, the shield is a major structure that strongly influences the overall appearance and shape of the body. Lastly, in larvae and adults the shield plays a major role for understanding the ecology of the individuals.

For both larvae and adults, the shield is the major structure of the functional exoskeleton. For zoea larvae the shield and its specializations lower sinking rates, therefore helping, particularly the rather large larvae, to remain at a sufficient depth in the water column and maintain their life in the plankton (*e.g.*, Young, 1995 and references therein). For zoea larvae of Hippidae at least, the shield appears to also form a major defensive structure (Rudolf *et al.*, 2016). This might also be a possible function for the larval shield in the two other ingroups of Hippoidea. For adults, the morphology of the shield is important for allowing the individual to "submerge" into the sand (*e.g.*, Borradaile, 1904; Faulkes and Paul, 1997; Harvey *et al.*, 2014). For both of these life phases selection seems to act strongly on shield morphology, with the shield shape reflecting aspects of their ecology. Therefore, the shield is likely a good proxy for the diversity of ecology in the groups of Hippoidea.

3) Our approach only gathers two-dimensional (2D) shape data, yet shields are three-dimensional entities. It is possible to perform shape analysis on three-dimensional (3D) data, however, here we again meet the challenge of data availability. If there were at least two different perspectives for each shield, for example dorsal and lateral, we could include three-dimensional aspects of the shields into the data analysis. However, such data are mostly absent in the literature. In order to have at least a reasonable sample size, we therefore have to fall back to 2D-analysis so that we can use data from the literature as well as data from direct examination of specimens.

This specific limitation explains some aspects of the results. Larvae of Albuneidae and Hippidae can be well differentiated based on shield shape, as the postero-lateral spines are far dorsal in larvae of Albuneidae, but far ventral in larvae of Hippidae (see Figs. 2, 3; Knight, 1967; Harvey *et al.*, 2014). However, as this information is only available in 3D, it can currently not be included into our analysis, rendering separation of the two groups less accurate.

Despite the above listed limitations of the approach, we can observe some patterns in the data, providing information on Hippoidea. As often in science, the underlying data of an analysis is not optimal. That is also applicable for our approach, which has the potential to be improved by better and greater amounts of data. However, this does not indicate that the results have no meaning. It rather means that results are less "sharp" and improving the underlying data will likely improve the results and conclusions that can be drawn from them.

Categorization of life phases: the late zoea stages

Prominent among the different ontogenetic stages are later stages of the zoea phase, essentially all zoea stages after the first one. These appear to fulfil all criteria that are generally considered to characterize larvae (summarized in Haug, 2020b and in App. 4):

1) They differ strongly in overall morphology from their corresponding adults (morpho-larva *sensu lato*). Based on the present data this difference is not only apparent qualitatively, but also quantitatively in the shield shape. Quantifiable aspects of the morphology may prove an interesting tool for identifying a larval form as such (for first stage zoeae it is not possible to see the difference quantitatively, but rather qualitatively).

2) The zoea stages possess distinct structures absent in the later adult (morpho-larva *sensu stricto*) such as the prominent spines on the shield and all structures related to swimming-type locomotion on the maxillipeds (thorax appendages 1–3; *e.g.*, compare Stuck and Truesdale, 1986 and Faulkes, 2017 their fig. 5; our Figs. 1–4). In addition, for Hippidae, all structures necessary for defensive enrolment, present in later stage zoea larvae (Fig. 4), are likewise absent (reduced) in later stages (Haig, 1974; Rudolf *et al.*, 2016). Defensive enrolment is a behavior where the trunk is bent forward under the anterior body, therefore protecting the ventral body of the larva (Haug and Haug, 2014).

3) The later zoea larvae also differ markedly in ecology from their corresponding adults (eco-larva *sensu lato*), as they are part of the plankton, feeding on other planktic organisms, while the adult is a digging (fossorial) benthic inhabitant (Faulkes and Paul, 1997; Harvey *et al.*, 2014). Also, the rather large zoea type larva is clearly a specialized dispersal-stage (eco-larva *sensu stricto*; Johnson and Lewis, 1942).

4) Finally, the transition from last zoea stage to the megalopa involves a drastic restructuring of the overall morphology (*e.g.*, Knight, 1967; Siddiqi and Ghory, 2006). This moult is therefore generally accepted as a metamorphic moult (metamorph-larva), even though there is no absolute criterion for distinguishing between a metamorphic and a non-metamorphic moult (Haug and Haug, 2013). This is also easily recognized by the quantitative morphological data of the shield. The distance in the morphospace, mostly in the first principal component (PC1; Fig. 7A), between the later stage zoea larvae and subsequent stages is quite prominent.

5) In an evolutionary context the late zoea stages clearly possess numerous characters that are apomorphic (apo-larva). These characters are most likely coupled to the challenges of a rather large organism being able to remain in the plankton, such as the long spines to reduce sinking rates.

Categorization of life phases: the megalopa

The ambiguity of the megalopa stage has a long-standing tradition in research on Decapoda. In the past this stage has also been called 'post-larva', among other names (Gurney, 1942; Felder *et al.*, 1985) and has been considered to represent a larva (Gurney, 1942), but also not to represent a larva (Felder *et al.*, 1985). Interestingly, even in cases in which it was not considered a larva it was recognized as a specific stage differing from earlier and later ones (Felder *et al.*, 1985).

Our quantitative analysis resolved the megalopa stages to be different from zoea stages but not markedly different from adults, although the megalopa do differ in certain aspects from the later stages. However, it seems unlikely that this difference will be largely accepted as sufficient for considering the megalopa as a morpho-larva (*sensu lato*). A notable difference is the setae on the pleopods, as megalopae swim with their pleopods. These structures are usually better equipped with setae than later stages, where setae will be reduced. However, this will most likely not convince many people that the megalopa should be considered a morpho-larva *sensu stricto.*

Concerning their ecology, the megalopa differs from the later adult in being a transitory stage, mediating the change in mode of life from planktic to benthic (Harvey *et al.*, 2014). However, it could be considered an eco-larva *sensu lato*, however not in the strict sense, as it does not represent a dispersal stage (Johnson, 1939). As the morphological changes of the moult to the next stage are very minor it is generally categorized as non-metamorphic, therefore the megalopa is unlikely to be recognised as a metamorph-larva.

The evolutionary framework provides an interesting view. In many lineages of meiuran crustaceans, the megalopa clearly represents a plesio-larva, retaining many plesiomorphic traits that will be lost in later stages. Such characters are prominent on the pleon and especially apparent in eubrachyuran ("true") crabs, but also in hermit crabs. In both lineages the megalopa retains a more ancestral morphology of the pleon, while the next stage ("crab 1") has the more apomorphic adult-type condition (*e.g.*, Provenzano, 1968; Martin *et al.*, 1984; Brodie and Harvey, 2001; Negreiros-Fransozo *et al.*, 2009). This seems quite different in hippoideans where the megalopa already appears to possess the highly derived adult morphology, including specializations of the pleon, but also of the appendages which are adapted for burrowing (Faulkes and Paul, 1997). Hippoideans might be unique in this aspect and a larger-scale future comparison should focus on the degree of differentiation between megalopa and later stages in more lineages of Meiura.

Categorization of life phases: the early zoea stages

For the early zoea stages most of the considerations provided for the later zoea stages apply as well, although not yet as strongly expressed on the shield. The differentiation of the mouth parts as swimming-type appendages, overall structure of shield and telson and other characters clearly provide a good argument for considering these larvae as morpho-larva *sensu lato* and morpho-larva *sensu stricto* as well as eco-larvae *sensu lato* and eco-larvae *sensu stricto*.

The aspect of metamorphosis again reveals a problem of terminology. The zoea phase as a whole is ended with a moult, generally accepted as a metamorphic one, yet the transition between the individual zoea stages are generally not considered metamorphic. Therefore, one could argue that only the last zoea stage is a metamorph-larva. Here, we face differences in research traditions. Within Insecta the term 'metamorphosis' is not applied to a single moult, but to the overall ontogenetic change during post-embryonic development (*e.g.*, Bishop *et al.*, 2006). Within Decapoda, the transition between phases seems to be considered separately, leading to the recognition of at least two metamorphosis events: from zoea to megalopa and from megalopa to the following stage (juvenile), at least in many meiurans (Haug, 2020a and references therein).

Despite these terminological issues it is worth noting that the quantitative analysis resolves some early zoea stages as much closer to the megalopa stages than the later zoea stages (Fig. 6A). This most likely reflects that the apomorphic characters of the later zoea stages, such as the very long spines on the shield, are not yet fully expressed in these early forms. In this aspect the early zoea stages may be considered as representing a more ancestral type of morphology than the later ones, although the case is much less clear than in other examples of identifying plesio-larvae (Haug, 2020b).

Categorization of life histories

The special position of late stage zoea larvae in life history is obvious, when looking at the quantitative analysis of the shield morphology of Hippoidea (Fig. 7A). The plot reveals that the late zoea stages are a true derivation, or "detour", during development. A hypothetical pattern outlined by early zoea stages, megalopa, and adults would describe a straight developmental trajectory (a downward line). This hypothetical pattern would qualify as being categorized as 'direct development'. However, the actual observed pattern, including the late zoea stages, clearly shows a pattern that needs to be considered as 'indirect' (cf. discussion in Haug, 2019).

When considering all representatives of Hippoidea, this pattern is admittedly not extremely apparent, which is likely due to varying sample sizes and different within-group variation, both in developmental and phylogenetic groups (Fig. 7A). However, when singling out the group with the largest sample size, Hippidae, this pattern becomes obvious (Fig. 7B).

In any case, the late stage zoea larvae show a group mean for PC1 that is different and more positive from other developmental stages (Fig. 7). The larger positive values for PC1 are due to their slim and long-spined shields separating them qualitatively from other developmental stages. This qualitative and quantitative "developmental detour" in late stage zoea can probably be explained by their specialization to a planktic lifestyle. The larvae sometimes need to stay in

the plankton for a long time, waiting for chemical cues to trigger settling to the substrate, which is achieved by moulting to the next stage (Harvey *et al.*, 2014).

Staying longer in the plankton has the side effect that the body size of the larvae also increases during this time. The radius of an organism, as a factor, is squared in Stokes' law for calculating sinking rates of spherical objects and therefore sinking rate increases four-fold with a linear growth of the body (Stokes, 1851; Gorski and Dodson, 1996). The larvae that stay in the plankton and grow larger over time are therefore in need of additional hydrostatic uplift, not provided by the body morphology of an early stage zoea. Specializing the shield form might therefore have become a necessity and explain why the late stage zoea larvae deviate from the 'path of direct development' roughly drafted here. Their comparatively lower variation in PC1 compared to other developmental stages also may be an indication that these selective pressures (*e.g.*, sinking rates) acting on the planktic larvae are very similar for all species of Hippoidea. Therefore, neither of the ingroups lose or change any of the features of the "ancestral zoea" of the stem species (≈ ancestor) of Hippoidea.

Phenotypic plasticity in Hippoidea

Upon inspection of the results, we found another pattern within the late zoea stages that is not explained by group affiliation: specimens originating from wild-caught samples plot quite differently from specimens originating from lab-reared samples (Fig. 8). Along the second principal component (PC2), wild-caught zoea larvae of later stages appear to exhibit larger negative values. These negative values indicate that the animals possess a more triangular shield and the spines protrude more laterally than posteriorly (see App. 2). The lab-reared zoea larvae on the other hand are mostly showing larger positive values for PC2, which indicates more slim and elliptical shields, elongated in an anterior-posterior axis. When comparing wild-caught zoea shields with lab-reared zoea shields qualitatively, the former show more laterally than posteriorly protruding spines, and these spines seem to be more strongly developed (longer in comparison). However, there are still some lab-reared zoea larvae with large protruding spines, some of them even directed laterally.

Difference in sample sizes, and therefore "missing out" on existing diversity, would be one explanation but this seems unlikely as the number of lab-reared *vs.* wild-caught specimens is 22 to 21, respectively. A more likely explanation for the differences in shield morphology between lab-reared late stage zoea larvae and wild-caught late stage zoea is phenotypic plasticity.

The term "phenotypic plasticity" refers to the ability of a single genotype to produce different phenotypes in response to stimuli from the environment (Stearns, 1989; DeWitt *et al.*, 1998; Pigliucci, 2001). It is based on the concept that the phenotype of an organism is the result of its genetic information being expressed under specific environmental influences and is, therefore, variable and adaptable. This variability and adaptability can be found in numerous traits including behavior, life history, and morphology (Miner *et al.*, 2005). This is not a new concept applied to representatives of Eucrustacea. For example, brachyurans of the species *Cancer productus* Randall, 1840 have been shown to adapt the size and strength of their chelipeds in response to their prey type. In an experimental setting, crabs fed with shelled prey developed larger chelipeds, than crabs fed with shell-less prey (Smith and Palmer, 1994; Lee, 1995). In more recent studies it has been shown that environmental change already affects the dispersal capacities of larvae, leading to shorter larval phases in response to change (*e.g.*, Bashkevin *et al.*, 2020. For further examples of phenotypic plasticity in Crustacea see Criales and Anger (1986), Chucholl (2012), and Ma *et al.* (2016)).

Coming up with an explanation for the possible case of phenotypic plasticity in hippoidean larvae led us to two hypotheses that have been proposed previously in the literature (*e.g.*, Anger, 2001; chapters 2.5, 10.1.6.1). First, there is the possibility that larger spines are a defensive mechanism against predation by fish. This has previously been shown to be the case in brachyuran zoea larvae which produce larger spines in habitats that are under high predation pressure by planktivorous fish (Morgan, 1990). Earlier stage zoea of hippoideans, which are smaller in size, mostly do not have these large spines yet. In brachyuran zoea

larvae, this has been shown to be correlated with their offshore dispersal and therefore lower exposure to predation (Morgan, 1990). A similar scenario is possible in the present case.

The second hypothesis is that the spines are needed for increasing effective buoyancy, or more precisely, lowering sinking rates. It could be the case that spines are larger in wild-caught zoeae, because these larvae become larger in size than the ones reared in the laboratory, consequently requiring those prominent spines for additional hydrostatic uplift. Spines protruding away from the body have been shown to decrease sinking rates (Anger, 2001; chapter 10.1.6.1), although they have also been shown to not decrease sinking rates (Morgan, 1987; 1990; 1992). As discussed earlier, sinking rates should increase four-fold with linear body growth. It has been shown in earlier studies that larvae reared in the laboratory are generally smaller than their wild-caught counterparts (Knight, 1967). That would explain why early stage zoea larvae do not have strongly developed spines yet, as they are smaller and do not need them for hydrostatic uplift. Additionally, it has been shown in Chinese mitten crabs (*Eriocheir sinenis* H. Milne-Edwards, 1853) that spine length is negatively correlated with water density and longer spines are formed at lower salinity levels (Furigo, F. and Anger, K., pers. comm.).

In general, lab-reared larvae have been found to deviate from the ontogenetic process shown in the wild (Gurney, 1942; Knight, 1967). However, the literature is not in complete agreement on this topic as larval stages have been described both as variable (Knight, 1967) and not variable (Stuck and Truesdale, 1986). Furthermore, larval stages from the wild have been described to be further developed than their lab-reared counter parts (based on timing of appearance of thorax limb buds and pleopods; Rees, 1959). This could be another possible explanation for the condition of the spines, meaning that larger spines are a sign of the individual being further developed. This would hint at the transition to a benthic life being the reason for phenotypic plasticity. Larvae will stay in the plankton, and therefore zoea stage, until they get a trigger to settle down (Harvey *et al.*, 2014). If this trigger does not occur, they will simply stay in the plankton for a longer time. It may therefore be possible that rearing in the laboratory provides an early settling trigger, prohibiting larvae ever getting to the large late stages.

In any case, the results indicate that the morphology of hippoidean larvae can be variable at times. This also means that the complete morphological diversity of this group cannot be fully grasped when larvae are only observed under laboratory conditions. This is important to keep in mind, as morphological diversity is generally still an underrated factor when it comes to assessing overall biological diversity. After all, the morphology of an organism influences how it can impact its environment and therefore morphology impacts the ecology of the organism.

Although it has been mentioned before (Gurney, 1942; Knight, 1967), we still see a certain lack of consideration towards museum material. We therefore want to again express the importance of wild-caught material, widely available in plankton samples in collections all over the world (recent examples include Kutschera *et al.*, 2012; Haug and Haug, 2014; Eiler *et al.*, 2016; Rudolf *et al.*, 2016; Haug *et al.*, 2016a; 2016b; 2018; Gundi *et al.*, 2020) for fully understanding and describing the ontogenetic processes of decapodan crustacean species.

Conclusion

The applied quantitative analysis method has some difficulties of application but when performed, can unravel morphological patterns that were previously unknown, or not even considered in a study. The later zoea stages of Hippoidea can be categorized as morpho- and eco-larva *sensu stricto* as outlined by Haug (2020b). The same cannot be clearly stated for the megalopa stage, however a clear categorization into a zoea or adult stage cannot be made either and it should therefore be considered as its own entity. The data on Hippoidea larvae in the literature is limited and lacks any degree of detail to fully make use of modern morphological analysis techniques. Still, the species of Hippoidea show a distinct morphological pattern during their ontogenetic process, which includes a detour at the late stage zoea level, and therefore is considered as 'indirect'. The late zoea stages also show a possible case of phenotypic plasticity, but again do not answer the question as to whether buoyancy or

predation defence is the selective pressure for long spines in decapod larvae. Lastly, we want to spread awareness of the often-overlooked potential offered by museum larval material, not only in Hippoidea but also for other decapod groups.

Acknowledgements

We thank Zen Faulkes (University of Texas Rio Grande Valley, Edinburg) and one anonymous reviewer for their comments on our manuscript, which improved it greatly in our opinion. We thank Laure Corbari (MNHN Paris), Jørgen Olesen, Tom Schiøtte, Danny Eibye-Jacobsen, and Jens T. Høeg (all ZMUC Copenhagen), who helped with work in the collections, equipment, and curation of material. Gideon T. Haug (Neuried, Germany) assisted with the photography. Research visits to MNHN Paris and ZMUC Copenhagen by CH and JTH were made possible by grants from the European Commission's (FP 6) Integrated Infrastructure Initiative programme SYNTHESYS (FR-TAF-5175, FR-TAF-5181, DK-TAF-2591). Martin Schwentner (NHM Wien) and Martin Husemann (CeNak Hamburg) are thanked for their support during a student trip to Hamburg. We thank all students helping during this trip as well as the Faculty of Biology, LMU Munich and the Lehre@LMU program for support. This study was funded by the German Research Foundation under Ha 6300/3-2 (project 'Palaeo-Evo-Devo of Malacostraca') and by the Volkswagen Foundation in the form of a Lichtenberg-Professorship. We also thank J. Matthias Starck (LMU Munich) for long-term support. We are grateful to all people providing free or low-cost software for their general support of science.

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Appendix 1. Table of material used in this study with literature source citation and or specimen location.

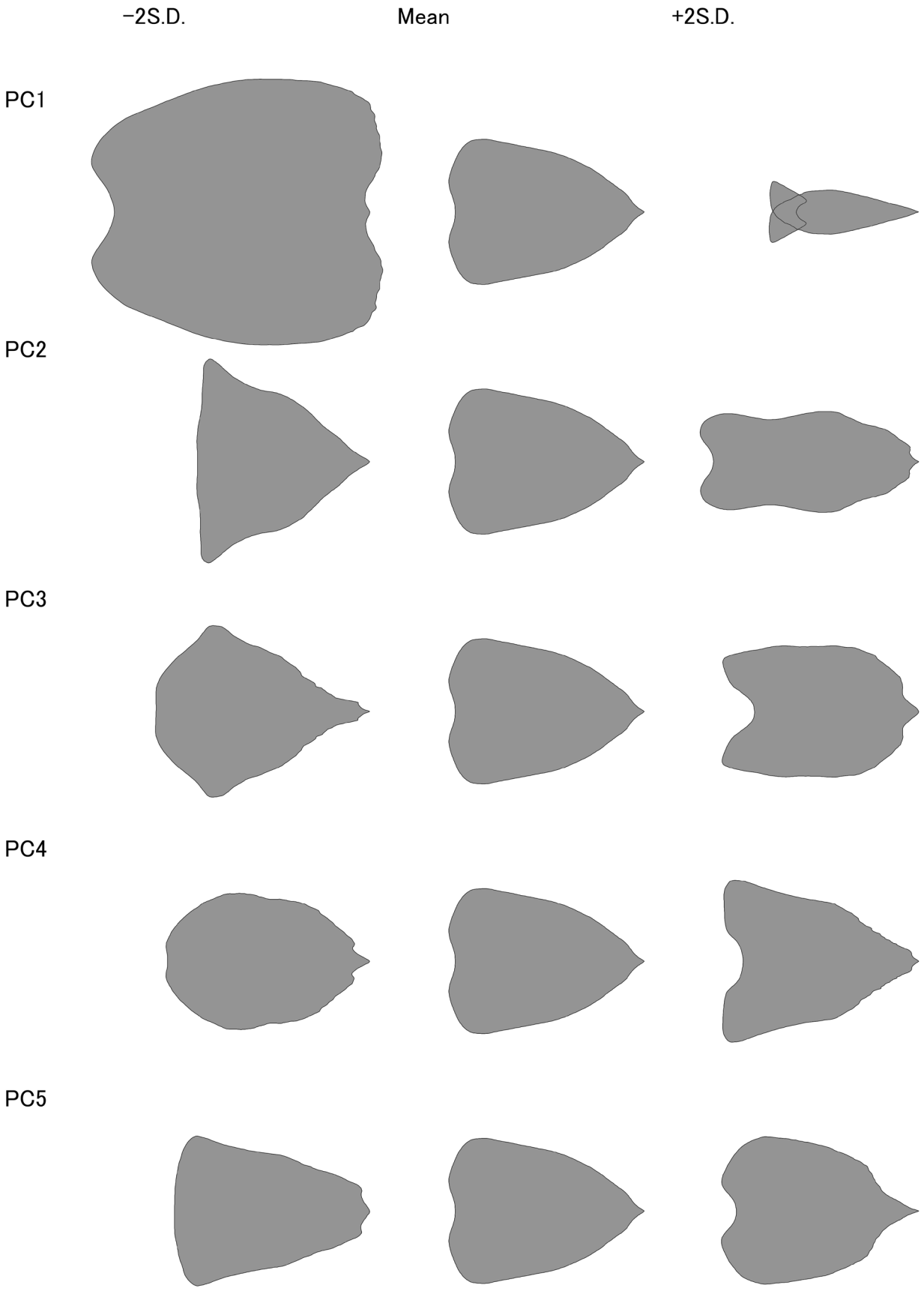
no.	major group	species group	species	size class	lab/ wild	author	year	figure	accession number	museum	geographic information	cruise	further info
1	Hippidae	*Emerita*	*analoga*	adult		Uribe *et al.*	2013	*Emerita analoga* (Stimpson, 1857)					
2	Hippidae	*Emerita*	*analoga*	late zoea	lab	Puls	2001	fig. 18C					
3	Hippidae	*Emerita*	*brasiliensis*	adult		Scelzo	2004	fig. 1B					
4	Hippidae	*Emerita*	*emiritus*	adult		Gomalanon	2016	fig. 6					
5	Hippidae	*Emerita*	*holthuisi*	adult		Sankolli	1965	fig. 1A					
6	Hippidae	*Emerita*	*holthuisi*	megalopa		Harvey *et al.*	2014	fig. 53.6B					
7	Hippidae	*Emerita*	*holthuisi*	early zoea	lab	Harvey *et al.*	2014	fig. 53.1C					
8	Hippidae	*Emerita*	*holthuisi*	late zoea	lab	Siddiqi and Ghory	2006	fig. 2A					
9	Hippidae	*Emerita*	*holthuisi*	late zoea	lab	Harvey *et al.*	2014	fig. 53.1D					
10	Hippidae	*Emerita*	*holthuisi*	late zoea	lab	Siddiqi and Ghory	2006	fig. 4A					
11	Hippidae	*Emerita*	*holthuisi*	late zoea	lab	Siddiqi and Ghory	2006	fig. 5A					
12	Hippidae	*Emerita*	*holthuisi*	late zoea	lab	Siddiqi and Ghory	2006	fig. 6A					
13	Hippidae	*Emerita*	*portoricensis*	adult		Schmitt	1935	fig. 72B					
14	Hippidae	*Emerita*	*rathbunae*	megalopa		Knight	1967	fig. 36					
15	Hippidae	*Emerita*	*rathbunae*	late zoea	wild	Knight	1967	fig. 7					
16	Hippidae	*Emerita*	*rathbunae*	late zoea	lab	Knight	1967	fig. 8					
17	Hippidae	*Emerita*	sp.	megalopa		Fonghoy	2015	fig. 37A					
18	Hippidae	*Emerita*	sp.	early zoea	lab	Fonghoy	2015	fig. 25A					
19	Hippidae	*Emerita*	sp.	late zoea	lab	Fonghoy	2015	fig. 27A					
20	Hippidae	*Emerita*	sp.	late zoea	lab	Fonghoy	2015	fig. 29A					
21	Hippidae	*Emerita*	sp.	late zoea	lab	Fonghoy	2015	fig. 31A					
22	Hippidae	*Emerita*	sp.	late zoea	lab	Fonghoy	2015	fig. 33A					
23	Hippidae	*Emerita*	sp.	late zoea	lab	Fonghoy	2015	fig. 35A					
24	Hippidae	*Emerita*	*taiwanensis*	adult		Hsueh	2015	fig. 2A					
25	Hippidae	*Emerita*	*talpoida*	adult		Gomalanon	2016	fig. 9					
26	Hippidae	*Hippa*	*adactyla*	adult		Boyko and McLaughlin	2010	fig. 1G					
27	Hippidae	*Hippa*	*granulatus*	adult		Borradaile	1904	fig. 1A					

no.	major group	species group	species	size class	lab/ wild	author	year	figure	accession number	museum	geographic information	cruise	further info
28	Hippidae	*Hippa*	*indica*	adult		Haig *et al.*	1986	fig. 1A					
29	Hippidae	*Hippa*	*marmorata*	adult		Boyko and McLaughlin	2010	fig. 1H					
30	Hippidae	*Hippa*	*ovalis*	adult		Boyko and McLaughlin	2010	fig. 1I					
31	Hippidae	*Hippa*	*strigillata*	adult		Miers	1878	fig. 3					
32	Hippidae	*Hippa*	*truncatifrons*	adult		Boyko and McLaughlin	2010	fig. 1J					
33	Hippidae	*Hippa*	*truncatifrons*	megalopa		Kato and Suzuki	1992	fig. 7A					
34	Hippidae	*Mastigochirus*	*gracilis*	adult		Miers	1878	fig. 7					
35	Hippidae	*Mastigochirus*	*quadrilobatus*	adult		Miers	1878	fig. 8					
36	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 4A–B	MNHN-IU-2014-5475A	MNHN Paris	3°38'S 9°22'E, west of Gabun	Ombango 1960, c. 12, station 301	leg. 03.05.1960
37	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 4C–D	MNHN-IU-2014-5475B	MNHN Paris	3°38'S 9°22'E, west of Gabun	Ombango 1960, c. 12, station 301	leg. 03.05.1960
38	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 3E–F	MNHN-IU-2014-5524A	MNHN Paris	23°07'S 43°11'W, south of Brazil	Calypso 1961-62, station 108	–
39	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 3G–I	MNHN-IU-2014-5524B	MNHN Paris	23°07'S 43°11'W, south of Brazil	Calypso 1961-62, station 108	–
40	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 3J–L	MNHN-IU-2014-5526	MNHN Paris	24°03'S 46°22'W, south of Brazil	Calypso 1961-62, station 139	–
41	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 3A–D	ZMH-K07448A	CeNak Hamburg	20°S 73°W, west of Chile	–	leg. H. Nissen, 23.04.1907
42	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 4E–H	ZMH-K07448B	CeNak Hamburg	20°S 73°W, west of Chile	–	leg. H. Nissen, 23.04.1907
43	Hippidae	Unknown	Unknown	zoea	wild	This paper		Fig. 1	ZMH-K16356	CeNak Hamburg	Sansibar	–	
44	Hippidae	Unknown	Unknown	zoea	wild	Rudolf *et al.*	2016	fig. 5	MNHN-IU-2014-5468	MNHN Paris			
45	Hippidae	Unknown	Unknown	zoea	wild	Rudolf *et al.*	2016	fig. 5	SMF-Mu_267	Senckenberg Naturmuseum Frankfurt			
46	Hippidae	Unknown	Unknown	zoea	wild	Rudolf *et al.*	2016	fig. 5	ZMUC-CRU-8679	NHMD Copenhagen			
47	Hippidae	Unknown	Unknown	zoea	wild	Rudolf *et al.*	2016	fig. 5	ZMUC-CRU-8680	NHMD Copenhagen			

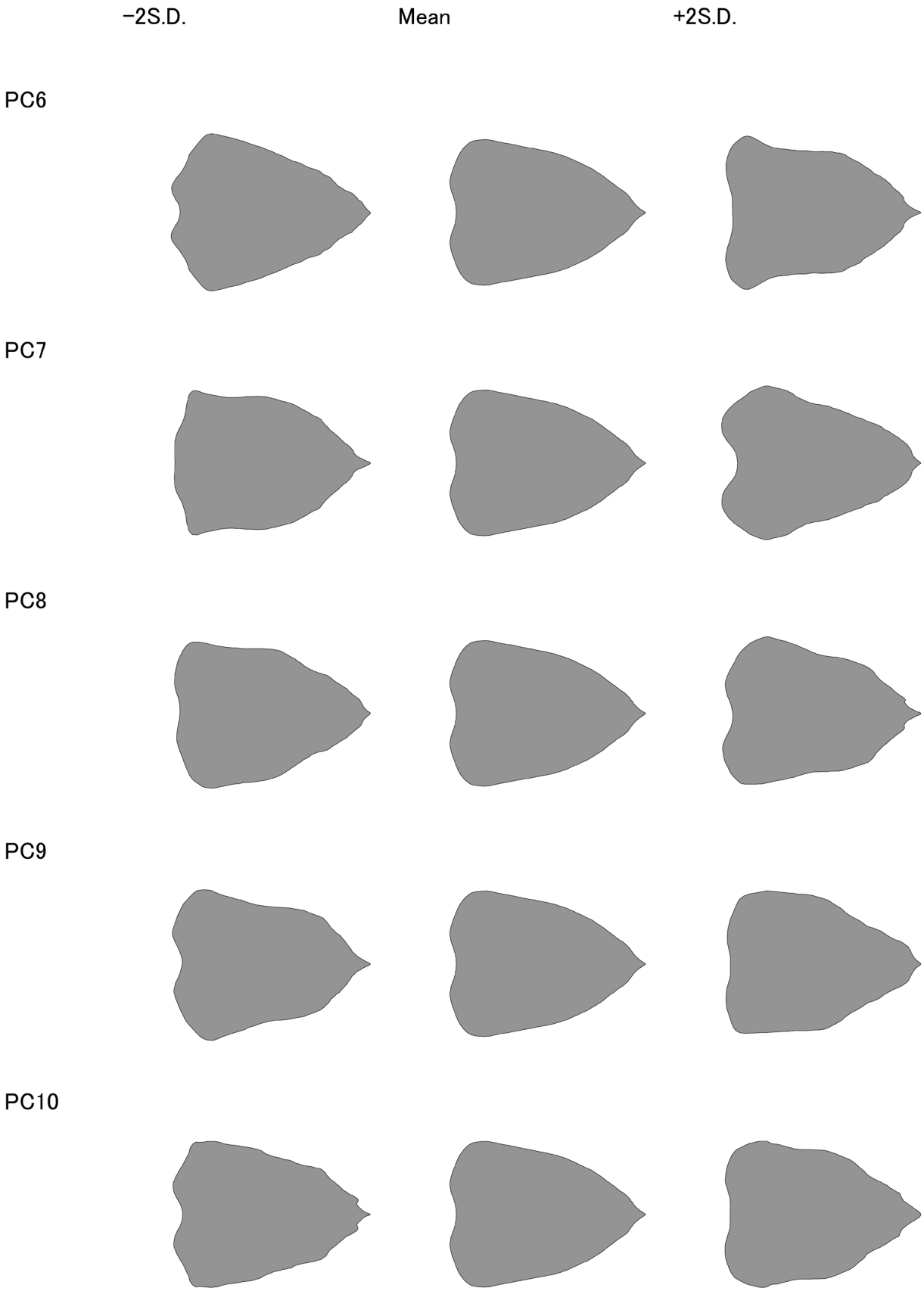
no.	major group	species group	species	size class	lab/ wild	author	year	figure	accession number	museum	geographic information	cruise	further info
48	Hippidae	Unknown	Unknown	zoea	wild	Rudolf *et al.*	2016	fig. 5	ZMUC-CRU-8682	NHMD Copenhagen			
49	Hippidae	Unknown	Unknown	zoea	wild	Rudolf *et al.*	2016	fig. 5	ZMUC-CRU-8683	NHMD Copenhagen			
50	Hippidae	Unknown	Unknown	zoea	wild	Rudolf *et al.*	2016	fig. 5	ZMUC-CRU-8684	NHMD Copenhagen			
51	Albuneidae	*Albunea*	*carabus*	adult		Abdelsalam and Ramadan	2017	fig. 2A					
52	Albuneidae	*Albunea*	*carabus*	early zoea	wild	Seridji	1988	fig. 1A					
53	Albuneidae	*Albunea*	*carabus*	late zoea	wild	Seridji	1988	fig. 2A					
54	Albuneidae	*Albunea*	*carabus*	late zoea	wild	Seridji	1988	fig. 3A					
55	Albuneidae	*Albunea*	*elioti*	adult		Boyko and McLaughlin	2010	fig. 1A					
56	Albuneidae	*Albunea*	*occulta*	adult		Boyko and McLaughlin	2010	fig. 1B					
57	Albuneidae	*Albunea*	*symmysta*	adult		Mashar *et al.*	2015	fig. 3A					
58	Albuneidae	*Austrolepidopa*	*schmitti*	adult		Efford and Haig	1968	fig. 1					
59	Albuneidae	*Austrolepidopa*	*trigonops*	adult		Efford and Haig	1968	fig. 5					
60	Albuneidae	*Lepidopa*	*benedicti*	adult		Faulkes	2017	fig. 5B (right)					
61	Albuneidae	*Lepidopa*	*benedicti*	adult		Faulkes	2017	fig. 5B (middle)					
62	Albuneidae	*Lepidopa*	*benedicti*	adult		Faulkes	2017	fig. 5B (left)					
63	Albuneidae	*Lepidopa*	*benedicti*	megalopa		Harvey *et al.*	2014	fig. 53.6A					
64	Albuneidae	*Lepidopa*	*benedicti*	early zoea	lab	Stuck and Truesdale	1986	fig. 1B					
65	Albuneidae	*Lepidopa*	*benedicti*	late zoea	lab	Stuck and Truesdale	1986	fig. 2A					
66	Albuneidae	*Lepidopa*	*benedicti*	late zoea	lab	Stuck and Truesdale	1986	fig. 3A					
67	Albuneidae	*Lepidopa*	*benedicti*	late zoea	lab	Stuck and Truesdale	1986	fig. 4B					
68	Albuneidae	*Lepidopa*	*websteri*	adult		Boyko and McLaughlin	2010	fig. 1C					
69	Albuneidae	*Paraleucolepidopa*	*myops*	adult		Boyko and McLaughlin	2010	fig. 1D					
70	Albuneidae	*Paraleucolepidopa*	*myops*	late zoea	lab	Harvey *et al.*	2014	fig. 53.1A					
71	Albuneidae	*Stemonopa*	*insignis*	adult		Efford and Haig	1968	fig. 8					
72	Albuneidae	Unknown	Unknown	zoea	wild	This paper		Fig. 2G–H	MNHN-IU-2014-5518	MNHN Paris	–	Calypso 1961-62, station 153	–

no.	major group	species group	species	size class	lab/ wild	author	year	figure	accession number	museum	geographic information	cruise	further info
73	Albuneidae	Unknown	Unknown	zoea	wild	This paper		Fig. 2A–C	MNHN-IU-2014-5523	MNHN Paris	08°25'S 34°48'W, east of Brazil	Calypso 1961-62, station 26	–
74	Albuneidae	Unknown	Unknown	zoea	wild	This paper		Fig. 2D–F	MNHN-IU-2014-5527	MNHN Paris	24°03'S 46°22'W, south of Brazil	Calypso 1961-62, station 139	–
75	Blepharipodidae	*Blepharipoda*	*doelloi*	adult		Schmitt	1942	fig. 1					
76	Blepharipodidae	*Blepharipoda*	*doelloi*	adult		Schmitt	1942	fig. 3					
77	Blepharipodidae	*Blepharipoda*	*doelloi*	megalopa		Harvey *et al.*	2014	fig. 53.6C					
78	Blepharipodidae	*Blepharipoda*	*liberata*	adult		Shen	1949	Plate XIV					
79	Blepharipodidae	*Blepharipoda*	*occidentalis*	adult		Boyko and McLaughlin	2010	fig. 1E					
80	Blepharipodidae	*Blepharipoda*	*occidentalis*	early zoea	lab	Harvey *et al.*	2014	fig. 53.1E					
81	Blepharipodidae	*Blepharipoda*	*occidentalis*	late zoea	lab	Johnson and Lewis	1942	Plate IV, fig. 1					
82	Blepharipodidae	*Blepharipoda*	*spinosa*	adult		Milne Edwards and Lucas	1841	Plate XXVIII, fig. 1					
83	Blepharipodidae	*Lophomastix*	*japonica*	adult		Duruflé	1889	*Blephacopoda Japonica* (Duru.) [*sic*]					
84	Blepharipodidae	*Lophomastix*	*japonica*	megalopa		Konishi	1987	fig. 7A					

Appendix 2. Factor loadings of the first five principal components resulting from the PCA performed on the results of the elliptic Fourier Analysis computed on the shield shapes of 84 hippoidean specimens.



Appendix 3. Factor loadings of the principal components six to ten resulting from the PCA performed on the results of the elliptic Fourier Analysis computed on the shield shapes of 84 hippoidean specimens. Anterior of the shield is always facing towards the right.



Appendix 4. Short summary of terms for different types of larvae and criteria for applying them as compiled in Haug (2020b).

Morpho-larva s. l.	Immature that differs in its morphology from that of the adult.
Morpho-larva *s. str.*	Immature that differs in its morphology from that of the adult and possesses structures that get reduced later in ontogeny.
Eco-larva *s. l.*	Immature that differs significantly in its ecological niche from that of the adult.
Eco-larva *s. str.*	Immature that differs significantly in its ecological niche from that of the adult and fulfils the specific function of dispersal.
Metamorph-larva	Immature that transforms into non-larval stage by metamorphosis.
Apo-larva	Immature that fulfils at least one of the above criteria and possesses evolutionary new structures for this specific stage.
Plesio-larva	Immature that fulfils at least one of the above criteria and possesses no evolutionary new structures for this specific stage.