

## REVIEW

# Going underwater: multiple origins and functional morphology of piercing-sucking feeding and tracheal system adaptations in water scavenger beetle larvae (Coleoptera: Hydrophiloidea)

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Larvae of water scavenger beetles (Coleoptera: Hydrophiloidea) are adapted to a wide variety of aquatic habitats, but little is known about functional and evolutionary aspects of these adaptations. We review the functional morphology and evolution of feeding strategies of larvae of the families Hydrophilidae and Epimetopidae based on a detailed scanning electron microscope (SEM) analysis, analysis of video records of feeding behaviour and observations of living larvae. There are two main types of feeding mechanisms: chewing and piercing-sucking. The character mapping using the latest phylogenetic hypothesis for Hydrophiloidea infers the chewing system as the ancestral condition. The piercing-sucking mechanism evolved at least four times independently: once in Epimetopidae (*Epimetopus*) and three times in Hydrophilidae (Berosini: *Berosus* + *Hemiosus*; Laccobiini: *Laccobius* group; Hydrobiusini: *Hybogralius*). The piercing-sucking apparatus allows underwater extra-oral digestion and decreases the dependence of larvae on an aerial environment. A detailed study of the tracheal morphology of the piercing-sucking lineages reveals four independent origins of the apneustic respiratory system, all of them nested within lineages with piercing-sucking mouthparts. We conclude that piercing-sucking mouthparts represent a key innovation, which allows for the subsequent adaptation of the tracheal system, influences the diversification dynamics of the lineages and allows the shift to new adaptive zones.

**ADDITIONAL KEYWORDS:** functional morphology – insecta – prey capture – morphological comparison – character evolution – evolution – feeding behaviour – larval development – aquatic adaptation.

## INTRODUCTION

The origin and evolution of aquatic insects has been a topic of discussion for many years. The fossil record, evolutionary history and morphological evidence

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suggest that most aquatic lineages evolved from terrestrial ancestors and have secondarily adapted to a life in a freshwater environment (Wootton, 1988; Pritchard *et al.*, 1993; Labandeira *et al.*, 1988; Grimaldi & Engel, 2005; Merritt *et al.*, 2008; Chapman *et al.*, 2012). This transition of habitat has occurred several times independently in different insect orders, at least in one stage of their life cycle. In the evolutionary history of beetles, at least eight major shifts from terrestrial to aquatic habitats have been recorded and the actual number may be more than twice that (Bilton *et al.*, 2019). Approximately 30 of the 170 families have aquatic or semi-aquatic representatives, although most water beetles belong to the families Dytiscidae, Hydraenidae, Hydrophilidae, Elmidae, Scirtidae and Gyrinidae (Jäch & Balke, 2008; Bilton *et al.*, 2019).

Hydrophiloidea is one of the most diverse aquatic beetle groups on Earth, constituting more than 28% of described species (Jäch & Balke, 2008; Short, 2018). Because of their key role in freshwater environments, comparative studies of the highly diverse morphology and biology found within the group are of major significance for understanding the evolution of aquatic beetles. They inhabit a wide range of environments from aquatic (flowing and standing water, wet rocks at waterfalls and seepages, phytotelmata, subterranean habitats and saline and thermal waters) to entirely terrestrial (organic matter in decomposition, leaf litter, mammalian dung, ant nests and flowers) (Short & Fikáček, 2013). Many authors agree that they were ancestrally aquatic and have repeatedly shifted between aquatic and terrestrial habitats along their evolutionary history (Bernhard *et al.*, 2006; Bloom *et al.*, 2014; Archangelsky *et al.*, 2016a). Bloom *et al.* (2014) inferred that at least three independent transitions from aquatic to terrestrial habitats, eight independent transitions from aquatic to semi-aquatic habitats and two secondary returns of terrestrial lineages to the aquatic environment occurred within Hydrophilidae. The transition between habitats requires physiological and morphological adaptations, such as thermo- and osmoregulation, gas exchange, feeding and locomotion (Dijkstra *et al.*, 2014; Pallarés *et al.*, 2015, 2017). These adaptations were largely studied in adults (Balfour-Browne, 1910; Balduf, 1935; Thorpe & Crisp, 1949). Although knowledge of morphological data and behaviour of immatures has increased significantly in recent years (Richmond, 1920; Wilson, 1923; Balduf, 1935; Bøving & Henriksen, 1938; Bertrand, 1950, 1972; Van Tassell, 1966; Perkins, 1972; Archangelsky, 1997; Fikáček *et al.*, 2017, 2018), it is still lacking in comparison with the available knowledge of adults of the superfamily. Considering that different lifestyles occur across the hydrophiloid tree of life, one would expect a high diversity of morphological configurations in water scavenger beetles' feeding and

respiratory systems, as well as convergences to similar functional solutions. However, there are few papers summarizing this information on pre-imaginal stages of hydrophiloids, and knowledge of this structural diversity is fragmentary and concentrated in few taxa. One might think that larvae of Hydrophilidae use similar strategies to feed and breathe in water. However, when studied in detail, larvae of different aquatic or semi-aquatic lineages have shown a great diversity of adaptations to the variety of ecological niches present in water. We will, herein, focus on feeding and gas exchange, because these factors are important in habitat utilization.

#### FEEDING

Most larvae of Hydrophiloidea have a chewing feeding system and cannot ingest solid particles, as their proventriculus is reduced (Archangelsky, 1997). Hence, they perform extra-oral digestion of the prey, which is held above the water surface to reduce the dilution of the digestive fluids (Richmond, 1920; Wilson, 1923; Balduf, 1935; Bøving & Henriksen, 1938; Bertrand, 1972; Archangelsky, 1997). The pre-digested tissue is then absorbed as a liquid. Two alternative feeding strategies are less frequent. (1) Few groups have developed a series of morphological adaptations for an entirely submerged life, including a complex underwater feeding system (piercing-sucking mechanism), which was partially described for *Berosus* Leach, 1817 (Bertrand, 1950; Van Tassell, 1966; Perkins, 1972). The structures involved in food processing were not studied in detail and the precise feeding mechanism remains unknown. (2) Larvae of the family Spercheidae are partial filter-feeders, with mouthparts adapted for filtering small particles from the water surface and the digestive tract adapted for ingestion of solid particles (including the presence of a large muscular proventriculus). Details on the filtration mechanism and food processing also remain unknown (Archangelsky, 2001).

#### RESPIRATION

Most larvae of Hydrophilidae, and all Hydrochidae and Spercheidae, have a metapneustic respiratory system (Richmond, 1920; Wilson, 1923; Balduf, 1935; Bøving & Henriksen, 1938; Bertrand, 1972; Archangelsky, 1997): only the last pair of spiracles, situated on the eighth abdominal segment, are enlarged and functional, situated in a special pocket formed by abdominal segments VIII and IX. The larvae take up oxygen from the atmosphere by exposing the terminal spiracles out of water. Recently, Fikáček *et al.* (2017) found that some hydrophilid larvae are peripneustic, with one pair of thoracic and eight pairs of abdominal spiracles

functional. This resembles the peripneustic system present in the Helophoridae and Georissidae, in which the spiracles of the eighth abdominal segment are not enlarged and specialized. In contrast, closed (apneustic) respiratory systems have been described for few lineages of the Hydrophilidae (*Epimetopus* Lacordaire 1854, *Berosus* + *Hemiosus* Sharp, 1882, *Laccobius* Erichson 1837 subg. *Yateberosus* Satô, 1966 and *Hybograalius* d'Orchymont, 1942), all of which have piercing-sucking mouthparts. Fikáček *et al.* (2018) briefly discussed the parallel evolution of these systems, hypothesizing that changes of head morphology allowed for modification of the breathing strategy.

We review here the main morphological configurations of the feeding system of Hydrophiloidea larvae and reconstruct their evolution, focusing primarily on the piercing-sucking model and associated adaptations. We infer how many times the different piercing-sucking morphologies have evolved, and from which ancestral morphologies they are derived. Furthermore, we explain the feeding behaviour and complex functional mechanics of the piercing-sucking feeding system for the first time. Additionally, we present a survey of the respiratory system of Hydrophiloidea larvae, with an emphasis on the evolutionary innovations that appeared in larvae with the piercing-sucking feeding system.

## MATERIAL AND METHODS

### MATERIAL STUDIED

The examined material is listed in [Table 1](#), specimens are deposited in the following institutions: Laboratory of Entomology, Buenos Aires University, Argentina (LEBA); Department of Entomology, National Museum, Praha, Czech Republic (NMPC) and Natural History Division, Kitakyushu Museum of Natural History and Human History, Kitakyushu-shi, Japan (KMNH). It should be noted that only a few microscopic slides of the third-instar larvae of *Hybograalius* were available for study. Therefore, some structures were difficult to observe and photograph. Additional data were taken from literature ([Supporting Information, Table S1](#)).

### LIGHT MICROSCOPY

Larvae were killed with boiling water and then preserved in 96% ethyl alcohol. Specimens were cleared in cold lactic acid for several days, dissected and mounted on glass slides in polyvinyl-lacto-glycerol or Hoyer's medium. Some larvae were stained with a saturated solution of Chlorazol black in 70% ethyl alcohol for the observation of tiny and membranous

structures. Pictures were taken with an Olympus LC30 digital camera mounted on an Olympus CX41 compound microscope or with a Leica DMLB compound microscope equipped with a Leica digital camera. Extended focal range images were composed with CombineZP free software.

### SCANNING ELECTRON MICROSCOPY

Larvae were superficially cleaned with a soft brush and placed in a drop of concentrated commercial detergent for 2–5 min. Subsequently, the specimens were sonicated in a warm water + detergent solution for 2–5 min and both steps were repeated until the larvae were clean. The specimens were then sonicated with commercial window cleaner for 2–5 min (warning: the ammonium of the window cleaner can disintegrate the larvae if they are not well sclerotized or are badly fixed). Specimens were then dehydrated in an ethanol series of increasing concentration (50%, 70%, 80%, 96% and 100%), infiltrated with hexamethyldisilazane and air dried overnight. Afterwards, the samples were mounted on stubs using copper tape, sputter-coated with gold-palladium and scanned with a Carl Zeiss NTS SUPRA 40 or a JEOL JSM-6380LV scanning electron microscope.

### VIDEO RECORDING

Larvae were placed in a Petri dish with water and filter paper as a bottom. Food was offered to them with tweezers; shaking the prey usually triggered the larval feeding response. The movements of the mouthparts during feeding were recorded immediately after the larvae started feeding using a Leica digital camera mounted on a Leica MZ stereo-microscope. Videos were edited with Adobe Premiere Pro CC 2019. Frame sequences of the videos showing feeding behaviour were obtained with free software 'Video to JPG converter' ([DVDVideoSoft, 2020](#)).

### CHARACTER EVOLUTION

To reconstruct the evolution of the feeding and respiratory behaviour we performed Bayesian stochastic character mapping, which uses a Markov chain Monte Carlo (MCMC) approach to sample character histories from their posterior probability distribution. Two characters were coded for each lineage: feeding strategy (chewing or piercing-sucking or filter-feeding) and spiracular system development [open (= peripneustic or metapneustic) or closed]. We used the R package *phytools* ([Revell, 2012](#)) for the estimation of ancestral character states of discrete valued traits. The analysis was performed under an

**Table 1.** List of the specimens used in the study, methods and repository

Taxon	Methods	Repository
<b>Hydrophilidae</b>		
Hydrophilinae		
Berosini		
<i>Allocotocerus punctatus</i> (Blackburn, 1888)	LM	LEBA
<i>Berosus adustus</i> Knisch, 1922	LM, SEM	LEBA
<i>Berosus decolor</i> Knisch, 1924	LM, SEM, VR	LEBA
<i>Berosus pallipes</i> Brullé, 1841	LM, SEM	LEBA
<i>Berosus patruelis</i> Berg, 1885	LM, SEM	LEBA
<i>Berosus</i> indet.	SEM	LEBA
<i>Hemiosus bruchi</i> Knisch, 1924	LM, SEM	LEBA
<i>Hemiosus dejeanii</i> (Solier, 1849)	LM, SEM, VR	LEBA
<i>Hemiosus multimaculatus</i> (Jensen-Haarup, 1910)	LM, SEM	LEBA
<i>Derallus paranensis</i> Oliva, 1981	LM, SEM	LEBA
<i>Derallus angustus</i> Sharp, 1882	LM, SEM	LEBA
<i>Derallus</i> indet.	SEM	LEBA
<i>Regimbartia attenuata</i> (Fabricius, 1801)	LM	LEBA, KMNH
Laccobiini		
<i>Laccobius kunashiricus</i> Shatrovskiy, 1984	SEM	KMNH
<i>Laccobius minutoides</i> Orchymont, 1942	LM	LEBA
<i>Laccobius</i> ( <i>Microlaccobius</i> ) sp.	SEM	KMNH
<i>Laccobius hammondi</i> Gentili, 1984	SEM	NMPC
<i>Oocyclus iguazu</i> (Oliva 1996)	LM, SEM	LEBA
<i>Oocyclus sapphirus</i> Short and García, 2010	SEM	LEBA
<i>Oocyclus magnifica</i> Hebauer & Wang, 1998	VR	NMPC
Hydrophilini		
<i>Hydrochara affinis</i> (Sharp, 1873)	SEM	KMNH
<i>Hydrophilus</i> ( <i>Dibolocelus</i> ) <i>palpalis</i> Brullé, 1837	LM, SEM, VR	LEBA
<i>Hydrophilus ensifer</i> Brullé, 1837	LM, SEM	LEBA
<i>Hydrophilus acuminatus</i> Motschulsky, 1853	SEM	KMNH
<i>Tropisternus acaragua</i> Bachmann, 1969	LM, SEM	LEBA
<i>Tropisternus setiger</i> Germar, 1824	LM, SEM	LEBA
<i>Tropisternus latus</i> Brullé, 1837	LM, SEM, VR	LEBA
<i>Tropisternus</i> indet.	SEM	LEBA
Hydrobiusini		
<i>Hybograalius hartmeyeri</i> (Régimbart, 1908)	LM	LEBA
Enochrinae		
<i>Enochrus variegatus</i> (Steinheil, 1869)	LM	LEBA
<i>Enochrus vulgaris</i> (Steinheil, 1869)	LM	LEBA
<i>Enochrus</i> indet.	SEM	LEBA
Acidocerinae		
<i>Helochares ventricosus</i> Bruch, 1915	LM, SEM	LEBA
<i>Helochares oculatus</i> Sharp, 1882	LM, SEM	LEBA
<i>Helochares</i> indet.	SEM	LEBA
Sphaeridiinae		
Coelostomatini		
<i>Dactylosternum cacti</i> (LeConte, 1855)	SEM	LEBA
<i>Hydroglobus puncticolle</i> Bruch, 1915	SEM	LEBA
<i>Phaenonotum exstriatum</i> (Say, 1835)	LM	LEBA

Table 1. Continued

Taxon	Methods	Repository
Megasternini <i>Cercyon quisquilius</i> (Linnaeus, 1761)	SEM	LEBA
<b>Epimetopidae</b> <i>Epimetopus mendeli</i> Fikáček <i>et al.</i> 2011	SEM	NMPC

Abbreviations: LM, light microscopy; SEM, scanning electron microscopy; VR, video recording.

equal rate (ER) model, which allows character states to change in both directions ( $0 > 1$  and  $1 > 0$ ) with equal probability. We used a molecular tree of the superfamily Hydrophiloidea published in Toussaint & Short (2018), with *Spercheus* Kugelann, 1798 placed as a sister-clade to the Hydrophilidae based on a genome-based phylogeny by McKenna *et al.* (2019).

## RESULTS

### MOUTHPART MORPHOLOGY AND FEEDING BEHAVIOUR

#### General morphology

The larval head and mouthparts in Hydrophilidae show two morphotypes related to the evolution of the mandibles (Supporting Information, Fig. S1). The first morphotype is characterized by a wide nasale, epistomal lobes of moderate size, symmetrical to asymmetrical mandibles without sucking channel and the well-developed labium (Figs 1A–C, 2, 5, 9A, B; Supporting Information, Fig. S1). This morphological design is found in most groups of Hydrophilidae and corresponds to the ancestral condition for the whole superfamily. Usually, larvae with this type of chewing feeding system digest prey extra-orally while holding it out of water and manipulating it with the mouthparts to form an alimentary bolus.

The second morphotype is characterized by a well-developed nasale, enlarged forward-projecting epistomal lobes developed either on the left side only or on both sides, asymmetrical mandibles (left one with sucking channel), epistomal-mandibular coupling system and reduced labium (Figs 1D–I, 3, 4, 6–8, 9C, D; Supporting Information, Figs S1–3). This morphological design was observed in *Berosus* and *Hemiosus* (Berosini), *Laccobius* and *Oocyclus* Sharp, 1882 (*Laccobius*-group *sensu* Short & Fikáček, 2013; Toussaint & Short, 2018) and *Hybogralius* (Hydrobiusini). Outside the Hydrophilidae, the piercing-sucking feeding mechanism is only present in the larvae of the family Epimetopidae.

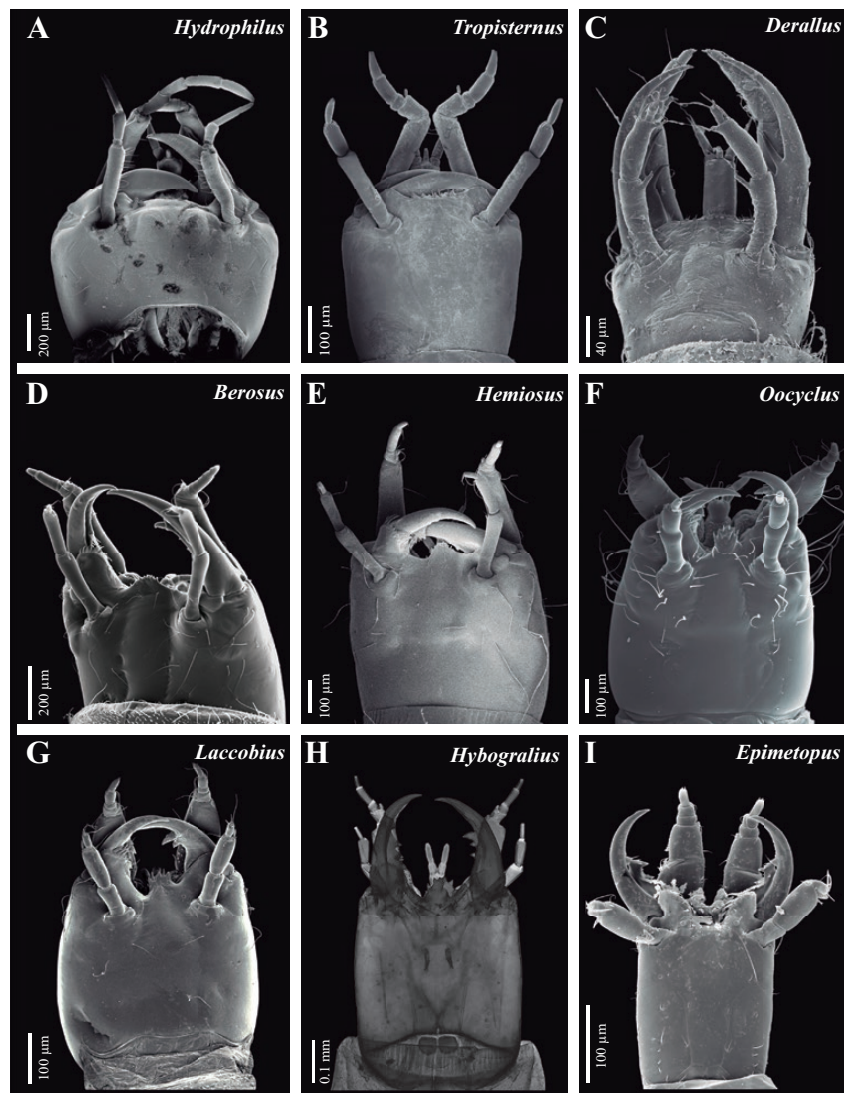
In the following sections, we summarize the general morphology of the mouthparts and describe in detail

the structural modifications of the piercing-sucking feeding system emphasizing functionally relevant differences.

### MANDIBLES

#### Groups with chewing feeding system

Within Hydrophilidae the ancestral mandibular morphology is associated with predatory behaviour and prey-tissue processing. Usually, the mandibles are elongated, acuminate, with a wide incisor edge, have one to three inner (retinacular) teeth and lack mola, protheca and penicillum (Fig. 2). The mandibles are mostly symmetrical, although in some genera they can be slightly asymmetrical (in shape and number of retinacular teeth) (Fig. 2A–D). The closing of asymmetrical mandibles is more accurate, locking and fitting into or onto each other to cut and grind prey tissue (Fig. 2B–D). It is possible that this asymmetry improves the efficiency of the chewing mechanism. This type of mandibular morphology is present with little modifications in most hydrophiloid taxa. An interesting modification is that of some *Hydrophilus* Müller, 1764 larvae (Inoda *et al.*, 2003, 2015; Sato *et al.*, 2017; pers. obs.) in which the left mandible is short and stout with one retinacular tooth, whereas the right mandible is at least 1.5× longer and bears two retinacular teeth (Fig. 2D). Although these larvae are generalists, they prey mainly on aquatic gastropods and the modifications of mandibles improves the ability to break snail shells. Other morphological variations can be observed in the tribes Megasternini and Sphaeridiini (Sphaeridiinae). The left mandible of these groups is strongly acuminate, lacks retinacular teeth and usually bears fine cuticular pubescence at midlength, whereas the right mandible is slightly shorter and usually bears a strongly serrated retinacular tooth (Fig. 2E, F) (Archangelsky, 1997, 2016, 2018; Archangelsky *et al.*, 2016b; Fikáček, 2019). This may be an adaptation for preying on dipteran larvae, which are known to be the preferred prey at least of *Sphaeridium* Fabricius, 1775 larvae (Sowig *et al.*, 1997).



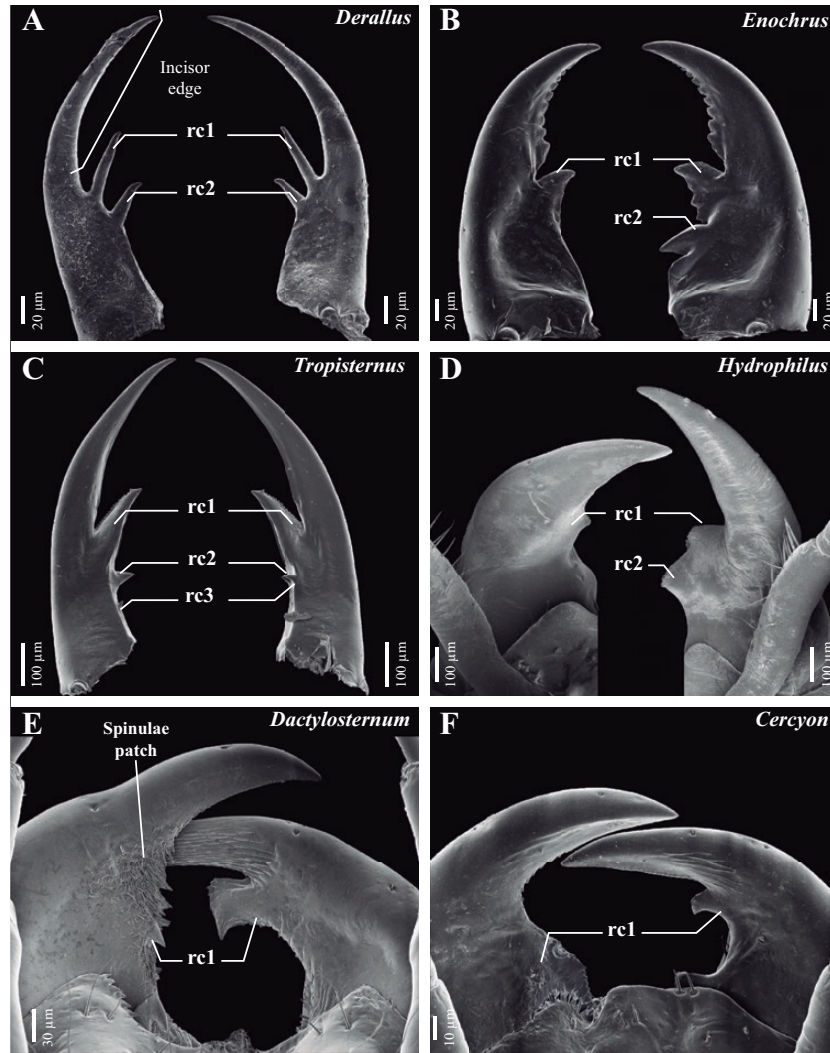
**Figure 1.** Head capsule of larvae with chewing (A–C) and piercing-sucking (D–I) feeding system, SEM micrograph, dorsal view. A, *Hydrophilus (Dibolocelus) palpalis* Brullé, 1837, second-instar larva. B, *Tropisternus setiger* Germar, 1824, first-instar larva. C, *Derallus paranensis* Oliva, 1981, first instar larva. D, *Berosus* sp., third-instar larva. E, *Hemiosus bruchi* Knisch, 1924, third-instar larva. F, *Oocyclus iguazu* (Oliva 1996), third-instar larva. G, *Laccobius kunashiricus* Shatrovskiy, 1984, third-instar larva. H, *Hybograalius hartmeyeri* (Régimbart, 1908), third-instar larva, light microscope photograph. I, *Epimetopus mendeli* Fikáček et al. 2011, first-instar larva.

#### Groups with piercing-sucking feeding system

Larvae of the following groups exhibit modifications in the mandibular morphology for piercing and sucking the food. In all, except *Epimetopus*, the left mandible is modified, whereas the right one corresponds to the general mandibular morphology in chewing larvae.

***Berosus* + *Hemiosus*:** The mandibles are strongly asymmetrical (Fig. 3A–C). The right mandible is longer, more slender and more acuminate than the left one, and usually bears three retinacular teeth. The first retinacular tooth is longer, curved and

acuminate, and the basal two retinacular teeth, when present, are smaller and triangular (Fig. 3C). On the contrary, the left mandible is shorter and stouter, and is characterized by the presence of a deep lateral mandibular groove (Fig. 3A, B). The margins of the groove are close to each other at the midlength of the mandible but they do not touch, hence forming an open sucking channel. The mandibular teeth are very complex and are involved not only in prey manipulation, but also in an epistomal-mandibular coupling system (see ‘Feeding strategies’ section below). The most distal tooth is large, subtriangular, with several long multifid

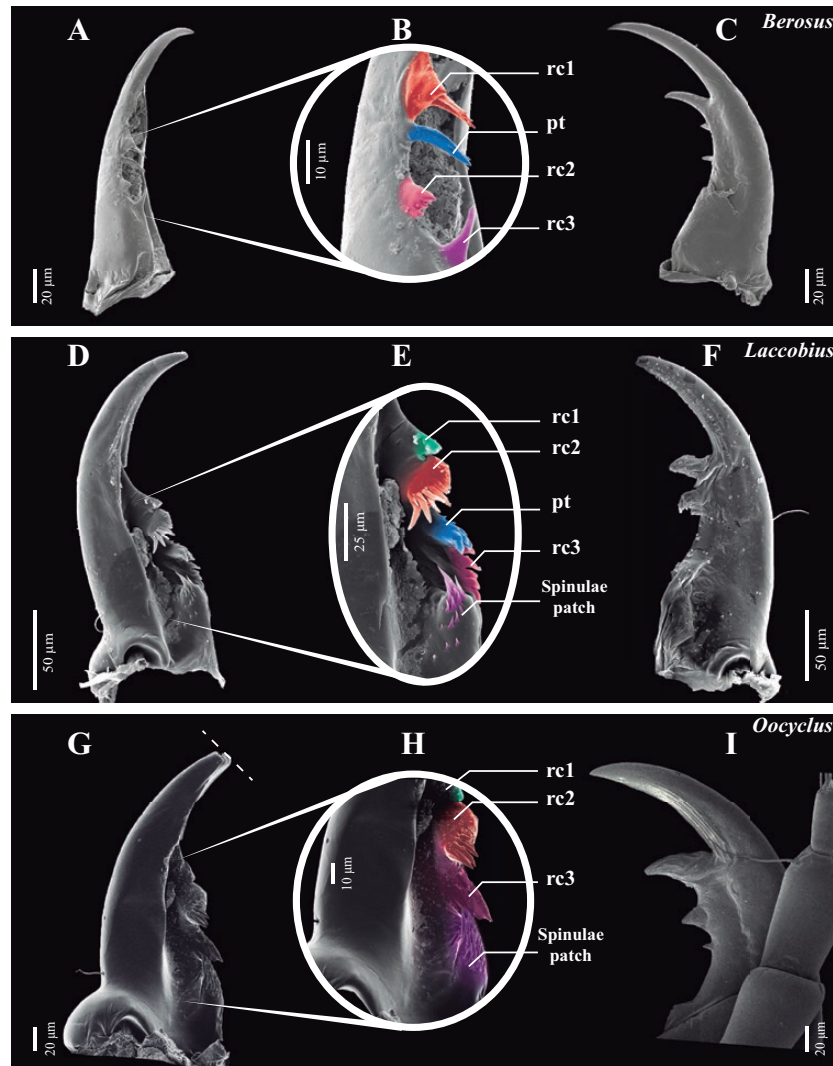


**Figure 2.** Chewing mandibles, SEM micrograph, dorsal view. A, *Derallus* sp., first-instar larva. B, *Enochrus* sp., first-instar larva. C, *Tropisternus* sp., second-instar larva. D, *Hydrophilus* (*Dibolocelus*) *palpalis* Brullé, 1837, first-instar larva. E, *Dactylosternum cacti* (LeConte, 1855), third-instar larva. F, *Cercyon quisquilius* (Linnaeus, 1761), third-instar larva. Abbreviations: rc1, first retinaculum; rc2, second retinaculum; rc3, third retinaculum.

projections on the proximal edge (rc1 in Fig. 3B). The second-most distal tooth is smaller and has a serrated proximal edge (rc2 in Fig. 3B). The proximal tooth (rc3 in Fig. 3B) is similar in size to the previous one but its tip is oriented towards the mandibular apex. Also, a hyaline large multifid process (prostheca) (pt in Fig. 3B) is situated between the first and the second tooth. The molar area is smooth and straight.

*Laccobius* + *Oocyclus*: The mandibles are strongly asymmetrical (Fig. 3D–I). In *Oocyclus*, the right mandible has three closely aggregated retinacular teeth. The distal-most retinacular tooth is slightly longer and more acuminate than the second retinacular tooth, whereas the third retinacular

tooth is much smaller and triangular (Fig. 3I). In *Laccobius*, usually only the first two retinacular teeth are present. However, some species can have a small third retinacular tooth (Fig. 3F). The left mandible is shorter and stouter than the right mandible in both genera and the groove is slightly shallower than in *Berosus* and *Hemiosus*. The first tooth from the apex is small, triangular and points mediad. The second tooth is larger, acuminate, with several leaf-like projections on the proximal edge. The third tooth is smaller, points downward and has several conspicuous toothlets on the proximal edge in *Laccobius* (rc3 in Fig. 3E) and several spinulae in *Oocyclus* (rc3 in Fig. 3H). Additionally, a group of strong spinulae (with their tips oriented towards the mandibular apex) are located



**Figure 3.** Piercing-sucking mandibles. A–C, *Berosus patruelis* Berg, 1885, first-instar larva, SEM micrograph: A, left mandible, ventral view; B, detail of mandibular teeth, ventral view; C, right mandible, dorsal view. D–F, *Laccobius hammondi* Gentili, 1984, third-instar larva, SEM micrograph, dorsal view: D, left mandible; E, detail of mandibular teeth; F, right mandible. G–I, *Oocyclus iguazu* (Oliva, 1996) third-instar larva, SEM micrograph, dorsal view; G, left mandible; H, detail of mandibular teeth; I, right mandible.

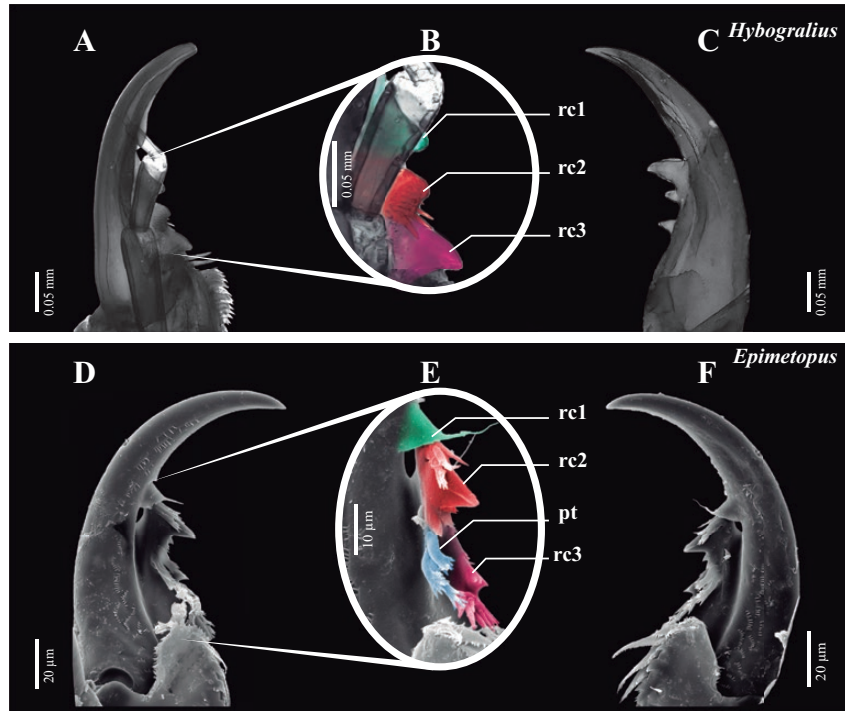
dorsally near its base. The prostheca is present only in *Laccobius* and the molar area has spinulae pointing downward along the margin (Fig. 3E, H).

*Hybogralius*: The mandibles are strongly asymmetrical (Fig. 4A–C). The right mandible is slightly larger than the left mandible and bears three retinacular teeth. The first retinacular tooth is longer, curved and acuminate, and the basal two retinacular teeth are smaller and triangular (Fig. 5C). The left mandible has three retinacular teeth (Fig. 5A, B). The first tooth is large, almost of the same size as the second tooth (see rc1 in Fig. 4B). The second retinacular tooth is blunt with several leaf-like projections on the proximal

edge (see rc2 in Fig. 4B). The first and the second retinacular teeth are not so closely aggregated as in *Laccobius* and *Oocyclus*. The third tooth is of similar size to the other retinacular teeth but its apex is blunt and points downward. The prostheca is absent, and the molar area is smooth and slightly rounded.

*Epimetopus*: The mandibles are symmetrical (Fig. 4D–F). Both mandibles are short and stout with a shallow groove. The first retinacular tooth is small, the base is triangular and extends towards the apex in a thin spine (see rc1 in Fig. 4E). The second retinacular tooth is larger, anvil-shaped, with stout multifid projections at the base, arranged parallel





**Figure 4.** Piercing-sucking mandibles. A–C, *Hybograllius hartmeyeri* (Régimbart, 1908), third-instar larva, light microscope photographs, dorsal view: A, left mandible; B, detail of mandibular teeth; C, right mandible. D–F, *Epimetopus mendeli* Fikáček *et al.* 2011, first-instar larva, SEM micrograph, dorsal view: D, left mandible; E, detail of mandibular teeth; F, right mandible. Abbreviations: rc1, first retinaculum; rc2, second retinaculum; rc3, third retinaculum; pt, prostheca.

to the proximal edge (see rc2 in Fig. 4E). The third retinacular tooth is smaller, triangular, with posterior series of digitiform projections (see rc3 in Fig. 4E). The prostheca is located internally, between the second and third retinacular teeth, and is formed by three large stout hand-shaped projections. The basal field of the mandible bears a group of strong spinulae, which are oriented with their tips towards the mandibular apex. In addition, groups of spinulae arranged in a semicircle cover the dorsal surface of the mandibles. The molar area has several conspicuous toothlets pointing downward along the outer margin.

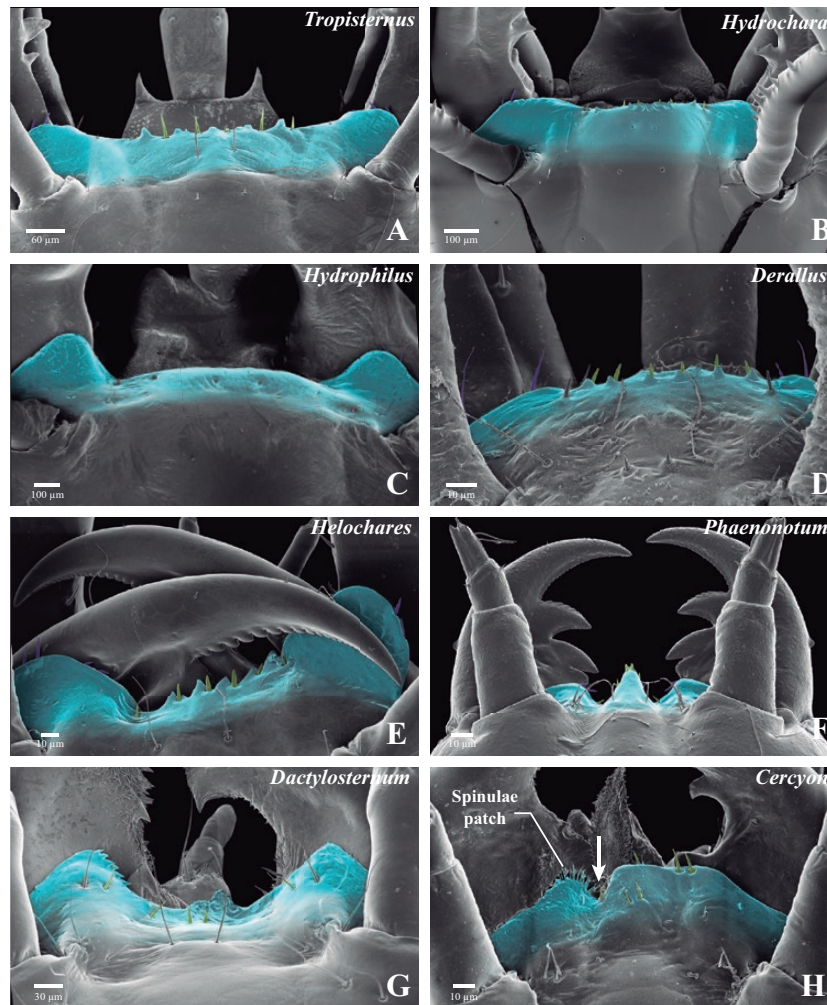
#### LABROCLYPEAL REGION

The description of the labroclypeus was divided in two parts: one details the general morphology and the other the primary sensory system (chaetotaxy).

In the labroclypeal region, two structures can be differentiated: the nasale, i.e. mesal projection of labroclypeus, and lateral lobes, i.e. the right and left one. The nasale usually bears six short bristle-like sensilla (gFR1), a character well conserved among hydrophiloids. The chaetotaxy of the epistomal lobes (gFR2) (Fig. 7A) is variable both in number and shape of the sensilla.

#### Groups with chewing feeding system

Most hydrophilid taxa have a broad anterior labroclypeal margin (Fig. 5). The epistomal lobes are generally symmetrical and poorly developed, and the nasale is wide and sometimes slightly protruding (e.g. *Allocotocerus* Kraatz, 1883, *Amphiops* Erichson, 1843, *Derallus* Sharp, 1882, *Enochrus* Thomson, 1859, *Paracymus* Thomson, 1867, *Regimbartia* Zaitzev, 1908, *Sphaerocetus* Fikáček, 2010, *Tormus* Sharp, 1884, *Tritonus* Mulsant, 1844 and *Tropisternus* Solier, 1834) (Fig. 5A–E). In some larvae, the inner margin of the epistomal lobes has strong spinulae or a large cuticular pubescent area (Fig. 5G–F). The nasale can have strong teeth on the anterior margin (e.g. *Crenitis* Bedel, 1881, *Cymbiodyta* Bedel, 1881, *Enochrus*, *Guyanobius* Spangler, 1986, *Helochares* Mulsant, 1844, *Hydrobius* Leach, 1815, *Notionotus* Spangler, 1972, *Paracymus*, *Sperchopsis* LeConte, 1861 and *Tropisternus*) (Fig. 5A, E) or the teeth can be reduced (e.g. *Allocotocerus*, *Derallus*, *Helobata* Bergroth, 1888, *Hydrobiomorpha* Blackburn, 1888, *Hydrochara* Berthold, 1827, *Hydrophilus* and *Regimbartia*) (Fig. 5B–D). The Sphaeridiinae lineages bear additional modifications (Fig. 5F–H): larvae of the tribe Coelostomatini have a subtriangular nasale (Fig. 5F, G). In the tribe Sphaeridiini, the nasale is also subtriangular and the



**Figure 5.** Labroclypeal region of larvae with chewing feeding system, SEM micrograph, dorsal view. A, *Tropisternus acaragua* Bachmann, 1969, first-instar larva. B, *Hydrochara caraboides* (Linnaeus, 1758), first-instar larva. C, *Hydrophilus (Dibolocelus) palpalis* Brullé, 1837, second-instar larva. D, *Derallus paranensis* Oliva, 1981, first instar larva. E, *Helochares ventricosus* Bruch, 1915, first-instar larva. F, *Hydroglobus puncticolle* Bruch, 1915, third-instar larva. G, *Dactylosternum cacti* (LeConte, 1855), third-instar larva. H, *Cercyon quisquilius* (Linnaeus, 1761), third-instar larva, white arrow indicates labroclypeal notch. Colours: light blue, frontoclypeal region; green, gFR1, group of sensilla of nasale; violet, gFR2, group of sensilla of epistomal lobe.

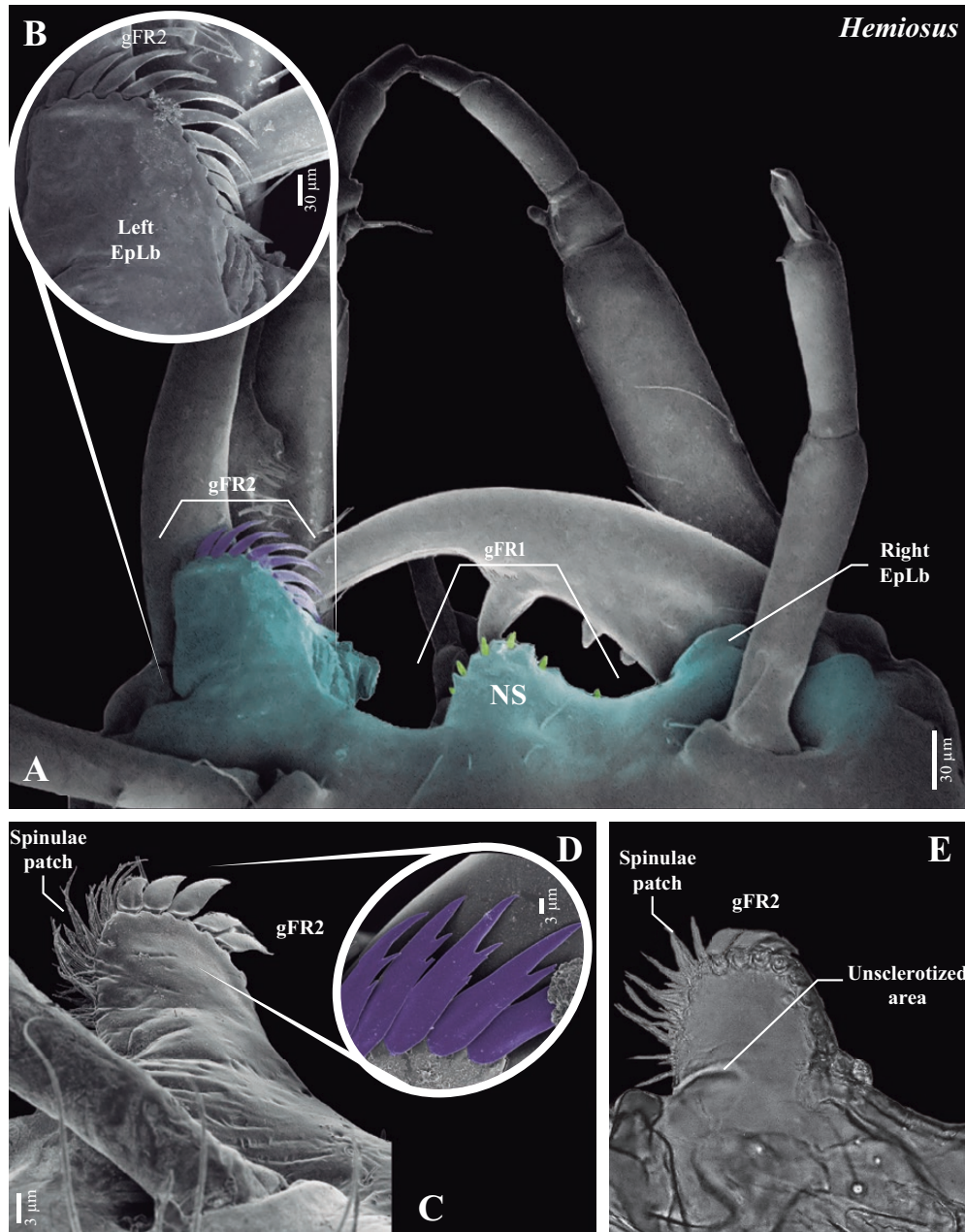
left epistomal lobe has a dense cuticular pubescence. In the Megasternini, the left epistomal lobe is rounded and a deep pubescent notch is present between the left epistomal lobe and the nasale (Fig. 5H).

The chaetotaxy of the epistomal lobes is symmetrical in most taxa (Fig. 5). These larvae usually have two to six setae on each epistomal lobe (e.g. *Derallus*, *Hydracara* Knisch, 1925 and *Hydrobius*), although two to four setae are most frequently found (e.g. *Anacaena* Thomson, 1859, *Enochrus*, *Helobata*, *Notionotus*, *Paracymus* and *Tropisternus*). These sensilla, regardless of their size, are always bristle-like and sparse.

#### *Groups with piercing-sucking feeding system*

The anterior labroclypeal margin is more complex in the lineages with a piercing-sucking feeding system. This involves an enlargement of one or both epistomal lobes to cover at least the basal-third of the mandibles, an area to increase the flexibility of the lobe, and the nasale becoming narrower and more prolonged (Figs 6–8; Supporting Information, Figs S2–3).

*Berosus* + *Hemiosus*: The labroclypeus is strongly asymmetrical (Figs. 6; Supporting Information, Fig. S2). The width and length of the nasale varies depending on the species (Minoshima & Hayashi,



**Figure 6.** Labroclypeal region of *Hemiosus* larvae. A, B, *Hemiosus bruchi* Knisch, 1924, third-instar larva, SEM micrograph, dorsal view: A, labroclypeus; B, left epistomal lobe. C–E, *Hemiosus multimaculatus* (Jensen-Haarup, 1910), third-instar larva, dorsal view: C, left epistomal lobe, SEM micrograph; D, detail of gFR2 serrated setae, SEM micrograph; E, left epistomal lobe, light microscope photograph. Abbreviations: EpLb, epistomal lobe; NS, nasale. Colours: light blue, frontoclypeal region; green, gFR1, group of sensilla of nasale; violet, gFR2, group of sensilla of epistomal lobe.

2015; Rodriguez *et al.*, 2015, 2018; Deler-Hernández & Fikáček, 2016): it can be narrow and long (Fig. 6A), rounded and shorter (Supporting Information, Fig. S2) or totally reduced. The left epistomal lobe is well developed, whereas the right epistomal lobe is highly reduced, with the right part of the labroclypeus usually straight or slightly rounded

(Fig. 6). On its inner margin, the left epistomal lobe bears a membranous structure with strong spinulae that extend ventrally (Fig. 6A; Supporting Information, Fig. S2). This structure is flexible and closes the mandibular channel mesally. A triangular unsclerotized area (Fig. 6E; Supporting Information, Fig. S2) appears on the outer margin near the base of

the left epistomal lobe. The weakly sclerotized cuticle provides a greater mobility to the epistomal lobe and, consequently, also to the mandible, since both structures work as a functional unit. The aperture increases the mobility of the epistomal lobe by *c.* 15° (Fig. 6E; Supporting Information, Fig. S2).

The chaetotaxy of the epistomal lobes is asymmetrical (Fig. 6). The left epistomal lobe has nine to 18 sensilla, depending on the species. Usually, the outer setae are bristle-like and the remaining setae, oriented towards the inner side of the lobe, are flat and acuminate (Fig. 6B–D; Supporting Information, Fig. S2). In *Berosus* and *Hemiosus*, one to two outer setae are short bristles and the remaining setae are flat with none to one (*Berosus* and *Hemiosus maculatus*-group) or two to three toothlets (*Hemiosus dejeanii*-group) (Fig. 6D; Supporting Information, Fig. S2; Rodriguez *et al.* 2018: figs 3C, 5C). The ventral surface of the left epistomal lobe has three minute additional setae. These three sensilla are in contact with the dorsal surface of the left mandible and most likely sense the distance between both structures. The right epistomal lobe does not bear sensilla (Supporting Information, Fig. S2).

*Laccobius + Oocyclus*: In these lineages, both left and right epistomal lobes are enlarged but only the left one has an active function in feeding (Fig. 7; Supporting Information, Fig. S3). The left epistomal lobe is much wider than the right one, covering dorsally the basal-third of the mandible, whereas the right epistomal lobe narrows towards the apex (Fig. 7A). The outer margin of the left epistomal lobe is expanded laterally and bears a deep notch on the base that increases the flexibility of the lobe (Fig. 7C; Supporting Information, Fig. S3). The nasale is prominent and narrow, reaching at least two-thirds of the epistomal lobes (Fig. 7A; Supporting Information, Fig. S3).

The chaetotaxy of the epistomal lobes is asymmetrical (Fig. 7; Supporting Information, Fig. S3). The left epistomal lobe has seven to eight sensilla, depending on the species (Fig. 7B–D; Supporting Information, Fig. S3). In these taxa, two bristles are located below the lateral cuticular expansion of the epistomal lobe (Supporting Information, Fig. S3), followed by a single row of five to six flat sensilla with a toothlet (Fig. 7B, C). The inner margin bears a group of seta-like projections arranged in two or three rows, which are serrated in *Laccobius* and short and simple in *Oocyclus* (Fig. 7E; Supporting Information, Fig. S3). The chaetotaxy of the right epistomal lobe is reduced to two minute setae in *Oocyclus*, and is completely absent in *Laccobius*.

*Hybograhius*: The labroclypeus is similar to that of *Laccobius* and *Oocyclus*. Both epistomal lobes are enlarged, but neither a notch nor an unsclerotized

area is observed at the base of the left epistomal lobe (Fig. 8A, B).

The chaetotaxy of the epistomal lobes is asymmetrical (Fig. 8). The left epistomal lobe has 12 sensilla, six outer setae are short bristle-like and the remaining are slightly flat with several toothlets on the inner margin, in third-instar larvae (Fig. 8A). The innermost ones are flat seta-like projections distributed in at least three rows (Fig. 8B). The right epistomal lobe has a group of 12 short bristle-like setae on the outer margin.

*Epimetopus*: The labroclypeus is symmetrical, both epistomal lobes are enlarged and have the same morphology, covering the basal-quarter of both mandibles (both of which have the sucking groove) (Fig. 8C). The epistomal lobes are wide and have a deep notch at the base. In addition, a series of short digitiform projections is present along the outer margin (Fig. 8D). The nasale is much narrower than in hydrophilids and only reaches the basal-third of the epistomal lobes.

The chaetotaxy of the epistomal lobes is symmetrical (Fig. 8C, D). Both epistomal lobes have four flat serrated setae, strongly bent downwards, intercalated between conspicuous cuticular teeth (Fig. 8D).

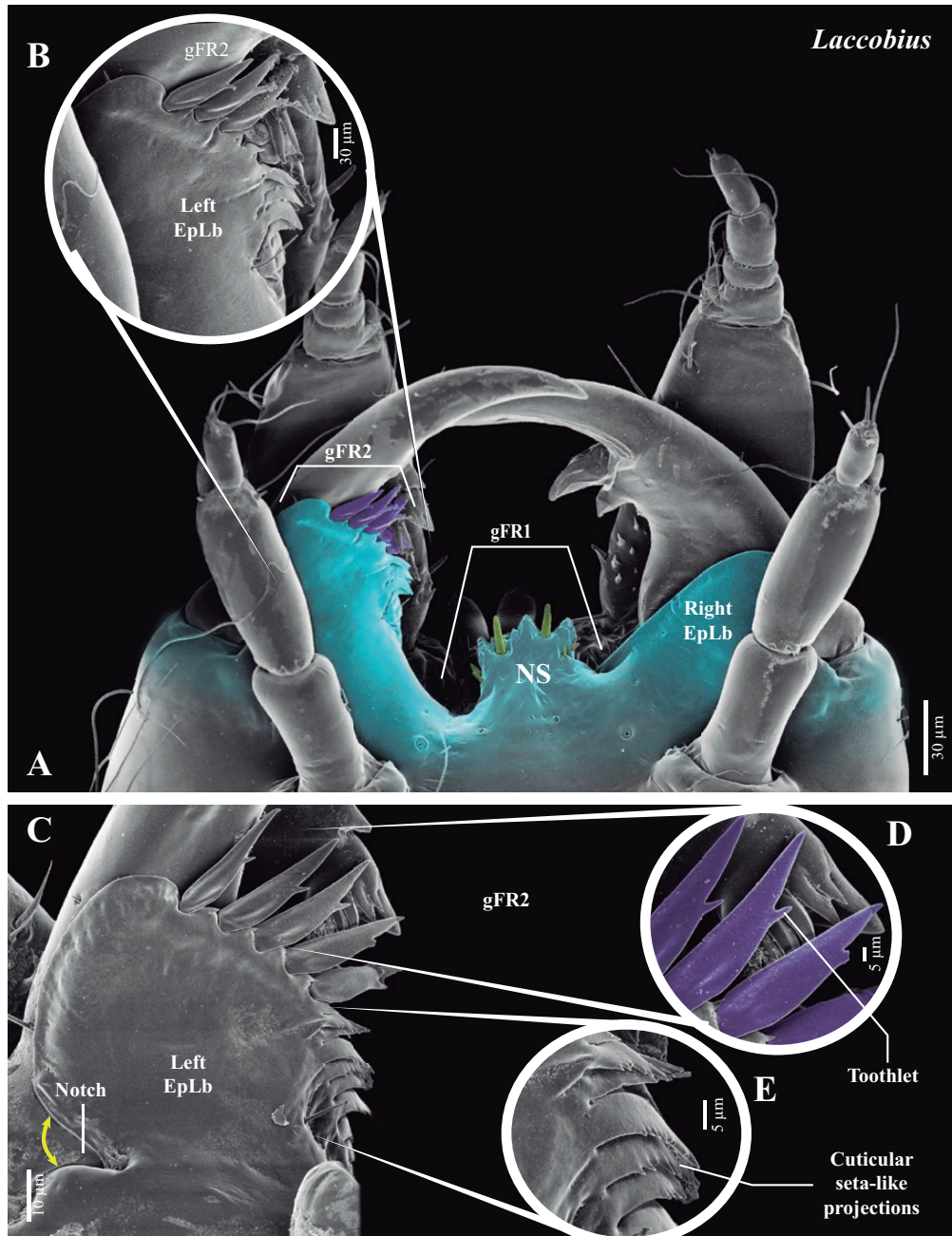
## LABIUM

### *Groups with chewing feeding system*

Larvae with chewing apparatus have a large labium armed with strong setae and spinulae (Fig. 9A, B). The mentum is usually subrectangular or subquadrangular, strongly sclerotized, with several robust cuticular spines on the dorsal surface and the ligula is well developed (Fig. 9A, B). It becomes smaller (with ligula reduced or absent) in derived lineages of Sphaeridiinae, but retains the conspicuous cuticular pubescence on the dorsal surface. Many groups of Sphaeridiinae (all Megasternini and Sphaeridiini, some Coelostomatini) and some Cylominae (*Austrotypus*) bear an additional structure, the large hypopharyngeal lobe developed on the left side and densely covered by cuticular pubescence. It seems that the lobe helps in the absorption of pre-orally digested food and filters small particles that could be mixed up with food (Archangelsky, 1999).

### *Groups with piercing-sucking feeding system*

*Berosus + Hemiosus*: The labium is reduced and lacks cuticular spines on its dorsal surface (Fig. 9C). The mentum is reduced, subpentagonal and has a large membranous area that allows the partial retraction of

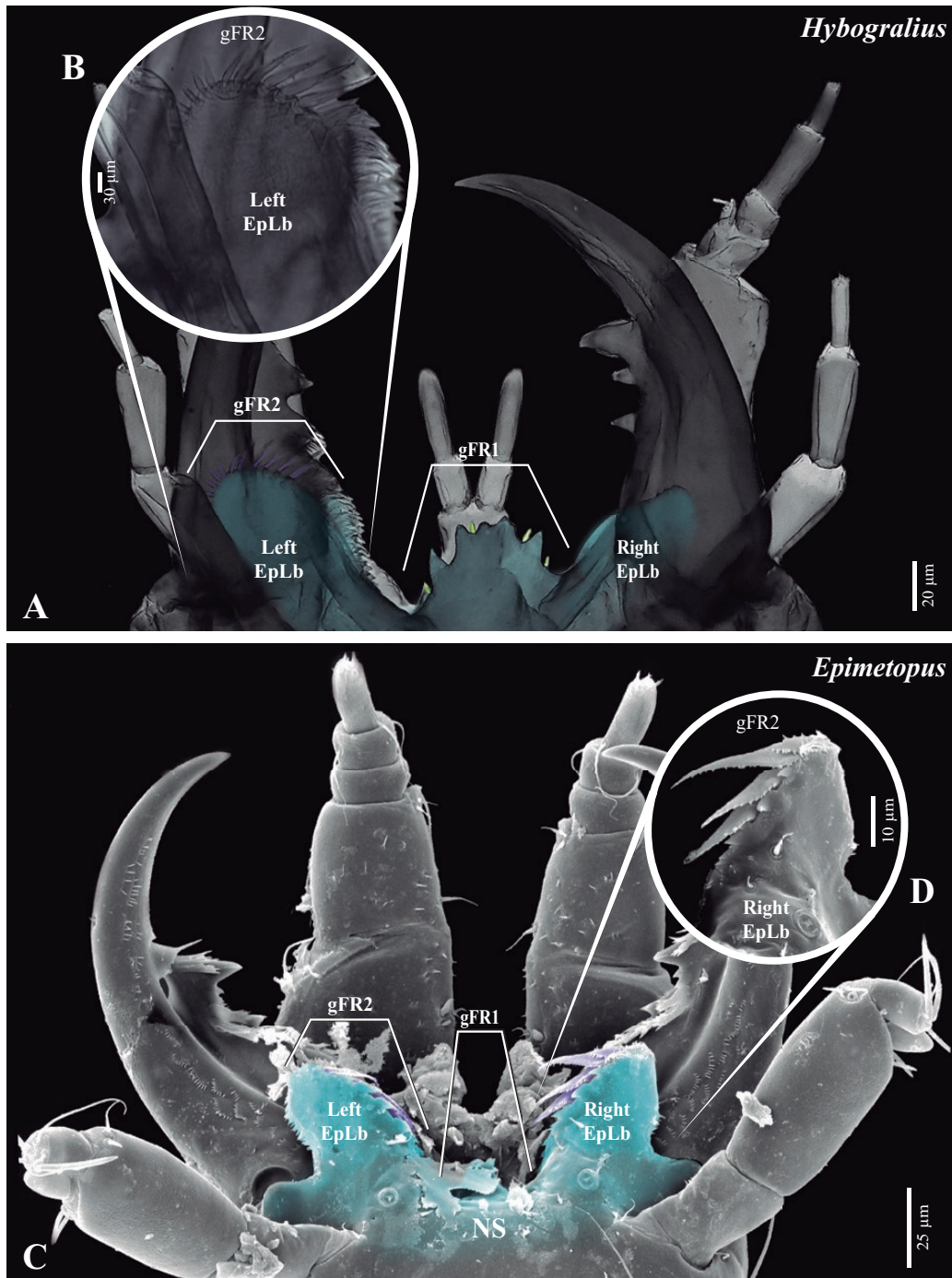


**Figure 7.** Labroclypeal region of *Laccobius* larvae. A, B, *Laccobius kunashiricus* Shatrovskiy, 1984, third-instar larva, SEM micrograph, dorsal view: A, labroclypeus; B, left epistomal lobe. C–E, *Laccobius (Microlaccobius)* sp., third-instar larva, SEM micrograph, dorsal view: C, left epistomal lobe; D; detail of gFR2 setae; E, seta-like cuticular projections of the latero-ventral membranous lobe. Abbreviations: EpLb, epistomal lobe; NS, nasale. Colours: light blue, frontoclypeal region; green, gFR1, group of sensilla of nasale; violet, gFR2, group of sensilla of epistomal lobe.

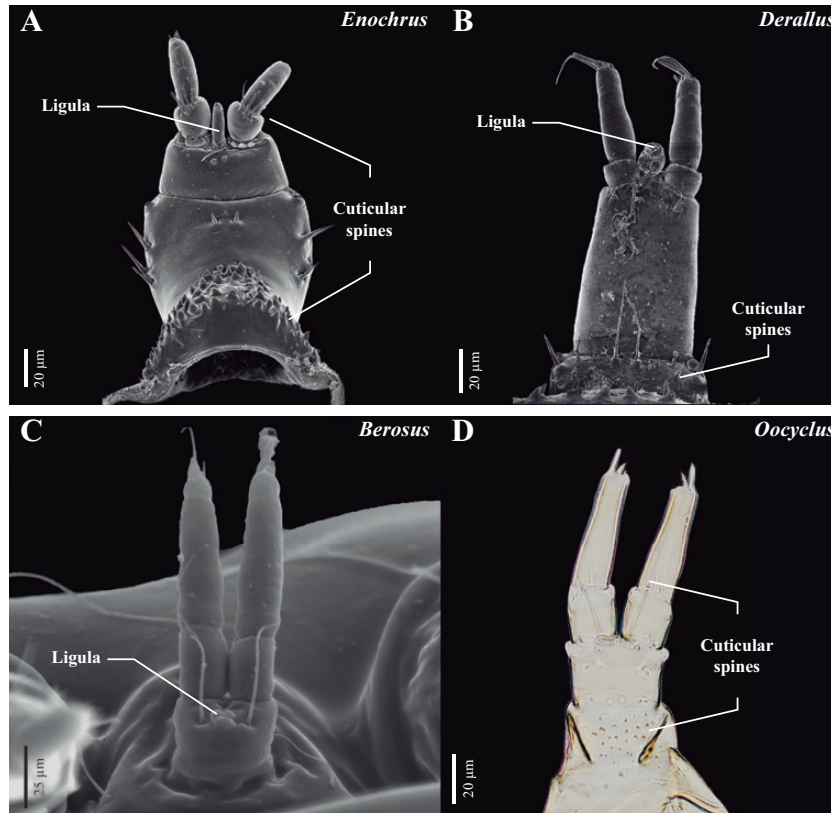
the labium inside the head capsule. The ligula is small in *Hemiosus* and completely reduced in most *Berosus* species.

*Laccobius* + *Oocyclus*: The labium is much reduced. In *Laccobius* species the prementum and mentum are

weakly sclerotized and lack cuticular spines, whereas in *Oocyclus* they are more sclerotized and the mentum bears few sparse cuticular spines (Fig. 9D). In some species of *Laccobius*, long cuticular spinulae are present on the anterior margin of the first palpomere; in *Oocyclus* these spinulae are also present on the



**Figure 8.** Labroclypeal region. A, B, *Hybogralius hartmeyeri* (Régimbart, 1908), third-instar larva, light microscope photographs, dorsal view: A, labroclypeus; B, left epistomal lobe. C, D, *Epimetopus mendeli* Fikáček *et al.* 2011, first-instar larva, SEM micrograph, dorsal view: C, labroclypeus; D, right epistomal lobe. Abbreviations: EpLb, epistomal lobe; NS, nasale. Colours: light blue, frontoclypeal region; green, gFR1, group of sensilla of nasale; violet, gFR2, group of sensilla of epistomal lobe.



**Figure 9.** Labium of larvae with chewing (A–B) and piercing-sucking (C–D) feeding system, dorsal view. A, *Enochrus* sp., first-instar larva, SEM micrograph. B, *Derallus* sp., first-instar larva, SEM micrograph. C, *Berosus* sp., third-instar larva, SEM micrograph. D, *Oocyclus sapphirus* Short & García, 2010, first-instar larva, light microscope photograph.

anterior margin of the prementum. The ligula is reduced to a small membranous lobe in both genera.

*Hybograhius*: The labium is reduced. The mentum has few sparse cuticular spines on its dorsal surface and a group of stout spines on the anterolateral angles. In addition, the anterior margin of the prementum and the anterior margin of the first labial palpomere bear long spinulae. The ligula is more developed than in the other taxa with piercing-sucking morphology, being almost of the same length as the first palpomere.

*Epimetopus*: The labium is very small and lacks cuticular spines or spinulae. The submentum is trapezoidal and is not fused with the head as in hydrophilids. The mentum and prementum are small and transverse. The ligula is absent.

#### FEEDING STRATEGIES

##### *Groups with chewing feeding system*

Chewing larvae have two foraging strategies: ambush or active hunting. Ambush predators use a sit-and-wait strategy, waiting in hiding for potential prey and

catching them with a rapid surprise attack, whereas active predators move in the environment searching for, or pursuing, prey. Despite the type of foraging strategy, all Hydrophilidae must perform a partial extra-oral digestion of prey tissue, a typically terrestrial feeding mode (Cohen, 1995). To prevent dilution of the digestive fluids in the water, the prey needs to be processed above the water surface (Fig. 10A; Supporting Information, Video S1). This is allowed by the flexion of the head backwards at an angle of almost 40° in respect to the body axis. Archangelsky (2008) mentioned that most Hydrophilidae use their mouthparts to form an open funnel or channel, using the labium as the base and the maxillae and mandibles on the sides, through which the digested tissues of the prey enter the pre-oral cavity (Supporting Information, Video S1). In this context, the dorsal surface of the labium is the only structure that gives support to the prey and avoids the loss of predigested food. This explains why the labium is large in the groups with chewing feeding, and why its dorsal face bears a strongly sclerotized dorsal cuticular armature. The mandibles cut the tissue and help to form the alimentary bolus while pushing it towards the pre-oral cavity.



**Figure 10.** Frame sequences of videos showing feeding behavior. A, *Tropisternus latus* Brullé, 1837, note that the larvae raise the head out of water while feeding. B, *Hydrophilus (Dibolocelus) palpalis* Brullé, 1837. C, *Hemiosus dejeanii* (Solier, 1849). D, *Oocyclus magnifica* Hebauer & Wang, 1998. See also [Supporting Information, Videos S1–S4](#).

Specific adaptations of the chewing feeding mechanism for processing specific prey are present in some hydrophilid lineages. For example, *Hydrophilus* larvae prefer to feed on gastropods (snails) and the mandibles have evolved to manipulate and crush gastropod shells. The longer right mandible holds the snail, whereas the shorter left one breaks the operculum and crushes the inner columella of the shell exposing the soft tissue (Sato *et al.*, 2017). Larvae of other *Hydrophilus* species are specialist predators of planorbid snails, bending the head backwards and holding the snail against the dorsal surface of the abdomen, and opening the planispiral shell following the direction of the coiling (without crushing the columella) to gain access to the soft tissue (Fig. 10B; [Supporting Information, Video S2](#)).

The prey is processed inside its shell, which prevents the dilution of the digestive fluids, and is hence processed underwater. Another variation of chewing feeding is found in terrestrial Sphaeridiinae, which raise their head very little, or not at all, while feeding (Archangelsky, 1999). This is likely a consequence of the shift to terrestrial habitat, since the prey does not need to be held above the water surface for digestion. Moreover, the absorption of the predigested food is facilitated by the hypopharyngeal lobe in many of these groups.

#### *Groups with piercing-sucking feeding system*

This feeding mechanism is an adaptation for underwater food processing and besides the morphology



outlined above, it also affects feeding behaviour: the capture, manipulation and processing of the prey differ from groups with chewing feeding. *Berosus* and *Hemiosus* larvae move slowly and, most of the time, remain immobile in the substrate or near the bottom (Supporting Information, Video S3). The larval body is covered by a fine cuticular pubescence that favours the adhesion of detritus particles and enables an efficient camouflage in the environment. Only the head remains exposed and the prey is captured by ambush. The larvae remain motionless and when a prey item approaches; they then forcefully close their mandibles and bend their body, pressing the prey against the substrate (Fig. 10C; Supporting Information, Video S3). Then the larvae pierce the prey integument with the left mandible and inject digestive enzymes through the sucking channel. The right mandible and maxillae manipulate the prey and hold it in place. In some cases, the right mandible also pierces the prey tissue for manipulation purposes, or it remains completely closed resting on the labroclypeal margin. While feeding, the larvae repeatedly open and close the left mandible inside the prey. When the mandible is open, the predigested liquid is pumped from the prey into the sucking channel and to the oral cavity. This process is repeated until the larva ingests all predigested prey tissues. The labium does not actively participate in prey processing, as in chewing lineages, and remains partially retracted within the cephalic capsule. A similar feeding behaviour has been observed in *Oocyclus* larvae (Fig. 10D; Supporting Information, Video S4), which inhabit the thin layer of water that covers the rocks near waterfalls. As in *Berosus* and *Hemiosus*, the left mandible pierces the integument of the prey and injects digestive fluids. Then the left mandible repeatedly closes and opens inside the prey tissue, pumping the predigested food until the prey is completely consumed. The right mandible and maxillae actively participate in feeding, manipulating the prey and pushing tissue closer to the left mandible. None of these larvae raise the head out of water while feeding; in fact, the larvae hold the food item against the bottom with the head bent downward, thus feeding inside the water film (Fig. 10D). Little is known about the feeding behaviour of *Laccobius* larvae, although Perkins (1972) mentioned that it is similar to that observed in *Berosus* larvae. The feeding behaviour was not observed in *Hybograllius* and *Epimetopus*, but the morphology of their mouthparts suggests a similar feeding strategy as in *Berosus*, *Hemiosus*, *Laccobius* and *Oocyclus*.

#### MORPHO-FUNCTIONAL INTERPRETATION OF PIERCING-SUCKING MECHANISM

Three main structures are related, directly or indirectly, with the piercing-sucking feeding mechanism in the Hydrophilidae and Epimetopidae (Figs 11, 12):

1. Partly open sucking channel on the left mandible.
2. Epistomal-mandibular coupling system formed by the left epistomal lobe and the teeth of the left mandible.
3. A notch or weakly sclerotized area at lateral portion of the left epistomal lobe, which provide the lobe and consequently the mandible with more mobility.

#### *Sucking channel*

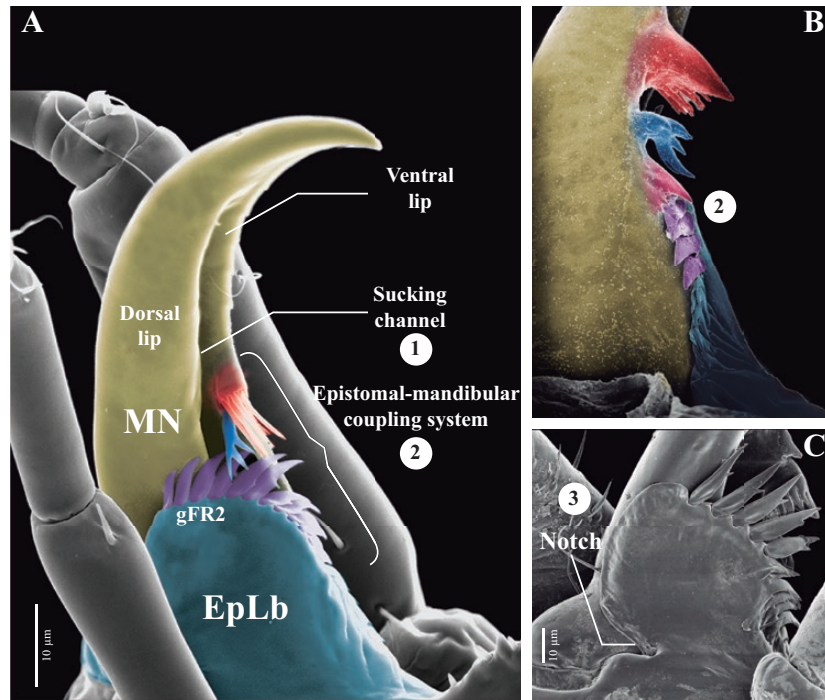
The left mandible with a deep groove is used for a rapid seizure of the prey (but not for its mechanical processing), injection of the digestive fluids into the prey and for sucking the predigested liquid food back to the pre-oral cavity (Figs 3, 4). The groove opens mesally in *Berosus* and *Hemiosus*, and dorsomesally in remaining groups. Its edges (lips) are not fused and form an open channel. The ventromesal edge bears the retinacular teeth and prostheca in *Berosus*, *Epimetopus* and *Hemiosus*.

#### *Lobular-mandibular coupling system*

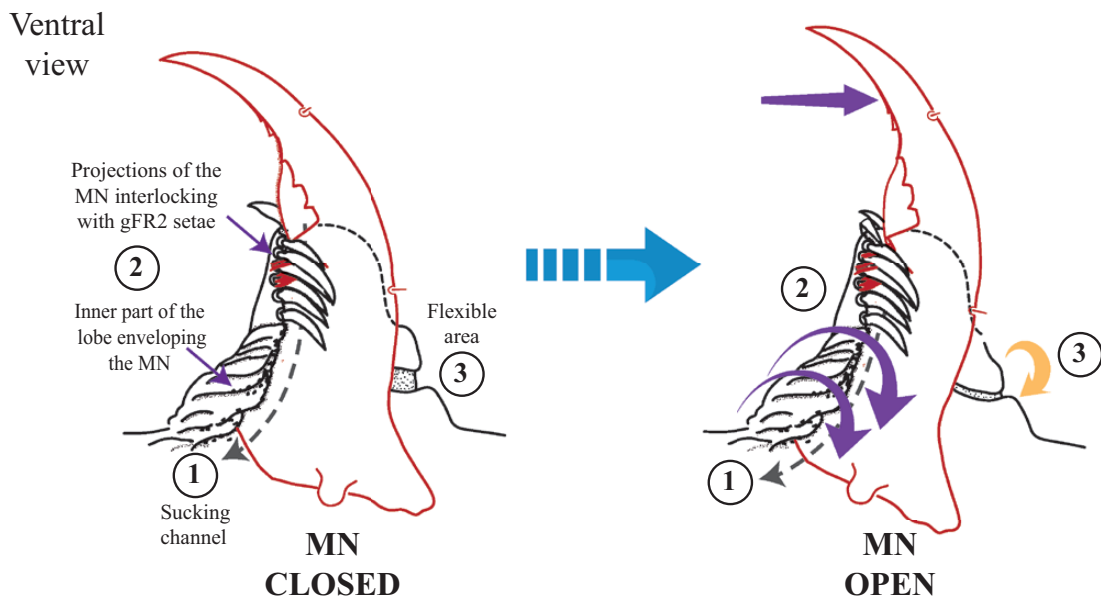
The left epistomal lobe is enlarged and covers the left mandible dorsally. The lobe and the mandible are kept together by a coupling system (Figs 11A, B, 12) consisting of interlocking inner projections of the mandible and the cuticular projections and setae of the epistomal lobe. This mechanism holds the epistomal lobe tightly attached to the mandible during the prey processing, i.e. in the position in which it closes the sucking channel and hence allows processing of the food underwater. Distally, the coupling is maintained by the flat, hooked setae of the epistomal lobe (gFR2; each seta may have additional denticles) interlocking with the proximal teeth of the mandible and with mandibular prostheca (in *Berosus* + *Hemiosus*, *Laccobius* and *Epimetopus*) (Fig. 11A, C). More basally, the second and third retinacular teeth of *Berosus* and *Hemiosus* function as a clamp that maintains the epistomal lobe attached to the mandible (Fig. 11B). In *Epimetopus*, *Hybograllius*, *Laccobius* and *Oocyclus* this function is accomplished by the basal retinacular tooth and a group of dorsal spinulae that are oriented towards the mandibular apex (Fig. 3E, H). At the mandibular base, the inner membranous part of the lobe envelopes the mesal part of the mandible (Figs 11B, 12) and the coupling is facilitated by basal spinulae (Fig. 3E, H) and/or projection (Figs 3B, 4B, E) of the mandible.

#### *Lateral notch of the left epistomal lobe*

The left epistomal lobe keeps tightly attached to the left mandible during the whole prey processing, although



**Figure 11.** Summary of the main structures related with piercing-sucking feeding mechanism, SEM micrograph. A, B, *Berosus* sp., third-instar larva: A, lobular-mandibular coupling system, dorsal view; B, detail of lobular-mandibular coupling system, ventral view. C, *Laccobius* (*MicroLaccobius*) sp., third-instar larva, left epistomal lobe, dorsal view.



**Figure 12.** Schematic drawing of the piercing-sucking feeding mechanism: 1, sucking channel; 2, epistomal-mandibular coupling system; 3, flexible area.

the mandible moves (opens and closes) slightly (see [Supporting Information, Videos S1–S4](#)). This would be not possible without a slight mobility of the epistomal

lobe in the mesal-lateral direction. The mobility of the lobe is possible due to a non-sclerotized membranous area (in *Berosus* and *Hemiosus*) (Figs 6E, 12) or a deep

notch (in *Epimetopus*, *Laccobius* and *Oocyclus*) (Figs 7C, 8D, 11C; Supporting Information, Fig. S3) laterally on the lobe. Both structures increase the flexibility of the epistomal lobe, allowing for a greater opening of the mandible (Fig. 12).

#### RESPIRATORY SYSTEM IN HYDROPHILID LARVAE

##### *General morphology and behaviour*

Larvae of Hydrophiloidea exhibit two alternative modifications of the tracheal system corresponding to their strategies to obtain oxygen:

1. Open tracheal system: oxygen is taken from the atmosphere above water through open spiracles.
2. Closed tracheal system: oxygen is absorbed directly from the water, through the cuticle or special respiratory organs (gills).

##### *Groups with chewing feeding system*

An open tracheal system is widespread in Hydrophiloidea, both in aquatic and terrestrial lineages. The tracheal system consists of a pair of dorsal tracheae that extend from the terminal abdominal spiracles to the head capsule. The terminal spiracles are enlarged, annular and open inside a chamber formed by the abdominal segments VIII–IX (Fig. 13). Internally, the spiracles consist of an atrium and the closing apparatus (Fig. 13A, B). The atrium is a long, tubular cavity formed by the section of the main trachea that is located between the closing apparatus and the external opening of the spiracle; it lacks structural reinforcements (taenidia) and is covered by spinulae acting, probably, as a dust filter (Fig. 13A, B, F). The closing apparatus consists of an atrial valve that strangles the trachea and blocks the passage of the air. The valve is formed by a fold of the tracheal wall in which the posterior side extends in a long cuticular rod (lever) (Fig. 13A, B). Short perpendicular spiracular tracheae arise from the main tracheal trunk and connect to the mesothoracic and abdominal spiracles. These spiracles are biforous and are located on the tip of laterodorsal tubercles (Fig. 13C, D, G, H, J). In the first and second instars, they are much reduced and generally non-functional. However, the spiracular tracheae are clearly visible and do not seem collapsed, and the spiracles have an atrium with spinulae and the same type of closing apparatus as observed in the terminal spiracles (Fig. 13C, D).

##### *Groups with piercing-sucking feeding system*

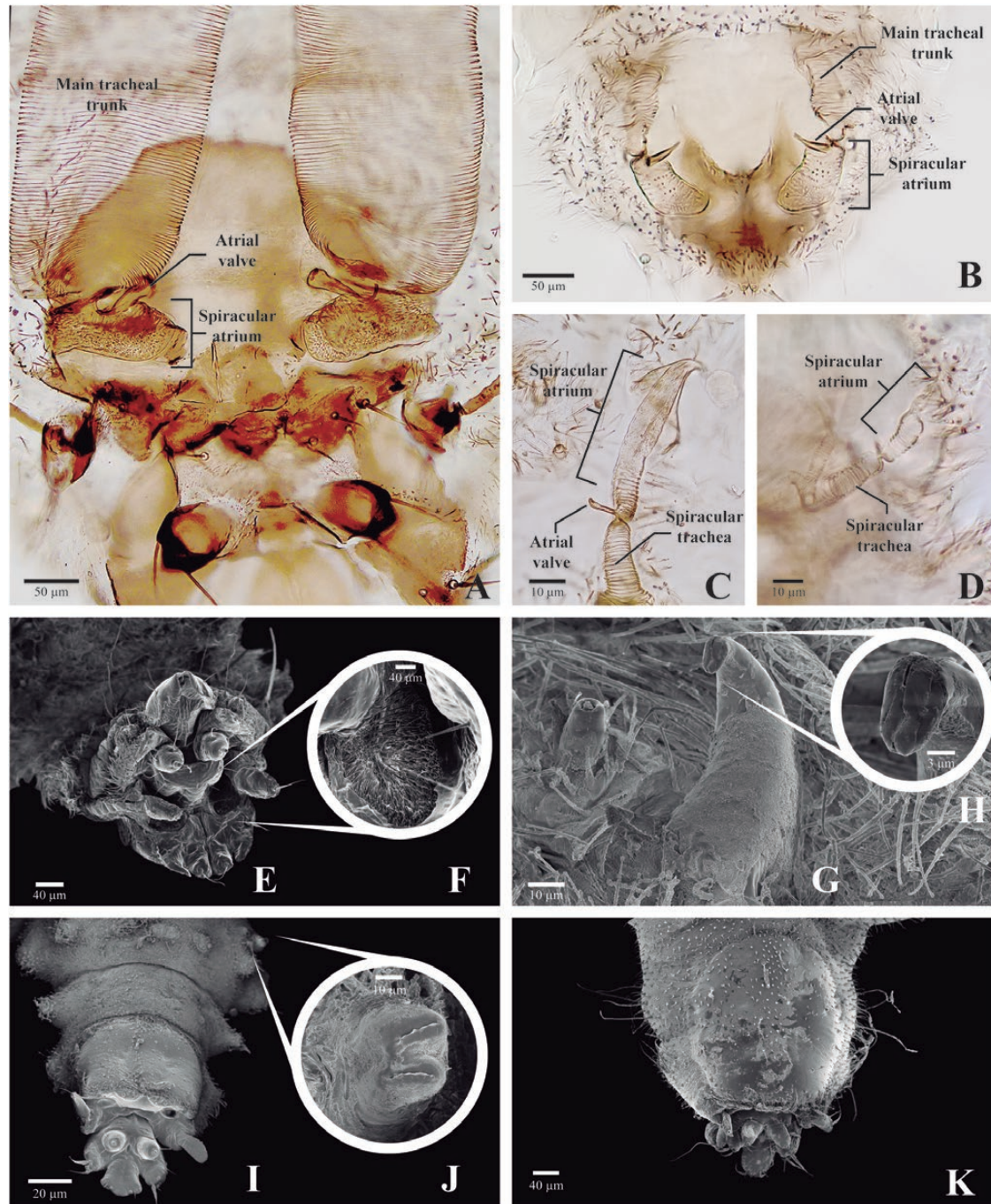
*Berosus* + *Hemiosus*: The tracheal system is closed and tracheal gills are present (*Berosus*) or absent (*Hemiosus*). The external spiracular chamber is reduced (*Hemiosus*) or absent (*Berosus*) (Fig. 14E, I, K).

The main tracheal trunks taper toward the terminal part of the body and end in blind tubes (Fig. 14B). As for the terminal spiracles, the spinulae in the atrium and the vestigial closing apparatus are still present, but the connection with the exterior is completely lost. *Berosus* larvae usually have one pair of gills on each of abdominal segments I–VII. The tracheation has only minor deviations from the tracheal arrangement of larvae without gills: a short lateral trachea arises from the tracheal trunk and bifurcates into (1) spiracular trachea and (2) gill trachea (Fig. 14C). Each gill has a single trachea in the axial position, which extends from the base to the tip, tapering towards the apex (Fig. 14A). The spiracular trachea is collapsed and looks like a solid cord connected to the metathoracic and abdominal spiracles, which are much reduced and difficult to observe. The spiracles and spiracular trachea are well developed in third-instar larvae; spiracles are located on dorsal tubercles, near the base of the gill (Fig. 14H, J). The spiracles and the spiracular tracheae are well developed and hollow, but they lack the closing apparatus and only the cuticular ornamentation of the dust filter remains (Fig. 14D). Mature larvae of *Berosus* can spend long periods of time outside the water (up to two weeks under laboratory conditions; pers. obs.), which indicates that the spiracles are functional, at least in mature larvae.

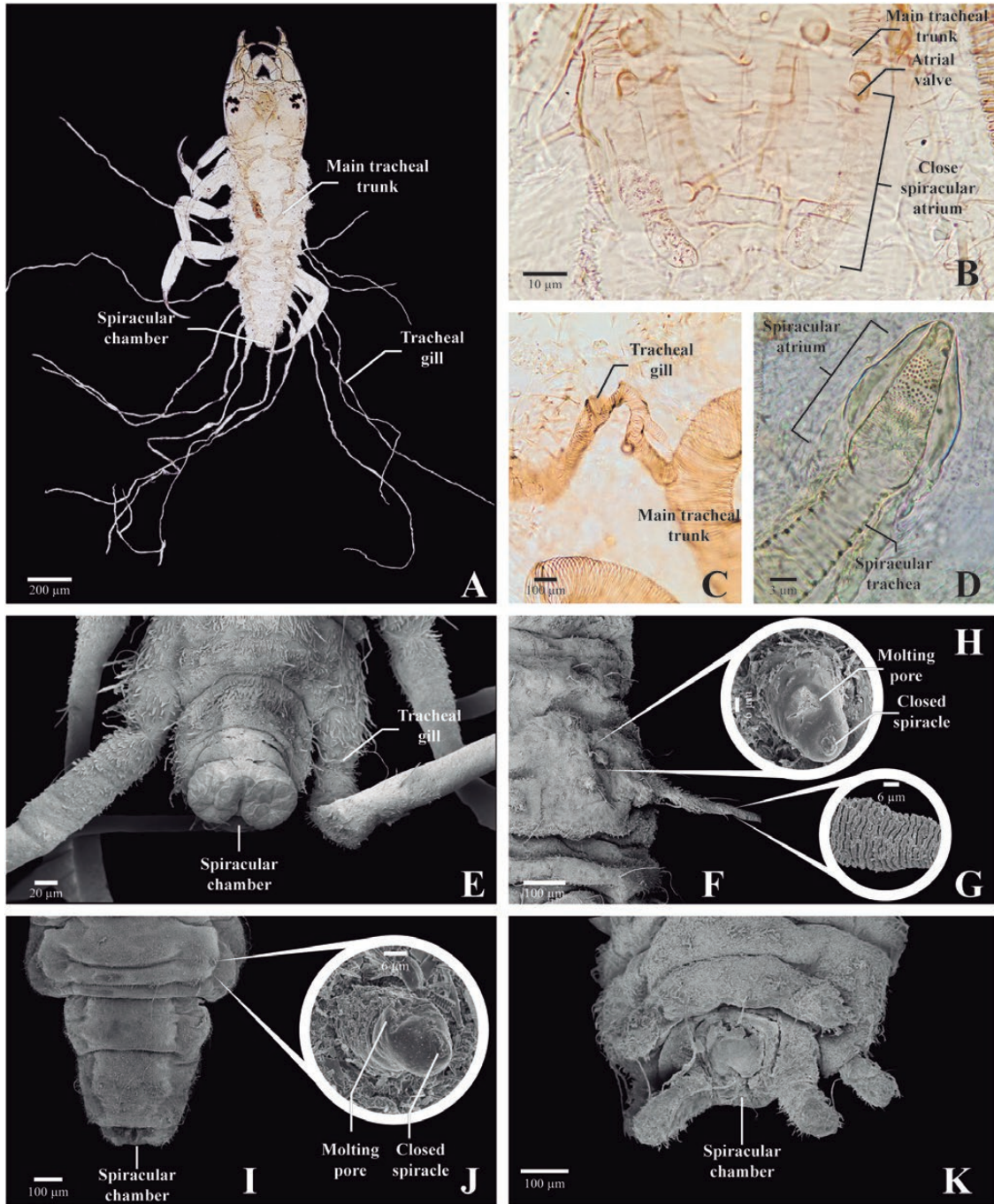
*Laccobius* + *Oocyclus*: The tracheal system is open and not modified in all *Oocyclus* and most *Laccobius* (Fig. 13K). The only known exception is *Laccobius* (*Yateberosus*), which has a closed tracheal system and bears nine long digitiform gills in abdominal segments VIII and IX to obtain oxygen by diffusion from the water (Fikáček *et al.*, 2018: figs 1, 4C). The spiracles are reduced and non-functional, and the spiracular chamber is not developed.

*Hybograalius*: The tracheal system is closed. The metathoracic and abdominal spiracles are non-functional and the spiracular chamber is absent. These larvae lack specialized respiratory organs and gas exchange probably occurs through the cuticle.

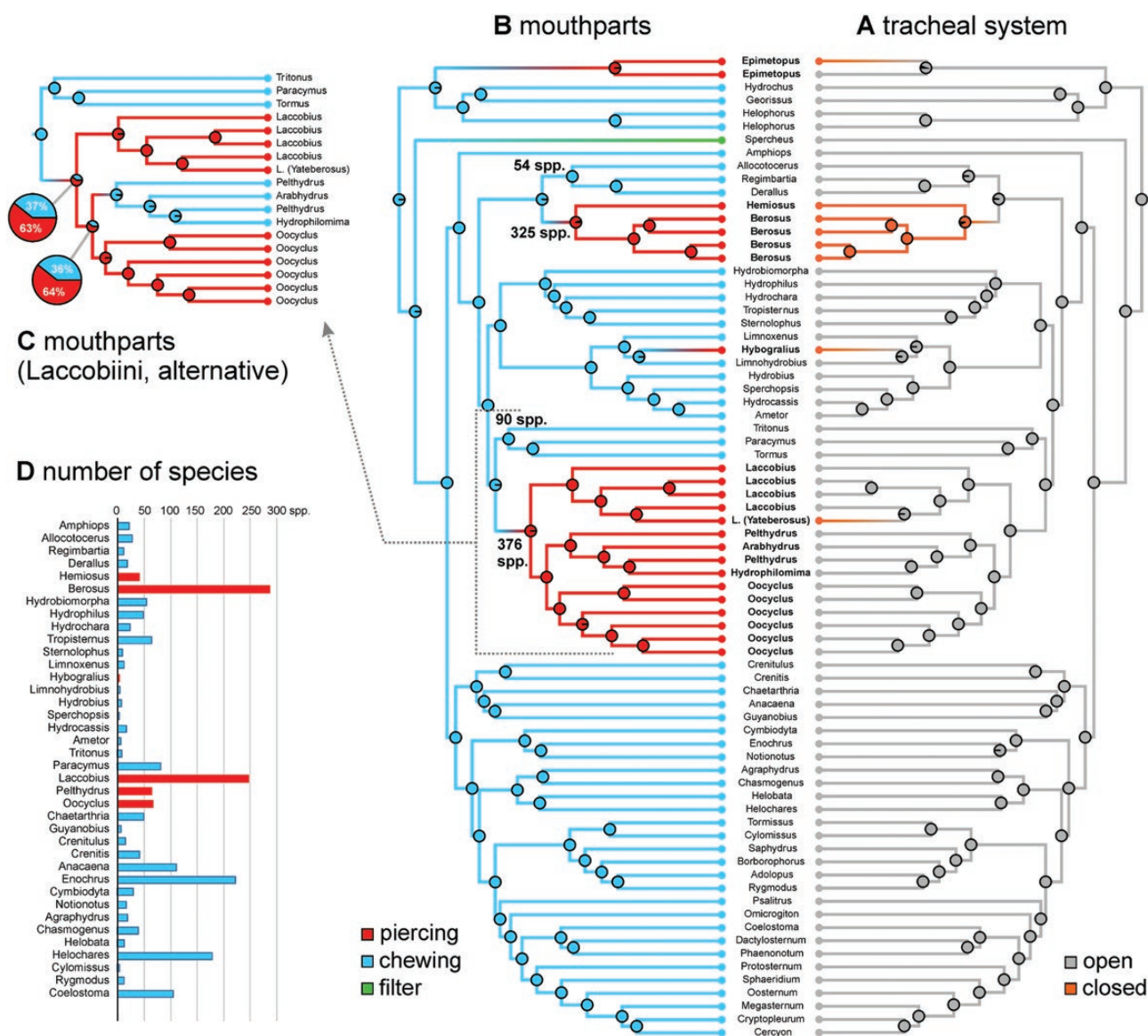
*Epimetopus*: The tracheal system is likely closed. Spiracles are biforous but strongly reduced, the spiracular chamber is absent and the larvae bear a pair of digitiform gills on abdominal segments VIII and IX. In some species, an extra pair of gills can be found ventrally on abdominal segment IX (Fikáček *et al.*, 2011: figs 9, 10).



**Figure 13.** Summary of the main structures related with metapneustic respiratory system. A, *Tropisternus latus* (Brullé, 1837), spiracular chamber, first-instar larva, light microscope photograph, dorsal view. B, *Helochares ventricosus* Bruch, 1915, spiracular chamber, first-instar larva, light microscope photograph, dorsal view. C, *Tropisternus latus* (Brullé, 1837), spiracular chamber, first-instar larva, light microscope photograph, dorsal view. D, *Helochares ventricosus* Bruch, 1915, abdominal spiracle, first-instar larva, light microscope photograph, dorsal view. E–H, *Tropisternus setiger* Germar, 1824, SEM micrograph: E, spiracular chamber, third-instar larva, ventral view; F, detail of the terminal spiracle with dust filter, third-instar larva, ventral view; G, abdominal spiracle, first-instar larva, dorsal view; H, detail of the closed abdominal spiracles, first-instar larva, dorsal view. I, J, *Oocyclus iguazu* (Oliva 1996) third-instar larva, SEM micrograph: I, spiracular chamber, dorsal view; J, biforous abdominal spiracle, dorsal view. K, *Laccobius kunashiricus* Shatrovskiy, 1984, spiracular chamber, third-instar larva, SEM micrograph, dorsal view.



**Figure 14.** Summary of the main structures related with apneustic respiratory system. A–C, *Berosus decolor* Knisch, 1924, light microscope photograph: A, habitus, first-instar larva, dorsal view; B, terminal spiracle, third-instar larva, dorsal view; C, detail of the abdominal spiracular trachea and tracheal gill, dorsal view. D, *Berosus pallipes* Brullé, 1841, abdominal spiracle, third-instar larva, dorsal view. E–H, *Berosus* sp., third-instar larva, SEM micrograph: E, spiracular chamber, ventral view; F, first abdominal segment bearing tracheal gill, dorsal view; G, detail of tracheal gill surface; H, abdominal spiracle. I, J, *Hemiosus bruchi* Knisch, 1924, third-instar larva, SEM micrograph: I, last abdominal segments, dorsal view; J, abdominal spiracle. K, *Hemiosus multimaculatus* (Jensen-Haarup, 1910), spiracular chamber, third-instar larva, ventral view.



**Figure 15.** Phylogeny of the Hydrophiloidea with mapped evolution of tracheal system (A) and mouthparts (B, C). Two alternative ancestral state reconstructions of mouthparts, considering mouthparts of the *Pelthydrus*-group as: B, piercing-sucking; C, chewing (only tribe Laccobiini shown). D, number of species of aquatic genera of Hydrophilidae with known larvae. Colors of branches/bars/pie-charts indicate functional morphology of mouthparts (red = piercing-sucking, blue = chewing, green = filter-feeding) and development of the tracheal system (grey = open; orange = closed).

#### ANCESTRAL RECONSTRUCTION

Stochastic character mapping revealed the chewing feeding system as ancestral for Hydrophiloidea larvae (Fig. 15B). The piercing-sucking system evolved independently four times within Hydrophiloidea (three times in Hydrophilidae: Berosini: *Berosus* + *Hemiosus* clade; Laccobiini: *Laccobius* group; Hydrobiusini: *Hybograllius*; once in Epimetopidae: *Epimetopus*) (Fig. 15B), with similar morphology and equivalent function. Larvae of the *Pelthydrus*-clade (i.e. genera *Arabhydrus* Hebauer, 1997, *Pelthydrus* d'Orchymont, 1919 and

*Hydrophilomima* Hansen & Schödl, 1997 in the *Laccobius*-group) are unknown. We hence performed an alternative analysis considering them bearing the chewing mouthparts (Fig. 15C). This analysis also resulted in reconstructing the *Laccobius* group as ancestrally bearing piercing-sucking mouthparts (PP = 0.63) (Fig. 15C).

The presence of an open spiracular system was reconstructed as the ancestral character state for Hydrophiloidea. Four independent origins of the closed spiracular system are inferred: in *Berosus* + *Hemiosus* clade, *Hybograllius*, in the subgenus

*Yateberosus* (Laccobiini: *Laccobius*) and in *Epimetopus* (Epimetopidae), i.e. in the same groups that evolved the piercing-sucking feeding system (Fig. 15A). In the *Laccobius* group, the closed spiracular system is only present in a small internal clade, not in all species with piercing-sucking mouthparts. A similar situation may be present in *Epimetopus*, but more studies are needed to understand the diversity of the tracheal system in this genus.

## DISCUSSION

### EVOLUTIONARY MORPHOLOGY

#### *Mouthparts*

The head and mouthparts design in coleopteran larvae reflects a considerable variety of adaptations to feeding habits and to prey-capture behaviour, in particular (Gorb & Beutel, 2000). In Hydrophiloidea, two general morphologies were found: chewing and piercing-sucking. The chewing feeding system is the ancestral state and represents a ground plan for hydrophiloid feeding structures (Figs 1A–C, 2, 5, 9A, B; Supporting Information, Fig. S1):

1. Frontoclypeal region symmetrical, epistomal lobes small or not developed.
2. Nasale wide, sometimes slightly protruded.
3. Mandibles with wide incisor edge; retinacular area developed, with one to three sharp teeth for cutting and slicing food; penicillium and molar area absent.
4. Mandibles symmetrical or slightly asymmetrical, without inner groove.
5. Labium developed, dorsally armed with strong spinulae for food processing.
6. Labium, mandibles and maxilla forming a funnel involved in concentrating and directing the food to the pre-oral cavity.

This basic chewing mouthparts' configuration is slightly modified in groups with specialized prey preferences and many modifications are observed in the Sphaeridiinae. Larvae and adults of Sphaeridiinae inhabit decaying organic matter, such as rotting mushrooms and cacti, compost piles, carrion, moss, dung, litter or corpses (Archangelsky, 1997, 1999; Clarkson *et al.*, 2014; Fikáček *et al.*, 2015; Arriaga-Varela *et al.*, 2017; Minoshima, 2018, 2019; Fikáček, 2019). The trend to colonize terrestrial habitats within Sphaeridiinae corresponds with behavioural and morphological adaptations. The morphology of the head and mouthparts is largely affected, especially the shape of mandibles, the addition of setose structures on the maxilla and labium, and the presence of strong spinulae on most feeding-related structures (Archangelsky, 1999, 2016; Archangelsky

*et al.*, 2016b; Minoshima, 2018, 2019; Fikáček, 2019). Archangelsky (1999, 2016) and Fikáček *et al.* (2013) suggest that the setose maxillary stipes and the hypopharyngeal lobe act as a sponge improving the absorption of predigested liquid food in these groups. The modification of the head and mouthparts may be also related to locomotive functions during burying and digging in solid substrate, as it is known in other insect larvae (Striganova, 1967).

Although the larvae of many hydrophiloid genera are still unknown and some clades need more research, a clear pattern of multiple origin of the piercing-sucking feeding system is revealed (Fig. 15B, C). Piercing-sucking is a specialized feeding mode that allows underwater feeding using extra-oral digestion. Four independent origins were revealed, irrespective of whether the unknown larvae of the *Pelthydrus* clade are considered chewing or piercing-sucking (Fig. 15C). Bertrand (1972, 1974) assumed that the supposed *Pelthydrus* larvae had a chewing morphology. If this were true, a reversal to the ancestral condition would have occurred in the common ancestor of the *Pelthydrus* clade. However, no reversals have been observed in piercing-sucking clades so far. The piercing-sucking feeding requires substantial modifications and a high specialization of mouthparts, indicating a return to a chewing feeding system in larvae of the *Pelthydrus* clade as improbable.

Although the piercing-sucking system configuration is functionally similar in unrelated taxa, their detailed morphology is different, as expected from their independent origins (see Table 2). However, there is great consistency in the repetitive emergence of certain structures (Figs 1D–I, 3, 4, 6–8, 9C, D; Supporting Information, Figs S1–3) in the Hydrophilidae:

1. Left epistomal lobe developed, covering the basal-third of the left mandible.
2. Groups of specialized sensilla on epistomal lobes: flat, spine-like, curved downward.
3. Epistomal lobes with a deep notch or membranous area on the outer margin that increases its flexibility.
4. Nasale developed, projecting forward, involved in holding the prey underwater against the substrate.
5. Left mandible with an open channel for the injection of digestive fluids into preys and pumping predigested food back to the pre-oral cavity.
6. Left mandible with teeth modified in brush- or comb-like structures, with a patch of spinulae and/or a medial hyaline lobe (prosthema) to prevent food from escaping from the processing area.
7. Mandible and epistomal lobe coupled during the prey processing.
8. Labium strongly reduced, without or with few spinulae on dorsal surface.

**Table 2.** Comparative table of selected morphological characters among larvae of Hydrophilidae

Character	<i>Berosus</i>	<i>Hemiosus</i>	<i>Oocyclus</i>	<i>Laccobius</i>	<i>Hybograhius</i>
<b>Labroclypeus</b>					
Epistomal lobes	Left enlarged	Left enlarged	Right and left enlarged	Right and left enlarged	Right and left enlarged
Lobe flexibility	Membranous area	Membranous area	Notch	Notch	Not observed
Inner membranous area of left epistomal lobe	With short spinulae	With short spinulae	With 2–3 rows of seta-like projections	With 2–3 rows of serrated seta-like projections	With at least 3 rows of seta-like projections
Right epistomal lobe sensilla	Absent	Absent	Present (reduced)	Absent	Present (reduced)
<b>Left mandible</b>					
Prostheca	Present	Present	Absent	Present	Absent
Teeth projections	Multifid	Multifid	Unifid	Unifid	Unifid
Basal patch of spinulae	Absent	Absent	Present	Present	Absent
<b>Labium</b>					
Dorsal spinulae	Absent	Absent	Present	Present	Present
Ligula	Reduced	Small	Reduced	Reduced	Large

Larvae of Epimetopidae share many features with the piercing-sucking model of the Hydrophilidae. However, the structures are symmetrical: both mandibles have a shallow sucking channel and are coupled with the enlarged left and right epistomal lobes (Fig. 12). The head morphology of Helophoridae and Georissidae larvae are similar to those of Epimetopidae, e.g. in large epistomal lobes and simple nasale (Archangelsky, 1997; Fikáček *et al.*, 2011; Angus *et al.*, 2016; Minoshima & Watanabe, 2020) but both latter groups feed in the chewing way. The features shared by Epimetopidae, Georissidae and Helophoridae were probably present in their common ancestor and may be considered exceptions. They originally evolved for a more general use and acquired functions in the piercing-sucking feeding in the epimetopid larvae.

Unlike the larvae of Epimetopidae, there is a clear trend towards the asymmetry of the frontoclypeal region and mandibles in Hydrophilidae, related to the evolution of the piercing-sucking feeding apparatus. The degree of asymmetry of the epistomal lobes and their primary chaetotaxy varies according to the genus. The head and mouthparts are probably under strong selective pressure for improvement of performance, and the observed diversity may reflect different stages of optimization in different piercing-sucking lineages. In contrast to other structures, the difference between the left and right mandibles is markedly developed in all three hydrophilid lineages: the left one is used for sucking predigested food, whereas the right mandible is involved in the capture and retention of the prey.

Such a conserved directional asymmetry in mandibles and associated structures in three unrelated lineages may indicate strong morphological constraints, possibly due to the slightly asymmetrical mandibular articulation in all hydrophilid larvae. The right mandible is situated below the left mandible when mandibles close and cross. The larger space between the right mandible and the right epistomal lobe may have constrained the evolution of the epistomal-mandibular coupling system. Surprisingly, the same articulation asymmetry did not constrain the evolution of the coupling mechanism between the right mandible and right epistomal lobe in *Epimetopus*, possibly because a large epistomal lobe was already present in the chewing ancestors.

Modified sucking mouthparts have evolved independently in other aquatic beetle families. Mandibles with grooves occur in several aquatic Adephagan families, such as Dytiscidae, Gyrinidae and Haliplidae (De Marzo & Nilsson, 1986; Lawrence *et al.*, 2011). In Dytiscidae, the mandibular groove is formed by an invagination of the integument of each mandible. The dorsal and ventral lips of this invagination can: (1) run parallel without approaching each other (open mandibular channel, e.g. *Agabetes* Crotch, 1873 and *Ilybius* Erichson, 1832); (2) approach each other in a section of their length, without touching each other (partially closed mandibular channel, e.g. *Colymbetes* Clairville, 1806, *Rhantus* Dejean, 1833 and *Laccophilus* Leach, 1815); and (3) be in contact with each other in a section of their length, with a more or



less complex coaptation (closed mandibular channel, e.g. *Dytiscus* Linnaeus, 1758, *Hydaticus* Leach, 1817 and *Hyphydrus* Illiger, 1802) (De Marzo, 1976, 1977). Within Polyphaga, the internally perforated mandibles are present in elateroid families Brachypsectridae, Drilidae, Phengodidae, Rhagophthalmidae and Lampyridae (Cicero, 1994; Costa *et al.*, 2006; Lawrence, 2011; Fu *et al.*, 2012). Of these families, only fireflies have representatives with aquatic or semi-aquatic larvae. The mandibular channel of lampyrids is closed, internal and forms already during the embryonic stage (Cicero, 1994). All mentioned adaphagan and polyphagan groups perform extra-oral digestion of the prey tissues by injecting histolytic substances through the mandibular channel, and subsequently sucking the predigested liquids (Haddon, 1915; De Marzo, 1979; Fu & Meyer-Rochow, 2012), i.e. the same way as in the Hydrophilidae. The structural similarities of these feeding systems are convergences gained as adaptations to an aquatic (submerged) lifestyle.

### Respiratory system

An open spiracular system is ancestral for larvae of Hydrophiloidea (Fig. 15A). The larvae of Epimetopidae, Georissidae and Helophoridae are riparian and have nine pairs of functional spiracles (peripneustic system), i.e. the ancestral respiratory system of the superfamily (Archangelsky, 2007). Families with aquatic larvae (Hydrochidae, Hydrophilidae and Spercheidae) are metapneustic: the last (eighth abdominal) pair of spiracles opens in a spiracular chamber that serves to connect the tracheal system of the submerged larva with the above-water atmosphere. The Sphaeridiinae and Cylominae lineages left the water and invaded humid terrestrial habitats, but their larvae have retained the spiracular atrium and the metapneustic tracheal system. The spiracular chamber became an adaptation for life in decomposed organic matter: posterior spiracles maintain the connection with the atmospheric air while the larva burrows and the thoracic and abdominal spiracles one to seven are covered by the substrate (Archangelsky, 1999). Fikáček *et al.* (2017) found that some hygropetric and terrestrial larvae (*Tormus* and *Tritonus*) have all spiracles open and likely functional, and the last pair is still situated in the spiracular atrium; the same authors have suggested that the completely open (peripneustic) tracheal system is probably more widespread in Hydrophilidae.

Apneustic larvae, with all the spiracles reduced to functionless vestiges, are relatively rare in Coleoptera, but occur, e.g. in Hygrobiidae, Haliplidae, Gyrinidae, Elmidae, Limmichidae and Psephenidae (Crowson, 1981; Yee & Kehl, 2015). A particular case is that of some Helophoridae larvae (*Helophorus* Fabricius, 1775

subgenus *Lihelophorus* Zaitzev, 1908) in which tracheal gills combined with functional spiracles were found (Angus *et al.*, 2016). A closed respiratory system is a derived trait in all beetle lineages. In Hydrophiloidea, it is tightly connected with modifications of mouthparts. A piercing-sucking feeding mode seems to be a prerequisite for the evolution of the closed tracheal system. The piercing-sucking feeding strategy allows the larvae to feed and perform extra-oral digestion underwater. However, they still depend on atmospheric air for gas exchange. Some of them have gone a step further and have developed an apneustic respiratory system (e.g. *Berosus*, *Hemiosus*, *Hybograllius*, *Laccobius* (*Yateberosus*) and *Epimetopus* (Epimetopidae)), which allows them to obtain oxygen by diffusion from the water. The combination of piercing-sucking mouthparts and a closed tracheal system makes them completely independent of the above-water environment.

### DIVERSIFICATION IN PIERCING-SUCKING LINEAGES

Hydrophilidae shows a great disparity in species richness between principal clades. Some lineages are exceptionally species rich, while others are relatively species-poor (Bloom *et al.*, 2014). We found that most piercing-sucking lineages show an unusually high species richness and a wide distributional range (Fig. 15D). Niche breadth, body size, population abundance, environmental variability, colonization and extinction dynamics, and dispersal ability may determine species richness and geographical range (Ribera, 2008). The acquisition of an evolutionary novelty may enable the occupation of a new ecological zone and trigger a boom of taxonomic diversity (= adaptive radiation) (Assis & de Carvalho, 2010). This connection between key innovations and adaptive radiations could explain the high number of species in the piercing-sucking lineages.

With 380 described species and many more awaiting description, the *Laccobius*-group (*sensu* Short & Fikáček, 2013) is one of the most diverse clades within Hydrophilidae. According to Bloom *et al.* (2014), the common ancestor of the *Laccobius*-group was aquatic. The members of the clade are adapted to an exceptionally broad range of ecological niches, including lotic, lentic and semi-aquatic (hygropetric) habitats. *Oocyclus* species show a transition to semi-aquatic habitats and represent one of the major hygropetric radiations within Hydrophilidae (Toussaint & Short, 2018). The acquisition of a piercing-sucking feeding mechanism may have facilitated the colonization of the hygropetric habitats and hence the diversification of the lineage. The piercing-sucking system evolved as an adaptation for underwater feeding but may be an effective adaptation for food processing in

hygropetric habitats. These environments are usually almost vertical with a thin layer of flowing water, which can make the ancestral feeding behaviour (especially holding and processing the prey above water) impossible. In contrast, the piercing-sucking mode allows feeding inside the water film and enable the larvae to exploit the rich seepage insect fauna as food source.

The sister-taxa *Berosus* and *Hemiosus* exhibit a great disparity in their species richness and distribution. The genus *Berosus* comprises 287 described species distributed almost worldwide and is the most speciose genus of the family Hydrophilidae (Hansen, 1999; Short & Fikáček, 2011; Oliva & Short, 2012). Conversely, only 38 species of *Hemiosus* have been described so far, all with an exclusively American distribution (Oliva & Short, 2010; Short & Fikáček, 2011; González-Rodríguez *et al.*, 2019). The larvae of both genera are similar and are adapted both to feed and breathe underwater. Both genera have a closed tracheal system. *Hemiosus* larvae lack specialized organs for breathing and are restricted to running-water bodies with high oxygen concentration and low water temperature (e.g. streams or rivers). The lack of specialized organs, and hence the occurrence in running waters, is also the ancestral condition for the *Berosus*–*Hemiosus* lineage. In contrast, *Berosus* larvae have tracheal gills that increase the respiratory surface and help to meet the metabolic requirements when the oxygen concentration is low or the temperature is high (Merritt *et al.*, 2008). Acquisition of tracheal gills likely facilitated the colonization of standing waters (lentic habitats). Lentic habitats are more ephemeral and lentic species tend to have a higher dispersal ability, lower extinction rates, broader fundamental niches and, therefore, larger geographic ranges (Ribera & Vogler, 2000; Ribera *et al.*, 2001, 2003; Vogler & Ribera, 2003; Ribera, 2008; Abellán *et al.*, 2009; Damm *et al.*, 2010; Arribas *et al.*, 2012; Dijkstra *et al.*, 2014; Hjalmarsson *et al.*, 2015; Letsch *et al.*, 2016). The evolution of tracheal gills may have allowed *Berosus* species to colonize a much wider array of microhabitats, including standing waters poor in oxygen, which are not suitable for *Hemiosus*. Wider species ranges might have also enabled *Berosus* to acquire a worldwide distribution. The benthic life style, independent of the above-water atmosphere thanks to piercing-sucking mouthparts combined with acquisition of specialized breathing organs, likely opened *Berosus* species novel ecological as well as geographic adaptive zones, and may be responsible for the current high species diversity of the genus.

*Hybograllius* is the only hydrophilid lineage with piercing-sucking mouthparts that is species-poor. It comprises a single species known from a small area of the Darling Range in Western Australia

(Fikáček, 2019). The closed tracheal system and lack of specialized gills makes *Hybograllius* a specialized inhabitant of winter and spring seasonal streams. It is likely that the genus always had a limited distribution, as is typical for stream-inhabiting species. Its diversity was likely affected also by the massive Australian aridification during the Late Miocene and Pliocene, pushing the ranges of humidity-demanding fauna into small refugia (Byrne *et al.*, 2011).

The effect of piercing-sucking food processing and a closed tracheal system on the species richness of *Epimetopus* require further studies. The larvae are not known for the Asian (*Eupotemus*) and African (*Eumetopus*) representatives of the Epimetopidae, which are relatively species-poor (seven and five species, respectively; Jäch, 2002; Skale & Jäch, 2003; Fikáček, unpublished data). In contrast, the American *Epimetopus* with piercing-sucking larvae has at least 60 species (Perkins, 2012; Perkins, pers. comm.).

## CONCLUSIONS

Our morphofunctional analysis provides the first detailed insight into the feeding behaviour and morphological adaptations of benthic hydrophilid larvae, and is a starting point for further investigating the evolution of adaptations to the aquatic environment, with an emphasis on feeding and respiratory mechanisms. Three principal feeding strategies have appeared in the evolution of the Hydrophiloidea. The highly specialized piercing-sucking mechanism has evolved independently in four lineages within the superfamily and has allowed these taxa to adapt for underwater feeding and to diversify in benthic, as well as hygropetric, habitats. The recurrent appearance of grooved mandibles along with an epistomal-mandibular coupling system suggests that these structures have a key role in underwater feeding. We found a strong correlation between this type of feeding mechanism and modifications of the tracheal system, although more thorough studies of the tracheal system are needed. The piercing-sucking mouthparts and tracheal gills are putative key innovations that may be responsible for the high species diversity of the *Laccobius* group and the *Berosus*–*Hemiosus* clade in Hydrophilidae and of *Epimetopus* in Epimetopidae. These adaptations have the potential to affect rates of speciation and extinction, but further tests need to be performed once DNA data of more species of these clades become available. The results of our study raise further evolutionary and functional questions, such as: (1) how do these adaptations affect the habitat specificity, (2) how do the morphological innovations relate to the colonization of new adaptive zones, (3) the possible role of specialized respiratory organs in dispersal and

diversification of species in aquatic environments and (4) how do the adaptations to similar environments result in a similar morphology in different taxa?

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#### REFERENCES

- Abellán P, Millán A, Ribera I. 2009.** Parallel habitat driven differences in the phylogeographical structure of two independent lineages of Mediterranean saline water beetles. *Molecular Ecology* **18**: 3885–3902.
- Angus RB, Jia F, Chen Z, Zhang Y, Vondraček D, Fikáček M. 2016.** Taxonomy, larval morphology and cytogenetics of *Lihelophorus*, the Tibetan endemic subgenus of *Helophorus* (Coleoptera: Hydrophiloidea). *Acta Entomologica Musei Nationalis Pragae* **56**: 109–148.
- Archangelsky M. 1997.** Studies on the biology, ecology, and systematics of the immature stages of New World Hydrophiloidea (Coleoptera: Staphyliniformia). *Ohio Biology Survey Bulletin New Series* **12**: 1–207.
- Archangelsky M. 1999.** Adaptations of immature stages of Sphaeridiinae (Staphyliniformia, Hydrophiloidea, Hydrophilidae) and state of knowledge of preimaginal Hydrophilidae. *The Coleopterists Bulletin* **53**: 64–79.
- Archangelsky M. 2001.** A new Neotropical species of *Spercheus* Kugelann, and its larval stages (Coleoptera: Hydrophiloidea: Spercheidae). *Studies on Neotropical Fauna and Environment* **36**: 199–204.
- Archangelsky M. 2007.** Escenarios evolutivos en larvas y adultos de Hydrophiloidea (Coleoptera) analizados en función de diferentes hipótesis filogenéticas. *Revista de la Sociedad Entomológica Argentina* **66**: 103–111.
- Archangelsky M. 2008.** Phylogeny of Berosini (Coleoptera: Hydrophilidae, Hydrophilinae) based on larval and adult characters, and evolutionary scenarios related to habitat shift in larvae. *Systematic Entomology* **33**: 635–650.
- Archangelsky M. 2016.** Chaetotaxy and larval morphometry of *Cercyon praetextatus* (Say) and *C. quisquilius* (Linnaeus) (Coleoptera: Hydrophilidae: Sphaeridiinae) and their phylogenetic implications. *Arthropod Systematics & Phylogeny* **74**: 177–193.
- Archangelsky M. 2018.** Larval chaetotaxy and morphometry of *Oosternum costatum* (Coleoptera: Hydrophilidae) including a discussion of larval characters with phylogenetic relevance. *Acta Entomologica Musei Nationalis Pragae* **58**: 499–511.
- Archangelsky M, Beutel RG, Komarek A. 2016a.** **Hydrophiloidea Latreille, 1802.** In: Beutel RG, Leschen RAB, eds. *Handbook of zoology. Arthropoda: Insecta. Coleoptera, beetles, Vol. 1: morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim), 2nd edn.* Berlin: Walter De Gruyter, 232–272.
- Archangelsky M, Rodriguez G, Torres PLM. 2016b.** Primary chaetotaxy and larval morphometry of *Phaenonotum exstriatum* and *Dactylosternum cacti* (Coleoptera: Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **56**: 167–193.
- Arriaga-Varela E, Seidel M, Deler-Hernández A, Senderov V, Fikáček M. 2017.** A review of the *Cercyon* Leach (Coleoptera, Hydrophilidae, Sphaeridiinae) of the Greater Antilles. *ZooKeys* **681**: 39–93.
- Arribas P, Velasco J, Abellán P, Sánchez-Fernández D, Andújar C, Calosi P, Millán A, Ribera I, Bilton DT. 2012.** Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *Journal of Biogeography* **39**: 984–994.
- Assis LCS, de Carvalho MR. 2010.** Key innovations: further remarks on the importance of morphology in elucidating systematic relationships and adaptive radiations. *Evolutionary Biology* **37**: 247–254.
- Balduf WV. 1935.** *The bionomic of entomophagous Coleoptera.* St. Louis: John S. Swift Co.
- Balfour-Browne F. 1910.** On the life-history of *Hydrobius fuscipes* L. *Transactions of the Royal Society of Edinburgh* **47**: 317–340.
- Bernhard D, Schmidt L, Korte A, Fritsch G, Beutel RG. 2006.** From terrestrial to aquatic habitats and back again - molecular insights into the evolution and phylogeny of Hydrophiloidea (Coleoptera) using multigene analyses. *Zoologica Scripta* **35**: 597–606.
- Bertrand H. 1950.** Observations biologiques sur les larves des Hydrophilides. *Bulletin de la Société Zoologique de France* **75**: 96–103.
- Bertrand H. 1972.** *Larves et nymphes des Coléoptères aquatiques du globe.* Abbeville: F. Paillart.
- Bertrand H. 1974.** Larves et nymphes des Coléoptères aquatiques (2e note). *Bulletin de la Société Entomologique de France* **79**: 53–63.

- Bilton DT, Ribera I, Short AEZ. 2019.** Water beetles as models in ecology and evolution. *Annual Review of Entomology* **64**: 359–377.
- Bloom DD, Fikáček M, Short AE. 2014.** Clade age and diversification rate variation explain disparity in species richness among water scavenger beetle (Hydrophilidae) lineages. *PLoS One* **9**: e98430.
- Bøving AG, Henriksen KL. 1938.** The developmental stages of the Danish Hydrophilidae (Ins., Coleoptera.). *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i København* **102**: 27–162.
- Byrne M, Steane DA, Joseph L, Yeates DK, Jordan GJ, Crayn D, Aplin K, Cantrill DJ, Cook LG, Crisp MD, Keogh JS, Melville J, Moritz C, Porch N, Sniderman JMK, Sunnucks P, Weston PH. 2011.** Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography* **38**: 1635–1656.
- Chapman EG, Przhiboro AA, Harwood JD, Foote BA, Hoeh WR. 2012.** Widespread and persistent invasions of terrestrial habitats coincident with larval feeding behavior transitions during snail-killing fly evolution (Diptera: Sciomyzidae). *BMC Evolutionary Biology* **12**: 175.
- Cicero JM. 1994.** Composite, haustellate mouthparts in netwinged beetle and firefly larvae (Coleoptera, Cantharoidea: Lycidae, Lampyridae). *Journal of Morphology* **219**: 183–192.
- Clarkson B, Albertoni FF, Fikáček M. 2014.** Taxonomy and biology of the bromeliad-inhabiting genus *Lachnodacnum* (Coleoptera: Hydrophilidae: Sphaeridiinae). *Acta Entomologica Musei Nationalis Pragae* **54**: 157–194.
- Cohen AC. 1995.** Extra-oral digestion in predaceous terrestrial Arthropoda. *Annual Review of Entomology* **40**: 85–103.
- Costa C, Vanin SA, Lawrence JF, Ide S, Branham MA. 2006.** Review of the family Brachypsectridae (Coleoptera: Elateroidea). *Annals of the Entomological Society of America* **99**: 409–432.
- Crowson RA. 1981.** Water beetles. In: Crowson RA, ed. *The biology of the Coleoptera*. London: Academic Press, 429–456.
- Damm S, Dijkstra K-DB, Hadrys H. 2010.** Red drifters and dark residents: the phylogeny and ecology of a Plio-Pleistocene dragonfly radiation reflects Africa's changing environment (Odonata, Libellulidae, *Trithemis*). *Molecular Phylogenetic Evolution* **54**: 870–882.
- De Marzo L. 1976.** Studi sulle larve dei coleotteri ditiscidi. VI. Studio per fini sistematici del comportamentodei caratteri delle mandibole nelle larve di alcune specie de la subf. Colymbetinae. *Entomologica (Bari)* **12**: 179–198.
- De Marzo L. 1977.** Studi sulle larve dei coleotteri ditiscidi. VII. Considerazioni sul significato evolutivo del comportamento dei caratteri delle mandibole nelle larve di alcune specie della subf. Dytiscinae. *Entomologica (Bari)* **13**: 71–84.
- De Marzo L. 1979.** Studi sulle larve dei coleotteri ditiscidi. X. Anatomia e funzionamento dell'apparato succhiante cibario-faringeo in alcune forme larvali delle subf. Dytiscinae, Colymbetinae, Laccophilinae e Hydroporinae. *Entomologica (Bari)* **15**: 1–72.
- De Marzo L, Nilsson AN. 1986.** Morphological variation and fine structure of some head structures in larvae of Dytiscidae (Coleoptera). *Entomologica Basiliensia* **11**: 29–42.
- Deler-Hernández A, Fikáček M. 2016.** Larval morphology and chaetotaxy of three Caribbean *Berosus* Leach, 1817 with revised adult diagnosis of *Berosus undatus* (Fabricius, 1792) (Coleoptera: Hydrophilidae). *Aquatic Insects* **37**: 99–113.
- Dijkstra KD, Monaghan MT, Pauls SU. 2014.** Freshwater biodiversity and aquatic insect diversification. *Annual Review of Entomology* **59**: 143–163.
- DVDVideoSoft. 2020.** Free video to JPG converter. Available at: <https://www.dvdvideosoft.com/products/dvd/Free-Video-to-JPG-Converter.htm>. (date last accessed, 20 October 2020).
- Fikáček M. 2019.** Neotropical leaf litter beetle genus *Motonerus* (Coleoptera: Hydrophilidae): new species, distribution data, and description of third instar larva. *Neotropical Entomology* **48**: 788–808.
- Fikáček M, Barclay MVL, Perkins P. 2011.** Two new species of the *Epimetopus mendeli* species group and notes on its adult and larval morphology (Coleoptera: Hydrophiloidea: Epimetopidae). *Acta Entomologica Musei Nationalis Pragae* **51**: 477–504.
- Fikáček M, Minoshima Y, Vondráček D, Gunter N, Leschen RAB. 2013.** Morphology of adults and larvae and integrative taxonomy of Southern Hemisphere genera *Tormus* and *Afrotormus* (Coleoptera: Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **53**: 75–126.
- Fikáček M, Maruyama M, Komatsu T, von Beeren C, Vondráček D, Short AEZ. 2015.** Protosternini (Coleoptera: Hydrophilidae) corroborated as monophyletic and its larva described for the first time: a review of the myrmecophilous genus *Sphaerocetum*. *Invertebrate Systematics* **29**: 23–36.
- Fikáček M, Gustafson GT, Short AEZ. 2017.** On wet rocks with snorkels: immature stages of *Tritonus* cascade beetles with unusual modification of spiracles (Coleoptera: Hydrophilidae: Laccobiini). *Annales Zoologici* **67**: 91–107.
- Fikáček M, Minoshima Y, Jäch MA. 2018.** Larval morphology of *Yateberosus*, a New Caledonian endemic subgenus of *Laccobius* (Coleoptera: Hydrophilidae), with notes on 'Berosus-like' larvae in Hydrophiloidea. *Acta Entomologica Musei Nationalis Pragae* **58**: 195–206.
- Fu X, Meyer-Rochow VB. 2012.** An investigation into the morphological and behavioral adaptations of the aquatic larvae of *Aquatica leii* (Coleoptera: Lampyridae) to prey upon freshwater snails that serve as intermediate hosts for the liver fluke. *Biological Control* **62**: 127–134.
- Fu X, Ballantyne L, Lambkin C. 2012.** The external larval morphology of aquatic and terrestrial Luciolinae fireflies (Coleoptera: Lampyridae). *Zootaxa* **3405**: 1–34.
- González-Rodríguez LM, García-Hernández AL, Clark B. 2019.** Two new species of the water scavenger beetle genus *Hemiosus* Sharp (Coleoptera: Hydrophilidae) from Colombian Andes. *Zootaxa* **4565**: 496–498.
- Gorb S, Beutel RG. 2000.** Head-capsule design and mandible control in beetle larvae: a three-dimensional approach. *Journal of Morphology* **244**: 1–14.

- Grimaldi D, Engel MS. 2005.** *Evolution of the insects*. New York: Cambridge University Press.
- Haddon K. 1915.** On the methods of feeding and the mouth-parts of the larva of the glow-worm (*Lampyris noctiluca*). *Proceedings of the Zoological Society of London* **85**: 77–82.
- Hansen M. 1999.** *World catalogue of insects, Vol. 2. Hydrophiloidea (Coleoptera)*. Stenstrup: Apollo Books.
- Hjalmarsson AE, Bergsten J, Monaghan MT. 2015.** Dispersal is linked to habitat use in 59 species of water beetles (Coleoptera: Adephegata) on Madagascar. *Ecography* **38**: 732–739.
- Inoda T, Hirata Y, Kamimura S. 2003.** Asymmetric mandibles of water-scavenger larvae improve feeding effectiveness on right-handed snails. *The American Naturalist* **162**: 811–814.
- Inoda T, Inoda Y, Rullan K. 2015.** Larvae of the water scavenger beetle, *Hydrophilus acuminatus* (Coleoptera: Hydrophilidae) are specialist predators of snails. *European Journal of Entomology* **112**: 145e150.
- Jäch MA. 2002.** First record of Epimetopidae in Laos (Coleoptera: Epimetopidae). *Koleopterologische Rundschau* **72**: 161–164.
- Jäch MA, Balke M. 2008.** Global diversity of water beetles (Coleoptera) in freshwater. *Hydrobiologia* **595**: 419–442.
- Labandeira CC, Beall BS, Hueber FM. 1988.** Early insect diversification: evidence from a Lower Devonian bristletail from Québec. *Science* **242**: 913–916.
- Lawrence JF, Ślipiński A, Seago AE, Thayer MK, Newton AF, Marvaldi AE. 2011.** Phylogeny of the Coleoptera based on morphological characters of adults and larvae. *Annales Zoologici* **61**: 1–217.
- Letsch H, Gottsberger B, Ware JL. 2016.** Not going with the flow: a comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. *Molecular Ecology* **25**: 1340–1353.
- McKenna DD, Shin S, Ahrens D, Balke M, Beza-Beza C, Clarke DJ, Donath A, Escalona HE, Friedrich F, Letsch H, Liu S, Maddison D, Mayer C, Misof B, Murin PJ, Niehuis O, Peters RS, Podsiadlowski L, Pohl H, Scully ED, Yan EV, Zhou X, Ślipiński A, Beutel RG. 2019.** The evolution and genomic basis of beetle diversity. *Proceedings of the National Academy of Sciences of the United States of America* **116**: 24729–24737.
- Merritt RW, Cummins KW, Berg MB. 2008.** *An introduction to the aquatic insects of North America*. Dubuque: Kendall Hunt.
- Minoshima YN. 2018.** Larval morphology of *Armotus ohyamatensis* Hoshina and Satô (Coleoptera: Hydrophilidae: Megasternini). *The Coleopterists Bulletin* **72**: 767–779.
- Minoshima YN. 2019.** First known larva of omicrine genus *Psalitrus* d'Orchymont (Coleoptera, Hydrophilidae). *Deutsche Entomologische Zeitschrift* **66**: 107–118.
- Minoshima Y, Hayashi M. 2015.** Description of the larval stages of the berosine genera *Berosus* and *Regimbartia* based on Japanese species *B. japonicus* and *R. attenuata* (Coleoptera: Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **55**: 47–83.
- Minoshima YN, Watanabe R. 2020.** Morphology of immature stages of *Helophorus* (*Gephelophorus*) *auriculatus* (Coleoptera, Helophoridae). *Acta Entomologica Musei Nationalis Pragae* **60**: 319–332.
- Oliva A, Short AEZ. 2010.** A remarkable new species of *Hemiosus* Sharp from Amazonian Peru. *Koleopterologische Rundschau* **80**: 139–142.
- Oliva A, Short AEZ. 2012.** Review of the *Berosus* Leach of Venezuela Coleoptera, Hydrophilidae, Berosini) with description of fourteen new species. *ZooKeys* **206**: 1–69.
- Pallarés S, Arribas P, Bilton DT, Millán A, Velasco J. 2015.** The comparative osmoregulatory ability of two water beetle genera whose species span the fresh-hypersaline gradient in inland waters (Coleoptera: Dytiscidae, Hydrophilidae). *PLoS One* **10**: e0124299.
- Pallarés S, Arribas P, Bilton DT, Millán A, Velasco J, Ribera I. 2017.** The chicken or the egg? Adaptation to desiccation and salinity tolerance in a lineage of water beetles. *Molecular Ecology* **26**: 5614–5628.
- Perkins PD. 1972.** *A study of the Hydraenidae and Hydrophilidae (Coleoptera) of the San Gabriel River, with emphasis in larval taxonomy*. Unpublished M.A. Thesis, California State University.
- Perkins PD. 2012.** A revision of *Epimetopus* Lacordaire, the New World hooded shore beetles (Coleoptera: Epimetopidae). *Zootaxa* **3531**: 1–95.
- Pritchard G, McKee MH, Pike EM, Scrimgeour GJ, Zloty J. 1993.** Did the first insects live in water or in air? *Biological Journal of the Linnean Society* **49**: 31–44.
- Revell LJ. 2012.** Phytools: an R package for phylogenetic comparative biology (and other things). *Methods for Ecology and Evolution* **3**: 217–223.
- Ribera I. 2008.** Habitat constraints and the generation of diversity in freshwater macroinvertebrates. In: Lancaster J, Briers RA, eds. *Aquatic insects: challenges to populations*. Wallingford: CAB International Publishing, 289–311.
- Ribera I, Vogler AP. 2000.** Habitat type as a determinant of species range sizes: the example of lotic-lentic differences in aquatic Coleoptera. *Biological Journal of the Linnean Society* **71**: 35–52.
- Ribera I, Barraclough TG, Vogler AP. 2001.** The effect of habitat type on speciation rates and range movements in aquatic beetles: inferences from species-level phylogenies. *Molecular Ecology* **10**: 721–735.
- Ribera I, Foster GN, Vogler AP. 2003.** Does habitat use explain large scale diversity patterns in European water beetles? *Ecography* **26**: 145–152.
- Richmond EA. 1920.** Studies on the biology of aquatic Hydrophilidae. *Bulletin of the American Museum of Natural History* **42**: 1–94.
- Rodriguez G, Archangelsky M, Torres PLM. 2015.** Description of immatures of *Berosus decolor* Knisch, 1924 (Coleoptera: Hydrophilidae: Berosini), with emphasis on chaetotaxy and morphometry. *Zootaxa* **3981**: 577–591.
- Rodriguez G, Archangelsky M, Michat MC, Torres PLM. 2018.** Comparative analysis of diagnostic and phylogenetic chaetotaxic characters of *Berosus* Leach and their implications

- on the relationships of the tribe Berosini (Coleoptera: Hydrophilidae). *Zoologischer Anzeiger* **277**: 203–217.
- Sato S, Inoda T, Niitsu S, Kubota S, Goto Y, Kobayashi Y. 2017.** Asymmetric larval head and mandibles of *Hydrophilus acuminatus* (Insecta: Coleoptera, Hydrophilidae): Fine structure and embryonic development. *Arthropod Structure & Development* **46**: 824–842.
- Short AEZ. 2018.** Systematics of aquatic beetles: current state and future directions (Coleoptera). *Systematic Entomology* **43**: 1–18.
- Short AEZ, Fikáček M. 2011.** World catalogue of the Hydrophiloidea (Coleoptera): additions and corrections II (2006–2010). *Acta Entomologica Musei Nationalis Pragae* **51**: 83–122.
- Short AEZ, Fikáček M. 2013.** Molecular phylogeny, evolution and classification of the Hydrophilidae (Coleoptera). *Systematic Entomology* **38**: 23–752.
- Skale A, Jäch MA. 2003.** A new species of *Eumetopus* Balfour-Browne, 1949 from Nepal (Insecta: Coleoptera: Epimetopidae). In: Hartmann M, Baumbach H, eds. *Biodiversität und Naturlandschaft im Himalaya*. Erfurt: Verein der Freunde & Förderer des Naturkundemuseums, 195–196.
- Sowig P, Himmelsbach R, Himmelsbach W. 1997.** Predator-prey relationship between insect larvae: growth of *Sphaeridium* larvae (Coleoptera: Hydrophilidae) under time constraints through predation on *Musca autumnalis* maggots (Diptera: Muscidae). *Canadian Journal of Zoology* **75**: 2069–2076.
- Striganova BR. 1967.** Morphological adaptations of the head and mandibles of some coleopterous larvae burrowing solid substrates (Coleoptera). *Beiträge zur Entomologie* **17**: 639–649.
- Thorpe WH, Crisp DJ. 1949.** Studies on plastron respiration: IV. Plastron respiration in the Coleoptera. *Journal of Experimental Biology* **26**: 219–260.
- Toussaint EFA, Short AEZ. 2018.** Transoceanic stepping-stones between Cretaceous waterfalls? The enigmatic biogeography of pantropical *Oocyclus* cascade beetles. *Molecular Phylogenetics and Evolution* **127**: 416–428.
- Van Tassell ER. 1966.** *Taxonomy and biology of the subfamily Berosinae of North and Central America and the West Indies (Coleoptera: Hydrophilidae)*. Unpublished D. Phil. Thesis, The Catholic University of America, Washington.
- Vogler AP, Ribera I. 2003.** Evolutionary analysis of species richness patterns in aquatic beetles: why macroecology needs a historical perspective. In: Blackburn TM, Gaston KJ, eds. *Macroecology: concepts and consequences*. Oxford: Blackwell Science, 17–30.
- Wilson CB. 1923.** Water beetles in relation to pondfish culture with life-histories of those found in fishponds at Fairport, Iowa. *Bulletin of the Bureau of Fisheries* **39**: 231–345.
- Wootton RJ. 1988.** The historical ecology of aquatic insects: an overview. *Palaeogeography Palaeoclimatology and Palaeoecology* **62**: 477–492.
- Yee DA, Kehl S. 2015.** Order Coleoptera. In: Thorp JH, Rogers DC, eds. *Thorp and Covich's freshwater invertebrates, 4th edn*. Boston: Academic Press, 1003–1042.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Table S1.** Publications from which we adopted data on larval morphology for hydrophiloid species mentioned but not examined in this study.

**Figure S1.** General head morphology of larvae with chewing and piercing-sucking feeding system, SEM micrograph, dorsal view. A, *Tropisternus acaragua* Bachmann, 1969, first-instar larva. B, *Hemiosus multimaculatus* (Jensen-Haarup, 1910), third-instar larva. Abbreviations: EpLb, epistomal lobe; LA, labium; MN, mandible; NS, nasale.

**Figure S2.** Labroclypeal region of *Berosus* larvae. A–F, *Berosus adustus* Knisch, 1922, first-instar larva, SEM micrograph, dorsal view: A, labroclypeus; B, gFR2 setae; C, right epistomal lobe; D, left epistomal lobe; E, detail of gFR2 setae; F, latero-ventral membranous lobe. G, *Berosus pallipes* Brullé, 1841, left epistomal lobe, third-instar larva, light microscope photograph, dorsal view. Abbreviations: EpLb, epistomal lobe; NS, nasale. Colours: light blue, frontoclypeal region; green, gFR1, group of sensilla of nasale; violet, gFR2, group of sensilla of epistomal lobe.

**Figure S3.** Labroclypeal region of *Oocyclus* larvae. A–D, *Oocyclus iguazu* (Oliva 1996) third-instar larva, SEM micrograph: A, labroclypeus, dorsal view; B, left epistomal lobe, dorsal view; C, left epistomal lobe, ventral view; D, seta-like cuticular projections of the latero-ventral membranous lobe, ventral view. Abbreviations: EpLb, epistomal lobe; NS, nasale. Colours: light blue, frontoclypeal region; green, gFR1, group of sensilla of nasale; violet, gFR2, group of sensilla of epistomal lobe.

**Videos S1, S2, S3, S4:** available in:

<https://doi.org/10.5281/zenodo.3988364>

**Video S1.** Typical feeding behaviour of chewing larvae. *Tropisternus latus* Brullé, 1837 first-instar larva. Note that feeding occurs above water surface.

**Video S2.** Alternative chewing feeding strategy of moluscivorous larvae. *Hydrophilus (Dibolocelus) palpalis* Brullé, 1837 second-instar larva feeding.

**Video S3.** Piercing-sucking feeding behaviour of *Hemiosus dejeanii* (Solier, 1849) third-instar larva. Note that feeding occurs under water surface.

**Video S4.** Piercing-sucking feeding behaviour of *Oocyclus magnifica* Hebauer & Wang, 1998. Note that feeding occurs inside water film.