Morphology and biology of the flower-visiting water scavenger beetle genus *Rygmodus* (Coleoptera: Hydrophilidae)

Yûsuke N. MINOSHIMA[1], Matthias SEIDEL[2,3], Jamie R. WOOD[4], Richard A. B. LESCHEN[5], Nicole L. GUNTER[6] and Martin FIKÁČEK[2,3]

[1]Natural History Division, Kitakyushu Museum of Natural History and Human History, Kitakyushu-shi, Japan, [2]Department of Zoology, Faculty of Science, Charles University in Prague, Praha, Czech Republic, [3]Department of Entomology, National Museum, Praha, Czech Republic, [4] Long-term Ecology Lab, Manaaki Whenua, Lincoln, New Zealand, [5] New Zealand Arthropod Collection, Manaaki Whenua, Auckland, New Zealand and [6] Department of Invertebrate Zoology, Cleveland Museum of Natural History, Cleveland, U.S.A.

Correspondence: Yûsuke N. Minoshima, Natural History Division, Kitakyushu Museum of Natural History and Human History, 2-4-1 Higashida, Yahatahigashi-ku, Kitakyushu-shi, 805-0071 Japan.
Email: minoshima@kmnh.jp
Martin Fikáček, Department of Entomology, National Museum, Cirkusová 1740, CZ-193 00 Praha 9 - Horní Počernice, Czech Republic.
Email: mfikacek@gmail.com

Abstract

Hydrophilidae (water scavenger beetles) is well known as an aquatic beetle family; however, it contains ca. 1,000 secondarily terrestrial species derived from aquatic ancestors. The New Zealand endemic genus *Rygmodus* White is a member of the hydrophilid subfamily Cylominae, which is the early-diverging taxon of the largest terrestrial lineage (Cylominae + Sphaeridiinae) within the Hydrophilidae. In this paper we demonstrate that *Rygmodus* beetles are pollen-feeding flower visitors as adults, but aquatic predators as larvae. Based on analyses of gut contents and a summary of collecting records reported on museum specimen labels, adult *Rygmodus* beetles are generalists feeding on pollen of at least 13 plant families. *Rygmodus* adult mouthparts differ from those of other (saprophagous) hydrophilid beetles in having the simple scoop-like apex and mola with roughly denticulate surface, resembling the morphology found in pollen-feeding staphylinid beetles. Larvae were found along the sides of streams, under stones and in algal mats and water-soaked moss; one collected larval specimens was identified using DNA barcoding of two molecular markers, mitochondrial cytochrome oxidase 1 (*cox1*) and nuclear histone 3 (H3). Larvae of two species, *Rygmodus modestus* and *Rygmodus* sp., are described in detail and illustrated; they closely resemble ambush-type predatory larvae of the hydrophilid tribe Hydrophilini in the head morphology. *Rygmodus* is the only known hydrophilid beetle with adults and larvae inhabiting different environments.

Key words: Cylominae, ecological divergence, gut contents, immature stages, larval morphology, mouthparts, pollen-feeding.

INTRODUCTION

Larval stages of holometabolous insects frequently exhibit very different life styles from that of the conspecific adults, both in habitat and in food preferences. This ability to occupy multiple niches during the life of a single specimen, or 'ecological divergence', is often considered as one of the reasons for increased diversification rates of Holometabola and for its immense species diversity (Yang 2001; Mayhew 2007; Rainford *et al.* 2014; but see Condamine *et al.* 2016). Numerous exceptions to the above pattern may be found, and in reality, the degree of 'ecological divergence' varies within particular groups of hemimetabolous and holometabolous insects. For example, in many groups of hemimetabolous stoneflies (Plecoptera), larvae are aquatic and predatory or detritivorous, whereas terrestrial adults feed on lichens, fungal spores, pollen or arthropod corpses (e.g. Fenoglio & Tierno de Figueroa 2003; Rúa & Tierno de Figueroa 2013). In contrast, larvae and adults of aquatic beetles often co-occur in the same habitat and feed on the same or very similar food source. For example, Dytiscidae with predatory adults and larvae, Elmidae with adults and larvae often living alongside on the same substrate, feeding on algae and detritus scraped from surface, or on microorganisms from decaying wood (Brown 1987; Balke 2005; Kodada & Jäch 2005).

Hydrophilidae (water scavenger beetles) is well known as an aquatic beetle family; however, one-third of its members are secondarily terrestrial species derived from aquatic ancestors (Bernhard *et al.* 2006; Short & Fikáček 2013). Although habitat transitions have occurred independently in multiple lineages across Hydrophilidae, the majority of the terrestrial species are assigned to a single clade comprised of the subfamilies Cylominae and Sphaeridiinae (>1,000 species), which includes ca. 100 aquatic species. (Short & Fikáček 2011, 2013; Fikáček *et al.* 2013; Minoshima *et al.* 2015; Girón & Short 2017). Hunt *et al.* (2007) reported that habitat shift from terrestrial to aquatic environments would have occurred at least ten times in the evolutionary history of Coleoptera. However, the reverse shifts from aquatic to terrestrial habitats are mostly known in the Hydrophiloidea (e.g. Toussaint *et al.* 2016; Ruta *et al.* 2017). Adults and larvae of the Hydrophilidae almost co-occur in the same (micro)habitat regardless of aquatic or terrestrial (leaf litter, mammal excrements or ant nests) environments (e.g. Fikáček *et al.* 2013, 2014, 2015; Minoshima *et al.* 2013, 2015; Clarkson *et*

al. 2014; Arriaga-Varela *et al.* 2017). This allows relatively easy identification of conspecific adults and larvae by DNA barcoding on a limited number of candidate species (e.g. Fikáček *et al.* 2013, 2015; Minoshima *et al.* 2013, 2015). On the other hand, if the ontogenetic habitat shift has evolved, accompanied morphological adaptations (i.e. convergence) to respective habitats should make it difficult to infer phylogenetic relationships morphologically and therefore to determine the conspecificity between larvae and adults (Archangelsky 1999; Bloom *et al.* 2014). In contrast to aforementioned Dytiscidae and Elmidae, food preferences differ between adults and larvae in Hydrophilidae: larvae are always predatory, feeding on various invertebrates, but adults are generally detritivorous, feeding on decaying organic matter, although precise food preferences are unknown for most taxa in either adult or larval stage (e.g. Archangelsky 1997).

Rygmodus White, 1846 is a New Zealand endemic genus of the hydrophilid subfamily Cylominae. This small subfamily is restricted in Australia, New Zealand, southern South America and South Africa, and is sister to the species-rich, largely terrestrial subfamily Sphaeridiinae (Short & Fikáček 2013; Minoshima *et al.* 2015; Seidel *et al.* 2016). Adults of *Rygmodus* have been reported to visit flowers (Thomson 1881; Broun 1886; Heine 1937; Primack 1983), which is an unusual life style in the family Hydrophilidae. No further information about the biology and life cycles of the genus is available, and adult food preferences and the habitat of their larvae remain unknown. Despite extensive collecting in New Zealand, including expeditions that targeted terrestrial water scavenger beetles, no hydrophilid larva of any kind has been found in flowers (with or without *Rygmodus* adults present). This suggests the larval habitat likely differs from that of adults in the genus.

In this study, we sequenced one larval specimen collected at side of a stream in northern South Island of New Zealand and compared its DNA barcodes with those of adult Hydrophilidae. In this way, the larva was surprisingly identified as *Rygmodus*, indicating the larvae of this flower-visiting hydrophilid beetle live in a totally different (aquatic) environment. We document some morphological and biological aspects of both adults and larvae: we investigate gut contents, mandibular morphology and host plants of *Rygmodus* adults, and describe the larval morphology based on detailed examination.

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MATERIALS AND METHODS

Adult and larval specimens examined

The majority of adults included in this study represent *Rygmodus modestus* White, 1846 collected in Te Urewera National Park in November 2012 by M. Fikáček, J. Hájek and A. Becker (see Table 1 for detailed collecting data). These specimens were used for dissection of mouthparts as well as for analyzing gut contents. In addition, we used freshly collected specimens of *R. modestus* and other *Rygmodus* species for DNA analyses. Currently, all these specimens are held in the entomological collection of National Museum, Prague (NMPC); however vouchers will be deposited in New Zealand Arthropod Collection, Auckland (NZAC) following a species-level taxonomic revision of the genus (M. Fikáček *et al*., in prep.). The host plant data listed in Table 2 were compiled from collecting records of these specimens plus all available historical specimens deposited in NZAC and the Museum of Natural History, London (BMNH; Broun and Sharp collections); host plant data were available for 27 collecting events in total.

Larval specimens described here were collected directly from aquatic habitats in 2010 (see detailed label data in respective parts of description) and preserved in ethanol. In addition, a few morphologically *Rygmodus*-like larval specimens were collected in 2012–2016, but we have failed to sequence DNA of these specimens for barcoding, and therefore they are only briefly mentioned and not described here in detail. The larval specimens used in this study are deposited in Kitakyushu Museum of Natural History and Human History, Kitakyushu, Japan (KMNH) and NMPC.

Identification of adults and larvae

The taxonomy of *Rygmodus* was reviewed by Hansen (1997), who constructed an identification key of adult *Rygmodus* based on the examination of type specimens deposited in BMNH. This key does not deal with sexual dimorphism in some body parts (shape of claws and antennal club) in some species and completely omits the male genitalia. We re-examined the type specimens deposited in BMNH, alongside the newly collected specimens, and

recognized that identification is straightforward for several species, i.e. *R. modestus*, *R. tibialis* Broun, 1893, *R. femoratus* Sharp, 1884, and *R. alienus* Broun, 1893 but that detailed revision is required for other species or species complexes such as the *R. cyaneus* Broun, 1881 species complex, *R. incertus* Broun, 1880, and *R. pedinoides* White, 1846. Therefore, the species identification for the latter group is provisional in this study and will be investigated in detail in a future study.

We identified a single larval specimen (voucher #: COL1804) by DNA barcoding based on two molecular markers: a 268 bp fragment of nuclear histone 3 (H3) and a 776 bp fragment of mitochondrial cytochrome oxidase 1 (*cox1*) (Table S2). The H3 and *cox1* sequences of the larva were compared with those from identified adult specimens of *Rygmodus* as well as of most New Zealand hydrophilid genera including all aquatic ones (Table S1). Most of the sequences were newly acquired; only one *cox1* sequence of *Rygmodus* (SLE0129) was taken from Short and Fikáček (2013). The sequence data were edited and aligned in Geneious 6.1 using MUSCLE algorithm. The aligned data were analysed using maximum likelihood with HKY+G (based on best fitting model selected by jModelTest 2.1.1; Guindon & Gascuel 2003; Darriba *et al.* 2012) in the MEGA7.0 software (Kumar *et al.* 2016); bootstrap values were calculated using 1,000 replicates in the same software.

Morphological studies of larvae and adults

For larval morphology, we largely followed the methods used by Minoshima and Hayashi (2011a). Given the limited larval specimens available for the study, our description is based on a few *R. modestus* larvae that were dissected and examined under compound light microscope. The specimens were, unfortunately, partially damaged and some characters could not be observed; we described and illustrated them where possible. To assess intra- and inter-generic variation, these larvae were briefly compared with the additional larvae of other species. The specimens were cleared using ca. 10% KOH solution, dissected and examined on H-S Slides (Shirayama *et al.* 1993) with lactic acid or glycerol. The examined larvae are preserved in 80% ethanol and stored within screw-cap vials. Observations and dissections were carried out using an Olympus SZX12 (Olympus Corp., Tokyo, Japan) and a Leica MZ16

(Leica Microsystems GmbH, Wetzlar, Germany) binocular microscopes and a Nikon E600 (Nikon Instech Co., Ltd., Tokyo, Japan) and an Olympus BX50 compound light microscopes. Illustrations were made with the aid of a drawing tube attached to the E600. Photographs were taken with an Olympus PEN Lite E-PL5 digital camera attached to the SZX12. Composite images were created using the Image Stacking Software Helicon Focus (Helicon Soft Ltd., Kharkov, Ukraine). The images were modified using Adobe Photoshop CC and Lightroom CC (Adobe Systems Inc., San Jose, USA) in needed cases.

Adult mouthparts of *R. modestus* were examined in detail. They were dissected after treating the specimen with 10% KOH solution and embedded in Euparal resin on a permanent microscopic slide following the protocol by Hanley and Ashe (2007). The mouthparts were photographed using Canon EOS1100D (Canon Inc., Tokyo, Japan) camera attached to an Olympus BX41 compound microscope; multilayer photographs were stacked using Helicon Focus software. Comparison with other cylomine genera was based on specimens dissected in the same way and deposited in NMPC. Detailed morphology of the mandible, especially the mandibular mola, was examined using an environmental SEM (Hitachi High-Technologies, Tokyo, Japan) after dissecting the mandibles from KOH-treated head and cleaning it from organic dirt by 10% hydrogen-peroxide solution. We examined the molar structure of four hydrophilid taxa for comparative purposes: *Rygmodus modestus* and *Saphydrus suffusus* Sharp, 1884 (both Cylominae, the former flower-visiting, the latter not; see Table 3), *Dactylosternum hydrophiloides* MacLeay, 1825 (Sphaeridiinae: Coelostomatini, terrestrial species feeding on decaying plant material) and *Helochares* (*Hydrobaticus*) sp. (Acidocerinae, saprophagous aquatic species).

The terminology of larval morphology follows Archangelsky (1997) and Minoshima *et al.* (2013). For the primary chaetotaxy of the larval head we refer to Fikáček *et al.* (2008) and Byttebier and Torres (2009). The following abbreviations are used for description: AN, antenna; FR, frontale; gAN, group of antennal sensilla; gAPP, group of sensilla on inner appendage of maxilla; gFR, group of sensilla on frontale; gLA, group of sensilla on labium; gMX, group of sensilla on maxilla; LA, labium; MN, mandible; MX, maxilla; PA, parietale; SE, sensorium. The terminology of adult mouthparts follows Lawrence and Ślipiński (2013) as adopted for Hydrophilidae by Fikáček *et al.* (2014). Classification follows Short and

Fikáček (2013) for Hydrophilidae, and Seidel *et al.* (2016) for Cylominae. Plant classification follows Stevens (2001 onwards) (see also Glenny *et al.* 2012 and Angiosperm Phylogeny Group 2016).

Analysis of gut contents

In order to test whether *Rygmodus* adults feed on tissues or pollen of flowers they are visiting, we examined mid-gut contents of 15 specimens of at least four *Rygmodus* species collected from nine host plants. All the examined specimens were collected from flowers directly and stored in 96% ethanol until dissection. Each specimen was dissected as follows: the abdomen was removed, and then the middle to posterior part of mid-gut and the hind-gut were dissected; but the mid-gut anterior part reaching into the thorax was not dissected in order to keep the remaining body parts of voucher specimen intact. One specimen was used to test for presence of pollen using safranine: the mid gut content was dissected on a slide with 0.25% safranine solution in glycerine (Jones 2012) and examined at 100–200× magnification of an Olympus BX41 compound microscopes. Remaining samples were sent to Landcare Research, Lincoln, New Zealand where contents of intestines were extracted and processed by washing in hot KOH for 10 min, followed by a wash in HCl and acetolysis. Pollen grains were concentrated from the acetylised material using flotation with lithium polytungstate (specific gravity 2.2), and stained with fuchsin-red and mounted on glass microscope slides in glycerol jelly. A minimum of 200 pollen grains were identified on each slide except where this number were not preserved (then all specimens on a slide were identified); samples were also checked for rare pollen grains which were indicated as <1 in Table 1. The pollen grains were identified to the family, genus or species level, based on a reference collection of pollen grains held at Landcare Research, Lincoln and using pollen and spore identification keys (Large & Braggins 1991; Moar 1993).

RESULTS

DNA barcoding

The molecular phylogenetic tree resulting from the H3 sequences has revealed that all the examined *Rygmodus* adult specimens form a strongly supported (bootstrap: 99%) clade, and that the focal larval specimen (COL1804) belongs to this clade (Fig. 1A). Furthermore, the *cox1* tree (Fig. 1B) has revealed that *R. modestus* is sister to the *R. cyaneus* complex (bootstrap: 100%), and that the larval specimen is nested inside of the *R. modestus* clade with a strong support (bootstrap: 98%). Hence, the larva was reliably identified as *R. modestus*.

The topology outside of the *R. modestus* + *R. cyaneus* clade was incongruent between the H3 and *cox1* trees. The topology of the *cox1* tree seems congruent to the differentiation in the genital morphology among *Rygmodus* species: *R. alienus*, which is a member of the *R. alienus - R. antennatus* (Sharp, 1884) species complex characterized by the wide and short median lobe of aedeagus, was placed as the early-diverging taxon sister to the other sampled species, which are all characterized by the rather uniform genital morphology with the narrow and elongate median lobe of aedeagus. On the other hand, the H3 tree supported the close relationships of *R. femoratus* and *R. tibialis*, which are extremely similar in morphology.

Biology of *Rygmodus*

Adults

Rygmodus modestus adults (Fig. 2D) examined in detail for this study were collected in the middle of November 2012 on flowering bushes of *Olearia* in full sun at a picnic area along Aniwaniwa Road in the *Nothofagus* forest at the banks of the Aniwaniwa Stream, Te Urewera National Park, North Island. Several hundred specimens were found crawling on the blossoms on a single *Olearia* bush (Fig. 2A–C) and smaller numbers were collected by beating flowering bushes of *Brachyglottis repanda* in the same area (e.g. Fig. 3F). In addition to *Olearia* and *Brachyglottis* tree daisies (Asteraceae), adults of various species of *Rygmodus* were collected from flowers of a wide spectrum of white-flowering native bushes across New Zealand, in particular those which have flowers accumulated in apparent inflorescences. Two additional host plants not recorded in our examined material were reported for *R. modestus* by Heine (1937): *Rubus australis* (Rosaceae) and *Euphrasia cuneata* (Orobanchaceae). A summary of all known host plants is provided in Table 2.

Two of the 15 specimens analyzed had the mid-gut empty. The gut contents of the remaining 13 specimens consisted almost exclusively of pollen grains (e.g. Fig. 2E–J), with a very small amount of fine unidentified particles; larger non-pollen particles were very rare (unidentified parts of plant tissue and fungal sporangia; Fig. 2K–L). In all the specimens, one pollen type formed the majority of the mid-gut contents (in four specimens only one pollen type was found), and one to five additional pollen types were found in much smaller amounts. In ten specimens, the main pollen type corresponded to the flower from which the specimen was collected. Three specimens of *R. modestus* collected together from flowering *Brachyglottis repanda* showed very different contents of mid-gut (rows 1–3 of Table 1); in two of them, the main pollen type did not correspond to the host plant from which they were collected. In total, the mid-guts of examined specimens contained pollen grains representing 14 plant families (Podocarpaceae, Apiaceae, Asteraceae, Asparagaceae, Campanulaceae, Cunoniaceae, Elaeocarpaceae, Ericaceae, Haloragaceae, Malvaceae, Myrtaceae, Plantaginaceae, Poaceae and Rubiaceae). Ten pollen types were identified to the genus level (*Dacrydium, Prumnopitys, Aciphylla, Aristotelia*, *Cordyline, Coprosma*, *Hebe, Hoheria*, *Leptospermum* and *Pratia*).

Pollen grains found in mid-guts of the dissected specimens largely agree with known plants on which museum specimens were collected (compare Tables 1 and 2: pollen grains of all known host plants except *Ranunculus* and *Pittosporum* were found). However, additional pollen types were recorded from the mid-guts: some of them (*Aristotelia*, *Coprosma*, and *Pratia*) were subdominant or even dominant in mid-guts of a few dissected specimens and likely resulted from previous feeding of the respective specimens. Other pollen types were rare and may be remnants from previous feeding (especially for species with apparent inflorescences, such as *Prumnopitys* and *Weinmannia*) or contaminants brought to host plant flowers by other insects or by wind (e.g. Haloragaceae, grasses and podocarps).

Larvae

Larvae of *Rygmodus* were first found in 2010 at two localities in the Marlborough region, northern South Island: from moss growing on wet rocks beside the small waterfall in the Pelorus Bridge Scenic Reserve (Fig. 3E) and algal mats at sites of the Dead Horse Creek at

Wakamarina Road south of Canvastown (Fig. 3G–H). The larvae collected in Dead Horse Creek were of a single morphotype. One (COL1804) of them was used for the species identification by DNA barcoding and identified as *R. modestus* (see above). These larvae were found in algal mats growing on exposed rocks with a thin film of water flowing over them, where they co-occurred with larvae of *Cylomissus glabratus* Broun, 1903 (see Minoshima *et al.* 2015 for details). In Pelorus Bridge, larvae of two morphotypes were found syntopically in the moss soaked by a thin film of water flowing through it. One type was morphologically identified as *R. modestus*, and the other as an unidentified *Rygmodus* species. The moss was inspected manually during the day and at night. At night, a single *Rygmodus* adult was found sitting on the moss in the waterfall; based on the abundance of *R. modestus* larvae at the same site, we suppose that it might have been a *R. modestus* female laying eggs there. During the later field work (performed between 2012–2016 by M. Fikáček, M. Seidel, R. Leschen and M. Gimmel), a few larval specimens were found at several occasions: in wet moss at side and below small waterfalls (Fig. 3F) of the Aniwaniwa Stream just below Aniwaniwa Falls, Te Urewera National Park, North Island, where numerous adults of *R. modestus* were collected as mentioned above, syntopically with adults and larvae of *Cylomissus glabratus*, in wet moss at side a tiny streamlet at Three Mile Pack Track south of Okarito, West Coast, South Island (no adults found in this area), and in water under stones at the side of a small stream in the alpine tussock area at Arthur's Pass, West Coast/Canterbury, South Island (Fig. 3I,J; adults of the *R. cyaneus* species group collected in the same area). In February 2016, three larval specimens of *Rygmodus* were sifted from leaf litter collected from two localities, Picnic Gully at the mouth of Taieri river south of Dunedin and along Matai Falls track in the Catlins area, in the very south of South Island. This area was very dry, without standing or flowing water, at the time of collection, and large amounts of leaf litter accumulated in depressions and along drains. Hence, it is likely that the sifted leaf litter was originally accumulated as flood debris beside streamlets flowing earlier in the season. No adults were collected in February 2016 from this area, but we suppose that the larvae may be of *R. opimus* Broun, 1880 which is the only *Rygmodus* species recorded so far from this area (M. Fikáček, unpubl. data).

Nothing is known about the length of larval development or the duration of adult stage. Our observations and label data from museum specimens suggest that adults appear mostly in spring and early summer (November–December) when the above-mentioned plant species bloom. In 2016 two adults were collected as late as at the end of January on *Hebe* bushes ending its bloom in the subalpine area below Borland Saddle, Fiordland, South Island.

Adult mouthparts of *Rygmodus*

Morphology of mouthparts of *R. modestus* (Figs 4A–G, S1) corresponds well with the general morphology of mouthparts of Hydrophilidae (Archangelsky *et al.* 2005; Fikáček & Vondráček 2014) and Cylominae (see Fikáček *et al.* 2014 for mouthparts of *Andotypus* Spangler, 1979 and *Austrotypus* Fikáček *et al.*, 2014). The following differences from other cylomine genera were found:

Labrum (Fig. S1B) moderately sclerotized, concealed under clypeus; transverse, ca. 2.6× wider than long, deeply concave on the anterior margin. Anterior margin with series of short setae mesally and very long setae on anterolateral corners; anterior third of dorsal portion bearing sparsely arranged very long setae.

Mandibles (Figs 4A–G, S1A) slightly asymmetrical, narrowly falcate in apical four fifths; mandibular angle very obsolete; apex simple, spoon-like. Inner face with fine long pubescence starting at apical fifth and reaching mola, mesal portion distally of mola membranous, finely pubescent. Mola large, hammer-shaped, strongly sclerotized on inner surface, asymmetrical; inner surface of both mandibles with strong denticles which are more massive on the left mandible, and finer (partly maybe abraded) in the right mandible.

Maxilla (Fig. S1D). Lacinia with sparse irregularly arranged long setae, sickle-shaped on distal parts; inner finger-like projection absent. Galea with a narrow basal sclerite extending over the inner surface; distal and outer surface membranous, bearing long irregularly arranged setae.

Labium (Fig. S1C). Mentum subquadrate, ca. 1.7× wider than long, lateral margins subparallel, with sparse setation, anterior margin bisinuate. Palpiger moderately sclerotized, rather narrow, partly concealed by mentum. Palpus with 3 palpomeres; basal palpomere minute, palpomeres II and III subequal in length, palpomere II with numerous fine sparsely

arranged setae in distal portion. Prementum in form of two strongly sclerotized lobes reaching basal part of palpomere II, bearing very long setae along inner face. Hypostome projecting into a crescent-like lobe on each side below the premental lobe reaching distal part of palpomere II, each lobe bearing dense series on inner face.

Description of larval morphology

Rygmodus modestus **White, 1846**

(Figs 3A–C, 5–7)

Material examined

1 L2 (KMNH), Dead Horse Cr, south of Canvastown, Marlborough, South Island, New Zealand, 41°19.599′S, 173°39.579′E, wet stones with algae and moss along a stream (scraped slime on rock), 30.xi.2010, M. Fikáček & R. Leschen leg, RL1513.

Description of second-instar larva

Body (Fig. 3A–C) slender, widest between abdominal segments III–V.

Color (Fig. 3A–C). Head capsule reddish brown with yellowish anterolateral part and posteromedian part; appendages reddish brown to yellowish. Thorax yellowish brown, sclerites darker. Abdomen yellowish brown; dorsal surface bicolored, with a regular pattern of dark areas on segments I–VII; ventral surface uncolored; sclerite of spiracular atrium brown.

Head. Head capsule (Fig. 7A) subtrapezoidal, slightly widened anteriorly. Cervical sclerite large, subquadrate. Frontal lines lyriform, coronal line short (Figs 5A, 7A). Surface of head capsule with minute microstructures distributed in posterior part of dorsal to lateroventral surface of parietale. Six stemmata on each anterolateral portion of head capsule. Posterior tentorial pits present on median part close to submental sulcus. Clypeolabrum almost symmetrical (Fig. 5C). Nasale with five large teeth; three median teeth smaller and more aggregated, both lateral teeth larger than median ones and more separated from them; all teeth subtriangular in shape. Nasale projecting slightly further than epistomal lobes. Lateral lobes of epistome present, almost symmetrical. Left lobe projecting anteriorly, rounded with widely obliquely truncate apex; right lobe similar to left lobe.

Chaetotaxy of head capsule. *Frontale* (Fig. 5A, C). Central part with three pairs of sensilla (FR1–3) slightly divergent posteriad; FR1 rather short seta; FR2 pore-like, situated

more anteriorly and more mesally to FR1; FR3 short seta, close and anterior to FR2. Irregularly arranged longitudinal row of short setae present along frontal line. Pore-like sensillum FR4 and setae FR5–6 located posteromesally to antennal socket; FR5 stout, rather short seta, posteromesally to FR6, FR6 rather long seta, laterally to FR4; FR4 mesally to FR5–6. FR7 rather short seta, situated on inner face of antennal socket. Setae FR9–10 closely aggregated mesally to antennal socket; FR9 rather long, FR10 rather short. Pore-like sensillum FR15 and seta FR8 situated mesally on clypeolabrum, behind nasale; FR15 anteriorly to FR8, distance between left and right FR15 subequal to distance between left and right FR8. Sensilla FR11–14 situated on epistome, anteromesally to antennal socket; FR11, 13–14 pore-like, FR12 short seta; FR11–13 forming triangular group situated on inner part of epistome, FR12 posteriorly to FR11 and FR13, FR11 mesally to FR12–13. FR14 located close to antennal socket, posterolaterally to FR11-13. Nasale with a group of six stout and short setae (gFR1) and two minute ventral setae laterally to median tooth of nasale. Epistomal lobe with nine setae on anterior margin; seven lateral ones moderately long, bearing subapical tooth; inner two short.

Parietale (Fig. 5A–B). Dorsal surface with a group of five sensilla (PA1–5) forming slightly irregular longitudinal row in posterior part; PA1–2 and 4–5 short setae, PA3 pore-like. PA6 pore-like, located posteromesally close to coronal line. Densely arranged short setae present along frontal lines and anterior half of dorsal and lateral face of parietale. Long setae PA7 and PA13 and short seta PA12 situated on median part of dorsal surface of parietale, PA7 and PA12 closer than PA12 and PA13; PA7 mesally to PA12–13, PA12 between PA7 and PA13. PA14–17 located in anterior third of lateral face of parietale, forming transverse row; PA14 and 16 long setae, PA15 and 17 pore-like, arranged in following order (from dorsal to ventral ones): PA14, 15, 16, 17. Pore-like sensilla PA10 and short seta PA11 located between anterior and posterior rows of stemmata; PA10 mesally to PA11. PA8–9 and PA19–22 on anterior corner of head capsule; PA8–9 and PA21 very long setae, PA20 and PA22 long setae, PA19 pore-like; PA8 situated posteriorly to lateral margin of antennal socket; PA9 laterally to antennal socket; PA19 situated between PA9 and PA20; PA20 and PA21 closely aggregated, PA20 situated dorsally to PA21; PA22 close and laterally to outer margin of ventral mandibular articulation. Pore-like sensilla PA23–25 on ventral mandibular

articulation; PA23 on outer margin; PA24–25 on inner part, closely aggregated. Sensillum PA18 present posteriorly to PA16–17, pore-like (but see Remarks). Pore-like sensillum PA30 posteromesally to PA18. PA26–28 aggregated, located on median part of anterior third of ventral surface of parietale, mesally to PA17 and PA18; PA26 possibly seta (seta missing, but socket of the same shape as PA28), anteriorly to PA27–28; PA27 pore-like, between PA26 and PA27; PA28 long seta. PA29 pore-like, situated posteromesally to PA28 and laterally to PA30, close to gular sulcus.

Antenna (Fig. 6A) 3-segmented, slender; surface of antenna smooth but a few very minute cuticular projections present on basal margin of dorsal surface of antennomere II. Antennomere I slightly longer than antennomeres II and III combined, antennomere I widest, antennomere III the shortest and narrowest. Approximate ratios of length of antennomeres I : II : III as follows: 1 : 0.6 : 0.2 (n = 1). Antennal sensorium present.

Chaetotaxy of antenna (Fig. 6A). Antennomere I with five pore-like sensilla (AN1–5) and 12 short setae sparsely distributed on dorsal surface. AN1 situated dorsolaterally on median part of inner surface; AN2 dorsally on distal fourth; AN3–5 subapical, AN3 on lateral face, AN4 on inner face, AN5 on inner part of ventral face. Antennomere II with one pore-like sensillum (AN6) situated dorsally on subapical part of sclerite. Setae AN7–8 and AN10–11 and sensorium (SE1) on intersegmental membrane between antennomeres II and III, AN9 absent; AN7–8 situated posteriorly and close to SE1, AN7 short, AN8 minute; AN10–11 on lateral face, AN10 very long, AN11 minute. Sensorium SE1 slender, as long as antennomere III, situated on outer face. Antennomere III with group of apical sensilla (gAN) in apical membranous area.

Mandibles (Fig. 6B) slender, almost symmetrical, right mandible slightly longer than left one. Each mandible with two inner teeth present on median part of inner face; apical inner tooth larger than proximal one. Incisor area of left mandible roughly serrated, that of right one serrated in basal part only.

Chaetotaxy of mandibles (Fig. 6B). Three pore-like sensilla (MN2–4) on median part; MN4 situated on dorsolateral face, anterolaterally to MN2–3, anteriorly to MN1; MN2 laterally to MN3; MN3 situated at base of apical inner tooth. Moderately long seta MN1 placed on lateral face, behind MN4; MN5 hard to distinguish, probably a minute seta situated

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lateral face of subapical part. Outer face of mandibles bearing numerous of short setae, except for apical part. MN6 undetectable.

Maxilla (Fig. 6C–D) slender, 6-segmented, longer than antenna. Cardo moderate in size, irregularly shaped. Stipes the longest, ca. 1.75 times as long as palpomeres I–IV combined; inner face smooth except of few hair-like cuticular projections basally, between MX7 and MX8. Maxillary palpus short, 4-segmented. Palpomere I widest, incompletely cylindrically sclerotized dorsally. Inner process sclerotized. Palpomere II short, wider than palpomeres III and IV; palpomere III longest, wider than palpomere IV; palpomere IV rather short and narrowest. Approximate ratios of length of palpomeres I to IV as follows: 1.0 : 0.55 : 1.74 : 1.0 (n = 1).

Chaetotaxy of maxilla (Fig. 6C–D). Cardo with one ventral seta (MX1). Inner face of stipes with a row of five stout setae (MX7–11), and seven short setae; MX7 at base, MX8–9 situated on subbasal part, MX8 behind MX9, MX10 ca. at midlength, MX11 anteriorly to MX10. Pore-like sensilla MX2–3 situated ventrally on ca. posterior two fifths; MX2 on lateral part, posterolaterally to MX3. Pore-like sensilla MX4 and setae MX5–6 situated subapically on lateral face. Lateral half of ventral surface to lateral surface bearing numerous setae; lateral face with several long setae, remaining ones short to rather short. Dorsal surface of palpomere I with one rather short, stout seta (MX16) situated basally on inner face. Three sensilla (MX12–14) located laterally on distal part of sclerite (Fig. 3D); MX12 dorsally to MX13–14, MX13 between MX14 and MX12. Pore-like sensilla MX15 and MX17 situated on membrane behind inner appendage, MX17 dorsally, MX15 ventrally. Inner appendage with one very long and a few short setae apically (gAPP). Palpomere II with two pore-like sensilla (MX18–19) and one minute seta (MX27); MX18 situated laterally on anterior margin of sclerite; MX27 behind MX18, laterally at basal margin of sclerite; MX19 on inner face of intersegmental membrane between palpomeres II and III. Palpomere III with two pore-like sensilla (MX20 and MX22), and long (MX21) and short (MX23) setae. MX22 located ventrally on subapical part of sclerite; MX20–21 and MX23 distally on borderline between sclerite and intersegmental membrane; MX21 on inner face, MX23 lateroventrally, MX20 laterodorsally. Palpomere IV with one rather long seta (MX24) situated at midlength of inner surface, and with digitiform (MX25) and pore-like (MX26) sensilla apically on outer face of

sclerite; MX25 dorsally, MX26 ventrally. Apical membranous area of palpomere IV with several minute setae (gMX).

Labium (Fig. 6E) developed. Submentum fused to head capsule, transverse; submental sulcus indistinct. Mentum trapezoid, widest at base. Dorsal and lateral surface densely covered with small cuticular teeth, dorsal surface with a pair of bare areas submedially. Prementum subquadrate, elongate, basal part slightly wider than apical part, without cuticular teeth; ca. 2.2 times longer than wide. Ligula slender, partly sclerotized medially, ca. as long as labial palpus. Labial palpus moderately long, palpomere I slightly wider than palpomere II; palpomere II long, parallel-sided, distinctly longer than palpomere I; intersegmental membrane between palpomeres I and II bearing a few short hair-like cuticular projections dorsally.

Chaetotaxy of labium (Fig. 6E). Submentum with two pairs of setae (LA1−2); LA1 very long on lateral margin, LA2 short on anterolateral corner. Mentum with a group of numerous rather short spiniform setae on dorsal to lateral surface of anterior corners; ventral face of anterior corners bearing a few short setae. Ventral surface of mentum with one pair of long setae (LA3) and pore-like sensilla (LA4) on anterior part; LA3 behind LA4, LA4 subapically on anterolateral corner. Prementum and its anterior membranous area with five pairs of sensilla (LA5−9), and a few short setae on lateral face of prementum. LA5−7 situated lateroventral surface of prementum, minute seta LA5 at base, long seta LA6 at midlength; pore-like sensillum LA7 apically on borderline between sclerite and membrane; LA8 subbasally on mesal part of dorsal surface. Sensillum LA9 situated laterally on anterior membranous area. Ligula with one pair of rather long setae (LA10) and two pairs of pore-like sensilla (LA11−12); LA10 situated on basal margin of sclerite; LA12 at apex, LA11 ventrally on subbasal part. Palpomere I with two sensilla (LA13−14); LA13 minute seta, situated ventrally at base; LA14 pore-like, dorsally on intersegmental membrane between palpomeres I and II. Palpomere II with one pore-like sensillum LA15 situated apically on outer face of sclerite; several minute setae of variable shape (gLA) on apical membranous area.

Thorax. Membranous parts very densely covered with hair-like cuticular projections. Prothorax wider than head capsule (Fig. 3A). Proscutum formed by one large plate subdivided by fine sagittal line, anterior and posterior margins weakly sclerotized; proscutal plate

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covered with several very long setae, and densely distributed rather short setae and fine cuticular projections. Prosternal sclerite (Fig. 7D) incompletely subdivided by fine sagittal line at base; bearing numerous short setae on lateral part of anterior margins and along sagittal line. Mesonotum with three pairs of dorsal sclerites (Fig. 7B); two pairs on anterior margin, median pair transverse, attached mesally, lateral pair small; one large pair behind anterior pairs, subpentagonal, attached mesally, subdivided by transverse ridge anteriorly, with transverse shallow groove medially, covered with short setae and densely arranged fine cuticular projections in posterior half. One pair of tubercles anteriorly on lateral face. Mesothoracic spiracles projecting laterally, forming a small finger-like projection. Metanotum with four pairs of dorsal sclerites (Fig. 7C); two pairs on anterior margin, median pair transverse, attached mesally, lateral pair narrow, hardly visible; one large pair behind anterior two pairs, transverse, subquadrate, attached mesally, subdivided by transverse ridge, with transverse shallow groove, covered with short setae and densely arranged fine cuticular projections in posterior part; last pair behind large sclerite, transverse, mostly covered with short setae and densely arranged fine cuticular projections. Legs (Fig. 7E–F) rather short and slender, 5-segmented; all three pairs similar in shape.

Abdomen. Abdomen (Fig. 3A–C) 10-segmented, widest at segments III and IV, then tapering posteriad; membranous parts covered with densely arranged hair-like cuticular projections. Segment I with two pairs of dorsal sclerites; anterior pair smaller than posterior pair, these two pairs closely aggregated, and may thus appear as one large semicircular sclerite subdivided by fine sagittal and transverse lines. One pair of spiracles on lateral part of dorsal surface, weakly tuberculate. Segments II–VII similar to segment I, but dorsal sclerites absent. Spiracular atrium (Fig. 7G): Segment VIII bearing short to moderately long hair-like cuticular projections; dorsal plate present but margins of the plate weakly defined and hardly visible; dorsal plate may be oblong oval, bearing minute but stout spine-like cuticular projections. Procercus short, inner part sclerotized. Segment IX trilobed, partly sclerotized. Lateral lobe of spiracular atrium large, partly sclerotized, narrowing apically, bearing rather long hair-like cuticular projections; acrocercus present but borderline between lateral lobe and acrocercus indistinguishable. Median lobe of spiracular atrium large, sclerotized dorsally,

widest apically; a pair of moderately long projections present between median and lateral lobes.

Remarks

We examined only one second-instar larva in detail. Although many additional/secondary sensilla were present especially on the frontale, almost all primary sensilla were detectable. A few sensilla which are problematic concerning the homology or morphology (pore-like vs. seta) are commented below:

PA18: After comparison with other cylomine larvae examined by us (*Andotypus* and *Austrotypus*: Fikáček *et al.* 2014; *Cylomissus* Broun, 1903 and *Anticura* Spangler, 1979: Minoshima *et al.* 2015), the pore marked PA18 in Fig. 5B is very likely the true PA18. Sensillum PA18 is a seta in all known hydrophilid larvae including Cylominae (e.g. Fikáček *et al.* 2008, 2014; Minoshima & Hayashi 2011b, 2012; Clarkson *et al.* 2014; Minoshima *et al.* 2015), hence it is possible that the seta has been broken before or during preparation.

PA26: We marked a pore closely aggregated with PA27–28 as PA26 (Fig. 5B), and assumed that this sensillum was originally a seta. This is based on the shape of the socket of PA26, which is very similar to that of PA28, and the fact that PA26 is a seta in *Rygmodus* sp. (Fig. S2B). PA26 and PA28 are usually setae in hydrophilid larvae (e.g. Fikáček *et al.* 2008, 2015; Minoshima & Hayashi 2011a; but see also Fikáček *et al.* 2008 and Minoshima *et al.* 2017 for arrangement of PA26–28 in Laccobiini).

MN5: Mandibular sensillum MN5 is undetectable from additional/secondary sensilla (Fig. 6B). To determine the precise position of the sensillum, examination of the first-instar larva is necessary. We treated an apical seta as MN5 for the moment.

MN6: MN6 seems to be absent (Fig. 6B), but usually this pore is hardly visible even when present (as in other cylomine larvae; Fikáček *et al.* 2014; Minoshima *et al.* 2015). The presence or absence of MN6 cannot be decided at the moment.

MX5–6: Maxillary sensilla MX5–6 are very often undetectable from closely situated secondary sensilla in second- and third-instar larvae (Minoshima & Hayashi 2011a); thus we labelled these with a question mark (Fig. 6C).

Rygmodus **sp.**

(Figs 3D, S2, S3)

We examined the following material: 1 L3 (KMNH), Pelorus Bridge, Marlborough, South Island, New Zealand, 41°30.490′S, 173°56.747′E, 29.x.2010, night collecting around waterfall splash zone, RL1509, M. Fikáček & R. Leschen leg. Description and figures are presented as Supporting Information (File S1).

DISCUSSION

Insect pollination in New Zealand

The flora of New Zealand is composed of a high diversity of plants that tend to have white flowers and thought to be pollinated by wind or little specialized insect groups such as flies, beetles, basal groups of Lepidoptera and short-tongued bees (e.g. Heine 1937; Webb & Kelly 1993; Swenson & Bremer 1997; Newstrom & Robertson 2005; Newstrom-Lloyd 2013), in agreement with that the New Zealand insect fauna is believed to have a paucity of specialized day-active pollinators. The low diversity of bees and butterflies in New Zealand led Primack (1978) to describe the pollinator fauna as 'unspecialized', which may be partly a consequence of the biogeographic history of New Zealand as a continental island (e.g. Buckley *et al.* 2015). However, Primack (1978) was especially interested in the montane fauna, and his conclusion should not be applied to the entire New Zealand fauna: though not all flower-feeding beetle lineages are present in New Zealand, there is a relatively rich fauna of galerucine beetles (Chrysomelidae), dasytine beetles (Melyridae) and weevils (Curculionidae: Curculioninae: Eugnomini) (e.g. Heine 1937; Kuschel 1990). There are also 'unusual' day-time flower visitors that are unique for New Zealand or occur only in the Australian region: species of *Rygmodus* (Hydrophilidae) treated in this paper, cryptophagid beetles (Leschen & Gimmel 2012), the erotylid beetle *Loberonotha olivascens* (Broun, 1893) (Leschen 2003), some oxypodine Aleocharinae (Leschen & Newton 2015), and the extraordinary high diversity of flower-visiting Scirtidae (Klimaszewski & Watt 1997). All these groups may be also pollen specialists and pollinators.

Pollen-feeding and pollination in *Rygmodus*

The early records of *Rygmodus* species from *Brachyglottis, Cordyline* and *Aciphylla* flowers were reported by Thomson (1881) and Broun (1886). Since then, a few faunistic studies (Heine 1937; Primack 1983) have listed *Rygmodus* species as 'pollinators', but without demonstrating that the beetles can deliver viable pollen and facilitate fertilisation. Our study is the first to demonstrate the specialized morphology of *Rygmodus* mandibles and confirm the presence of pollen in the guts of these beetles.

When *Rygmodus* mandibles are compared to those of other hydrophilid beetles, including members of the subfamily Cyclominae to which *Rygmodus* belongs, two differences are apparent: the simple (rather than bidentate) scoop-like apex of the mandible (Fig. 3B,E), and the denticulate microstructure of molae (Fig. 3B,D,F–G). Inner surfaces of molae consist of high vertical lamellae with fine comb-like edges (together composing a fine sieve-like structure) in all hydrophilids examined, i.e. aquatic *Helochares* Mulsant, 1844, a cylomine not visiting flowers (*Saphydrus* Sharp, 1884; Fig. 4H–J, Table 3) and terrestrial hydrophilids feeding on rotten plant material (*Dactylosternum* Wollaston, 1854; Fig. 4K) and excrements (*Sphaeridium* Fabricius, 1775; Holter 2004). All these hydrophilid groups likely feed on fine particles (16–19 μm in *Sphaeridium*; Holter 2004) extracted from water-soaked detritus, and the sieve may serve for drainage of superfluous fluid (Holter 2004) and/or size-selection of detritus particles. This "compression-sieve" involving complex molae is also present in other groups of beetles (e.g. scarabaeoids: Nel & Scholtz 1990; M. Seidel, pers. observ.). The molar structure of *Rygmodus* clearly differs from this pattern: it lacks the vertical lamellae and bears irregularly arranged denticles, resembling the molar structure of pollen-feeding omaliine staphylinid beetles (Betz *et al.* 2003). Compared to other spore-feeding or microphagous beetles, *Rygmodus* differs from them in having an internally scooped-shaped incisor lobe for gathering pollen and broad, transverse molae with denticulate surfaces and raised borders (compared to striate and somewhat flattened mola in many other spore-feeding groups) and utilized as a pollen press.

The spectrum of pollen grains found in the mid-gut (Table 1) and the list of flowers from which the beetles were collected (Table 2) indicate that *Rygmodus* are generalist pollen-feeders exploiting a wide range of available flowers. Feeding specialization seems habitat-

based: for example, species of the *R. cyaneus* complex mostly occur in subalpine habitats across North and South Islands of New Zealand, and are hence very frequently recorded from subalpine *Hebe* species. The pollen grains extracted from the mid-gut of *Rygmodus* are not punctured, abraded, or cracked as in other pollen-feeding beetles examined, e.g. the New Zealand cryptophagid *Paratomaria crowsoni* Leschen, 1996 collected from *Brachyglottis repanda* (R. A. B. Leschen, unpublished). *Paratomaria* Leschen, 1996 differs from *Rygmodus* in the smaller body length (less than 2 mm) and mandibles more typical for spore-feeding beetles, with the bidentate incisor lobe (not scoop-like), the relatively smaller prostheca compared to the body of the entire mandible and the striate mola. In contrast to the cryptophagid, it is possible that more pollen grains are gathered into the oral space of *Rygmodus* and their subtle compression by molae is enough to make them ready to extract nutrients. Moreover, the ancestors of *P. crowsoni* were likely mycophagous (Leschen 1996) rather than saprophagous; different ways of food processing in *Paratomaria* and *Rygmodus* may hence also reflect difference in evolutionary history between these taxa.

A large part of the ventral body surface of *Rygmodus* is covered with pubescence sparser than in other hydrophilid beetles which hold air bubble when submerged (e.g. Fikáček *et al.* 2012). Denser pubescence is only present on ventral surface of tarsi, on antennal club and on mouthparts (labrum, galea, prementum and hypopharynx). No pollen grains were found from these densely pubescent body parts in inspection with safranine staining nor electron microscopy; however, since the examined specimens were preserved in alcohol, we cannot rule out the possibility that pollen grains were washed out in the fixative fluid. We are hence unable to confirm that *Rygmodus* may serve as a pollinator at the moment, and additional observations of live specimens are necessary.

Habitat shifts in *Rygmodus*

The life style of *Rygmodus*, with flower-visiting, pollen-feeding adults and aquatic larvae, is unique within the Hydrophilidae; the larval habitat is different from the adult habitat, and adult's association with flowers is also unusual within the family. Only a few flower visitors have been reported within the Hydrophilidae: adults of *Pseudohydrobius* Blackburn, 1898 (Cylominae) come to flowers of *Leptospermum* (Lea 1919), adults of *Cycreon* Orchymont,

1919 and *Nitidulodes* Sharp, 1882 (Sphaeridiinae: Megasternini) are known to be associated with inflorescences of Araceae (Bloom *et al.* 2014; Low *et al.* 2016), and *Pelosoma* Mulsant, 1844 (Megasternini) with inflorescences of *Heliconia* (Archangelsky 1997).

Within the subfamily Cylominae, *Rygmodus* is moreover unusual in only larvae being aquatic. Cylominae contains 19 described genera (Short & Fikáček 2013; Seidel *et al.* 2016, in press) which usually inhabit forest leaf litter, rotten organic matter or aquatic environments as both adults and larvae (Table 3). The most recent common ancestor of the subfamily was estimated as having a terrestrial life style by Bloom *et al.* (2014), and subsequent unpublished analyses have reconstructed the cylomine ancestor as inhabiting decaying plant material in both larval and adult stages (V. Sýkora, unpubl. data). Hence, it can be hypothesized that habitat shifts from decaying plant material to the association with flowers in adults and to aquatic habitats in larvae occurred in the ancestor of *Rygmodus*. The association with flowers is also known in the Australian genus *Pseudohydrobius* (Lea 1919). *Pseudohydrobius* has been placed in the principal clade of Cylominae, along with *Rygmodus*, by preliminary phylogenetic analyses (V. Sýkora *et al.*, unpubl. data), but no detailed information about its biology and life cycle is available. Based on examination of slide-mounted specimens, *Pseudohydrobius* resembles *Rygmodus* in the simple mandibular apex, but the shape of its mandible is different (wider and shorter) and the mola seems to have the usual lamellate structure as shown in Figure 4H–K for other hydrophilids. It also lacks the basal tooth on tarsal claws, which is an autapomorphy for adult *Rygmodus* (Hansen 1997) and may facilitate crawling on plants/flowers. Additional research is therefore necessary to reveal whether *Rygmodus* and *Pseudohydrobius* have independently evolved the habit of association with flowers, or whether they are sister genera.

The association of *Rygmodus* larvae with aquatic habitats (stream edges and mossy spray zones) is secondary, and parallel to the terrestrial to aquatic transitions in the cylomine genera *Cylomissus* and *Anticura* (Short & Fikáček 2013; Bloom *et al.* 2014; Minoshima *et al.* 2015). Larval mouthparts of *Rygmodus* resemble those of the tribe Hydrophilini in having the long narrow falcate mandibles, long antenna-like maxillae and labium with far projecting long prementum, differing from all other known cylomine larvae. Hydrophilinae larvae are ambush-type predators feeding on a wide spectrum of aquatic invertebrates (e.g. Hosseinie

1976; Matta 1982; Formanowicz & Brodie 1988). From the morphological similarity of mouthparts, we may expect that *Rygmodus* larvae are also predatory. When compared to other known Cylominae larvae (Table 4), *Rygmodus* shares the nasale with five teeth, low almost symmetrical epistomal lobes, presence of coronal line, long antennal sensorium and presence of only five stout setae on inner face of the maxillary stipes with larvae supposedly belonging to the Australian genus *Borborophorus* Hansen, 1990 (Fikáček in press) and Chilean *Cylorygmus* Orchymont, 1933 (Seidel *et al.* in press); it however differs in all these characters from the genera *Cylomissus*, *Anticura*, *Andotypus* and *Austrotypus* (Fikáček *et al.* 2014; Minoshima *et al.* 2015). These differences correspond to the preliminary results of phylogenetic analyses (M. Fikáček & V. Sýkora, unpubl. data), in which *Rygmodus* , *Borborophorus* and *Cylorygmus* are members of the same principal clade but all the other aforementioned genera do not seem closely related to them, forming another big clade.

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REFERENCES

Angiosperm Phylogeny Group (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181**, 1–20.

Archangelsky M (1997) Studies on the biology, ecology, and systematics of the immature stages of New World Hydrophiloidea (Coleoptera: Staphyliniformia). *Bulletin of the Ohio Biological Survey, New Series* **12**, 1–207.

Archangelsky M (1999) Adaptations of immature stages of Sphaeridiinae (Staphyliniformia, Hydrophiloidea: Hydrophilidae) and state of knowledge of preimaginal hydrophilidae. *Coleopterists Bulletin* **53**, 64–79.

Archangelsky M, Beutel RG, Komarek A (2005) Hydrophiloidea. 10.1. Hydrophilidae. In: Beutel RG, Leschen RAB (eds) *Handbook of Zoology,Volum IV Arthropoda: Insecta. Part 38. Coleoptera, Beetles, Volume 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim)*, pp 157–183. Walter De Gruyter, Berlin.

Arriaga-Varela E, Seidel M, Deler-Hernández A, Senderov V, Fikácek M (2017) A review of the *Cercyon* Leach (Coleoptera, Hydrophilidae, Sphaeridiinae) of the Greater Antilles. *Zookeys* **681**, 39–93.

Balke M (2005) 7.6. Dytiscidae Leach, 1915. In: Beutel RG, Leschen RAB (eds), *Handbook of Zoology. Volume IV Arthropoda: Insecta. Part 38. Coleoptera, Beetles 1. Morphology and systematice (Archostemata, Adephaga, Myxophaga, Polyphaga partim)*, pp 90–116. Walter de Gruyter, Berlin.

Bernhard D, Schmidt C, Korte A, Fritzsch 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.

Betz O, Thayer MK, Newton AF (2003) Comparative morphology and evolutionary pathways of the mouthparts in spore-feeding Staphylinoidea (Coleoptera). *Acta Zoologica* **84**, 179–238.

Bloom DD, Fikáček M, Short AEZ (2014) Clade age and diversification rate variation explain disparity in species richness among water scavenger beetle (Hydrophilidae) lineages. *PLoS ONE* **9**, Article ID e98430. https://doi.org/10.1371/journal.pone.0098430

Broun T (1886) *Manual of the New Zealand Coleoptera. Part III*. Colonial Museum and Geological Survey Department, Wellington.

Brown HP (1987) Biology of riffle beetles. *Annual Review of Entomology* **32**, 253–273.

Buckley TR, Krosch M, Leschen RAB (2015) Evolution of New Zealand insects: summary and prospectus for future research. *Austral Entomology* **54**, 1–27.

Byttebier B, Torres PLM (2009) Description of the preimaginal stages of *Enochrus* (*Hugoscottia*) *variegatus* (Steinheil, 1869) and *E.* (*Methydrus*) *vulgaris* (Steinheil, 1869) (Coleoptera: Hydrophilidae), with emphasis on larval morphometry and chaetotaxy. *Zootaxa* **2139**, 1–22.

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.

Condamine FL, Clapham ME, Kergoat GJ (2016) Global patterns of insect diversification: towards a reconciliation of fossil and molecular evidence? *Scientific Reports* **6**, Article ID 19208. https://doi.org/10.1038/srep19208

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

Fenoglio S, Tierno de Figueroa JM (2003) Observations on the feeding of adults of some *Neoperla* and *Anacroneuria* species (Plecoptera : Perlidae). *African Entomology* **11**, 138–139.

Fikáček M (in press) 10D. Hydrophilidae Latreille, 1802. In: Lawrence JF, Ślipiński A (eds) *Australian Beetles Volume 2*. CSIRO Publishing, Melbourne.

Fikáček M, Vondráček D (2014) A review of *Pseudorygmodus* (Coleoptera: Hydrophilidae), with notes on the classification of the Anacaenini and on distribution of genera endemic to southern South America. *Acta Entomologica Musei Nationalis Pragae* **54**, 479–514.

Fikáček M, Archangelsky M, Torres PLM (2008) Primary chaetotaxy of the larval head capsule and head appendages of the Hydrophilidae (Coleoptera) based on larva of *Hydrobius fuscipes* (Linnaeus, 1758). *Zootaxa* **1874**, 16–34.

Fikáček M, Prokin A, Angus RB *et al.* (2012) Phylogeny and the fossil record of the Helophoridae reveal Jurassic origin of extant hydrophiloid lineages (Coleoptera: Polyphaga). *Systematic Entomology* **37**, 420–447.

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, Minoshima YN, Newton AF (2014) A review of *Andotypus* and *Austrotypus* gen. nov., rygmodine genera with an austral disjunction (Hydrophilidae: Rygmodinae). *Annales Zoologici* **64**, 557–596.

Fikáček M, Maruyama M, Komatsu T, Beeren C, von, 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.

Formanowicz DR, Brodie ED (1988) Prey density and ambush site changes in *Tropisternus lateralis* larvae (Coleoptera, Hydrophilidae). *Journal of the Kansas Entomological Society* **61**, 420–427.

Girón JC, Short AEZ (2017) Revision of the Neotropical water scavenger beetle genus *Quadriops* Hansen, 1999 (Coleoptera, Hydrophilidae, Acidocerinae). *Zookeys* **705**, 115–141.

Glenny D, James T, Cruickshank J, Dawson M, Ford K, Breitwieser I (2012) *Key to Flowering Plant Genera of New Zealand* [cited 19 June 2017]. Available from URL:

http://www.landcareresearch.co.nz/resources/identification/plants/flowering-plants-key

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704.

Hanley RS, Ashe JS (2007) Techniques for dissecting adult aleocharine beetles (Coleoptera: Staphylinidae). *Bulletin of Entomological Research* **93**, 11–18.

Hansen M (1990) Australian Sphaeridiinae (Coleoptera : Hydrophilidae): A taxonomic outline with descriptions of new genera and species. *Invertebrate Taxonomy* **4**, 317–395.

Hansen M (1997) Synopsis of the endemic New Zealand genera of the beetle subfamily Sphaeridiinae (Coleoptera: Hydrophilidae). *New Zealand Journal of Zoology* **24**, 351–370.

Heine EM (1937) Observations on the pollination of New Zealand flowering plants. *Transactions and Proceedings of the Royal Society of New Zealand* **67**, 133–148.

Holter P (2004) Dung feeding in hydrophilid, geotrupid and scarabaeid beetles: Examples of parallel evolution. *European Journal of Entomology* **101**, 365–372.

Hosseinie SO (1976) Comparative life histories of three species of *Tropisternus* in the laboratory (Coleoptera: Hydrophilidae). *Internationale Revue der gesamten Hydrobiologie und Hydrographie* **61**, 261–268.

Hunt T, Bergsten J, Levkanicova Z *et al.* (2007) A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science* **318**, 1913–1916.

Jones GD (2012) Pollen analyses for pollination research, unacetolyzed pollen. *Journal of Pollination Ecology* **9**, 96–107.

Klimaszewski J, Watt JC (1997) Coleoptera: family-group review and keys to identification. *Fauna of New Zealand* **37**, 1–194.

Kodada J, Jäch MA (2005) 18.2. Elmidae Curtis, 1830. In: Beutel RG, Leschen RAB (eds) *Handbook of Zoology. Volume IV Arthropoda: Insecta. Part 38. Coleoptera, Beetles 1. Morphology and systematice (Archostemata, Adephaga, Myxophaga, Polyphaga partim)*, pp 471–496. Walter de Gruyter, Berlin.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Kuschel G (1990) Beetles in a suburban environment: a New Zealand case study. *DSIR Plant Projection Report* **3**, 1–118.

Large MF, Braggins JE (1991) *Spore Atlas of New Zealand Ferns and Fern Allies.* SIR Publishing, Wellington.

Lawrence JF, Ślipiński A (2013) *Australian Beetles Volume 1. Morphology, Classification and Keys*. CSIRO Publishing, Melbourne.

Lea AM (1919) Notes on some miscellaneous Coleoptera, with descriptions of new species. Part V. *Transactions of the Royal Society of South Australia* **43**, 166–261, pls xxv–xxvii.

Leschen RAB (1996) Phylogeny and revision of the genera of Cryptophagidae (Coleoptera: Cucujoidea). *The University of Kansas Science Bulletin* **55**, 549–634.

Leschen RAB (2003) Erotylidae (Insecta: Coleoptera: Cucujoidea): Phylogeny and Review. *Fauna of New Zealand* **47**, 1–108.

Leschen RAB, Gimmel ML (2012) Catalogue of the tribe Picrotini (Coleoptera: Cryptophagidae: Cryptophaginae). *New Zealand Entomologist* **35**, 14–28.

Leschen RAB, Newton AF (2015) Checklist and type designations of New Zealand Aleocharinae (Coleoptera: Staphylinidae). *Zootaxa* **4028**, 301–353.

Low SL, Wong SY, Ooi IH *et al.* (2016) Floral diversity and pollination strategies of three rheophytic Schismatoglottideae (Araceae). *Plant Biology* **18**, 84–97.

Matta JF (1982) The bionomics of two species of *Hydrochara* (Coleoptera: Hydrophilidae) with descriptions of their larvae. *Proceedings of the Entomological Society of Washington* **84**, 461–467.

Mayhew PJ (2007) Why are there so many insect species? Perspectives from fossils and phylogenies. *Biological Reviews* **82**, 425–454.

Minoshima Y, Hayashi M (2011a) Larval morphology of the Japanese species of the tribes Acidocerini, Hydrobiusini and Hydrophilini (Coleoptera: Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **51 (supplementum)**, 1–118.

Minoshima Y, Hayashi M (2011b) Larval morphology of the genus *Hydrocassis* Fairmaire (Coleoptera: Hydrophilidae). *Journal of Natural History* **45**, 2757–2784.

Minoshima Y, Hayashi M (2012) Larval morphology of *Amphiops mater mater* Sharp (Coleoptera: Hydrophilidae: Chaetarthriini). *Zootaxa* **3351**, 47–59.

Minoshima Y, Hayashi M, Kobayashi N, Yoshitomi H (2013) Larval morphology and phylogenetic position of *Horelophopsis hanseni* Satô et Yoshitomi (Coleoptera, Hydrophilidae, Horelophopsinae). *Systematic Entomology* **38**, 708–722.

Minoshima YN, Fikáček M, Gunter N, Leschen RAB (2015) Larval morphology and biology of New Zealand-Chilean genera *Cylomissus* Broun and *Anticura* Spangler (Coleoptera: Hydrophilidae: Rygmodinae). *Coleopterists Bulletin* **69**, 687–712.

Minoshima YN, Iwata Y, Fikáček M, Hayashi M (2017) Description of immature stages of *Laccobius* (*Laccobius*) *kunashiricus*, with a key to genera of the Laccobiini based on larval characters (Coleoptera, Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **57**, 97–119.

Moar NT (1993) *Pollen Grains of New Zealand Dicotyledonous Plants*. Manaaki Whenua Press, Lincoln.

Nel A, Scholtz CH (1990) Comparative morphology of the mouthparts of adult Scarabaeoidea (Coleoptera). *Entomology Memoir, Republic of South Africa, Department of Agricultural Development* **80**, 1–84.

Newstrom-Lloyd LE (2013) Pollination in New Zealand. In: Dymond JR (ed) *Ecosystem Services in New Zealand : Conditions and Trends*, pp 408–431. Manaaki Whenua Press, Lincoln.

Newstrom L, Robertson A (2005) Progress in understanding pollination systems in New Zealand. *New Zealand Journal of Botany* **43**, 1–59.

Primack RB (1978) Variability in New Zealand montane and alpine pollinator assemblages. *New Zealand Journal of Ecology* **1**, 66–73.

Primack RB (1983) Insect pollination in the New Zealand mountain flora. *New Zealand Journal of Botany* **21**, 317–333.

Rainford JL, Hofreiter M, Nicholson DB, Mayhew PJ (2014) Phylogenetic distribution of extant richness suggests metamorphosis is a key innovation driving diversification in

insects. *PLoS ONE* **9**, Article ID e109085.

https://doi.org/10.1371/journal.pone.0109085

Rúa J, Tierno de Figueroa JM (2013) Adult feeding habits of three Perloidea species

(Plecoptera: Perlidae and Chloroperlidae). *Aquatic Insects* **35**, 99–104.

Ruta R, Klausnitzer B, Prokin A (2017) South American terrestrial larva of Scirtidae

(Coleoptera: Scirtoidea): the adaptation of Scirtidae larvae to saproxylic habitat is

more common than expected. *Austral Entomology*. Published online: 5 Jan 2017;

https://doi.org/10.1111/aen.12270

Seidel M, Arriaga-Varela E, Fikáček M (2016) Establishment of Cyalominae Zaitzev, 1908 as

a valid name for the subfamily Rygmodinae Orchymont, 1916 with an updated list of

genera (Coleoptera: Hydrophilidae). *Acta Entomologica Musei Nationalis Pragae* **56**,

159–165.

Seidel M, Minoshima YN, Arriaga-Varela E, Fikáček M (in press) Breaking a disjunct

distribution: a review of the Southern Hemisphere genera *Cylorygmus* and

Relictorygmus gen. nov. (Hydrophilidae: Cyalominae). *Annales Zoologici* **68**.

Shirayama Y, Kaku T, Higgins RP (1993) Double-sided microscopic observation of

meiofauna using an HS-slide. *Benthos Research* **44**, 41–44. (In Japanese with English

abstract.)

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**, 723–752.

Stevens PF (2001 onwards) *Angiosperm Phylogeny Website, Version 13* [cited 19th June

2017]. Available from URL: http://www.mobot.org/MOBOT/research/APweb/

Swenson U, Bremer K (1997) Patterns of floral evolution of four Asteraceae genera

(Senecioneae, Blennospermatinae) and the origin of white flowers in New Zealand.

Systematic Biology **46**, 407–425.

Thomson GM (1881) The flowering plants of New Zealand, and their relation to the insect

fauna. *Transactions of the Botanical Society of Edinburgh* **14**, 91–105.

Toussaint EFA, Hendrich L, Escalona HE, Porch N, Balke M (2016) Evolutionary history of a secondary terrestrial Australian diving beetle (Coleoptera, Dytiscidae) reveals a lineage of high morphological and ecological plasticity. *Systematic Entomology* **41**, 650–657.

Webb CJ, Kelly D (1993) The reproductive biology of the New Zealand flora. *Trends in Ecology & Evolution* **8**, 442–447.

Yang AS (2001) Modularity, evolvability, and adaptive radiations: a comparison of the hemi- and holometabolous insects. *Evolution & Development* **3**, 59–72.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

File S1 Description of the second instar larva of *Rygmodus* sp.

Figure S1 Mouthparts of *Rygmodus modestus*.

Figure S2 Head capsule of the third instar larva of *Rygmodus* sp. from Pelorus Bridge and its chaetotaxy.

Figure S3 Head appendages of the third instar larva of *Rygmodus* sp. from Pelorus Bridge.

Table S1 List of specimens used for molecular analyses, along with GenBank accession numbers of their sequences.

Table S2 Primers used for amplification of molecular data.

Table 1 Results of the analysis of mid-gut contents of *Rygmodus* adults

Species & collecting event	Collected from	Pollen type	Count[†]
R. modestus: GB: Te Urewera NP, Black Beech Tk, xi.2012[‡]	*Brachyglottis repanda*	*Aristotelia*	184
		Undetermined	1
R. modestus: GB: Te Urewera NP, Black Beech Tk, xi.2012[‡]	*Brachyglottis repanda*	Asteraceae	205
		Coprosma	39
		Pratia	27
		Prumnopitys taxifolia	6
R. modestus: GB: Te Urewera NP, Black Beech Tk, xi.2012[‡]	*Brachyglottis repanda*	*Aristotelia*	200
		Coprosma	<1
		Haloragaceae	<1
		Asteraceae	<1
		Hebe	<1
		Ericaceae	<1
R. cyaneus group: NC: Arthurs Pass, Temple basin Tk., i.2014	*Hebe*	*Hebe*	231
R. cyaneus group: NC: Arthurs Pass Village, i.2011	*Hoheria glabrata*	*Hoheria*	169
R. modestus: CL: Tapu, Coroglen Tk., xi.2009	*Cordyline australis*	*Cordyline*	200
		Cunoniaceae cf. *Weinmannia*	1
R. cyaneus group: WD: Otira, Kelly´s Creek, i.2011	*Hoheria glabrata*	*Hoheria*	115
R. femoratus: BR: Lewis Pass, xii.2012	*Aciphylla*	*Aciphylla*	200
		Haloragaceae	1
		Poaceae	<1
R. cyaneus group: KA: Mt. Fyffe Hut, i.2012	*Celmisia*	*Pratia* cf. *angulata*	177
		Leptospermum	1
R. alienus: BR: Whareata Mine, Denniston, i.2012	*Leptospermum scoparium*	*Leptospermum*	200
		Asteraceae	<1
		Dacrydium cupressinum	<1
R. cyaneus group: Arthurs Pass, Temple Basin Tk., i. 2011	*Hebe* + *Ranunculus*	*Hebe*	87
		Poaceae	1
R. modestus: GB: Te Urewera NP, Aniwaniwa Rd., xi.2012	*Olearia*	Asteraceae	19
R. modestus: GB: Te Urewera NP, Aniwaniwa Rd., xi.2012	*Olearia*	Asteraceae	200
		Coprosma	<1

[†] Pollen grain count: '<1' refers to rare pollen types found after inspecting the whole slide but not in the subsample used for counting dominant pollen types. [‡] Collected on the same inflorescence.

Table 2 List of plant taxa, based on collection records of *Rygmodus* adults from their flowers/inflorescences in the present study and for museum specimens examined

Family	Genus / species
Asparagaceae	*Cordyline* spp. (*C. australis*, *C. banksii*, *C. indivisa*)
Apiaceae	*Aciphylla* sp.
Asteraceae	*Olearia* spp.
	Brachyglottis repanda
	Celmisia sp.
Ericaceae	*Gaultheria* sp.
Malvaceae	*Hoheria* sp.
Myrtaceae	*Leptospermum scoparium*
Orobanchaceae	*Euphrasia cuneata*[†]
Pittosporaceae	*Pittosporum eugenioides*
Ranunculaceae	*Ranunculus* sp. (white large-flower subalpine species)
Rosaceae	*Rubus australis*[†]
Plantaginaceae	subalpine *Veronica* (*Hebe*) sp. (*Veronica subalpina* morphotype)

[†] After Heine (1937).

Table 3 Summary of larval and adult habitats in the representatives of Cylominae

Genus	Larval habitat	Adult habitat	References
Adolopus Sharp, 1884	terrestrial (leaf litter, rotten wood)	terrestrial (leaf litter, rotten wood)	M. Seidel & M. Fikáček, pers. observ.
Andotypus Spangler, 1979	terrestrial (leaf litter, baited traps)	terrestrial (leaf litter, baited traps)	Fikáček *et al.* (2014)
Anticura Spangler, 1979	aquatic	Aquatic (submerged moss)	Minoshima *et al.* (2015)
Austrotypus Fikáček *et al.*, 2014	terrestrial (rotten organic material)	terrestrial (rotten organic material)	Fikáček *et al.* (2014)
Borborophorus Hansen, 1990	? terrestrial (log and bark litter)	terrestrial (leaf litter, rotten wood)	Fikáček (in press)
Coelostomopsis Hansen, 1990	unknown	terrestrial (leaf litter, FIT traps)	Fikáček (in press)
Cyloma Sharp, 1872	terrestrial (leaf litter)	terrestrial (leaf litter, baited traps)	M. Seidel & M. Fikáček, pers. observ.
Cylomissus Broun, 1903	aquatic	Aquatic (submerged moss)	Minoshima *et al.* (2015)
Cylorygmus Orchymont, 1933	semiaquatic	semiaquatic	Seidel *et al.* (in press)
Eurygmus Hansen, 1990	unknown	terrestrial (FIT traps)	Fikáček (in press)
Exydrus Broun, 1886	unknown	terrestrial (leaf litter, baited traps)	M. Seidel & M. Fikáček, pers. observ.
Hydrostygnus Sharp, 1884	unknown	terrestrial (leaf litter, baited traps)	M. Seidel & M. Fikáček, pers. observ.
Petasopsis Hansen, 1990	unknown	terrestrial (leaf litter)	Hansen (1990)
Pseudohydrobius Blackburn, 1898	unknown	terrestrial (flower-visiting)	Fikáček (in press)
Relictorygmus Seidel, Minoshima, Arriaga-Varela & Fikáček, 2018	semiaquatic	unknown	Seidel *et al.* (in press)
Rygmodus White, 1846	aquatic / semiaquatic	terrestrial (flower-visiting)	this paper
Rygmostralia Orchymont, 1933	unknown	terrestrial (no more details known)	Fikáček (in press)
Saphydrus Sharp, 1884	terrestrial (leaf litter)	terrestrial (sweeping, baited traps)	M. Seidel & M. Fikáček, pers. observ.
Tormissus Broun, 1893	terrestrial (rotten organic material)	terrestrial (rotten organic material)	M. Seidel & M. Fikáček, pers. observ.

Table 4 Comparison of larval morphology among genera of the subfamily Cylominae

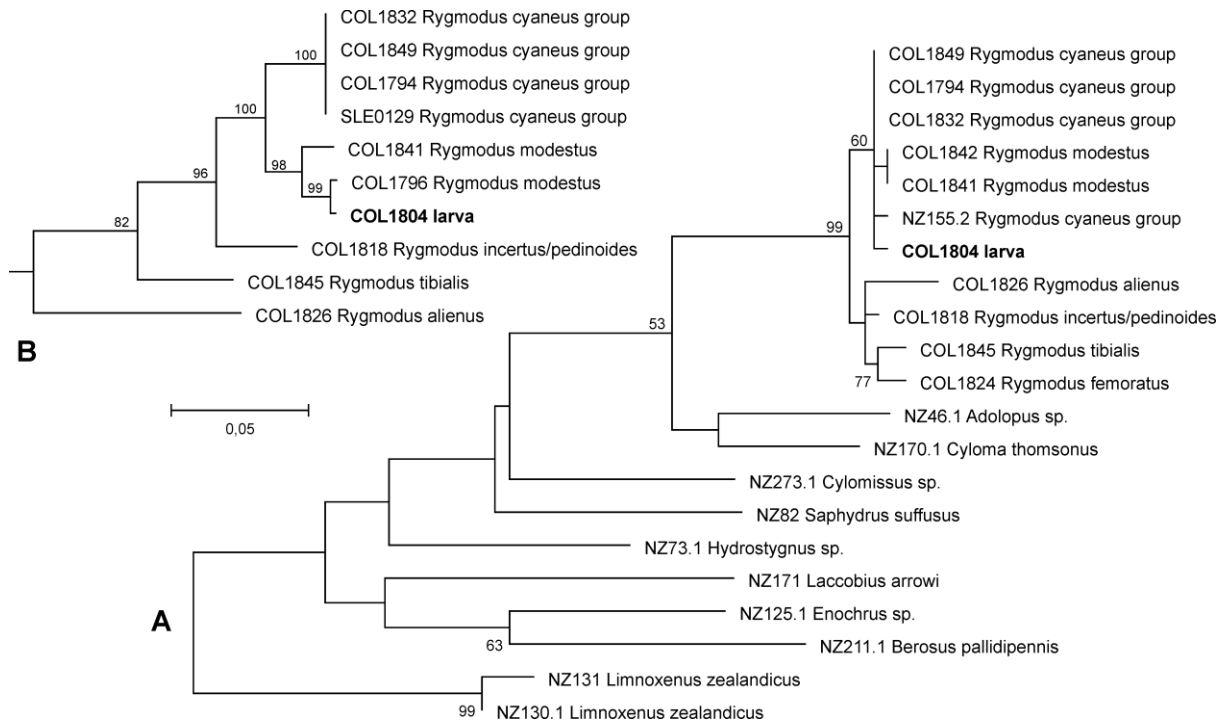| Character	*Andotypus*	*Anticura*	*Austrotypus*	*Cylomissus*	*Cylorygmus*	*Rygmodus*	? *Borborophorus*
Nasale	one small tooth	two teeth	one tooth	single or two teeth	five teeth	five teeth	five teeth
Epistomal lobe	left lobe projecting further than right lobe	almost symmetrical	left lobe projecting further than right lobe	almost symmetrical	almost symmetrical	almost symmetrical	almost symmetrical
Number of setae of gFR2 (left/right)	8/2	8–9/5–8	11/0	6/4	7–8/7–9	9/9	4/4
Coronal line	absent	present	absent	present	present	present	present
Mandible	asymmetrical / stout	symmetrical / stout	asymmetrical / stout	symmetrical / stout	symmetrical / stout	symmetrical / narrow	symmetrical / stout
Prementum	wider than long	wider than long	wider than long	wider than long	as long as wide or slightly wider than long	much longer than wide	wider than long
Ligula	reduced	developed	reduced	developed	developed	developed	developed
Hypopharyngeal lobe	absent	absent	present	absent	absent	absent	absent
Antennal sensorium (SE1)	slightly shorter than ant3, thick	very short, thick	very short, thick	half the length of ant3, stout	shorter than ant3, thick	as long as ant3, thin	as long as ant3, thick
gMX2	present	present	present	present	absent	absent	absent
Lateral projections abdominal segment VIII	present	absent	present	absent	absent	absent	absent
Dorsal plate on segment VIII	subdivided	simple	subdivided	simple	simple	simple	simple

Figure 1 DNA barcoding of a larva (COL1804) in comparison with adult specimens of New Zealand Hydrophilidae. (A) Maximum likelihood tree of nuclear H3 (histone 3) sequences of New Zealand hydrophilids, showing the monophyly of *Rygmodus*; (B) maximum likelihood tree of mitochondrial cox1 (cytochrome oxidase 1) sequences of *Rygmodus*, showing that *R. cyaneus* and *R. modestus* form a clade and that the larva is identified as *R. modestus*. Bootstrap support values larger than 50 are indicated above the branches.

Figure 2 Adults of *Rygmodus modestus* White, 1846. (A–C) Mass occurrence of *R. modestus* adults at Aniwaniwa Road, Te Urewera NP in November 2012 (A, *Olearia* bushes from which adults were collected; B–C, alive *R. modestus* adults at *Olearia* flowers, together with *Rhopalomerus picipennis* (Pascoe) and *R. monachus* (Broun) (Curculionidae: Eugnominae) in B); (D) adult in dorsal view; (E–L) safranine-dyed midgut contents of a specimen collected from *Brachyglottis repanda* at Black Beach Tk., Te Urewera NP (E–F, general view of the slide with dissected mid-gut contents; G–L, details of pollen grains found: G, Asteraceae gen. sp.; H, *Coprosma* sp.; I, *Pratia* sp.; J, *Prumnopitys taxifolia*; K–L, other rarely occurring particles: K, fungal sporangium; L, fragment of plant tissue).
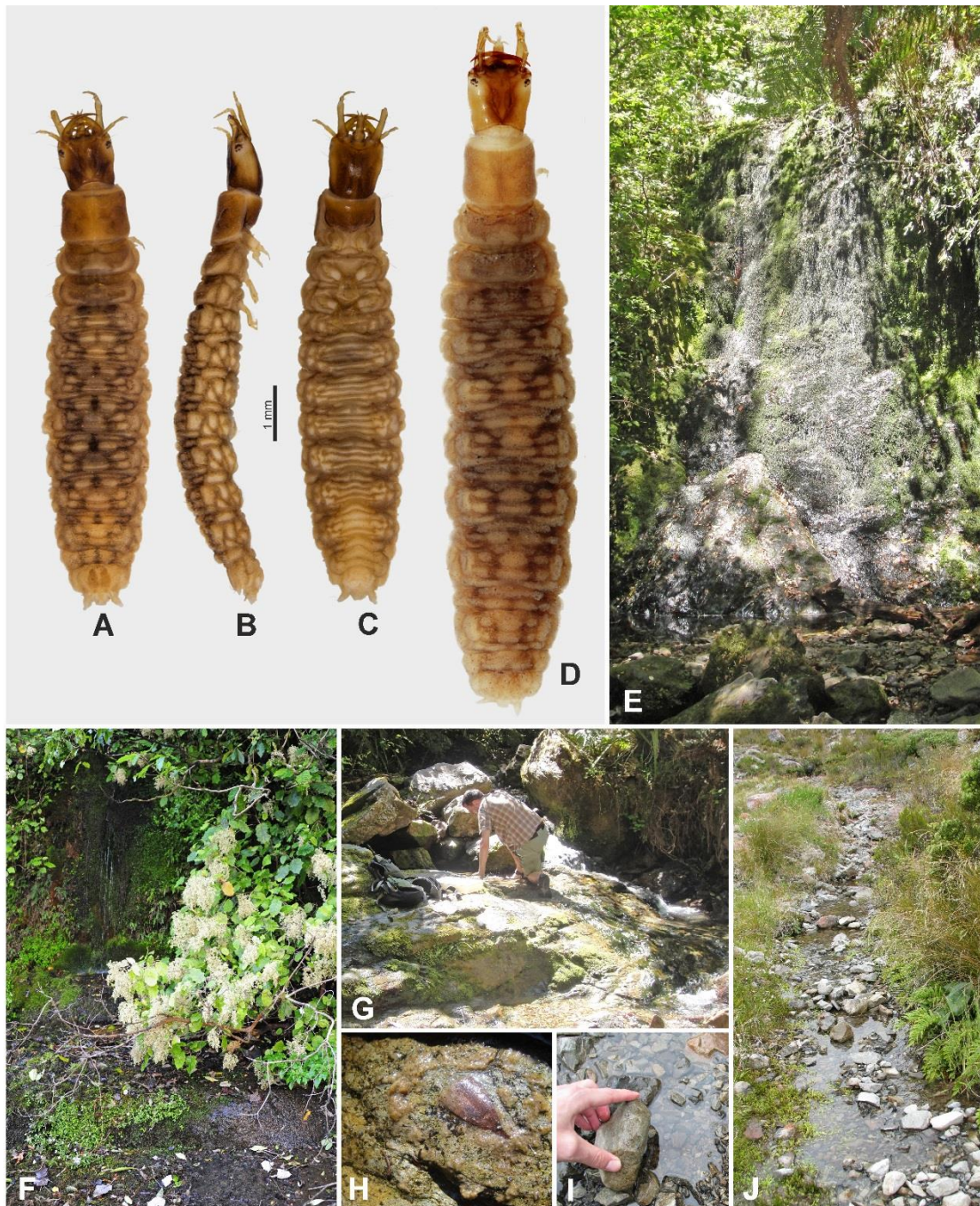
Figure 3 Larvae and larval habitats of *Rygmodus*. (A–C) Second-instar larva of *R. modestus* (dorsal, lateral and ventral view, respectively); (D) third-instar larva of *Rygmodus* sp. from Pelorus Bridge, dorsal view; (E–J) habitats of larvae: E, mossy waterfall in Pelorus Bridge (habitat of larvae of *Rygmodus* sp.); F, small waterfall flowing over moss rock (habitat of larvae of *R. modestus*) with flowering *Brachyglottis repanda* at side (from which adults of the same species were collected) in Te Urewera NP; G, H, Dead Horse Creek, with detail of algal mats from which larvae of *R. modestus* were collected; I, J, small stream in alpine tussock area at Arthur's Pass, with detail of stones under which larvae of *Rygmodus* were collected. Photos E and F by M. Fikáček, G and H by R. Leschen, I and J by M. Gimmel.
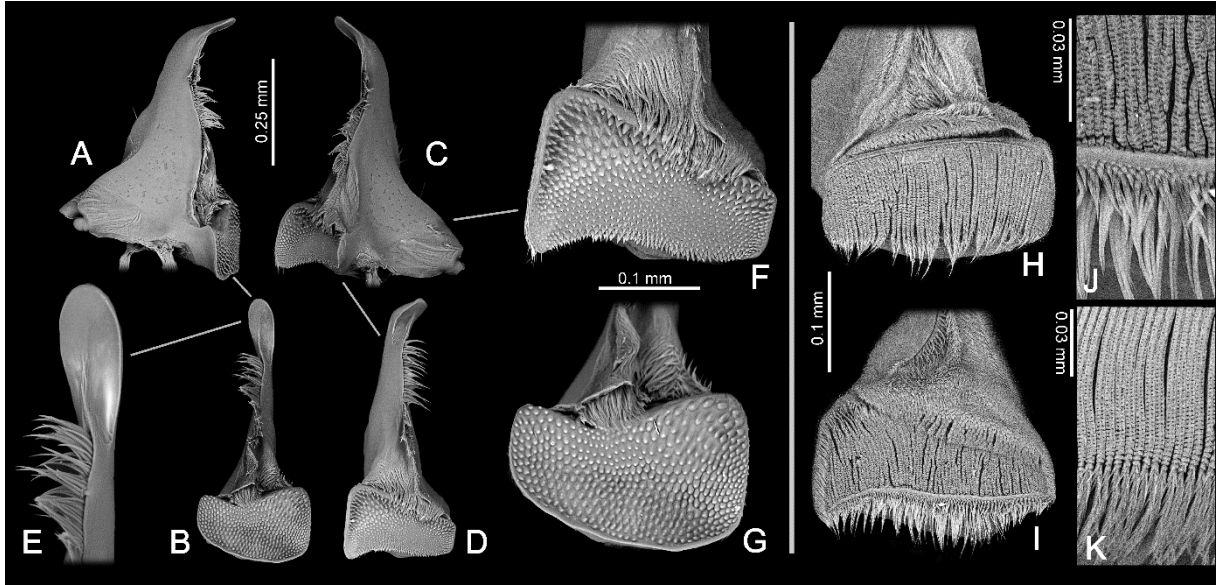
Figure 4 Mandibles of hydrophilid beetles. (A–G) *Rygmodus modestus*; (H–J) *Saphydrus suffusus* Sharp, 1884 (Cylominae); (K) *Dactylosternum hydrophiloides* (MacLeay, 1825) (Sphaeridiinae). A, C, Whole mandible, ventral view; B, D, whole mandible, mesal view; E, detail of mandibular apex in mesal view; F–I, detail of mola in mesal view; J–K, detail of fine structure of mola.
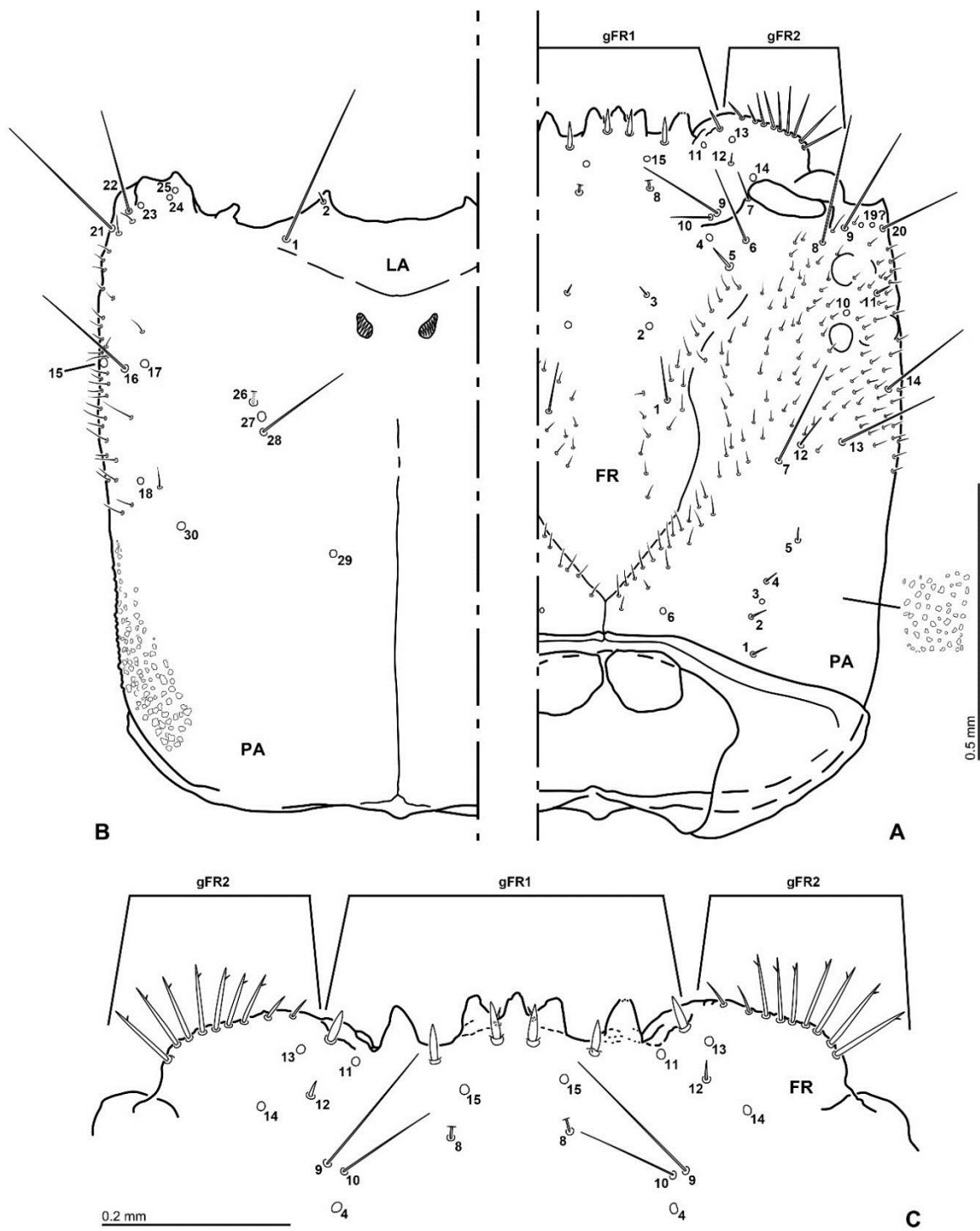
Figure 5 Head capsule of the second-instar larva of *Rygmodus modestus* and its chaetotaxy. (A) Dorsal view; (B) ventral view; (C) detail of clypeolabrum.
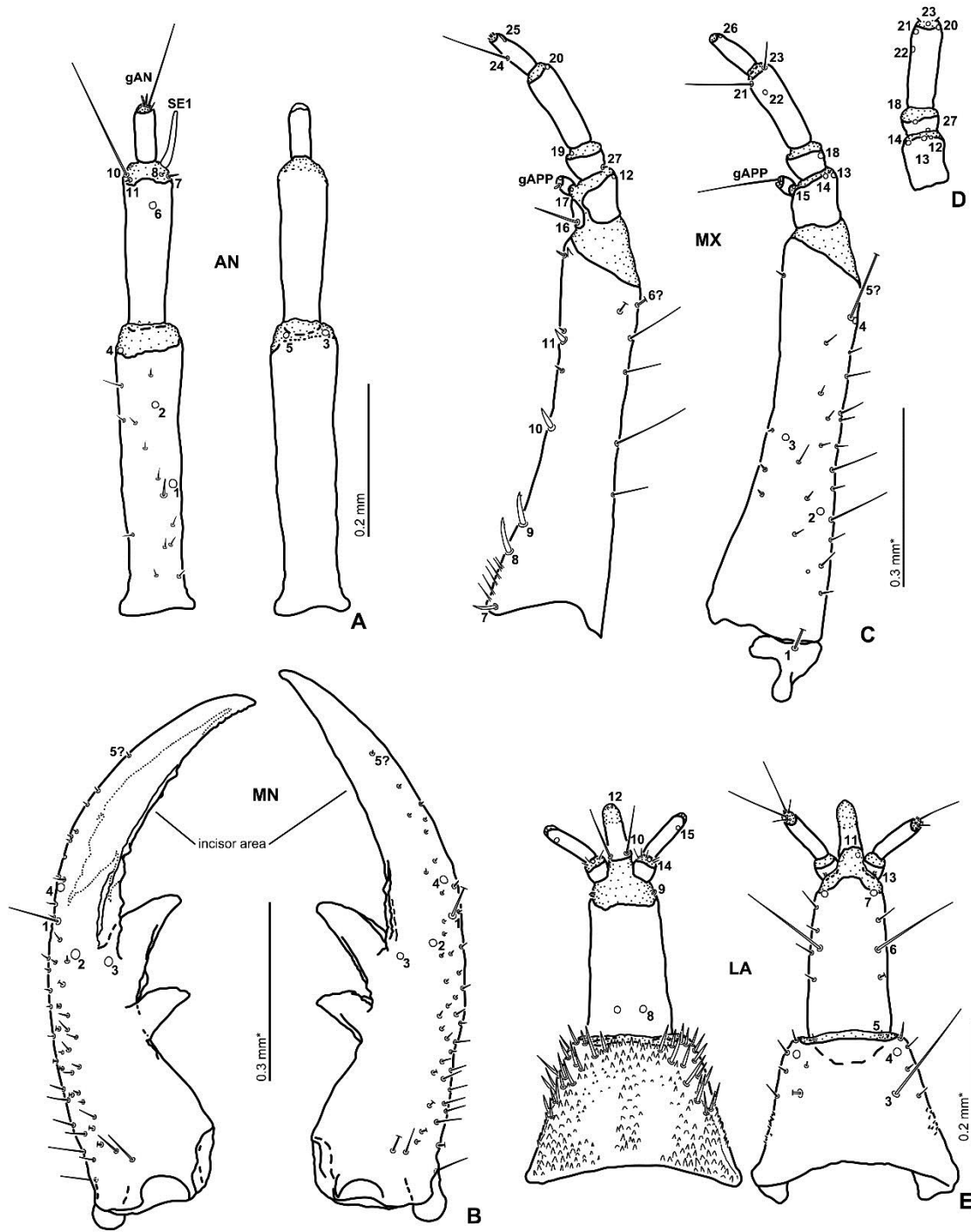
Figure 6 Head appendages of the second-instar larva of *Rygmodus modestus*. (A) Antenna, dorsal (left) and ventral (right) view; (B) mandibles, dorsal view; (C) maxilla, dorsal (left) and ventral (right) view; (D) maxillary palpomeres 1–3, lateral view; € labium in dorsal (left) and ventral (right) view.
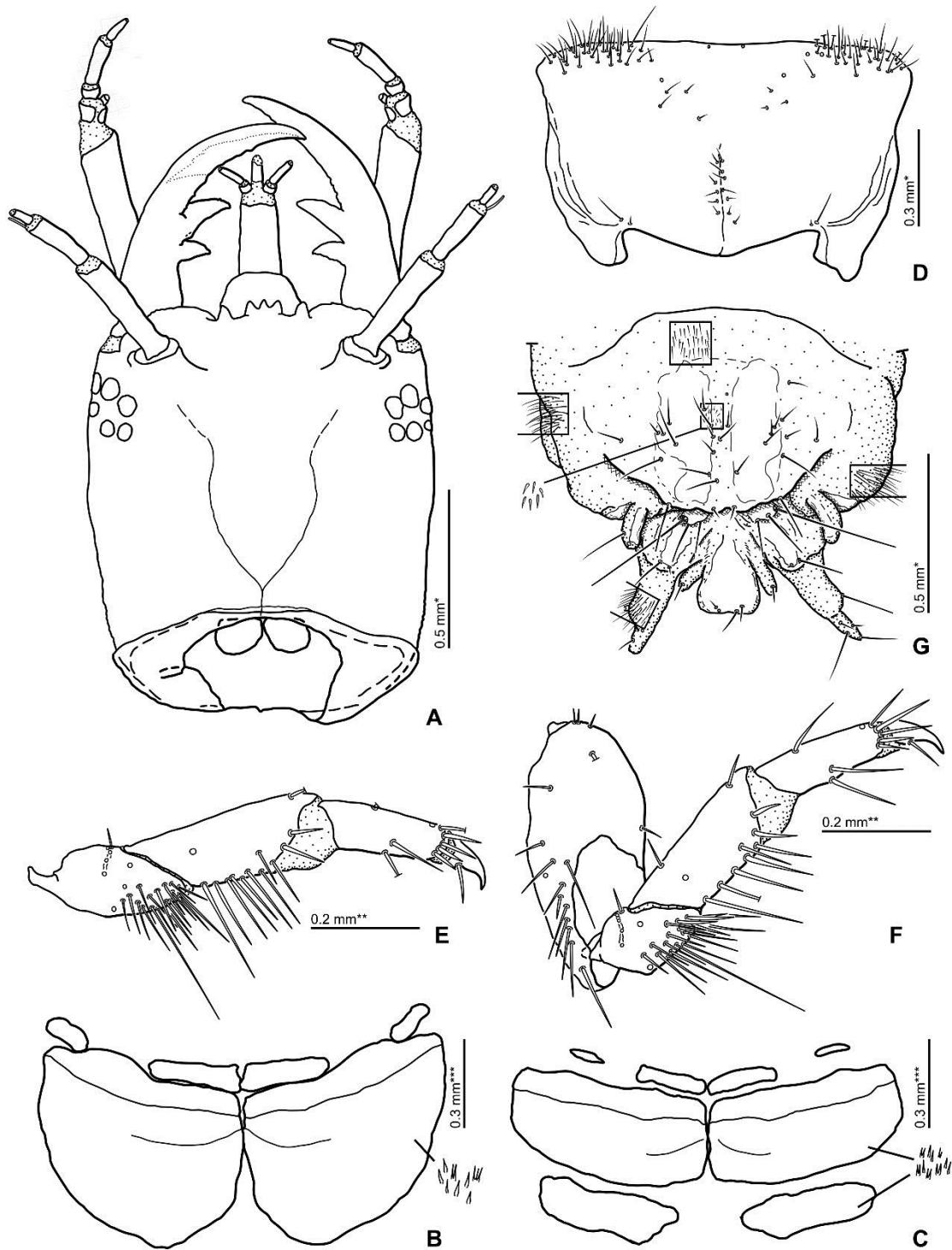
Figure 7 Details of larval morphology of the second-instar larva of *Rygmodus modestus*. (A) Head, dorsal view; (B) mesonotum sclerites, dorsal view; (C) metanotal sclerites, dorsal view; (D) prosternal plate, ventral view; (E) foreleg, anterior view; (F) mesoleg, anterior view; (G) abdominal apex with spiracular atrium.