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Description and molecular phylogeny of a new species of *Phoronis* (Phoronida) from Japan, with a redescription of topotypes of *P. ijimai* Oka, 1897

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Abstract

We describe *Phoronis emigi* **sp. n.** as the eighth member of the genus based on specimens collected from a sandy bottom at 33.2 m depth in Tomioka Bay, Amakusa, Japan. The new species is morphologically similar to *P. psammophila* Cori, 1889, but can be distinguished from the latter by the number of longitudinal muscle bundles in the body wall (56–72 vs. 25–50 in *P. psammophila*) and the position of the nephridiopores (situated level with the anus vs. lower than the anus in *P. psammophila*). Using sequences of the nuclear 18S and 28S rRNA genes and the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, we inferred the relationship of *P. emigi* to other phoronids by the maximum likelihood method and Bayesian analysis. The analyses showed that *P. emigi* is closely related to *P. hippocrepia* Wright, 1856 and *P. psammophila* Cori, 1889. We describe the morphology of the topotypes and additional material for *P. ijimai* Oka, 1897. Neither our morphological observations of *P. ijimai*, nor the phylogenetic analyses based on 18S and COI sequences, contradicts that *P. vancouverensis* Pixell, 1912 is conspecific with *P. ijimai*, a synonymy that has long been disputed.

Keywords
Lophophorata, 3D reconstruction, cladistic analyses, Japan, Misaki, Kyushu

Introduction

Phoronids, or horseshoe worms, are exclusively marine, sedentary, vermiform animals with a crown of ciliated tentacles, the lophophore, used in suspension feeding. They comprise the small phylum Phoronida, which currently contains two genera, *Phoronis* Wright, 1856 and *Phoronopsis* Gilchrist, 1907, with seven and three species, respectively (Emig 2007). Phoronid species are morphologically well defined, primarily on the basis of the arrangement and pattern of the body-wall musculature, nephridia, and lophophore in adults (e.g., Emig 1974, 1979, 1982). They produce characteristic actinotroch larvae, and most species have a cosmopolitan distribution (Emig 1982, Zimmer 1991).

For over the last half century, no new species of phoronids have been established, although the current species diversity is likely to have been underestimated (Santagata and Zimmer 2002), with *Phoronis pallida* Silén, 1952 and *Phoronopsis californica* Hilton, 1930 being the most recently described valid species in each genus (Silén 1952, Hilton 1930). More recently described nominal species have been regarded as invalid, junior synonyms of older names based on morphological concordance: *Phoronis svetlanae* Temereva & Malakov, 1999 as synonymous with *P. ijimai* Oka, 1897 (Emig 2007), and *Phoronopsis malakhovi* Temereva, 2000 with *Phoronopsis harmeri* Pixell, 1912 (Emig 2003). Since DNA sequence data have been obtained for almost all valid species in the phylum (e.g., Santagata and Cohen 2009, and references therein), sequences from *Phoronis svetlanae* and *Phoronopsis malakhovi* would have helped either to discriminate these species from congeners or to corroborate the proposed synonymies.

One of the unsettled taxonomic issues in phoronid systematics is whether or not *P. ijimai* Oka, 1897 (type locality: Misaki, Japan) is conspecific with *P. vancouverensis* Pixell, 1912 (type locality: Vancouver, Canada). Emig (1971a,b, 1974, 1977, 1982, 2007) synonymized these two nominal species based on similarity in various anatomical features in adults. Santagata and Zimmer (2002), however, avoided drawing a definitive conclusion on this synonymy, arguing that the late and competent larval stages described by Zimmer (1964) for *P. vancouverensis* were not recorded for *P. ijimai* in developmental observations by Ikeda (1901) and Wu and Sun (1980). Most of the DNA sequences from species in this complex currently deposited in GenBank are registered under the name *P. vancouverensis*, and all are derived from specimens collected in the northeastern Pacific, at localities closer to Vancouver than to Misaki: Friday Harbor, WA (Fuchs et al. 2009, Sperling et al. 2011); Monterey, CA (Cohen 2000, Mallatt and Winchell 2002); and Los Angeles, CA (Erber et al. 1998). For some sequences, the locality of origin is not reported in GenBank (Halanych et al. 1995, Passamaneck and Halanych 2006, Bourlat et al. 2008). On the other hand, no sequence data have been reported for *P. ijimai*, either from its type locality or a reasonably close locality in the northwestern Pacific. Undoubtedly, this has in part contributed to the continuing dispute over synonymy.

In this paper, we 1) describe a new phoronid species from Japan, which differs from all the previously known species in adult morphology; 2) reconstruct the phylogeny of representative phoronids, including the new species, based on DNA sequences of the nuclear 18S and 28S rRNA genes (hereafter, 18S and 28S, respectively), and the mitochondrial cytochrome *c* oxidase subunit I gene (COI); 3) describe topotypes of *P. ijimai* from Misaki, Sagami Bay, and discuss the synonymy with *P. vancouverensis* in the context of adult morphology and the molecular phylogeny; and 4) provide a key to the Japanese phoronid species.

Material and methods

Sampling

A sediment sample was obtained with a Smith-McIntyre grab having an aperture of 25 cm × 25 cm, from a sandy bottom at 33.2 m depth (32°32'27"N, 130°03'17"E) in Tomioka Bay, Amakusa, Kumamoto, Japan (Fig. 1A, 1B) on 26 November 2009 by Keiichi Kakui, Hiroshi Yamasaki, and Shushi Abukawa on board the research and training vessel *Seriola* of the Amakusa Marine Biological Laboratory (AMBL), Kyushu University. The sediment was agitated and stirred in a bucket with seawater and the supernatant was decanted; specimens suspended in the supernatant were collected with a sieve having a 0.3-mm mesh size. Of the 560 specimens obtained, most were fixed in 10% formalin seawater, and the rest were placed directly in 99% EtOH.

Topotypes of *Phoronis ijimai* were collected in Moroiso Bay, from a pier (≈35°09'28"N, 139°36'44"E) in front of the Misaki Marine Biological Station (MMBS), The University of Tokyo, Kanagawa, Japan (Fig. 1C) on 10 May 2012 by Hisanori Koutsuka, and from a rocky shore (≈35°09'32"N, 139°36'40"E) beside Arai Beach, Sagami Bay, near MMBS on 7 May 2012 by Mayumi Masuda. Additional specimens of *P. ijimai* were collected at Irukabana (≈34°13'42"N, 132°23'03"E), Etajima Island, Hiroshima, Japan (Fig. 1D) on 13 February 2011 by Daisuke Ueno.

Morphological observation

Measurements of the lophophore and body size were taken from digital photographs with ImageJ 1.37v software (Rasband 1997–2011, Abramoff et al. 2004). For observation of internal morphology, specimens were dehydrated in an ethanol series, cleared in *n*-butanol, embedded in paraffin, sectioned at a thickness of 5–6 μm, and stained with hematoxylin-eosin (HE). DeltaViewer 2.1.1 software (Wada et al. 2005) was used to construct three-dimensional images of the nephridium. All the type and voucher specimens have been deposited in the National Museum of Nature and Science, Tsukuba, Japan (NSMT).

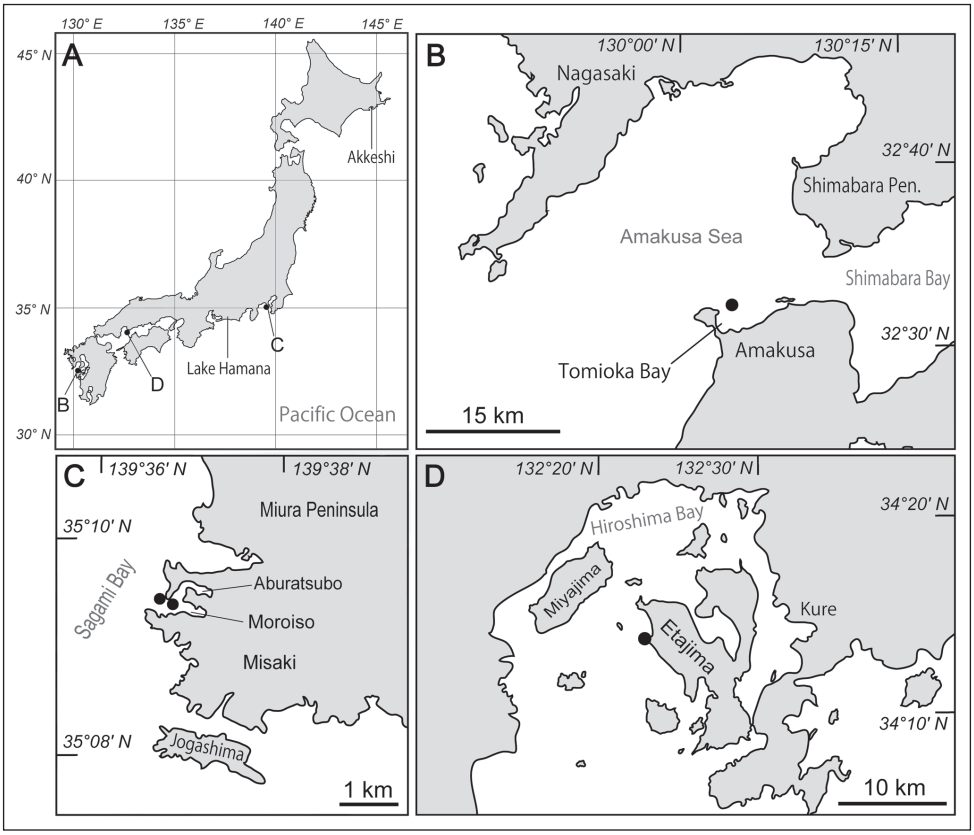

Figure 1. Maps showing the locations of collecting sites. **A** Map of Japan showing the collecting localities and the locations of Lake Hamana and Akkeshi **B** enlargement of west-central Kyushu, with the solid circle indicating the collecting site at Amakusa **C** enlargement of the southwestern part of the Miura Peninsula, with solid circles indicating the topotype collecting sites (type localities) of *P. ijimai* Oka, 1897 at Misaki, Sagami Bay **D** enlargement of Hiroshima Bay, with the solid circle indicating an additional collecting site for *P. ijimai* at Etajima.

DNA extraction and PCR amplification

Total genomic DNA was extracted from one of the ethanol-fixed specimens of the new species, as well as one of the topotypes of *P. ijimai* (NSMT-Te 881), using a DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol. The 18S gene was amplified with three primer sets: 1F/4R, 3F/18sbi, and 18Sa2.0/9R (Giribet et al. 1996, Whiting et al. 1997). The 28S fragment was amplified with primer set LSU5/LSU3 (Littlewood 1994). The COI fragment was amplified with the primer pair LCO1490/HCO2198 (Folmer et al. 1994). PCR reactions were performed with *ExTaq* (TaKaRa). Conditions for hot-start thermal cycling were 2 min at 94°C; 35 cycles of 45 sec at 94°C, 45 sec at 50°C, and 90 sec at 72°C; and 7 min at 72°C. PCR products were visualized on a 1% agarose gel and purified according to the method of

Table 1. Taxa included in the phylogenetic analyses and GenBank accession numbers for sequences. Sequences obtained in this study are in **bold**.

Species	COI	18S	28S	Reference
Phoronis emigi sp. n.	**AB621915**	**AB621913**	**AB621914**	this study
Phoronis architecta	AY368231.1	AF025946	EY334109	a (COI), b (18S), c (28S)
Phoronis australis (New Caledonia)	EU484457	AF202111	EU334110	c (COI, 28S), d (18S)
Phoronis australis (Japan)	EU484458	EU334122	EU334111	c
Phoronis australis (Australia)	—	EU334123	EU334112	c
Phoronis australis (Spain)	—	AF119079	—	e
Phoronis hippocrepia	EU484459	AF202112	AY839251	c (COI), d (18S), f (28S)
Phoronis ijimai	**AB752304**	**AB752305**	—	this study
Phoronis muelleri	EU484460	EU334125	EU334114	c
Phoronis ovalis	EU484461	EU334126	EU334115	c
Phoronis pallida	—	EU334127	EU334116	c
Phoronis vancouverensis/*ijimai*	EU484462	AF202113	AF342797	c (COI), d (18S), g (28S)
Phoronopsis californica	EU484463	EU334129	EU334118	c
Phoronopsis harmeri	EU484464	EU334130	EU334119	c
Phoronopsis viridis	EU484465	AF123308	EU334120	c
Novocrania anomala	—	AY842018	AY839245	f
Discinisca cf. *tenuis*	—	AY842020	AY839248	f
Glottidia pyramidata	—	U12647	AY839249	f (28S), h (18S)

a Helfenbein and Boore (2004); **b** Cohen et al. (1998); **c** Santagata and Cohen (2009); **d** Cohen (2000); **e** Giribet et al. (2000); **f** Cohen and Weydmann (2005); **g** Mallatt and Winchell (2002); **h** Halanych et al. (1995)

Boom et al. (1990) with some modifications (Kobayashi and Tachi 2009, Kobayashi et al. 2009). Cycle sequencing was performed with BigDye Terminator 3.1 (Life Technologies). The PCR primers were used for sequencing reactions, together with two additional 28S primers, D2F (Littlewood 1994) and a truncated version (Thollesson and Norenburg 2003) of 28z (Hillis and Dixon 1991). Both product strands were sequenced with an ABI 3130 Genetic Analyzer (Life Technologies). Chromatograms were edited and overlapping sequence fragments were assembled by using ATGC 4.0.6 (GENETYX). The sequences have been deposited with DDBJ/EMBL/GenBank under accession numbers AB621913–AB621915 for the new species and AB752304–AB752305 for *P. ijimai* (Table 1).

Morphological analyses

From the literature (Emig 1974, Santagata and Cohen 2009) and our own data, we tabulated 32 morphological and reproductive characters (Suppl. material 1) among 11 phoronid species. Based on this data matrix, we performed three different analyses using Mesquite version 2.75 (Maddison and Maddison 2011): 1) a cluster analysis with

single-linkage method based on distances between taxa calculated from the data matrix; 2) a morphology-based cladistic analysis; and 3) a most-parsimonious reconstruction of ancestral characters. For the cladistic analysis, a heuristic search was conducted with tree length criterion and rearrangement by subtree pruning and regrafting (SPR); all trees were rooted with *Phoronis ovalis* Wright, 1856 as the outgroup based on the results of Santagata and Cohen (2009). The ancestral character reconstruction was carried out based on the maximum-likelihood tree based on concatenated COI–18S–28S dataset (see below) for the 21 adult morphological characters.

Molecular phylogeny

We checked validity of the yielded COI sequences to prevent the isolation of nuclear encoded mitochondrial psuedogenes (NUMTS) instead of true mitochondrial sequences before phylogenetic analyses. We regarded the consistently yielded fine single peaks for all the analysed sites in chromatograms and including neither indel nor stop codon as the criteria for judging the safely rejection of the possibility for the contamination of NUMTS.

The COI, 18S, and 28S sequences obtained for the new species were aligned with those from other phoronids deposited in GenBank (Table 1) using Clustal W (Thompson et al. 1994) implemented in Seaview 4.2.5 (Gouy et al. 2010) and/or MEGA 5.05 (Tamura et al. 2011). The alignment was performed gene by gene, before concatenated data sets were generated. These sequences were analyzed both independently and as concatenated data sets.

Maximum likelihood (ML) analyses was performed with MEGA 5.05. For ML, the best-fit model for all data sets determined by the AICc implemented in MEGA 5.1 was GTR+G+I (general time reversible [Tavaré 1986] with gamma-distributed rates and invariant rates among sites). Optimal ML trees were found by a nearest neighbor interchanges (NNI) search, starting with a tree topology generated by the BIONJ method (Gascuel 1997) using maximum composite likelihood (MCL) distances (Tamura et al. 2004). One-thousand bootstrap pseudoreplicates were analyzed to obtain nodal support values.

Bayesian analyses were performed by using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The best-fit substitution model was GTR+G+I model, determined from AICc tests in MrModeltest 2.3 (Nylander 2004) and PAUP* 4.0b10 (Swofford 2003). A Markov-Chain Monte-Carlo (MCMC) search was performed with four chains, each of which was run for 1,000,000 generations. Trees were sampled every 100 generations, and those from the first 250,000 generations were discarded as burn-in, ensuring that a stable likelihood had been reached. Trace files generated by Bayesian MCMC runs were inspected in TRACER 1.5.0 (Rambaut and Drummond 2007) to check that the number of sampling generations and effective sample sizes were large enough for reliable parameter estimates. A consensus of sampled trees was computed, and the posterior probability for each interior node was obtained to assess the robustness of the inferred relationships.

The 18S and 28S trees were rooted with three brachiopods (*Novocrania anomala*, *Discinisca* cf. *tenuis*, and *Glottidia pyramidata*) as outgroup taxa (Cohen and Weydmann 2005, Halanych et al. 1995). The COI tree was rooted with *Phoronis ovalis* Wright, 1856 as the outgroup based on the results of Santagata and Cohen (2009).

Since most of the sequences used in this study were obtained from GenBank, we used the original specific names in GenBank given by the previous authors (Halanych et al. 1995, Cohen et al. 1998, Cohen 2000, Giribet et al. 2000, Mallatt and Winchell 2002, Helfenbein and Boore 2004, Cohen and Weydmann 2005, Santagata and Cohen 2009) in Table 1. However, to make the discussion clear, we also indicate taxonomically valid specific names in our results and discussion, i.e., *Phoronis ijimai* instead of *Phoronis vancouverensis*, *Phoronis psammophila* instead of *Phoronis architecta*, and *Phoronopsis harmeri* instead of *Phoronopsis viridis*.

Taxonomy

***Phoronis ijimai* Oka, 1897**
[Japanese name: Hime-houkimushi]
http://species-id.net/wiki/Phoronis_ijimai
Figures 2–7

Phoronis ijimai Oka, 1897, 147–148.
Phoronis vancouverensis Pixell, 1912, 257–271, figs 1–5.
Phoronis svetlanae Temereva & Malakov, 1999, 627–630, figs 1, 3, 4.
?*Phoronis hippocrepia*: Uchida and Iwata 1955, 1–3, text-figs 1, 2, pl. 1, figs A–D.

Material examined. Five series of transverse sections and 34 whole specimens. NSMT-Te 878, several specimens, fixed and preserved in 10% formalin, collected at Etajima Island; NSMT-Te 879, several individuals, fixed and preserved in 10% formalin, collected in Moroiso Bay, attached to the pier in front of MMBS; NSMT-Te 880, several individuals on a living shell of *Barbatia* sp. (Mollusca: Bivalvia), collected in Sagami Bay; NSMT-Te 881, same data as NSMT-Te 879; NSMT-Te 882, same data as NSMT-Te 880; NSMT-Te 883, 6-µm transverse section stained with HE, collected at Etajima Island; NSMT-Te 884, same data as NSMT-Te 883; NSMT-Te 885, 6-µm transverse sections stained with HE, collected in Moroiso Bay; NSMT-Te 886, same data as NSMT-Te 885; NSMT-Te 887, 6-µm transverse sections stained with HE, collected in Sagami Bay.

Description. Body except lophophore 2.40–16.83 mm in length (avg. 5.87±4.04 mm, n = 34; average of topotypes 9.55±4.78 mm, n = 12); 0.49–0.90 mm in diameter at ampula (avg. 0.64±0.11 mm, n = 34; average of topotypes 0.59±0.12 mm, n = 12); white and translucent in living state (Figs 2A, 2B, 3), yellowish white after fixation (Fig. 2C). Lophophore horseshoe-shaped, without significant coiling (Fig. 4); 0.87–3.11 mm in length (avg. 2.17±0.55 mm, n = 34; average of topotypes 1.66±0.49 mm, n = 12), 0.27–0.99 mm in diameter at its base (avg. 0.61±0.17 mm, n = 34; avg.

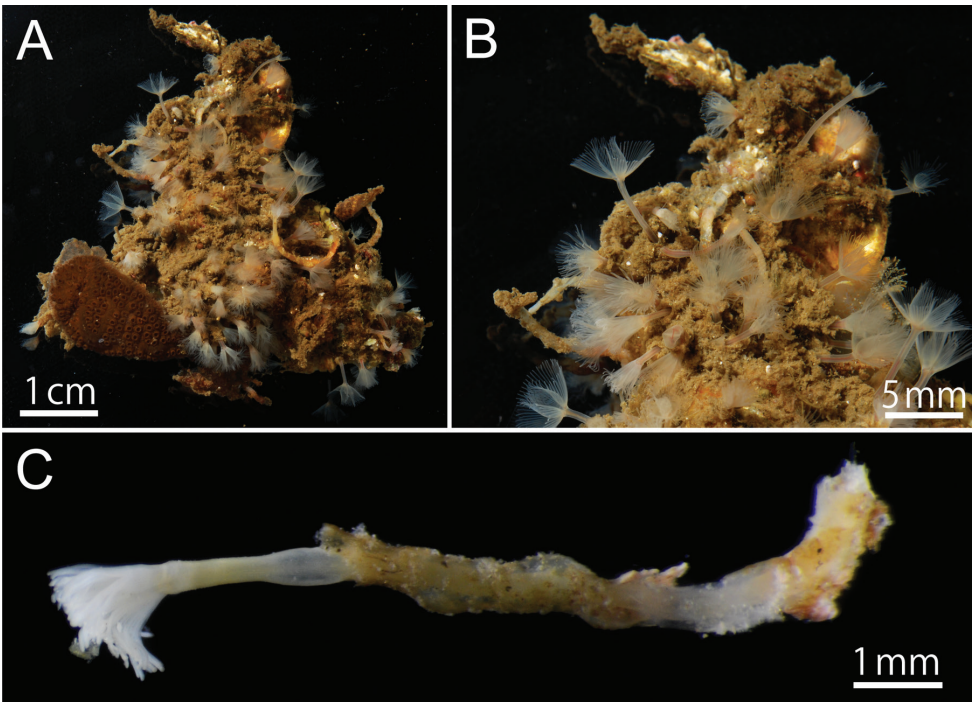

Figure 2. *Phoronis ijimai* Oka, 1897, NSMT-Te 879. **A** Living individuals collected from the pier of Misaki Marine Biological Station **B** enlargement of living individuals **C** preserved individual (10% formalin seawater) with a transparent cylindrical tube.

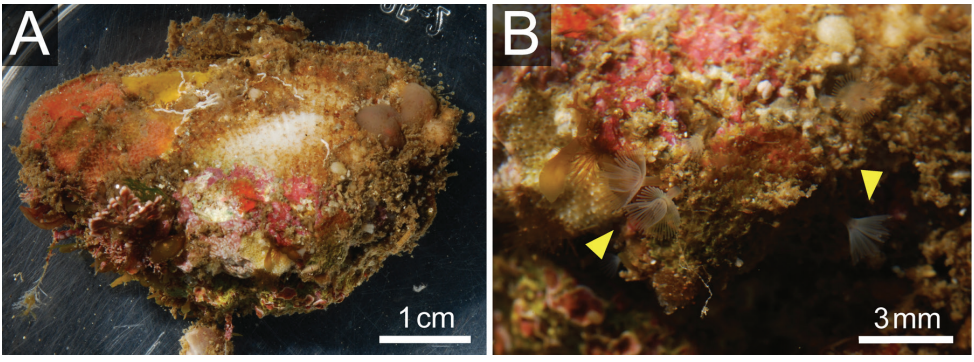

Figure 3. *Phoronis ijimai* Oka, 1897, NSMT-Te 880. **A** Living bivalve (*Barbatia* sp.) with various sessile organisms **B** Living *Phoronis ijimai* on the shell (arrowheads).

of topotypes 0.43±0.09 mm, n = 12); tentacles 106–151 in number (avg. 129±18, n = 7; avg. of topotypes 110±5, n = 3). Inhabits a transparent cylindrical tube either encrusting or burrowing in hard substrates (Fig. 2C).

Nephridium 162.00–204.00 μm in height (avg. 183.00±29.70 μm, n = 2), with straight nephridial papilla and curved ascending branch (Fig. 5A, 5B). Descending

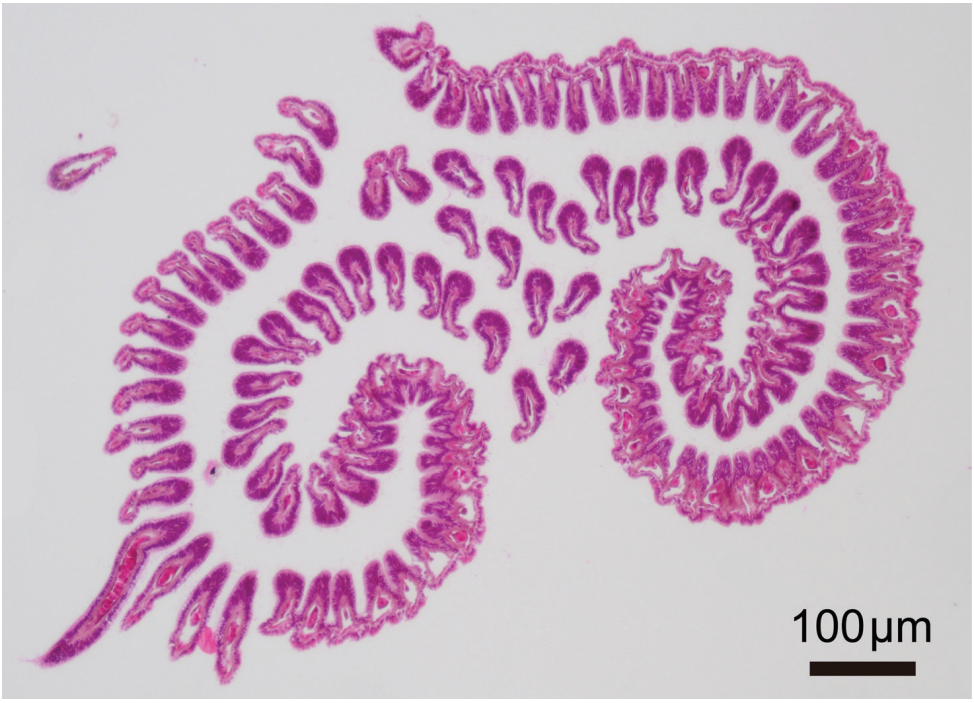

Figure 4. *Phoronis ijimai* Oka, 1897, NSMT-Te 885, transverse section through basal part of lophophore.

branch absent. Ascending branch with single chamber. Nephridial papilla situated beside anus, 294.24–324.91 µm in length (avg. 309.57±21.69 µm, n = 2); nephridiopore situated on nephridial papilla opening above (in living orientation) anus level (Fig. 5A, 5C). Ascending branch offset along body axis near intestine, with its lower end extending toward esophagus (Fig. 5B, 5D); 277.55–323.49 µm in length (avg. 300.52±32.49 µm, n = 2). Two nephridial funnels present; anal funnel larger than oral funnel. Anal funnel large (avg. 69.00±4.24 µm in height, 45.77±3.15 µm in width at base, 111.94±16.48 µm in maximum width at tip; n = 2), its aperture located at lower end of ascending branch. Oral funnel small (avg. 20.01±1.40 µm in diameter, n = 2), its aperture opening on lateral surface of ascending branch, situated slightly lower than anal funnel.

Body-wall longitudinal muscles of generally bushy type (Fig. 6A, 6B) but sometimes feathery in lower part of body; 45–50 in number, arranged in following formula (Selys-Longchamps 1907):

Composite formula Mean formula

$$[45\text{–}53]\ \frac{14-16}{5-9}\left|\frac{17-24}{5-9}\right. \qquad 49=\frac{15.0}{6.7}\left|\frac{20.9}{6.4}\right.$$ (n = 7 sections from 3 individuals)

Left and right lateral mesenteries present (Fig. 6A). Two giant nerve fibers present; left giant nerve fiber 3.16–10.61 µm in diameter (avg. 6.72±3.27 µm,

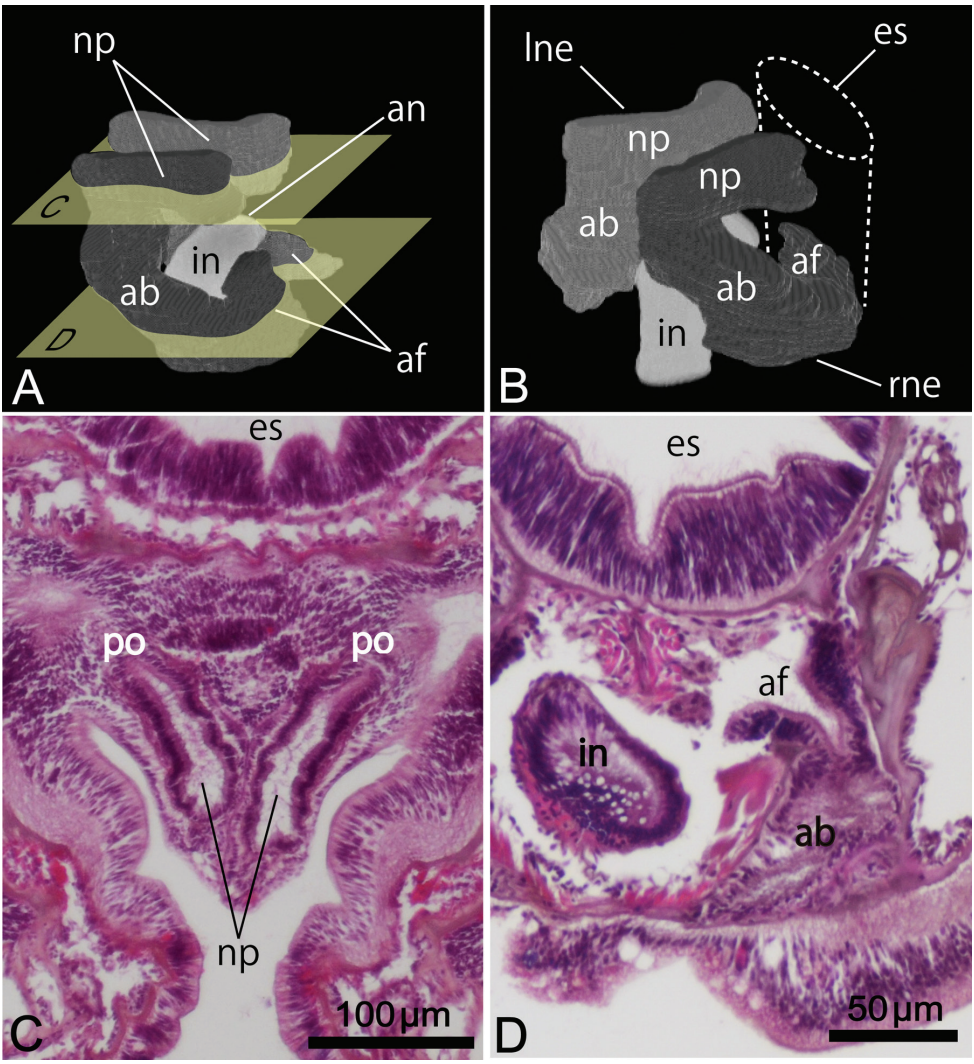

Figure 5. Reconstructed three-dimensional images and transverse sections of the nephridium of *Phoronis ijimai* Oka, 1897, from NSMT-Te 886 (**A, B, D**) and NSMT-Te 884 (**C**). **A** Lateral view, showing the long nephridial papillae above the anus **B** dorsolateral view, showing the offset arrangement of the nephridia, with the curved ascending branch and large anal funnel extending toward the esophagus **C** transverse section through the nephridial papilla, showing the nephridiopore **D** transverse section through the ascending branch, showing the large anal funnel opening toward the esophagus, Abbreviations: **ab** ascending branch; **af** anal funnel; **an** anus; **es** esophagus; **in** intestine; **lne** left nephridium; **np** nephridial papilla; **p** nephridiopore; **rne** right nephridium. Planes **C** and **D** in panel **A** indicate the positions of the transverse sections in **C** and **D**.

based on eight sections from different parts of the body, from two individuals), situated at base of left lateral mesentery (Fig. 6C); right giant nerve fiber 2.47–7.81 µm in diameter (avg. 4.55±2.15 µm, based on nine sections of different parts of the

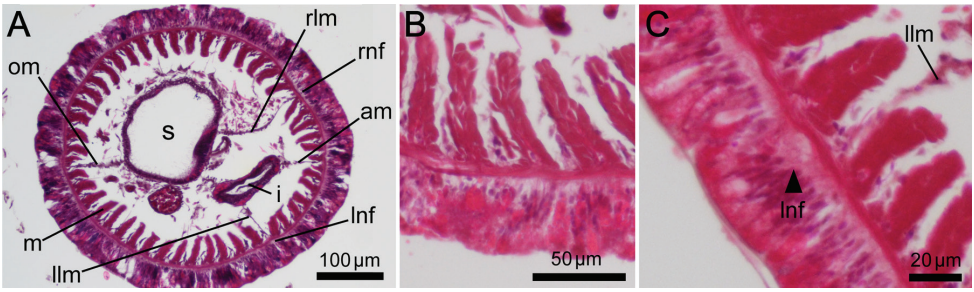

Figure 6. *Phoronis ijimai*. **A** NSMT-Te 886, transverse section through the posterior part of the body, showing four mesenteries and the position of the giant nerve fibers **B** NSMT-Te 885, enlargement showing longitudinal muscles of the bushy type **C** NSMT-Te 885, enlargement of the left giant nerve fiber situated at the base of the left lateral mesentery. Abbreviations: **am** anal mesentery; **i** intestine; **llm** left lateral mesentery; **lnf** left giant nerve fiber; **m** longitudinal muscle; **om** oral mesentery; **rlm** right lateral mesentery; **rnf** right giant nerve fiber; **s** stomach.

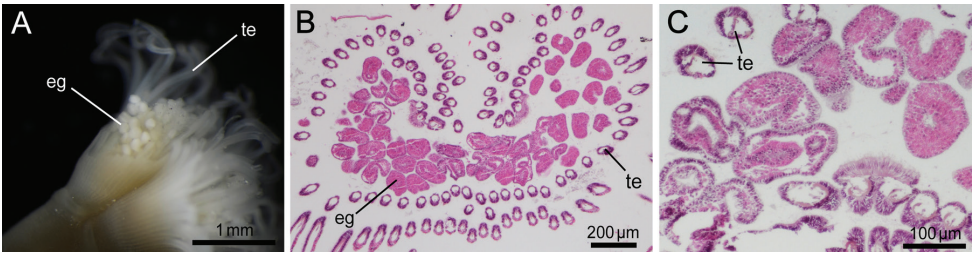

Figure 7. *Phoronis ijimai*. **A** NSMT-Te 878, eggs brooded in the lophophore (some tentacles have been removed) **B** NSMT-Te 884, transverse section through the basal part of the lophophore, showing mature eggs on the basal nidamental glands **C** NSMT-Te 883, enlargement of brooded eggs, showing various developmental stages. Abbreviations: **eg** egg; **te** tentacle.

body from two individuals), situated at base of right lateral mesentery. Esophageal valve absent.

Hermaphroditic; early-stage ova and spermatocytes found beside lateral blood vessel. Brooded eggs observed in specimens from Hiroshima (Fig. 7A, 7B, 7C); embryos of various developmental stages brooded on basal nidamental glands on lophophore (Fig. 7C).

Distribution and habitat. *Phoronis ijimai* is widely distributed in the North Pacific, along the coasts of North America, Canada, Japan, and Russia, including the Sea of Japan (Emig 1971a, 1974, Emig and Golikov 1990, Temereva and Malakhov 1999). *Phoronis ijimai* has been reported from hard substrates such as rocks, bivalve shells, and wood, and also from a sandy bottom; it often forms dense populations, up to about 15,000 individuals per m^2 (Emig 1974).

Remarks. Our topotype material of *P. ijimai* collected from Misaki perfectly agrees with previous morphological accounts of this species (Oka 1897, Emig 1971a, 1974) in the following characters: 1) the long nephridial papilla and the large anal funnel of

the nephridium, 2) the small diameter of the two giant nerve fibers, 3) the number of longitudinal muscles in the right oral and both anal coeloms, and 4) the brooding of embryos on lophophoral organs. These characters also agree with the description of *P. hippocrepia*, but differ in 1) the large number of longitudinal muscles in the right oral coelom, and 2) the single chamber in the ascending branch of the nephridium. Our topotypes of *P. ijimai* also match the description of *P. vancouverensis* (Pixell 1912, Emig 1971a, 1974). While our specimens have slightly fewer longitudinal muscles in the right anal and left oral coeloms compared to the original description of *P. vancouverensis* by Pixell (1912) and the revised description of *P. ijimai* by Emig (1974), respectively, the numbers are within the range of variation in *P. ijimai* (Emig 1974). The topotypes had fewer tentacles, probably due to the smaller size of the body and lophophore.

***Phoronis emigi* sp. n.**

[New Japanese name: Amakusa-houkimushi]
http://zoobank.org/51F10DA8-DE79-4537-86E7-DE2F1CBC1B56
http://species-id.net/wiki/Phoronis_emigi
Figures 8–11

Material examined. Eleven series of transverse sections and two series of longitudinal sections, and nine whole specimens. *Holotype*: NSMT-Te 714, 5-μm transverse sections stained with HE. *Paratypes*: NSMT-Te 703–708, seven intact specimens, fixed and preserved in 10% formalin seawater; NSMT-Te 711–713, 715–721, 5-μm transverse sections stained with HE; and NSMT-Te 722, 723, 5-μm longitudinal sections stained with HE. *Other material examined*: NSMT-Te 709, 710, two intact specimens.

Etymology. The specific name, a masculine noun in the genitive case, is in honor of the French researcher Dr. Christian C. Emig for his remarkable contributions to lophophorate systematics.

Description. Body except lophophore 4.42–20.06 mm in length (holotype 9.67 mm; avg. 10.87±4.70 mm, n = 10); 0.34–0.66 mm in diameter at ampula (holotype 0.39 mm; avg. 0.47±0.10 mm, n = 9); reddish in living state, yellowish white after fixation (Fig. 8). Lophophore horseshoe-shaped, without significant coiling (Fig. 9); 2.00–3.51 mm in length (holotype 3.18 mm; avg. 2.77±0.52 mm, n = 10), 0.54–0.76 mm in diameter at base (holotype 0.68 mm; avg. 0.67±0.07 mm, n = 10); tentacles 136–170 in number (holotype 137; avg. 147±13.17, n = 6).

Nephridium 205.00–324.00 μm in length (holotype 310 μm; avg. 276.78±38.69 μm, n = 5), with straight ascending branch (ab) and short descending branch (db) (Fig. 10A), ab/db length ratio 3.5 (n = 5). Ascending branch with single chamber (Fig. 10C). Nephridiopore situated on anal papilla. Tip of ascending branch (i.e., nephridiopore) lying against intestine. Nephridia slightly offset along body axis (Fig. 10B); left nephridiopore lower (in living orientation) than anus, right nephridiopore same level as anus. Single nephridial funnel present, with aperture at tip of descending branch (Fig. 10D).

Figure 8. *Phoronis emigi* sp. n., NSMT-Te 714 (holotype), photographed in the preserved state (10% formalin seawater) before sectioning.

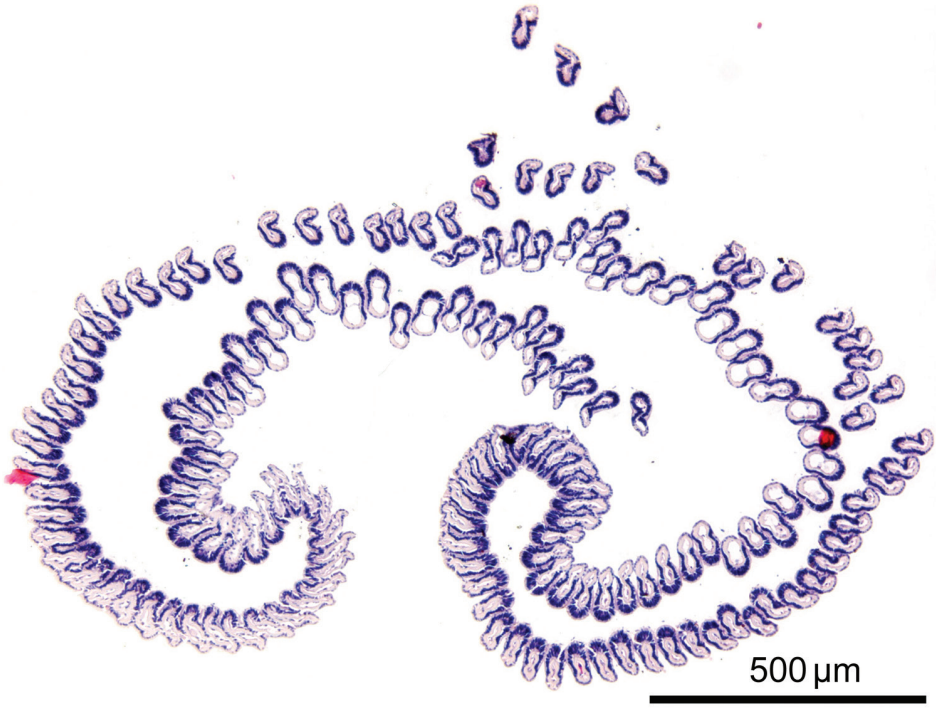

Figure 9. *Phoronis emigi* sp. n., NSMT-Te 713 (paratype), transverse section through the basal part of the lophophore.

Body-wall longitudinal muscles of feathery type (Fig. 11A, 11B); 56–72 (holotype 67) in number, arranged in following formula (Selys-Longchamps 1907):

Composite formula Mean formula

$$[56\text{–}72]\ \frac{18-23}{10-13}\Big|\frac{16-24}{11-13} \qquad 64.3 = \frac{20.4}{11.3}\Big|\frac{20.6}{11.9}$$ (n = 74 sections from 7 individuals)

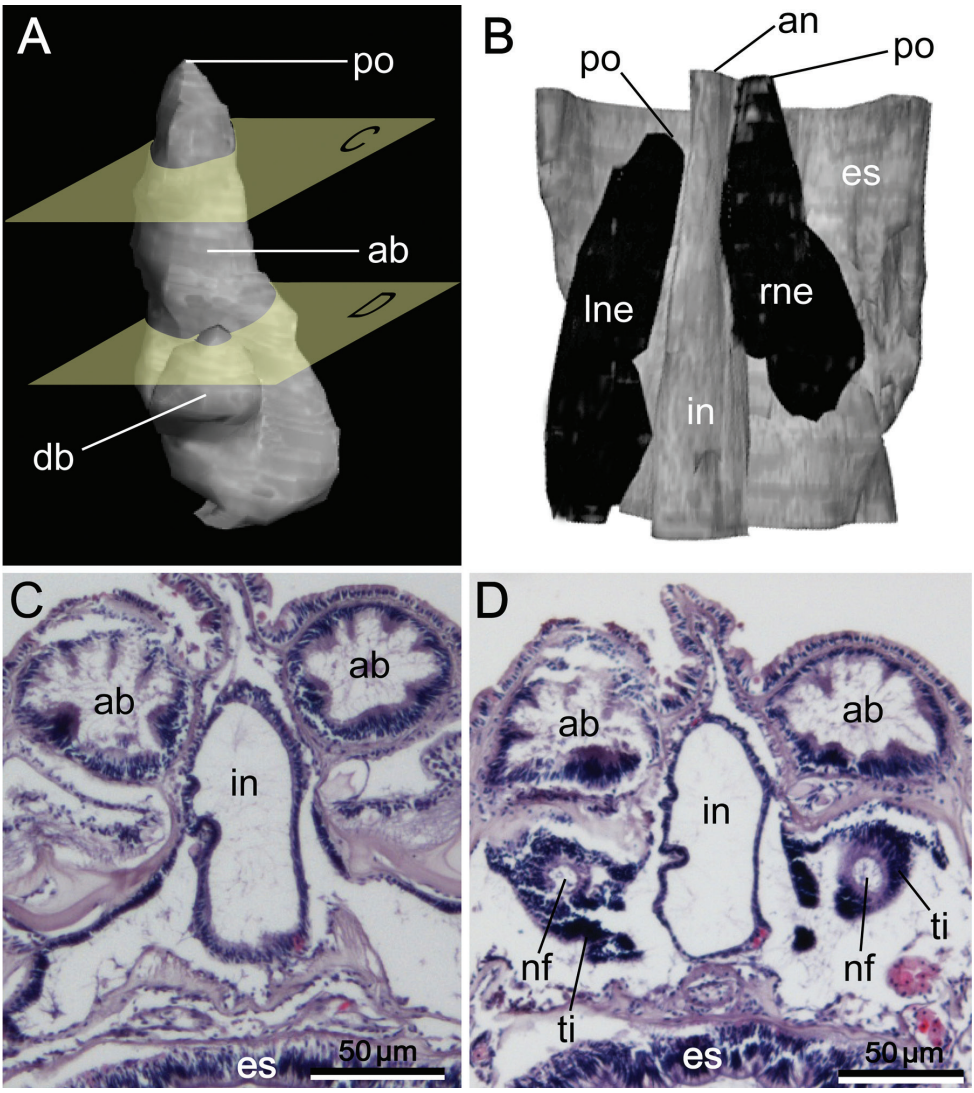

Figure 10. Reconstructed three-dimensional images and transverse sections of the nephridium of *Phoronis emigi* sp. n., based on NSMT-Te 721 (paratype). **A** Lateral view, showing the different lengths of the ascending and descending branches **B** dorsal view, showing the offset arrangement of the nephridia, with the nephridiopores at different levels along the body axis **C** transverse section through the ascending branch **D** transverse section through the tip of the descending branch, showing the nephridial funnels. Abbreviations: **ab** ascending branch; **an** anus; **db** descending branch; **es** esophagus; **in** intestine; **lne** left nephridium; **nf** nephridial funnel; **p** nephridiopore; **rne** right nephridium; **ti** funnel tissue. Planes **C** and **D** in panel **A** indicate the positions of the transverse sections in **C** and **D**.

Left and right lateral mesenteries present (Fig. 11A). Single giant nerve fiber, 15.98–36.03 µm in diameter (holotype avg. 27.40±6.29 µm, based on 5 sections from different parts of the body; avg. 25.93±6.05, based on 11 sections from different parts of the

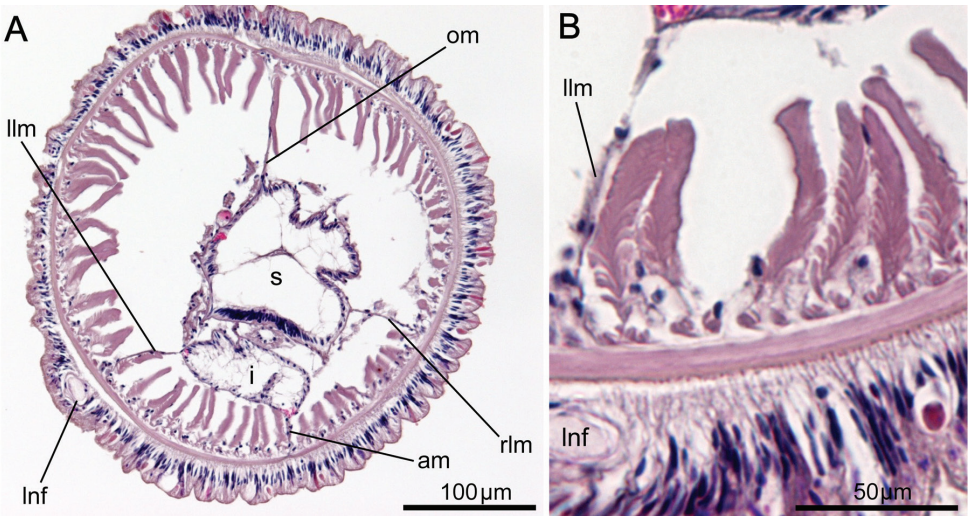

Figure 11. *Phoronis emigi* sp. n., NSMT-719 (paratype). **A** Transverse section through the posterior part of the body, showing four mesenteries and the position of the giant nerve fiber **B** enlargement of longitudinal muscles of the long feathery type. Abbreviations: **am** anal mesentery; **lnf** left giant nerve fiber; **i** intestine; **llm** left lateral mesentery; **om** oral mesentery; **rlm** right lateral mesentery; **s** stomach.

body, from five individuals [5 sections from holotype and 6 sections from 4 paratypes]), situated at base of left lateral mesentery (Fig. 11A, 11B). Esophageal valve absent.

Gonads not observed in any of our specimens; sex could thus not be determined.

Distribution and habitat. *Phoronis emigi* is known only from a sandy bottom in northern Tomioka Bay, Amakusa, Japan, where we detected densities of up to about 90 individuals per 100 cm^2. We observed no chitinous tubes after agitation and decantation during sampling, but the tubes would be fragile and might have been lost.

Remarks. *Phoronis emigi* sp. n. is morphologically most similar to *P. psammophila* Cori, 1889, with which it has in common 1) a long ascending branch of nephridium that is more than three times the length of the descending branch, 2) a single nephridial funnel, with the aperture situated at the tip of the descending branch, 3) a single giant nerve fiber situated on the left side, and 4) two lateral mesenteries. *Phoronis emigi* differs from *P. psammophila* in the number of longitudinal muscle bundles in the body wall (56–72 vs. 25–50 in *P. psammophila*) and the position of the right nephridiopores (at the same level as the anus vs. lower than the anus in *P. psammophila*) (cf. Andrews 1890, Selys-Longchamps 1907, Marsden 1959, Long 1960, Emig 1968, 1971b, 1979).

Naturally, *P. emigi* is morphologically similar to, but distinct from, the nominal *Phoronis architecta* Andrews, 1890, which is regarded as a junior synonym of *P. psammophila* (Emig 1971b, 1974). Based on the descriptions by Andrews (1890) and Brooks and Cowles (1905), Emig (1971b, 1974) noticed that *P. psammophila* and *P. architecta* are morphogically identical, with the exception of the differences in larval brooding type and the presence of nidamental gland. Subsequently, Emig (1977) found that *P. psammophila* shows a sympatric occurrence with *Phoronis muelleri* in the type locality of *P. architecta*;

therefore, he concluded that the larval brooding type and the absence of nidamental gland of *P. architecta* described in Brooks and Cowles (1905) came from a specimen of *P. muelleri*. On the other hand, some researchers have suggested the need of reexamination of the synonymy (Stancyk et al. 1976, Santagata and Zimmer 2002). Although we could not observe the larval brooding type of *P. emigi*, the present species is clearly different from any of these species, *P. psammophila*, *P. muelleri*, and nominal *P. architecta*, in the adult morphologies such as number of longitudinal muscle bundles.

The lack of gonads in our specimens was probably due to breeding seasonality. The breeding period of phoronid species previously studied is generally from spring to autumn (Rattenbury 1953, Emig 2003), whereas our material was collected at the end of November. Our specimens were likely in the post-breeding condition, following spawning and the relaease of embryos.

Morphological analyses

In the resulting cladogram from the cluster analysis (Fig. 12A), three major clades were retrieved: 1) *Phoronopsis harmeri* + *Ph. californica* + *Ph. albomaculata*; 2) *Phoronis emigi* + *P. psammophila* + *P. muelleri* + *P. pallida*; and 3) *P. hippocrepia* + *P. ijimai* + *P. australis*.

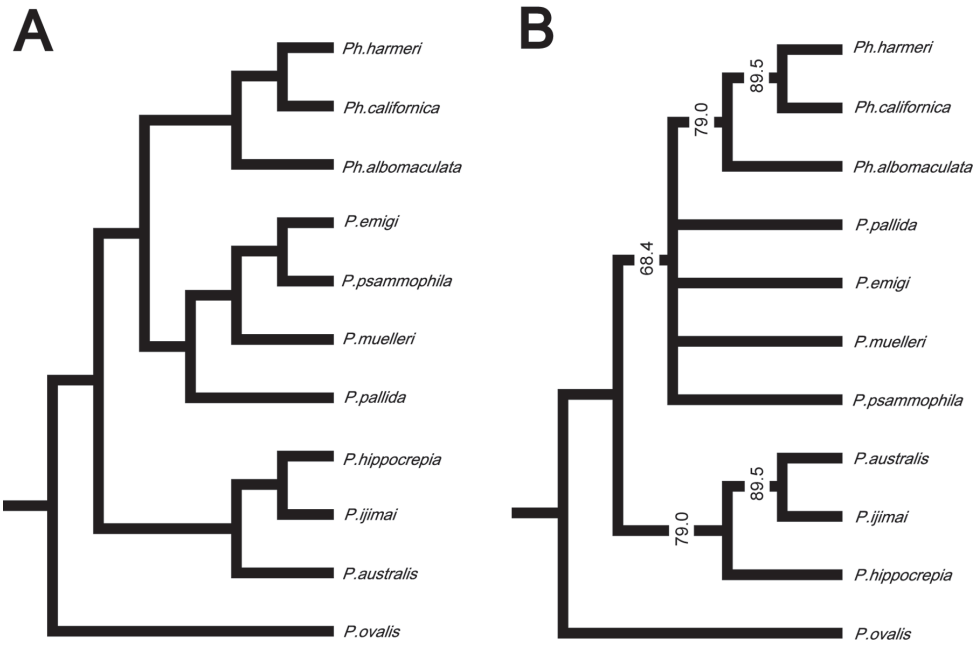

Figure 12. A Cladogram of single-linkage cluster analysis among 11 phoronid species based on 32 morphological characters **B** majority-rule consensus tree of 57 equally parsimonious tree obtained by cladistic analysis among 11 phoronid species based on 32 morphological characters. Numerals on nodes indicate frequency values.

It shows the morphological similarity of the new species *P. emigi* with *P. psammophila*, sharing 16 adult morphological characters. *Phoronis emigi* also resembles *P. muelleri* and *P. pallida*, with which it shares 15 and 12 characters, respectively (Fig. 12A; Suppl. material 1). We conducted another cluster analysis without nephridial characters (eliminating character 6–14 in Suppl. material 1) to test the influence of the large amount of nephridial characters. In the resulting cladogram (Appendix 1 - Supplementary Fig. S1A), the same three major clades mentioned above were also obtained, although the topology between/within the three clades changed.

Our cladistic analysis yielded 57 equally parsimonious trees. The majority-rule consensus tree of those (Fig. 12B) did not resolve the relationship between *P. emigi*, *P. psammophila*, *P. muelleri*, and *P. pallida*; these four species formed a large clade together with *Phoronopsis* spp., with low consensus frequency value (68.4%). Another clade including three species (*P. australis* + *P. ijimai* + *P. hippocrepia*) appeared as a sister group to this large clade; *P. australis* formed a clade with *P. ijimai* (89.5% in consensus frequency), to which *P. hippocrepia* was the sister taxon (79.0% in consensus frequency). A parsimony tree without nephridial characters (Appendix 1 - Supplementary Fig. S1B) was almost identical to the tree including nephridial characters, except that *P. emigi* appeared as sister to *Phoronopsis* (85.0% in consensus frequency), and *P. ijimai* formed a clade with *P. hippocrepia* (67.0% in consensus frequency).

Molecular phylogeny

In this study, most of the sites for both 18S and 28S were unambiguously aligned; therefore, we used the entire region excluding gap sites for our phylogenetic analyses. For the COI dataset, we used all the codon positions in our phylogenetic analyses.

The 18S dataset comprised 1756 bp aligned sites, with 208 variable sites, for 15 ingroup taxa. In the resulting ML tree (Fig. 13A) (log L = –4104.32), not all nodes are resolved or well supported. *Phoronis emigi* appears in a polytomous clade along with *P. architecta* (= *psammophila*) and a large, weakly supported clade that includes *P. ijimai* and nominal "*P. vancouverensis*" from California. Japanese *P. ijimai* is the sister taxon to nominal "*P. vancouverensis*" from California, with high nodal support (100/1.0). These species are embedded in a clade otherwise containing only *P. australis* from various localities, with Spanish *P. australis* the sister taxon to the *ijimai*/"*vancouverensis*" clade (nodal support, - /0.96). The Bayesian tree (log L = –4371.60) was identical in topology to the ML tree.

The 28S dataset comprised 1065 bp aligned sites, with 333 variable sites, for 13 ingroup taxa. Most nodes in the ML tree (Appendix 1 - Supplementary Fig. S2) (log L = –3898.29) are resolved, and many have high nodal support. *Phoronis emigi* forms a clade with *P. australis* from New Caledonia with moderate to high nodal support (97/0.71). *Phoronis australis* appears as polyphyletic, with nominal "*P. vancouverensis*" comprising the sister taxon to a well-supported but polytomous clade containing *P. australis* from Australia and Japan, and *P. muelleri*. We did not obtain a 28S sequence

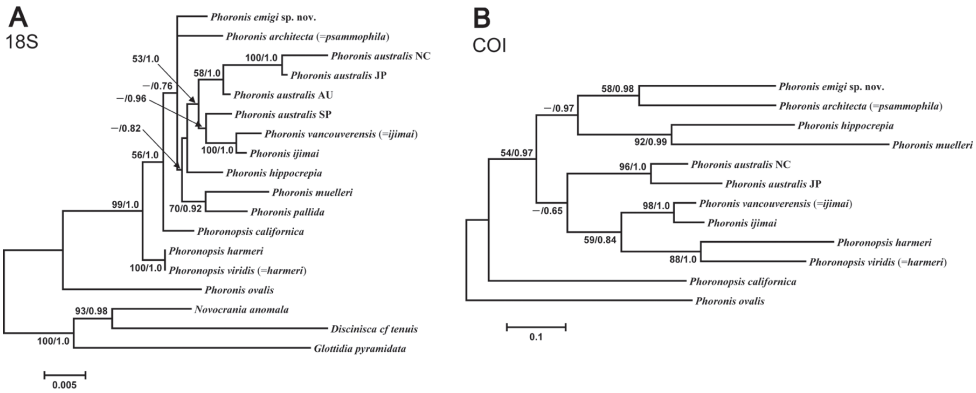

Figure 13. A Maximum-likelihood tree for 15 phoronid samples based on 18S data; three brachiopod species (*Novocrania anomala*, *Discinisca* cf. *tenuis*, and *Glottidia pyramidata*) are included as outgroup taxa **B** maximum-likelihood tree for 12 phoronid samples based on COI data; the tree is rooted with *Phoronis ovalis*. The scale bars indicate branch length in substitutions per site. Nodal support values are presented as the ML bootstrap value followed by the Bayesian posterior probability; only values >50% and 0.50, respectively, are shown.

for *P. ijimai*, which is thus missing from this analysis. The resulting Bayesian tree (log L = –4601.76) is topologically identical with the ML tree, but the clade containing *P. emigi* and New Caledonian *P. australis* is supported by lower Bayesian posterior probability (0.71).

The COI dataset comprised 621 bp aligned sites, with 253 variable sites, for 12 ingroup taxa (the tree was rooted with *P. ovalis*, which was the basal phoronid in all trees rooted with brachiopods). The resulting ML tree (Fig. 13B) (log L = –3633.85) is completely resolved, but with variable nodal support. The sister taxon to *Phoronis emigi* is *P. architecta* (= *psammophila*) rather than New Caledonian *P. australis* as in the 28S ML tree. The two *P. australis* samples inlcuded in the analysis form a clade with high support (96/1). *Phoronis ijimai* and nominal "*P. vancouverensis*" group together with high support (98/1), with this clade forming the sister group (nodal support, 59/0.84) to (*Phoronopsis harmeri* + *Ph. viridis*). *Phoronopsis* appeared polyphyletic, with *Ph. californica* the sister taxon to all other phoronids except *P. ovalis*. The resulting Bayesian tree (log L = –3772.71) was identical in topology to the ML tree.

The concatenated 18S–28S dataset comprised 2819 bp aligned sites, with 537 variable sites, for 13 ingroup taxa. The ML tree (Fig. 14A) (log L = –8247.64) was identical in topology to the 28S ML tree (Appendix 1 - Supplementary Fig. S2), except the unresolved trichotomy of AU and JP *P. australis* and *P. muelleri* in the latter is resolved in the 18S-28S tree. The Bayesian tree (log L = –9181.86) differs from the ML tree in that *P. emigi* forms a clade with *P. hippocrepia*, with New Caledonian *P. australis* the sister group to this clade.

The concatenated 18S–28S–COI dataset comprised 3440 bp aligned sites, with 555 variable sites, for 11 ingroup taxa (the tree was rooted with *P. ovalis*). The resulting ML

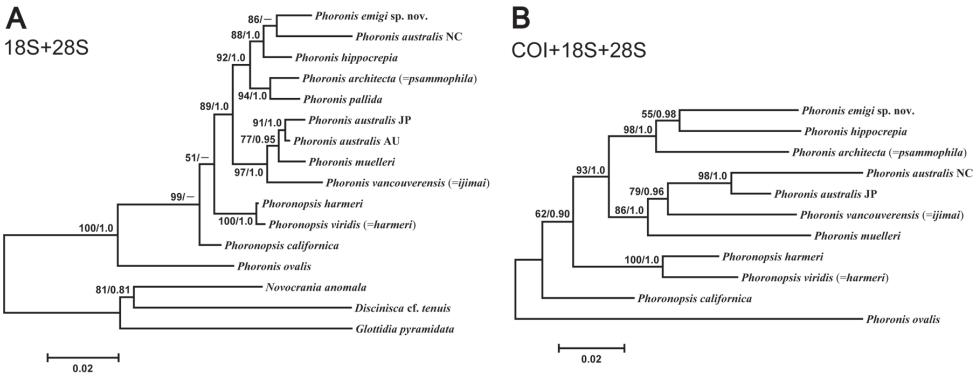

Figure 14. A Maximum-likelihood tree for 13 phoronid samples based on the combined 18S + 28S data set; three brachiopod species (*Novocrania anomala*, *Discinisca* cf. *tenuis*, and *Glottidia pyramidata*) are included as outgroup taxa **B** maximum-likelihood tree for 11 phoronid samples based on the combined COI + 18S + 28S data set; the tree is rooted with *P. ovalis*. Scale bars indicate branch length in substitutions per site. Nodal support values are presented as the ML bootstrap value followed by the Bayesian posterior probability; only values >50% and 0.50, respectively, are shown.

tree (Fig. 14B) (log *L* = –10594.85) differs from the 28S and 18S–28S trees in several ways. The sister taxon to *P. emigi* is *P. hippocrepia* (nodal support, 55/0.98) rather than New Caledonian *P. australis*. The positions of New Caledonian *P. australis* and *P. architecta* (= *psammophila*) are different in the 18S–28S–COI ML tree, but these changes in topology appear to some extent due to the omission of *P. pallida* from the 18S–28S–COI dataset. The topology within the "*P. vancouverensis*" / *P. australis* / *P. muelleri* clade also differs between 18S–28S–COI ML and the other trees that include 28S. The 18S–28S–COI Bayesian tree (log *L* = –10802.56), was identical to the ML tree in topology.

Discussion

Before our study, three species of phoronids had been recorded from Japan: *Phoronis ijimai*, *P. australis*, and *P. psammophila*. The former two were reported from Misaki (Oka 1897, Ikeda 1902), and the latter from Lake Hamana (Hirose et al. 2011). *Phoronis ijimai* was also reported from Akkeshi under the name *P. hippocrepia* (Uchida and Iwata 1955), but the taxonomic identity of this population is uncertain (Hirose et al. 2011). Bailey-Brock and Emig (2000) listed Tokyo Bay as a locality for *P. pallida*, with the note "coll. T. Furota", although they did not include any other details about the specimens. The known phoronid diversity in Japan thus remains low, with all specimens reported from sandy substratum. Investigations on rocky shores may yield additional species in the future.

Although the molecular phylogenetic trees (Figs 13A, 13B, 14A, 14B; Appendix 1 - Supplementary Fig. S2) produced by the various datasets differed in topology, our phylogenetic reconstructions suggest that most of the adult morphological characters

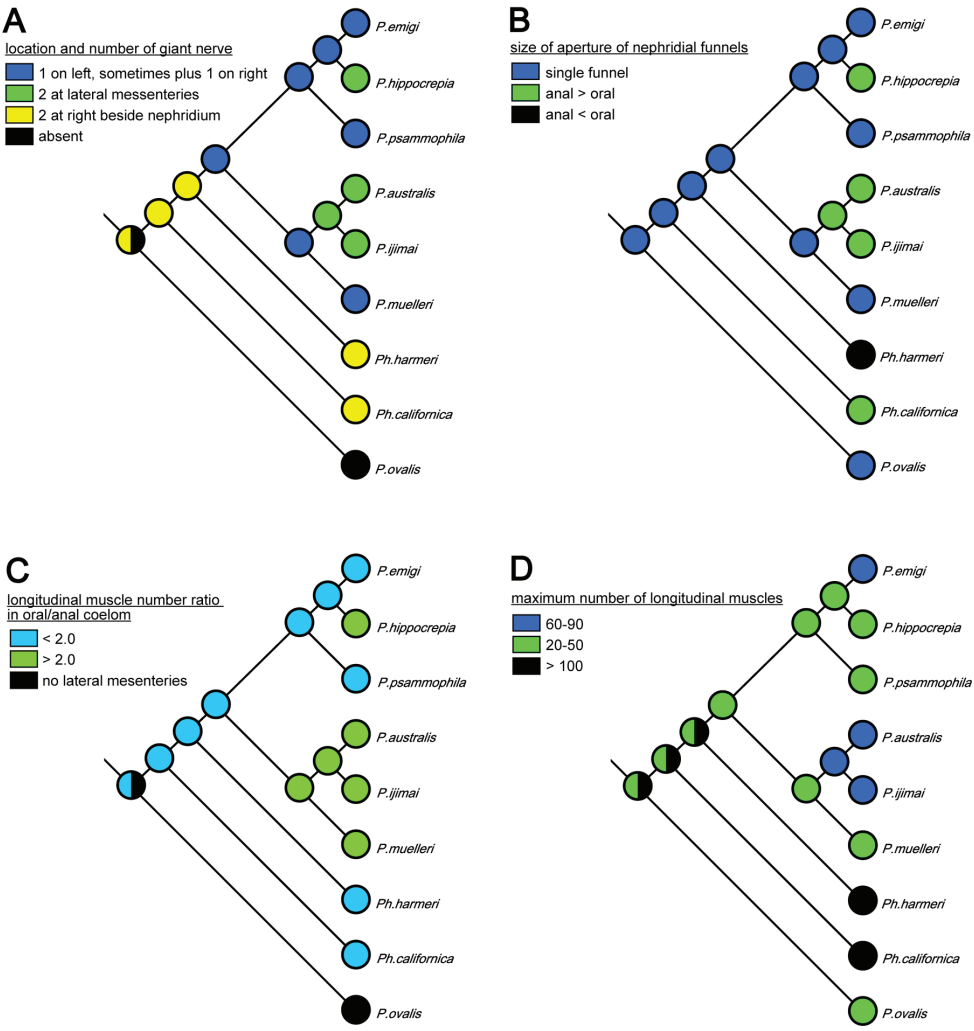

Figure 15. Parsimonious reconstruction of four adult morphological characters among nine phoronid species on the maximum-likelihood tree based on concatenated COI–18S–28S dataset.

used to date in phoronid taxonomy are highly homoplastic (Fig. 15A–D), and thus phylogenetically less informative than the molecular data. According to the character matrix and the cladogram based on 32 morphological and reproductive characters among 11 phoronid species (Suppl. material 1; Fig. 12A, 12B; Appendix 1 - Supplementary Figs S1A, S1B, S3 A–D, S4 A–D), *Phoronis emigi* comprise a group with *P. psammophila*, *P. muelleri*, and *P. pallida*. In none of our molecular trees (Figs 13A, 13B, 14A, 14B), however, did these four species alone comprise a clade. In the COI tree (Fig. 13B), *P. architecta* (= *psammophila*), *P. muelleri*, and *P. emigi* comprise a clade that also includes *P. hippocrepia*. In the COI–18S–28S tree (Fig. 14B), *P. emigi* and *P. architecta* (= *psammophila*) group with *P. hippocrepia*, to the exclusion of

Table 2. Pairwise genetic distances (K2P distances) based on 583 positions of COI sequences between *P. ijimai, P. emigi*, and the other species. The largest (*P. australis* JP and *P. muelleri*) and the lowest (*P. australis* NC and *P. vancouverensis*) interspecific distances are also listed. The analysis involved 12 phoronid sequences.

Species 1	Species 2	K2P Distance
Phoronis australis JP	*Phoronis muelleri*	0.287
Phoronis australis NC	*Phoronis vancouverensis*	0.164
Phoronis australis NC	*Phoronis australis* JAPAN	0.115
Phoronis ijimai	*Phoronis muelleri*	0.278
	Phoronis architecta	0.258
	Phoronopsis californica	0.258
	Phoronis ovalis	0.239
	Phoronis hippocrepia	0.222
	Phoronopsis viridis	0.216
	Phoronopsis harmeri	0.215
	Phoronis australis JAPAN	0.206
	Phoronis australis NC	0.179
	Phoronis vancouverensis	0.070
Phoronis emigi sp. n.	*Phoronis muelleri*	0.274
	Phoronopsis viridis	0.259
	Phoronopsis harmeri	0.252
	Phoronis ovalis	0.240
	Phoronis hippocrepia	0.239
	Phoronopsis californica	0.238
	Phoronis ijimai	0.235
	Phoronis vancouverensis	0.218
	Phoronis australis JAPAN	0.208
	Phoronis australis NC	0.205
	Phoronis architecta	0.202

P. muelleri, but no morphological or reproductive characters (Suppl. material 1; Fig. 15) appear to be synapomorphic for this clade, though character 19 (ratio of number of longitudinal muscles in oral coelom / anal coelom) in these three species is smaller than in other species of the genus except for *P. ovalis*, which lacks lateral mesenteries (Suppl. material 1).

Our molecular trees do not correspond with any of the subdivisions of phoronids suggested by previous researchers solely based on morphological characters (Silén 1952, Marsden 1959, Emig 1974). Within the phylum, Emig (1974) proposed five subgroups based on nephridial structure (Appendix 1 - Supplementary Fig. S5); most of these subgroups were identical to those in Silén's (1952) morphological categorization, except that Silén (1952) grouped *P. psammophila* with *P. ijimai* rather than *P. muelleri*. Although relationships within each group vary depending on the characters used in the analyses, our morphology-based cladograms (Fig. 12; Appendix 1 - Supplementary Figs S1, S3, S4) mostly correspond Emig's (1974) subgroup relationships; therefore, Emig (1974) would have been classified *P. emigi* in his "group 3" along with *P. psammophila*

and *P. muelleri* based on nephridial morphology. None of our molecular trees (Figs 13A, 13B, 14A, 14B; Appendix 1 - Supplementary Fig. S2), however, shows a clade comprising these three species alone. In the COI tree (Fig. 13B), these species form a clade that also includes *P. hippocrepia*.

Our morphological and molecular results do not contradict that "*P. vancouverensis*" is conspecific with *P. ijimai*, as proposed by Emig (1971a). Although we were not able to obtain a 28S sequence for *P. ijimai*, in the 18S and COI trees it always formed a clade with "*P. vancouverensis*" accompanied by high nodal support (Fig. 13A, 13B). The Kimura (1980) 2-parameter (K2P) distance between *P. ijimai* and "*P. vancouverensis*" for 583 bp of COI was 0.07, substantially below the value of the intraspecific distance 0.115 between *P. australis* NC and *P. australis* JAPAN (Table 2). On the other hand, the interspecific distances among phoronids ranged from 0.164 to 0.287; therefore, K2P divergence factor between 0.115 and 0.164 could be a threshold for discriminating phoronid species.

Taxonomic key to Japanese Phoronida

1 Inhabiting cerianthid tube-wall; lophophore multispiral; normally black in color ..... ***Phoronis australis*** **Haswell, 1883**

– Inhabiting cylindrical tube on hard substrate or soft sandy and muddy bottom; lophophore horseshoe-shaped without significant coiling; white or red in color **2**

2 Cylindrical tube constructed of small sand grains; tentacles fewer than 100 in number, with white spots..... ***Phoronis psammophila*** **Cori, 1889**

– Cylindrical tube obscure or not constructed of sand grains; tentacles more than 100 in number, without white spots **3**

3 Left giant nerve fiber more than 15 μm in diameter, right giant nerve fiber absent; longitudinal muscles of feathery type, more than 10 in number on each side of anal coelom; nephridium with single funnel, nephridial papilla absent, descending branch present ..... ***Phoronis emigi*** **sp. n.**

– Left giant nerve fiber less than 15 μm in diameter, right giant nerve fiber present; longitudinal muscles of bushy type, fewer than 10 in number on each side of anal coelom; nephridium with two funnels, nephridial papilla present, descending branch absent ..... ***Phoronis ijimai*** **Oka, 1897**

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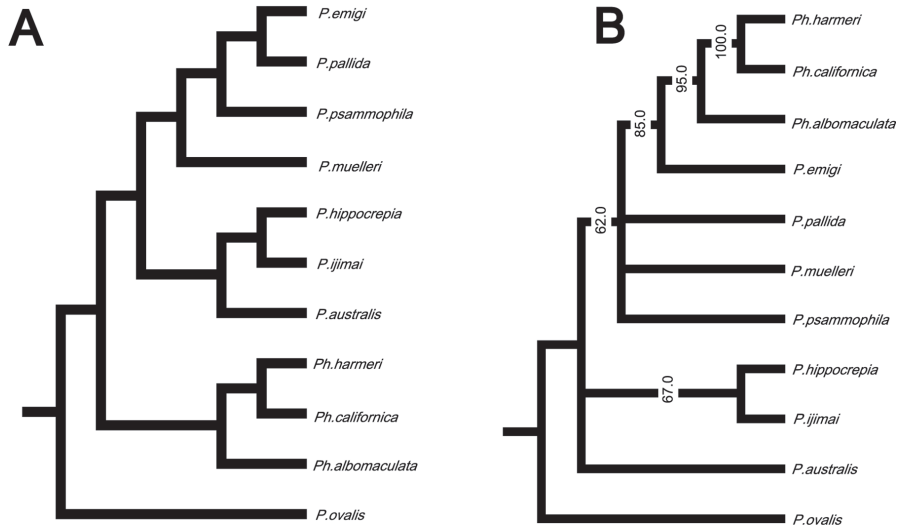
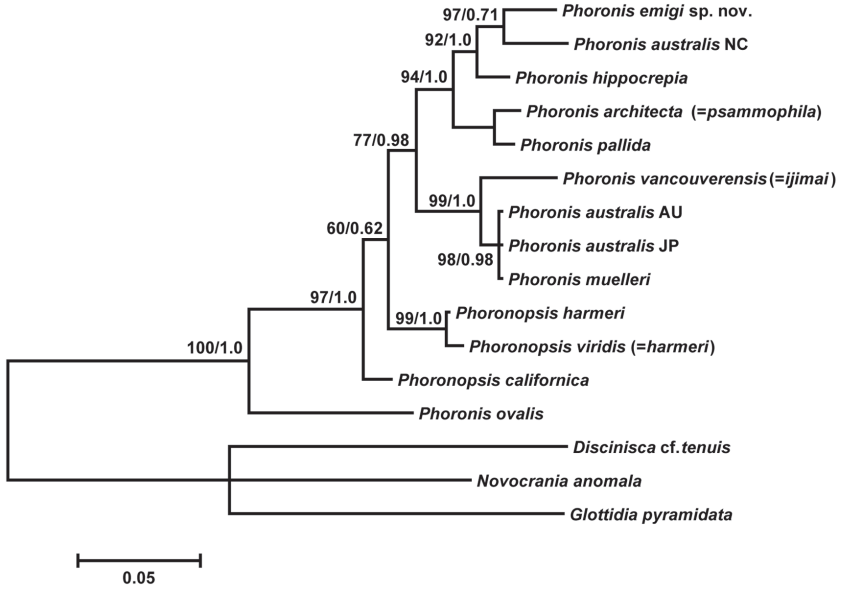
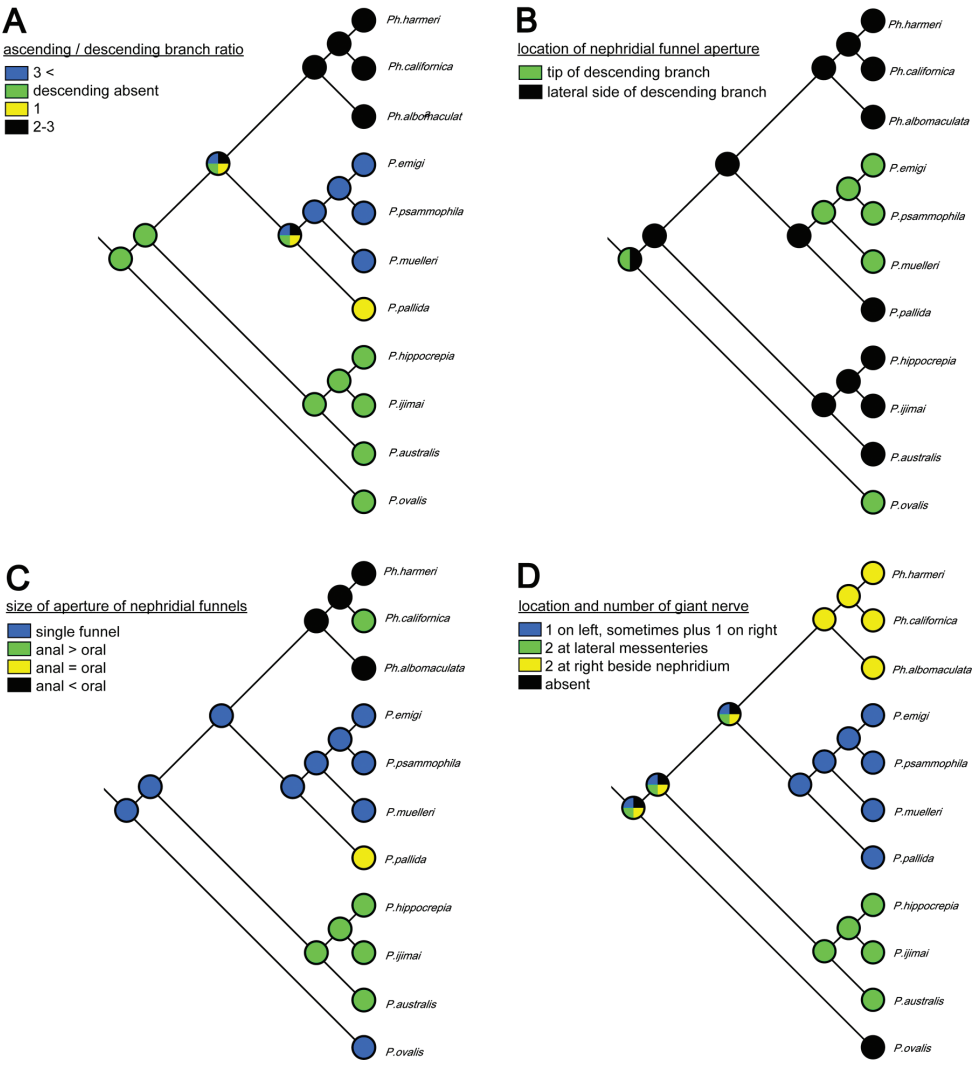
Appendix 1



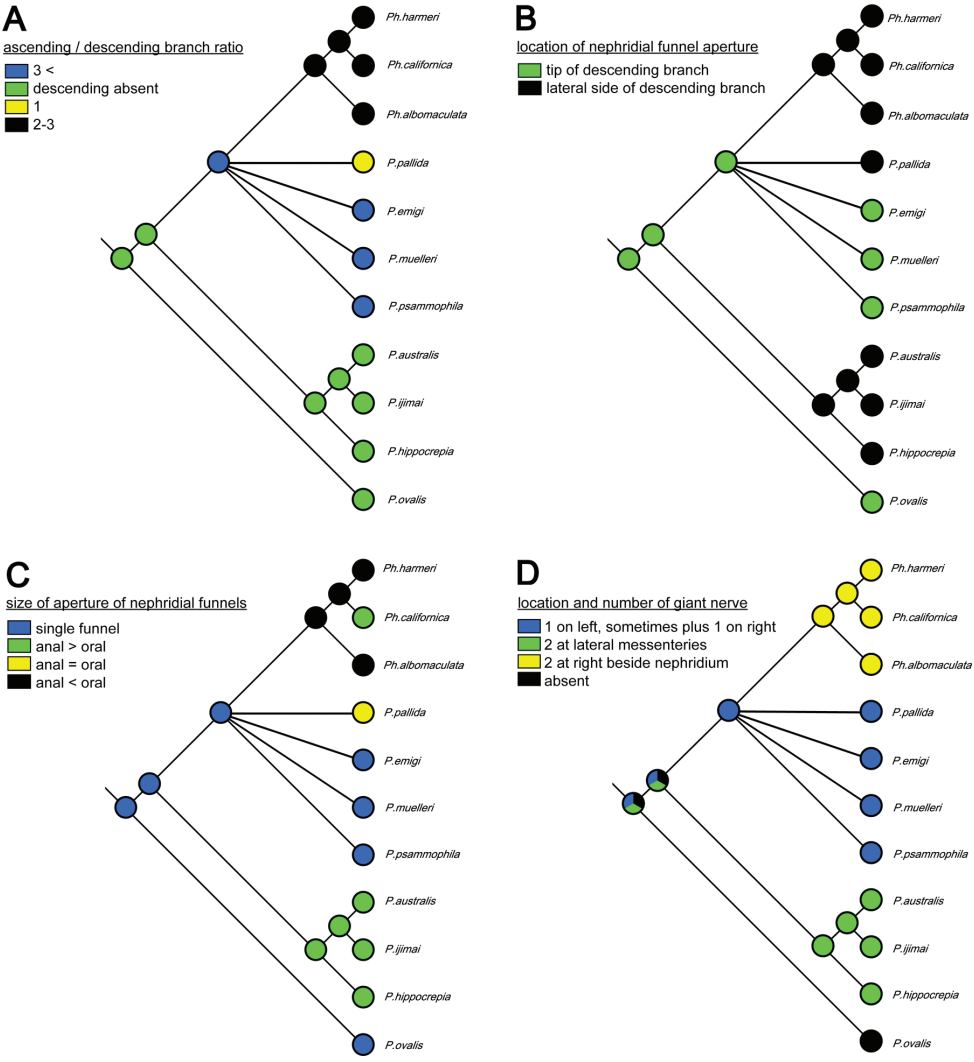
Supplementary Figure S1. A Cladogram of single-linkage cluster analysis among 11 phoronid species based on 23 morphological characters excluding nephridial characters **B** majority-rule consensus tree of 100 equally parsimonious tree obtained by cladistics analysis among 11 phoronid species based on 23 morphological characters excluding nephridial characters. Numerals on nodes indicate frequency values.



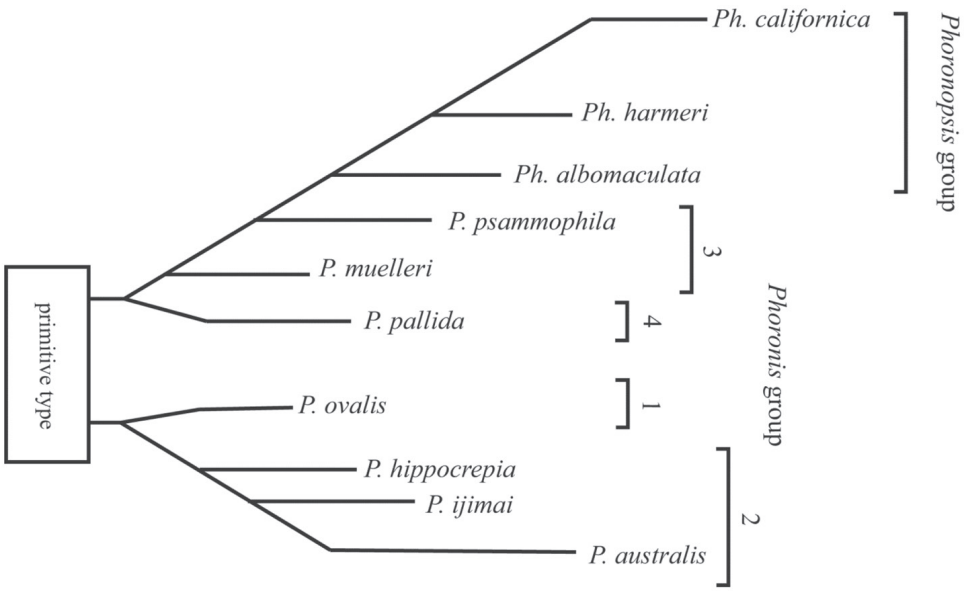
Supplementary Figure S2. Maximum-likelihood tree for 13 phoronid samples based on 28S data; three brachiopod species (*Novocrania anomala*, *Discinisca* cf. *tenuis*, and *Glottidia pyramidata*) are included as outgroup taxa. The scale bar indicates branch length in substitutions per site. Nodal support values are presented as the ML bootstrap value followed by the Bayesian posterior probability; only values >50% and 0.50, respectively, are shown.



Supplementary Figure S3. Parsimonious reconstruction of four adult morphological characters among 11 phoronid species on the cladogram of the cluster analyses based on 32 morphological characters.



Supplementary Figure S4. Parsimonious reconstruction of four adult morphological characters among 11 phoronid species on the parsimonious consensus tree based on 32 morphological characters.



Supplementary Figure S5. Emig's (1974) classification of five morphological categories within Phoronida, based on nephridial structure. Modified from Emig (1974).

Supplementary material 1

Character matrix of 32 morphological and reproductive characters among 11 phoronid species considered in the Discussion.

Authors: Masato Hirose, Ryuma Fukiage, Toru Katoh, Hiroshi Kajihara

Data type: character matrix

Link: doi: 10.3897/zookeys.398.5176.app1

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RESEARCH ARTICLE

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A review of the non-bulimulid terrestrial Mollusca from the Region of Atacama, northern Chile

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Abstract

Terrestrial mollusca are sparsely studied in Chile and, for the first time, a formal record of the diversity of land snails in northern Chile is reported. Coastal and desertic areas in the Region of Atacama, in the border of the Atacama desert and the Pacific Ocean, were surveyed with the aim to describe the presence and distribution of this poorly known fauna. Of the fourteen species recorded, the geographic distribution records for nine species are extended, and some taxa are recorded for the first time since their original descriptions. All, except one, of the fourteen terrestrial molluscan species occurring in the area are endemic to Chile; they are all terrestrial species, most of them have a restricted geographic distribution, and none of them is currently protected by law. The results reveal that the region of Atacama has one of the most diverse terrestrial snail biodiversity in Chile, ranking only after the Juan Fernandez Archipelago. Distribution records of all the studied species and a taxonomic key are also provided.

Keywords

Land snails, Chile, Charopidae, Bothriembryontidae, Ellobiidae, Pupillidae, Strophocheilidae

Introduction

Terrestrial molluscs are one of the least studied invertebrate groups in Chile, the first work compiling the records of land molluscan species is still extant (Stuardo and Vega 1985). Just a few subsequent studies have reviewed genera or families (Valdovinos and Stuardo 1988, Stuardo and Vargas-Almonacid 2000) or described new species, all

of them micromolluscs (Vargas-Almonacid 2000, Vargas and Stuardo 2007, Miquel and Barker 2009, Miquel and Cádiz-Lorca 2009, Miquel and Araya 2013). Studies considering species from northern Chile have been very scarce, like the work of Rehder (1945), which reviewed the subgenus *Peronaeus* and the work of Valdovinos and Stuardo (1988), reviewing the genus *Plectostylus* in Chile.

This work presents an overview, with distributions and illustrations, of all the land molluscan species found in the Region of Atacama, northern Chile. Ellobiidae species are also included, taking into account their terrestrial habitat in the country. The distribution range and a taxonomic key to all the studied taxa is also provided. The aim of this preliminary paper is thus to contribute to the knowledge of the land snail fauna in Chile.

Methods

Most of the sampling was made in the coastal desert areas around the port of Caldera (27°04'S, 70°50'W), and in specific localities in the Region of Atacama, northern Chile, during the summers of 2009 to 2012 and in August–December 2012. This region occupies the southern part of the Atacama desert and has an arid to hyper-arid climate, with low precipitation, mostly associated with the El Niño Southern Oscillation (ENSO) events. Detailed descriptions of the surveyed area, particularly of the flora and higher fauna are provided in Squeo et al. (2008). A synopsis of all the localities is given in Table 1. The surveys used a similar approach like Cowie and Robinson (2003) by also collecting litter for further sorting in the laboratory. The terminology of shell morphology is based upon Breure (1979). Original descriptions of all species were carefully reviewed, and the references included in the synonymies are mostly the ones that contained detailed descriptions or figures. Dimensions of the shells, measured with Vernier callipers (± 0.1 mm) are depicted in the Figure 1. Abbreviations used for repositories of material are: JFA-LG, private collection of the author section land Gastropoda, Santiago, Chile; MZUC, Museo de Zoología de la Universidad de Concepción, Concepción, Chile; RMNH.MOL, Naturalis Biodiversity Centre, The Netherlands, Mollusca collection; RCG, private collection of Ricardo Catalán, Sernapesca, Caldera, Chile.

Systematics

Family Bothriembryontidae Iredale, 1939

Genus *Plectostylus* Beck, 1837

Type species. *Bulimus peruvianus* Bruguière, 1789, by subsequent designation (Gray 1847).

The genus is extant and distributed in Chile and Argentina, its type species is endemic to Chile. All the species have a minutely rugose, granulate or striate protoconch.

Table 1. Sampling sites, arranged from north to south.

Locality	Coordinates/Altitude	Ecology	Ocurring species
Aguas Verdes	26°52'S, 70°48'W, 60 m	Low coastal hills with rocky outcrops, scarce vegetation.	*Plectostylus broderipii*, *Plectostylus coturnix*, *Sarnia frumentum*
Zoológico de Piedra	26°56'S, 70°47'W, 94 m	Rocky outcrop with sparse vegetation.	*Plectostylus broderipii*
Quebrada del León	26°57'S, 70°44'W, 378 m (Hill). 26°58'S, 70°45'W, 155 m (Plains).	Sandy plains and rocky hills with vegetation of cacti and desert bushes.	*Plectostylus broderipii*, *Stephacharopa calderaensis*
Plains NE Caldera.	27°04'22"S, 70°49'03"W, 135 m	Coastal plain, almost no vegetation and rocky hills with scarce vegetation.	*Plectostylus broderipii*, *Plectostylus coturnix*
Caldera Bay	27°04'S, 70°49'W, 54 m	Sandy plains with very scarce vegetation.	*Plectostylus coturnix*, *Cornu aspersum*, *Marinula pepita*, *Sarnia frumentum*
El Morro Hill	27°08'43"S, 70°55'42"W, 194 m	Steep rocky terrain, herbs and cacti, plentiful lichen communities.	*Plectosylus coturnix*, *Pupoides* (*I.*) *minimus*, *Stephacharopa calderaensis*
Chorrillos beach Area	27°09'37"S, 70°56'40"W, 64 m	Coquina cliffs and rocky outcrops.	*Plectostylus broderipii*, *Marinula pepita*
Copiapó	27°22'00"S, 70°19'00"W, 470 m	Small mountains, very scarce vegetation.	*Plectostylus broderipii*, *Chiliborus rosaceus*
Barranquilla beach Area	27°42'33"S, 71°01'03"W, 123 m	Sandy plains and rocky outcrops with scarce vegetation.	*Plectostylus elegans*
Chañaral de Aceituno	29°01'35"S, 71°26'20"W, 174 m	Sandy hills with scarce vegetation.	*Chiliborus pachychilus*

***Plectostylus broderipii* (Sowerby I, 1832)**
http://species-id.net/wiki/Plectostylus_broderipii
Figs 3.1–3.4, Table 2

Bulinus broderipii Sowerby I, 1832: 30, figs 1,1*. *Bulimus* (*Plectostylus*) *broderipii*: Beck 1837: 58. *Bulimus broderipii*: Reeve 1849: pl. 16, fig. 97; Hidalgo 1870: 117. *Bulimus* (*Plectostylus*) *broderipi*: Pilsbry 1897: 4, pl. 6, figs 79–83. *Plectostylus broderipi*: Stuardo and Vega 1985: 135; Valdovinos and Stuardo 1988: 121, figs 86–88, pl. 3, figs 28–30, Table 3; Valdovinos 1999: 151; Neubert and Janssen 2004: 203, Taf. 13, fig. 156; Köhler 2007: 141, fig. 69; Breure and Ablett 2012: 8, figs 4A–B, 4i.

Material examined. El Morro hill (27°08'43"S, 70°55'42"W) and Aguas verdes sector (26°52'S, 70°48'W), Commune of Caldera, JFA 100112, 35 specimens, RMNH. MOL.329662 (lot).

Diagnosis. Shells elongate-globose, imperforate, whorls convex with a pattern of axial and spiral brownish streaks. Last whorl ample, lip simple.

Distribution and remarks. From Iquique (20°30'S, 69°30'W) to Huasco (Valdovinos and Stuardo 1988). This species was moderately abundant in the area, living in sand near cacti, and in rocky outcrops.

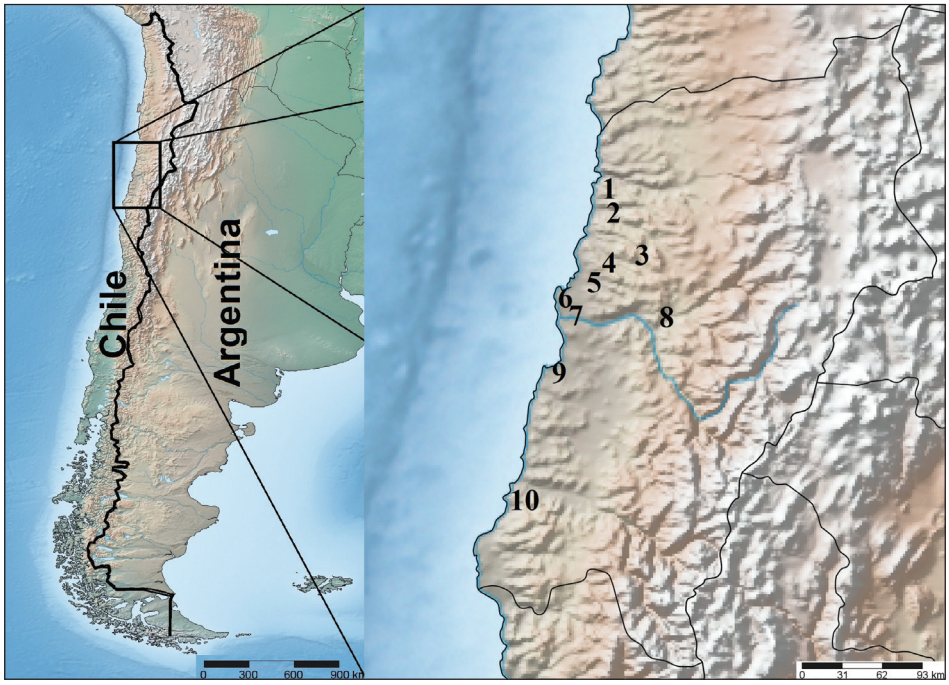

Figure 1. Map of sampling sites, arranged from north to south. **1** Aguas Verdes **2** Zoológico de Piedra **3** Quebrada del León **4** Plains NE Caldera **5** Caldera Bay **6** El Morro Hill **7** Chorrillos beach Area **8** Copiapó **9** Barranquilla beach Area **10** Chañaral de Aceituno.

***Plectostylus coturnix* (Sowerby I, 1832)**
http://species-id.net/wiki/Plectostylus_coturnix
Figs 3.5–3.9, Table 2

Bulinus coturnix Sowerby I, 1832: 30. *Bulimus coturnix*: Hupé in Gay 1854: 102, pl. 1, fig. 4; Hidalgo 1870: 115. *Bulimulus* (*Plectostylus*) *coturnix*: Pilsbry 1897: 3, pl. 6, figs 89–92. *Plectostylus coturnix*: Breure 1979: 89; Stuardo and Vega 1985: 136; Valdovinos and Stuardo 1988: 128–129, figs 86–88. Pl. 3, figs 25–27; Valdovinos 1999: 151; Neubert and Janssen 2004: 206, Taf. 13, fig. 157; Breure and Ablett 2012: 12, figs 4C–D, 4ii. *Plectostylus broderipii*: Köhler 2007: 142, fig. 71.

Material examined. El Morro hill (27°08'43"S, 70°55'42"W), Commune of Caldera, JFA 100113, 12 specimens. Hills near Vallenar (28°34'S, 70°45'W), October 2010, RCG (unnumbered), 25 specimens.

Diagnosis. Shells stout, elongate-globose, with convex or very convex whorls, decorated with axial and spiral brownish streaks and spots. Last whorl very ample, lip simple, rimate umbilicus.

Distribution and remarks. Huasco (28°20'S, 71°15'W) (Valdovinos and Stuardo 1988). This is the northernmost record for the species. This species is easily distin-

Table 2. Distribution range of terrestial molluscan taxa considered in this work.

Species	Distribution	References
Chiliborus bridgesii (Pfeiffer, 1842)	Caleta Pajonales (27°43'S, 71°02'W) to Freirina (28°30'S, 71°04'W).	Stuardo and Vega 1985 and this study
Chiliborus pachychilus (Pfeiffer, 1842)	Chañaral de Aceituno (29°01'35"S, 71°26'20"W) to Coquimbo (29°57'S, 71°20'W).	Stuardo and Vega 1985 and this study
Chiliborus rosaceus (King & Broderip, 1831)	Copiapó (27°22'00"S, 70°19'56"W) to Chiloé Island (42°52'S, 73°49'W).	Stuardo and Vega 1985 and this study
Cornu aspersum (Müller, 1774)	Worldwide, in Chile from Caldera (27°04'S, 70°49'W) to Chiloé Island (42°52'S, 73°49'W).	Valdovinos 1999 and this study
Marinula pepita King 1832	Caldera (27°04'S, 70°49'W) to Chiloé Island (42°52'S, 73°49'W), Chile and in Lima (12°02'S, 77°01'W), Peru.	Paredes et al. 2005 and this study
Plectostylus broderipii (Sowerby I, 1832)	Iquique (20°30'S, 69°30'W) to Huasco (28°20'S, 71°15'W).	Valdovinos and Stuardo 1988
Plectostylus coturnix (Sowerby I, 1832)	El Morro hill (27°08'43"S. 70°55'42"W) to Huasco (28°20'S, 71°15'W).	Valdovinos and Stuardo 1988 and this study
Plectostylus elegans (Pfeiffer, 1842)	Barranquilla beach (27°21'29"S, 70°20'24"W) and Huasco (28°20'S, 71°15'W).	Valdovinos and Stuardo 1988 and this study
Plectostylus moestai (Dunker, 1864)	Copiapó (27°22'00"S, 70°19'56"W).	Valdovinos and Stuardo 1988 and Köhler 2007
Plectostylus punctulifer (Sowerby I, 1833)	Paposo (25°05'S, 70°25'W) to Huasco (28°20'S, 71°15'W).	Valdovinos and Stuardo 1988
Plectostylus variegatus (Pfeiffer, 1842)	Paposo (25°05'S, 70°25'W) to Lomas de Huasco (28°20'S, 71°15'W).	Valdovinos and Stuardo 1988 and this study
Pupoides (Ischnopupoides) minimus (Philippi, 1860)	Paposo (25°05'S, 70°25'W) to La Serena (29°54'S, 71°015 W).	Stuardo and Vargas Almonacid 2000
Sarnia frumentum (Petit de Saussaye, 1842)	El Callao (12°02'S, 77°08'W), Peru to Aguas Verdes (26°52'S, 70°48'W), Chile.	Paredes et al. 2005 and this study
Stephacharopa calderaensis Miquel & Araya, 2013	Quebrada del León (26°57'S, 70°44'W) and El Morro hill (27°08'43"S. 70°55'42"W).	Miquel and Araya 2013

guished from *P. broderipii* due to the conspicuous rimate umbilicus, the more globose whorls, stouter shell and shorter spire.

***Plectostylus elegans* (Pfeiffer, 1842)**
http://species-id.net/wiki/Plectostylus_elegans
Figs 3.10–3.14, Table 2

Succinea elegans Pfeiffer, 1842: 56; Pfeiffer 1852: 187. *Bulimus elegans*: Hupé in Gay 1854: 104, pl. 3, fig. 2. *Bulimulus coquimbensis Var. elegans*: Pilsbry 1897: 11, pl. 8, figs 18–22. *Plectostylus coquimbensis perelegans*: Breure 1978: 201, pl. 9, fig. 14. *Plectostylus elegans*: Breure 1979: 9; Stuardo and Vega 1985: 136; Valdovinos and Stuardo 1988: 129, figs 86–88. Pl. 3, figs 34–36. *Plectostylus perelegans*: Val-

dovinos 1999: 151; Neubert and Janssen 2004: 222, Taf. 13, fig. 159; *Plectostylus broderipii*: Breure and Ablett 2012: 34, figs 5E–F, 5ii. (syn. n).

Material examined. Barranquilla beach (27°21'29"S, 70°20'24"W), Commune of Caldera, RCG (unnumbered), 5 specimens. Aguas verdes (26°52'S, 70°48'W), Commune of Caldera, 3 specimens. MZUC 39619 (lot).

Diagnosis. Shells thin, elongate-globose, with convex and slightly shouldered whorls, decorated with axial greyish, and brownish-reddish, streaks. Last whorl very ample, lip simple, periostracum shiny and transparent.

Distribution and remarks. Huasco (28°20'S, 71°15'W) (Valdovinos and Stuardo 1988). The specimens here studied constitute the northernmost record for this species. Breure and Ablett (2012) synonymized this species as *P. broderipii*. However, the shells here examined were much lighter, thinner and broader than *P. broderipii*. Shell patterns, which are contained in the thin outer shell layer, can easily differenciate *P. elegans* from *P. broderipii* in having axially marked reddish-brown lines, even in juvenile specimens. Only extensive comparative anatomy, including soft parts as well as shell morphology, would certainly help to establish its true identity.

***Plectostylus moestai* (Dunker, 1864)**
http://species-id.net/wiki/Plectostylus_moestai
Table 2

Bulimus moestai Dunker, 1864: 156. *Bulimulus* (*Plectostylus*) *moestai*: Pilsbry 1897: 6. *Plectostylus moestai*: Breure 1979: 90; Stuardo and Vega 1985: 136; Valdovinos and Stuardo 1988: 131; Köhler 2007: 142, fig. 72.

Material examined. no material seen.

Diagnosis. Shell subrimate, ovate-conic, thin, marked with irregular chestnut streaks. Whorls six, a little convex, apex obtuse, aperture oval, peristome simple (Valdovinos and Stuardo 1988).

Distribution and remarks. Cerro Bravo, Copiapó (Pilsbry 1897). Valdovinos and Stuardo (1988), in their review of the genus, could no locate specimens of this species. Although this species has been cited for the area, searches at the type locality were unsuccessful. This may represent an extinct taxon.

***Plectostylus punctulifer* (Sowerby I, 1833)**
http://species-id.net/wiki/Plectostylus_punctulifer
Figs 3.15–3.18, Table 2

Bulinus punctulifer Sowerby I, 1833: 36. *Bulimus punctulifer*: Reeve 1849: Pl. 16, fig. 92; Hidalgo 1870: 118.

Bulimulus (*Plectostylus*) *punctulifer*: Pilsbry 1897: 317, pl. 26, figs 67–69; Pl. 8, fig. 27; Breure 1979: 90. *Plectostylus punctulifer*: Stuardo and Vega 1985: 136; Valdovinos and Stuardo 1988: 135, figs 86–88, pl. 1, figs 1-3; Köhler 2007: 142, fig. 74. *Bulimulus* (*Plectostylus*) *punctulifer*: Breure and Ablett 2012: 33, figs 5A–B, 5i.

Material examined. Fray Jorge National Park (30°40'S, 71°40'W), Region of Coquimbo, July 2006, RCG (unnumbered), 3 specimens.

Diagnosis. This elongated *Plectostylus* species has a thin and somewhat fusiform shell, with an acute, long spire and five slightly convex whorls, sculptured with minute granules and growth lines. The aperture is narrow and descending, somewhat expanded in the anterior side. Periostracum is thin, opaque and yellowish.

Distribution and remarks. Valdovinos and Stuardo (1988) cited this species from Paposo (25°05'S, 70°25'W) to Huasco. This species was not found in the area under current study.

***Plectostylus variegatus* (Pfeiffer, 1842)**
http://species-id.net/wiki/Plectostylus_variegatus
Figs 3.19–3.21, Table 2

Succinea variegata Pfeiffer, 1842: 56; Pfeiffer 1843: 187. *Bulimus elegans*: Hupé *in* Gay 1854: 102, pl. 3, fig. 1. *Bulimulus* (*Plectostylus*) *variegatus*: Pilsbry 1897: 5, pl. 6, figs 86–88. *Plectostylus variegatus*: Breure 1978: 202, pl. 9, figs 17–18; Breure 1979: 202; Stuardo and Vega 1985: 136; Valdovinos and Stuardo 1988: 137, figs 86–88, pl. 2, figs 19–21; Neubert and Janssen 2004: 233, pl. 13, fig. 155. *Plectostylus broderipii*: Breure and Ablett 2012: 43, figs 5C–D, 5iii. (syn. n.)

Material examined. Hills near Vallenar (28°34'S, 70°45'W), RCG (unnumbered), 5 specimens.

Diagnosis. This relatively large species (up to 52 mm) has a thin but stout shell, with an acute, somewhat short spire and five slightly convex whorls sculptured by thin growth lines and fine spiral threads. The aperture is large, oval and slightly angulated in the columellar lip, which is completely white in its anterior part.

Distribution and remarks. Valdovinos and Stuardo (1988) cited this species from Paposo (25°05'S, 70°25'W) to Lomas de Huasco (28°20'S, 71°15'W). According to Breure and Ablett (2012) this species is a subjective synonym of *P. broderipii*. The specimens here examined seem slightly different; the shells are more elongated, with a larger aperture and a more acute spire. Some specimens have rimate shells, with a pseudo-umbilicus formed by the folding of the columellar lip. These specimens have a thin, opaque, persistent and delicate brownish periostracum.

Family Charopidae Hutton, 1884

Genus *Stephacharopa* Miquel & Araya, 2013

Type species. *Stephacharopa calderaensis* Miquel & Araya, 2013, by original designation (Miquel and Araya 2013).

The genus is extant and distributed in Chile and Argentina, its type species is restricted to the Region of Atacama, northern Chile. Protoconchs of species within the genus have 40–60 axial, smooth and low ribs.

***Stephacharopa calderaensis* Miquel & Araya, 2013**
http://species-id.net/wiki/Stephacharopa_calderaensis

Stephacharopa calderaensis Miquel & Araya, 2013: 227, figs 2–5

Material examined. El Morro hills (27°8'33"S, 70°55'35"W), Commune of Caldera, August 2012, JFA 100127, 37 specimens MZUC 39613 (lot), RMNH.MOL 329670 (lot). Quebrada del León sector (26°57'S, 70°44'W), JFA 100128, 12 specimens.

Diagnosis. This species has a tiny (largest specimen: 3.1 mm width), orbicular, low-spired shell, sculptured by numerous axial lamellae (about 90–95 in last whorl), with a depressed apex, a thin and brownish periostracum and an ample umbilicus. Live animals are unknown.

Distribution and remarks. According to Miquel and Araya (2013) this species has a patchy distribution, having been found only in the vicinites of the port of Caldera, Region of Atacama, Chile.

Family Ellobiidae H. & A. Adams in Pfeiffer, 1854

Genus *Marinula* King & Broderip, 1832

Type species. *Marinula pepita* King, 1832, by monotypy.

The genus is extant and distributed in South Africa, New Zealand and Chile, its type species is found from Ecuador to Chile.

***Marinula pepita* King, 1832**
http://species-id.net/wiki/Marinula_pepita
Table 2

Marinula pepita King,1832: 344; Keen 1971:850, fig. 849; Paredes et al. 2005: 74, fig. 8.

Material examined. Caldera Bay (27°04'S, 70°49'W), Commune of Caldera, July 12 2012, JFA 100501, 21 specimens.

Diagnosis. This species have small shells (up to about 11 mm), brownish or reddish in colour, higher than wider, of short spire, a large last whorl and an impressed suture. Aperture is simple, with a thin lip with a tooth in the inner external lip and three more in the columellar area. Animals are traslucent, with darker tentacles and a comparatively short foot.

Distribution and remarks. This species has been cited from Coquimbo to Chiloé Island, Chile (Keen 1971), and in Lima, Peru (Paredes et al. 2005). The specimens here examined constitute the northernmost record of this species in Chile. It has been found that this species feeds on remains of birds, fishes and sea urchins (Paredes et al. 2005).

Genus *Sarnia* H. & A. Adams in Pfeiffer, 1855

Type species. *Sarnia frumentum* Petit de Saussaye, 1842, by subsequent designation (H. A. Adams 1855).

The genus and its type species are extant and distributed in Chile and Peru.

Sarnia frumentum (Petit de Saussaye, 1842)

http://species-id.net/wiki/Sarnia_frumentum
Table 2

Auricula frumentum Petit de Saussaye, 1842: 105–106; Reeve 1878, vol. 20, Auricula, pl. 4, fig. 23. *Auricula avena* Petit de Saussaye, 1842: 106; Reeve 1878, vol. 20, Auricula, pl. 4, fig. 24. *Melampus avena* Dall, 1909: 204. *Sarnia frumentum* Keen, 1971: 850, fig. 2418; Marincovich 1973: 41, figs 92, 94; Paredes et al. 2005: 70, fig. 2.

Material examined. Aguas Verdes (26°52'S, 70°48'W), Commune of Caldera, August 2011, JFA 100502, 15 specimens.

Diagnosis. This is one of the smallest terrestrial snails found in northern Chile. They have small (up to about 7 mm) whitish-orangish shells, of subcylindrical shape, with a simple and sharp aperture, with three plyes in the columellar side.

Distribution and remarks. This species has been cited from El Callao, Peru to Chañaral, Chile (Paredes et al. 2005). This is the southernmost record for this species in Chile. It has been found that this species feeds on remains of birds, fishes and sea urchins (Paredes et al. 2005).

Family Pupillidae Turton, 1831

Genus *Pupoides* Pfeiffer, 1854

Subgenus *Ischnopupoides* Pilsbry, 1926

Type species. *Pupa hordacea* Gabb, 1866, by original designation.

The subgenus is extant and distributed in USA, northern Mexico, Cuba and Chile, its type species is restricted to southern USA.

***Pupoides* (*Ischnopupoides*) *minimus* (Philippi, 1860)**
http://species-id.net/wiki/Pupoides_minimus
Table 2

Bulimus minimus Philippi, 1860: 166, Pl. 7, fig. 12a–b. *Pupoides (Ischnopupoides) minimus minimus*: Biese 1960: 133. Taf. 13, figs 1–4. *Pupoides (Ischnopupoides) minimus*: Stuardo and Vega 1985: 127; Stuardo and Vargas Almonacid 2000: 176.

Material. El Morro hill (27°8'33"S, 70°55'35"W) and Zoológico de Piedra (26°56'20"S, 70°47'14"W), Commune of Caldera, September 2012 and January 2013, JFA 100126, 52 specimens, MZUC 39612 (lot), RMNH.MOL 329669 (lot).

Diagnosis. This species has a tiny (up to 6 mm), whitish and elongated shell, sculptured by widely separated axial lamellae, with a small aperture and a thin and brownish periostracum.

Distribution and remarks. Paposo to La Serena (Stuardo and Vargas-Almonacid 2000). Here the species seem to be narrowly distributed, with small but abundant communities found in elevated rocky areas facing the Pacific Ocean.

Family Strophocheilidae Pilsbry, 1902

Genus *Chiliborus* Pilsbry, 1926

Type species. *Bulinus chilensis* Sowerby I, 1833, by subsequent designation (Klappenbach and Olazarri 1970).

The genus and type species are extant and endemic to Chile; protoconchs of all species of the genus have a characteristic spiral striation.

Figure 2. *Chiliborus* shells. *Chiliborus bridgesii*, Pajonales Bay, Province of Copiapó, 20.3 mm: **1** Ventral view **2** Dorsal view **3** Detail of protoconch **4** Juvenile shell. *Chiliborus pachychilus*, Chañaral de Aceituno, Province of Huasco, 37.3 mm: **5** Ventral view **6** Dorsal view **7** Detail of protoconch **8** Detail of sculpture. *Chiliborus rosaceus*, Los Molles, Valparaiso Region, 61.9 mm: **9** Ventral view **10** Dorsal view **11** Detail of protoconch **12** Detail of suture and sculpture **13** Ventral view of an orangish specimen, Pichidangui, Valparaiso Region, 74. 5 mm **14** Preserved epiphragm **15** Detail of protoconch.

***Chiliborus bridgesii* (Pfeiffer, 1842)**
http://species-id.net/wiki/Chiliborus_bridgesii
Figs 2.1–2.4, Table 2

Bulimus bridgesii Pfeiffer, 1842: 43; Reeve 1848: 5, *Bulimus*, pl. 19, fig. 117; Hupé in Gay 1854: 107, Malacología pl. 3, fig. 4. *Strophocheilus* (*Borus*) *bridgesii*: Pilsbry 1895: 35, pl. 2, figs 4–6. *Strophocheilus* (*Chiliborus*) *bridgesii*: Pilsbry 1926: 6. *Strophocheilus* (*Chiliborus*) *bridgesii*: Bequaert 1948: 186, pl. 13, figs 2, 3; pl. 14, fig. 3. *Chiliborus bridgesii*: Stuardo and Vega 1985: 129.

Material examined. Caleta Pajonales (27°43'S, 71°02'W), Commune of Copiapó, September 2005, RCG (unnumbered), 4 specimens.

Diagnosis. This species have small (up to 23 mm), pale brown, thin ovate-oblong shells, minutely sculptured by fine spiral lines, with a reflexed and delicate thin lip and a comparatively large protoconch decorated by spiral threads. This is the smallest species in the Strophocheilidae.

Distribution and remarks. Freirina (28°30'S, 71°04'W) and Huasco (28°20'S, 71°15'W) (Stuardo and Vega 1985). This is the northernmost record for the species.

***Chiliborus pachychilus* (Pfeiffer, 1842)**
http://species-id.net/wiki/Chiliborus_pachychilus
Figs 2.5–2.8, Table 2

Bulimus pachychilus Pfeiffer, 1842: 48–49. *Bulimus pachycheilus*: Reeve 1848: 5, pl. 15, fig. 87. *Strophocheilus pachychilus*: Pilsbry 1895: 35, pl. 12, figs 63–64. *Strophocheilus* (*Chiliborus*) *pachychilus*: Pilsbry 1926: 6; Bequaert 1948: 184, pl. 8, figs 2, 4. *Chiliborus pachychilus*: Stuardo and Vega 1985: 129.

Material examined. Chañaral de Aceituno (29°01'35"S, 71°26'20"W), Commune of Freirina, February 2008, RCG (unnumbered), 33 specimens. MZUC 39615.

Diagnosis. This species has ovate-oblong, shells (up to 39 mm), with a thin, brownish periostracum and a slightly flattened apex. They are easily distinguished by their solid and thick whitish shell and their thickened, lamellate outer lip. On magnification the surface of the shell has a rugose appearance, especially in the subsutural area, due to very fine spiral threads crossed by thin axial lines (Fig. 2.8).

Distribution and remarks. Questa de Arenas, Huasco (28°20'S, 71°15'W) and Coquimbo (29°57'S, 71°20'W) (Stuardo and Vega 1985). The specimens studied here constitute the northernmost record for the species.

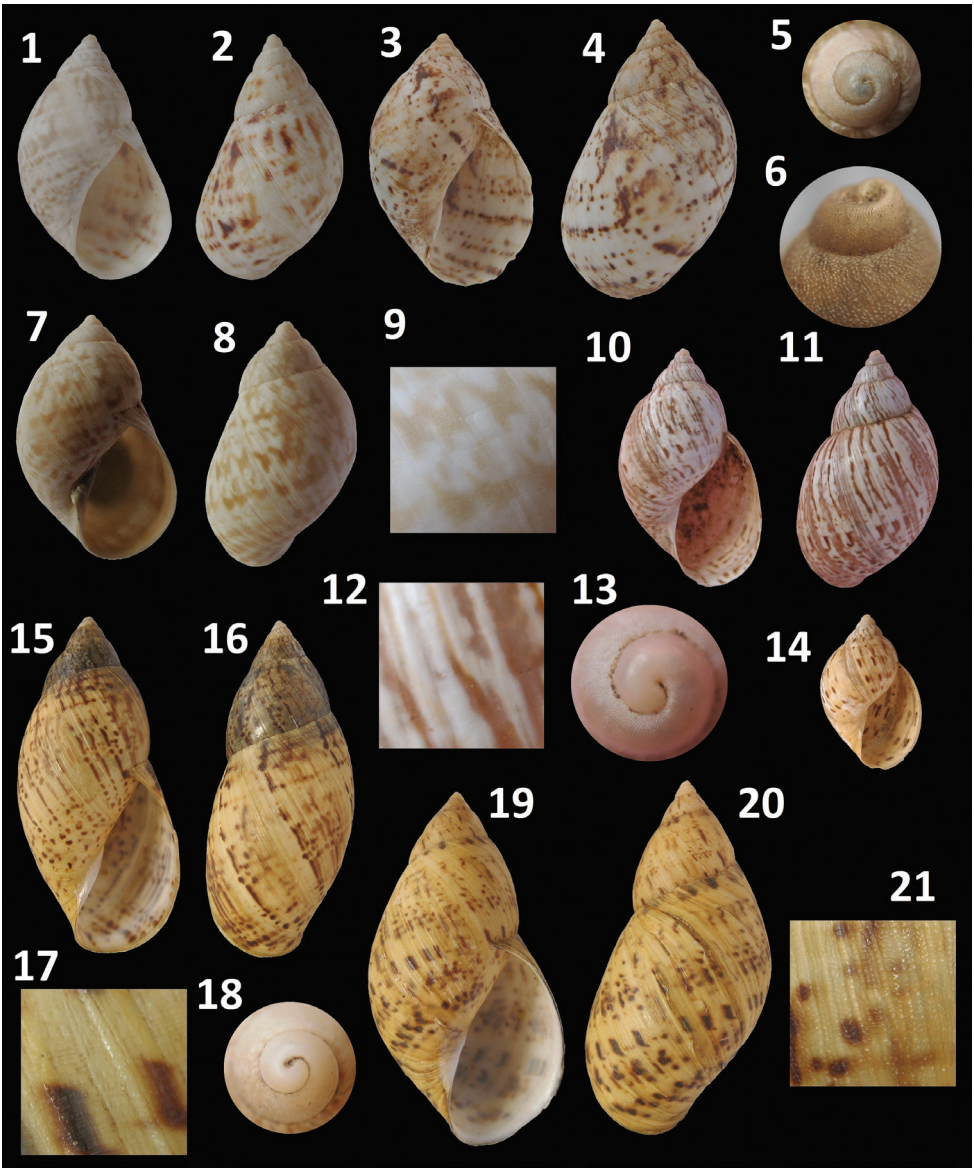

Figure 3. ***Plectostylus*** **shells.** *Plectostylus broderipii*, Aguas Verdes, Commune of Caldera, 24 mm: **1** Ventral view **2** Dorsal view. El Morro hill, Commune of Caldera, 28.8 mm: **3** Ventral view **4** Dorsal view **5** Detail of protoconch *Plectostylus coturnix*, El Morro hill, Commune of Caldera, 27.5 mm: **6** Detail of protoconch **7** Ventral view **8** Dorsal view **9** Detail of sculpture. *Plectostylus elegans*, Barranquilla, Commune of Caldera, 24 mm: **10** Ventral view **11** Dorsal view **12** Detail of sculpture. **13** Detail of protoconch. **14** Juvenile shell. *Plectostylus punctulifer*, Fray Jorge National Park, Coquimbo Region, Chile, 20.2 mm: **15** Ventral view **16** Dorsal view **17** Detail of sculpture. **18** Detail of protoconch. *Plectostylus variegatus*, Vallenar, Province of Huasco, 50.5 mm.: **19** Ventral view **20** Dorsal view **21** Detail of sculpture.

***Chiliborus rosaceus* (King & Broderip I, 1831)**
http://species-id.net/wiki/Chiliborus_rosaceus
Figs 2.9–2.15 , Table 2

Bulinus rosaceus King & Broderip, 1831: 341. *Bulimus* (*Bulimus*) *rosaceus*: Beck 1837: 52; Hidalgo 1870: 53. *Strophocheilus* (*Borus*) *rosaceus*: Pilsbry 1895: 33, pl. 5, fig. 26, pl. 6, figs 29–30. *Strophocheilus* (*Chiliborus*) *rosaceus*: Pilsbry 1926: 6; Bequaert 1948: 178, pl. 8, figs 2, 4. *Chiliborus rosaceus*: Stuardo and Vega 1985: 129.

Material examined. Rocky hills north of Copiapo (27°21'29"S, 70°20'24"W), Commune of Copiapó, April 4 2006, JFA 100101. Pichidangui (32°08'39"S, 71°31'15"W) and Los Molles (32°13'56"S, 71°29'23"W), Region of Valparaíso, 2008, RCG (unnumbered), 9 specimens.

Diagnosis. This species has large (up to 89 mm in examined specimens), brownish and elongate shells decorated with growth lines. Shells have crenulated sutures, a large protoconch and a thick lip. Animals have an orange or brownish body, with short grey tentacles.

Distribution and remarks. From Huasco to Chiloé Island (42° S, 73° W) (Stuardo and Vega 1985). This is the northernmost record for the species.

Family Helicidae Rafinesque, 1815

Genus *Cornu* Born, 1778

Type species. *Cornu copiae* Born, 1778 (= *Helix aspersa* Müller, 1774), by original designation. The genus is extant and native to Europe.

***Cornu aspersum* (Müller, 1774)**
http://species-id.net/wiki/Cornu_aspersum
Table 2

Helix (*Cryptomphalus*) *aspersa*: Stuardo and Vega 1985: 136; Valdovinos 1999: 151. *Cornu aspersum*: Landler and Nuñez 2012: 264.

Material examined. Mirador de Charito sector, Caldera city (27°3'45"S, 70°50'8"W), Commune of Caldera, July 2012, JFA 100129, 2 specimens.

Diagnosis. This very common species has a distinctive low-spired, brown shell with yellowish and brownish markings and four or five whorls.

Distribution and remarks. According to Valdovinos (1999) this species has records in Chile from La Serena (29°54'S, 71°15'W) to the Chiloé Island, and the Juan Fernandez Archipelago (33°38'S, 78°84'W). This is the northernmost record of this species in Chile and it is the only introduced land snail species found in the area.

Conclusions

The terrestrial molluscs found in the Region of Atacama encompasses five families: Bothriembryontidae, a Gondwanan family which in Chile is solely represented by the genus *Plectostylus*; Charopidae, a widely extended family of tiny snails; Ellobiidae, a family which includes conspicuous terrestrial species living in litoral areas, in mangroves and under rocks in salty conditions; Strophocheilidae, with conspicuously large snails and Bulimulidae, with 29 species in Chile, all in genus *Bostryx*. This last family is currently under study, with twenty three species represented in the Region of Atacama, and will be reviewed in a further work. Most of the species here considered occur in patchy distributions along the coastal desert of northern Chile, most of them with sparse records and very few have been found alive.

In summary, fourteen species of terrestrial molluscs are recorded in the Region of Atacama. All of them are ground dwellers, and only one introduced species, *Cornu aspersum,* has been found in the residential gardens of Caldera. *Chiliborus bridgesii*, *C. pachychilus*, *C. rosaceus*, *C. aspersum*, *Marinula pepita*, *Plectostylus coturnix*, *P. elegans*, *P. variegatus* and *Sarnia frumentum* are recorded from the Atacama region for the first time and thus they extend their distribution records in the country. Taking into account the twenty three species of Bulimulidae, which will be reviewed in another work, the number of species recorded in the region of Atacama make it one of the richest places in Chile in terms of terrestrial molluscan biodiversity. Intensive collections are needed for a further understanding of the biology and ecology of this group.

Key for the identification of terrestrial Mollusca from the Atacama region, based on shell characters

1	Shell orbicular, depressed, ample umbilicus	**2**
1a	Shell higher than wider, globose to turrited	**3**
2	Shell globose, up to 40 mm, variegated in brown-chestnut, very convex whorls, small umbilicus, ample aperture, external lip white internally	***Cornu aspersum* (Müller, 1774)**
2a	Minute shell (up to 3.5 mm), convex whorls, sculptured by numerous and fine axial lamellae, ample umbilicus and flat apex	***Stephacharopa calderaensis* Miquel & Araya, 2013**
3	Shell obese-ovate to elongated	**4**
3a	Shell very elongated or turrited, very small (up to 4.5 mm), elongate, corneous, shallow axial ribs, oval aperture	***Pupoides (Ischnopupoides) minimus* (Philippi, 1860)**
4	Presence of plyes or teeth inside aperture, shells very small (up to 11 mm), last whorl very large	**12**

4a Lip simple, protoconch rugose or decorated with spiral lines, shells 40 mm to 93 mm. Aperture comparatively large **5**

5 Protoconch not prominent or flattened, decorated with spiral lines **6**

5a Protoconch rugose or finely striated, thin shells of medium size (up to 55 mm), whitish to yellowish in colour, variegated with brown streaks and marks, aperture very large **8**

6 Shell medium sized (40 mm) to large (up to 89 mm), large protoconch, wavy suture, engrossed outer lip 7

6a Shell small (up to 23 mm), thin, caramel-brown in colour, suture simple, reflected outer lip, yellowish thin periostracum..... ***Chiliborus bridgesii*** **(Pfeiffer, 1842)**

7 Shell up to 42 mm, white to pale brown, thick, minutely sculptured by shallow spiral and axial lines, lamellated, accrescent outer lip..... ***Chiliborus pachychilus*** **(Pfeiffer, 1842)**

7a Shell large (65 mm to 89 mm), lightweight, pink-brownish, irregularly sculptured by growth marks, plicated sutures..... ***Chiliborus rosaceus*** **(King & Broderip, 1831)**

8 Shell ovate-oblong or slightly fusiform, thin, no umbilical ply..... **9**

8a Shell stout, globose whorls, comparatively small spire, noticeable pseudo-umbilicus, aperture wider in medium part...***Plectostylus coturnix*** **(Sowerby I, 1832)**

9 Ovate-globose shell, aperture wider in first tird of shell heigh **10**

9a Shell ovate-elongate to fusiform, yellowish to pale brownish, angulated columellar lip, surface of shell minutely granulated, yellowish periostracum.... **11**

10 Maximum width near half of last whorl, size up to 45 mm..... ***Plectostylus broderipii*** **(Sowerby I, 1832)**

10a Shell slightly elongated, acute spire, maximum width near first third of last whorl, size up to 55 mm, delicate brownish periostracum..... ***Plectostylus variegatus*** **(Pfeiffer, 1842).**

11 Shell elongate, decorated with axial brown streaks, acute spire, ovate-elongate aperture slightly flared in anterior side, yellowish periostracum..... ***Plectostylus punctulifer*** **(Sowerby I, 1833)**

11a Shell ovate, shiny, decorated with axial segmented redish-brown stripes, spire low, whorls slightly shouldered ..... ***Plectostylus elegans*** **(Pfeiffer, 1842)**

12 Shell brownish or reddish, short spire, impressed suture, thin lip with a tooth in the inner external lip and three more in the columellar area..... ***Marinula pepita*** **(King, 1831)**

12b Shell of subcylindrical shape, with a simple and sharp aperture and three plyes in the columellar area..... ***Sarnia frumentum*** **(Petit de la Saussaye, 1842)**

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RESEARCH ARTICLE

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The eastern swamp crayfish *Gramastacus lacus* sp. n. (Decapoda, Parastacidae) a new species of freshwater crayfish from coastal New South Wales, Australia

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Abstract

Gramastacus lacus **sp. n.**, is described from coastal lowlands of the Central and Mid North Coast regions of New South Wales, Australia. *Gramastacus lacus* has a restricted distribution in ephemeral habitats, being dependent on regular natural flooding and drying cycles, and burrows for survival during temporary dry cycles. Documented are population distributions in lowland habitats (3–48 m, a.s.l.) from Wamberal Lagoon, north along the coastal strip to Wallis Lake. The species is small, reaching a maximum weight of 7 grams and 21.3 mm OCL, and distinguished by a large male genital papilla, large raised post orbital ridges, laterally compressed carapace and elongated chelae.

Keywords

Gramastacus lacus, Australia, Central Coast, Mid North Coast, Parastacidae, Ramsar wetlands

Introduction

Gramastacus lacus sp. n., first came to my attention in 1984 when specimens were collected from the Ramsar Wetlands of Myall Lakes National Park for aquaculture trials. Since 2005 the Australian Crayfish Project (ACP) has been surveying eastern Australia to increase the knowledge base of all freshwater crayfish species. As part of this ongoing project, surveys of coastal New South Wales (NSW) over the last eight years have resulted in the discovery of isolated populations of this new *Gramastacus* species (Figures 1–3).

Riek (1972) erected the genus *Gramastacus* comprising two new species, *Gramastacus insolitus* Riek, 1972, and *Gramastacus gracilis* Reik, 1972, from the Grampians area of western Victoria. Zeidler and Adams (1990) revised the genus and concluded that *G. gracilis* was a junior synonym making the genus monotypic. This new species from coastal NSW, occurring 900 km northeast of *G. insolitus,* brings the species total back to two.

Schultz et al. (2007) included specimens of the then undescribed New South Wales *Gramastacus* in their study and concluded from a molecular perspective, that these specimens are clearly *Gramastacus*, despite their wide geographic separation from the only currently recognized species in this genus. In this paper I present a formal description of the new species, together with biological and distribution information.

Methods

Crayfish were collected using a variety of methods to suit the conditions at each survey site. Opera House traps (630 mm × 470 mm × 180 mm, 90 mm steel ring entrance hole) and box traps (430 mm × 260 mm × 260 mm, 50 mm steel ring entrance hole) were utilized, all baited with fresh fish. Scoop rakes and scoop nets were used to sample suitable sites with collection by hand used at many sites. Such collection included lifting structures like rocks and logs and excavating burrows, using hands and with the assistance of spades. Burrows were carefully and slowly excavated allowing the burrow and any branches to be followed. Careful burrow excavation provides information on species habitat requirements and burrow system structures.

In an effort to better understand the biology of freshwater crayfish throughout their ranges and over time, voucher material for further study was retained where appropriate (to confirm the distribution and occurrence of a species at a certain place at a certain time). All retained specimens were placed in plastic transport containers with a small amount of water and returned alive to the laboratory. Specimens were photographed and examined in the laboratory under dissecting microscopes; measured with digital calipers; and weighed to nearest gram. Specimens were euthanized by freezing for at least 24 hours and subsequently stored in clear, labelled specimen jars containing originally 70% ethanol (and now 100% ethanol to better preserve tissue integrity for our genetics collaborations with James Cook University and the Australian Museum).

Additionally, tissue samples from live animals were retained in cell lysis buffer from selected specimens for subsequent DNA analysis, as part of the broader ACP via our Carnegie Museum of Natural History genetics program.

Type material is deposited in the Australian Museum, Sydney (AM). Other voucher specimens are lodged in the ACP collection, the Australian Museum and the Carnegie Museum of Natural History (CMNH). Additionally, results of surveys are included in the Atlas of NSW Wildlife database.

At each site, the geographic position co-ordinates and altitude were recorded using a Magellan Explorist 510 and 600 handheld GPS. Notes were taken on exact location details, landforms, aquatic vegetation and stream conditions. Water quality information (flow, pH, temperature, salinity, visibility, DO, conductivity and TDS) was also recorded at selected sites. All specimen measurements are given in millimetres and all weights in grams. Morphological abbreviations and measurements follow Morgan (1986).

Abbreviations

a.s.l. above sea level
ACP Australian Crayfish Project
AdW abdominal width
AM Australian Museum
ArL areola length; measured along the midline from the cervical groove to dorsal posterior of carapace.
ArW areola width; minimum width of areola.
CD carapace depth; maximum depth at the deepest part, from dorsal carapace to ventral margin above legs.
CL carapace length; measured along the midline from apex of rostrum to the dorsal posterior margin of the carapace.
CMNH Carnegie Museum of Natural History
CW carapace width; taken arbitrarily, maximum width at the widest point.
DactL dactylus length of the first cheliped
LP lateral process
OCL occipital carapace length; measured from the posterior margin of the orbit to the dorsal posterior of carapace (Morgan 1986).
PropD first cheliped propodus depth; greatest thickness, measured between dorsal and ventral palm surfaces.
PropL first cheliped propodus length; measured from propodal base to apex propodal finger.
PropW first cheliped propodus width; greatest height, measured between proximal and distal edges of mesial margin of propodus.
RL rostral length
RW rostral width
Spread TAP minus TAA

SqL antennal squame length.
TAA teeth anterior to the margin of zygocardiac ossicle ear
TAL total abdomen length
TAP teeth anterior to posterior margin of zygocardiac ossicle ear
TEL tailfan; measured from posterior margin of abdominal somite 6 to the tip of the telson
TL total length; measured from the apex of the rostrum to the tip of the tailfan

Taxonomy

***Gramastacus lacus* sp. n.**
http://zoobank.org/FCA312BD-AB04-49B3-9177-C50C13D93F78
http://species-id.net/wiki/Gramastacus_lacus
Figures 1–3

Material examined. HOLOTYPE: AM P.89665, male, 18.63 mm OCL, 5.07 grams, Boomeri Swamp behind Boomeri camp ground, off Old Gibber Road, Myall Lakes National Park, 32.50910°S, 152.32143°E, altitude 12 m, 21 September 2012, R.B. McCormack.

ALLOTYPE: AM P.89663, berried female, 16.86 mm OCL, 3.17 grams, type locality, 21 September 2012, R.B. McCormack.

PARATYPES: AM P.89664, male, 17.62 mm OCL, 4.55 grams, type locality, 21 September 2012, R.B. McCormack. AM P.89666, berried female, 14.23 mm OCL, 2.06 grams, type locality, 21 September 2012, R.B. McCormack. AM P.89660, male, 20.22 mm OCL, 7 grams, tributary of Boolambayte Creek crossing Violet Hill Rd, Boolambatye, 32.41893°S, 152.28788°E, altitude 21 m, 19 August 2009, R.B. McCormack and S. Pavescki. AM P.89661, male, 16.76 mm, OCL, 4 grams, tributary Pourmalong Creek, under power lines, off Wyee Road, Wyee, 33.12770°S, 151.47438°E, altitude 36 m, 23 August 2009, R.B. McCormack. AM P.89662, male, 17.36 mm OCL, 4 grams, wetland swamp, Wamberal, 33.40927°S 151.45890°E, altitude 7 m, 10 June 2010, R.B. McCormack.

Other material examined. CMNH 37973.83, female, 14.47 mm OCL, Myall Lakes, May 2008, R.B. McCormack and K. Dawkins. CMNH 37973.84, male, 17.75 mm OCL, Myall Lakes, May 2008, R.B. McCormack and K. Dawkins. AM P.78514, Smiths Lake, May 1970, J. Paxton. ACP 179, male, 19.36 mm OCL, Myall Lakes, August 2005, R.B. McCormack. ACP 180, berried female, 15.27 mm OCL, Myall Lakes, August 2005, R.B. McCormack. ACP 181, male, 16.79 mm OCL, Myall Lakes, August 2005, R.B. McCormack. ACP 806, male, 2 g, 13.96 mm OCL, Myall Lakes, August 2007, R.B. McCormack. ACP 1147, male, 17.7 mm OCL, Budgewoi Lake, April 2008, R.B. McCormack. ACP 1148, male, 17.85 mm OCL, Myall Lakes, April 2008, R.B. McCormack. ACP 1169, male, 2 g, 14.65 mm OCL, Myall Lakes, May 2008, R.B. McCormack, K. Dawkins. ACP 1170, female, 2 g, 16.54 mm OCL, Myall

Figure 1. The eastern swamp crayfish *Gramastacus lacus* sp. n.

Lakes, May 2008, R.B. McCormack, K. Dawkins. ACP 1171, male, 2 g, 14.65 mm OCL, Myall Lakes, May 2008, R.B. McCormack, K. Dawkins. ACP 1172, male, 3 g, 15.7 mm OCL, Myall Lakes, May 2008, R.B. McCormack, K. Dawkins. ACP 1591, male, 14.06 mm OCL, Lake Macquarie, November 2008, R.B. McCormack. ACP 1598, female, 9.17 mm OCL, Lake Macquarie, November 2008, R.B. McCormack. ACP 1628, male, 13.06 mm OCL, Lake Macquarie, November 2008, R.B. McCormack. ACP 1630, male, 12.13 mm OCL, Lake Macquarie, November 2008, R.B. McCormack. ACP 1651, male, 14.7 mm OCL, Lake Macquarie, December 2008, R.B. McCormack, S. Pacevski, J. Moylan, T.A. Moylan. ACP 1652, male, 13.38 mm OCL, Lake Macquarie, December 2008, R.B. McCormack, S. Pacevski, J. Moylan, T.A. Moylan. ACP 2286, female, 3 g, 16.7 mm OCL, Myall Lakes, August 2009, R.B. McCormack, S. Pacevski. ACP 2300, berried female, 3 g, 16.74 mm OCL, Lake Macquarie, August 2009, R.B. McCormack. ACP 2306, female, 3 g, 15.88 mm OCL, Lake Macquarie, August 2009, R.B. McCormack. ACP 2307, berried female, 4 g, 17.56 mm OCL, Lake Macquarie, August 2009, R.B. McCormack. ACP 2309, berried female, 2 g, 15.21 mm OCL, Lake Macquarie, August 2009, R.B. McCormack. ACP 2313, female, 7 g, 21.32 mm OCL, Myall Lakes, August 2009, R.B. McCormack. ACP 2318, berried female, 1 g, 12.3 mm OCL, Myall Lakes, September 2009, R.B. McCormack. ACP 2349, male, 11.99 mm OCL, Smiths Lake, September 2009, R.B. McCormack. ACP 2354, female, 9.2 mm OCL, Wallis Lake, September 2009, R.B. McCormack. ACP 2363, male, 11.79 mm OCL, Wallis Lake, September 2009, R.B. McCormack. ACP 2368, female, 9.08 mm OCL, Wallis Lake, September 2009, R.B. McCormack. ACP 2369, female, 11.27 mm OCL, Wallis Lake, September 2009, R.B. McCormack. ACP 2376, female, 11.72 mm OCL, Wallis Lake, September 2009, R.B. McCormack. ACP 2378, berried female, 1 g, 13.4 mm OCL, Myall Lakes, September 2009, R.B. McCormack. ACP 2387, berried female, 2 g,

13.08 mm OCL, Myall Lakes, September 2009, R.B. McCormack. ACP 2403 berried female, 2 g, 14.1 mm OCL, Myall Lakes, September 2009, R.B. McCormack. ACP 2411, male, 2 g, 15.1 mm OCL, Budgewoi Lake, September 2009, R.B. McCormack. ACP 2422, berried female, 1.5 g, 13.68 mm OCL, Tuggerah Lake, September 2009, R.B. McCormack. ACP 2532, male, 13.53 mm OCL, Port Stephens, December 2009, R.B. McCormack. ACP 2925, mix x 2, Wamberal Lagoon, May 2010, R.B. McCormack. ACP 2926, male, 4 g, 15.57 mm OCL, Wamberal Lagoon, May 2010, R.B. McCormack. ACP 2928, male, 3 g, 14.35 mm OCL, Wamberal Lagoon, May 2010, R.B. McCormack. ACP 4059, male, 3.65 g, 17.67 mm OCL, Myall Lakes, July 2012, R.B. McCormack. ACP 4063, female, 2.37 g, 16.46 mm OCL, Myall Lakes, July 2012, R.B. McCormack. ACP 4072, male, 2.99 g, 16.13 mm OCL, Myall Lakes, July 2012, R.B. McCormack. ACP 4074, female, 2.84 g, 16.52 mm OCL, Myall Lakes, July 2012, R.B. McCormack. ACP 4079, male, 4.26 g, 17.7 mm OCL, Myall Lakes, July 2012, R.B. McCormack. ACP 4100, female, 1.21 g, 12.91 mm OCL, Myall Lakes, September 2012, R.B. McCormack. ACP 4101, male, 1.97 g, 14.48 mm OCL, Myall Lakes, September 2012, R.B. McCormack. ACP 4102, male, 2.56 g, 14.84 mm OCL, Myall Lakes, September 2012, R.B. McCormack. ACP 4109, male, 0.83 g, 10.81 mm OCL, Myall Lakes, September 2012, R.B. McCormack. ACP 4114, male, 0.61 g, 9.77 mm OCL, Myall Lakes, September 2012, R.B. McCormack. ACP 4116, female, 1.11 g, 12.10 mm OCL, Myall Lakes, September 2012, R.B. McCormack. ACP 4121, female, 1.94 g, 14.66 mm OCL, Myall Lakes, September 2012, R.B. McCormack. ACP 4130, male, 1.39 g, 12.24 mm OCL, Myall Lakes, September 2012, R.B. McCormack.

Comparative material examined. ACP 899, *Gramastacus insolitus,* male, 10 mm OCL, Moora Moora, Grampians, Victoria. ACP 927, *Engaeus laevis*, male, 15.81mm OCL, Thurra River, Croajingalong, Victoria. ACP 2884, *Engaeus lyelli*, male, 22.54 mm OCL, Eildon, Victoria.

Diagnosis. Rostrum long, narrow, reaching midlength or end of 3rd antennal segment, with spine at apex. Rostral carinae conspicuously raised and sharp, extending well back onto carapace just inside postorbital carinae. Carapace laterally compressed, deep, narrow. Antennal flagellum twice OCL. Antennal squame very long reaching end of 3rd antennal segment or beyond, widest at midlength, usually half as wide as long. Antennal basipodite spine variable, small to very large and sharp; coxopodite antennal spine absent. Interantennal spine wide, margins smooth and raised, with blunt to sharp spine. Areola very broad, width 0.5–0.7 times length, narrowest at centre. Telson U-shaped, margins gently converging to caudolateral corners, each with small sharp spine. Uropod outer ramus with 2 medium marginal outer spines with tuft of setae between. First chelae smooth and distinctly elongated. Males with large genital papilla.

Description. Size. Maximum OCL 21.32 mm, 7 gram. Maximum size animals extremely rare, mean weight of large adults 4-5 gram.

Rostrum. Long, narrow, reaching midlength or end of 3rd antennal segment, approximately 0.3 × OCL. Rostral apex variable, ranging from very small to a large sharp

Figure 2. *Gramastacus lacus* sp. n. Holotype male, AM P.89665, 18.63 mm OCL, 5.07 grams. **A** dorsal view **B** ventral view **C** anterior view **D** male genital papilla **E** lateral view. All measurements in centimetres.

conical acumen spine; apex upturned at 45–60° angle. Rostral carinae conspicuously raised and sharp, extending well back onto carapace, reaching beyond anterior end of postorbital carina; intracarinate region flat or slightly recessed, setose along mesial base of carinae with tuft of setae at apex of rostrum, carinae terminating at base of acumen in blunt process to large sharp conical rostral marginal spine upturned at 30° angle. OCL/CL 0.78–0.86. RW/OCL 0.09–0.14. RL/OCL 0.25–0.39.

Cephalon. Postorbital carinae conspicuously raised and prominent, anteriorly extremely variable from blunt to large sharp conical spine. Carinal length similar to rostral length. Suborbital spine small to medium. Antennal squame long, reaching to end of 3rd antennal segment or beyond, lateral margin slightly convex terminating in long, sharp conical spine, inflated at midlength. Cephalic setation variable, from slight to medium with scattered long bristle setae, densest towards lateral cephalic edge. SqL/OCL 0.18–0.32.

Thorax. Cervical groove deeply impressed, U-shaped. Postcervical groove lightly impressed, well separated from cervical groove, with postcervical groove diverging laterally; postcervical groove not continuous, vaguely extending just past the inner side of branchiocardiac groove. Branchiocardiac groove merging gradually with postcervical groove, with a distinct, short, transverse groove at posterior end of branchiocardiac groove. Areola very broad, 0.5-0.7 wide as long, narrowest towards centre. Carapace laterally compressed, deep, narrow, depth 0.67 × OCL, width 0.55 OCL. ArL/OCL 0.29–0.39. ArW/OCL 0.19–0.25. CW/OCL 0.51–0.59. CD/OCL 0.65–0.73.

Abdomen. Smooth, unarmed, with scattered long bristle setae. Some morphological differences between sexes (see Sexes). TAL average 1.66 × OCL. TAL/OCL 1.5-1.87. AdW/OCL 0.48–0.61 (average 0.54). TL/OCL 2.51-3.13 (average 2.84).

Tailfan. Telson U-shaped with sides gently converging to caudolateral corners, each with 1 small sharp standard spine. Outer uropod with 2 small standard marginal outer spines with tuft of setae between, 1 medium spine on longitudinal median carina on suture, longitudinal carina extends beyond suture, suture straight, 5–11 extra dorsolateral spines (outer) and 5–9 extra dorsolateral spines (inner) stopping just past half way. Outer uropod extends well past inner uropod and inner uropod extends just past caudal margin of telson. Inner uropod with medium standard spine at caudolateral corner, small upturned spine at base of longitudinal median carina (two specimens with 2 spines on one side). Tailfan with medium scattered long bristle setae. Coxopodite of the outer uropod terminating in rounded edge to medium conical spine, coxopodite of inner uropod terminating in small to medium conical spine. TEL/OCL; females 0.41–0.47 (average 0.45), males 0.38–0.47 (average 0.42).

Thoracic sternal keel. Sternal keel commencing as raised ridge posterior of LP1, thin sharp and recessed between LP1 and LP2. Rising at LP2 with slight dip between LP2 and LP3 with a small crest at LP3 and continuing straight as raised sharp ridge to LP4. LP4 with distinctive huge oval pores posterolaterally. Bullar lobes absent.

Gastric mill. TAP count 2.5–3.5. TAA 0.5–1.5. Spread 1–2. Urocardic ridges 2.

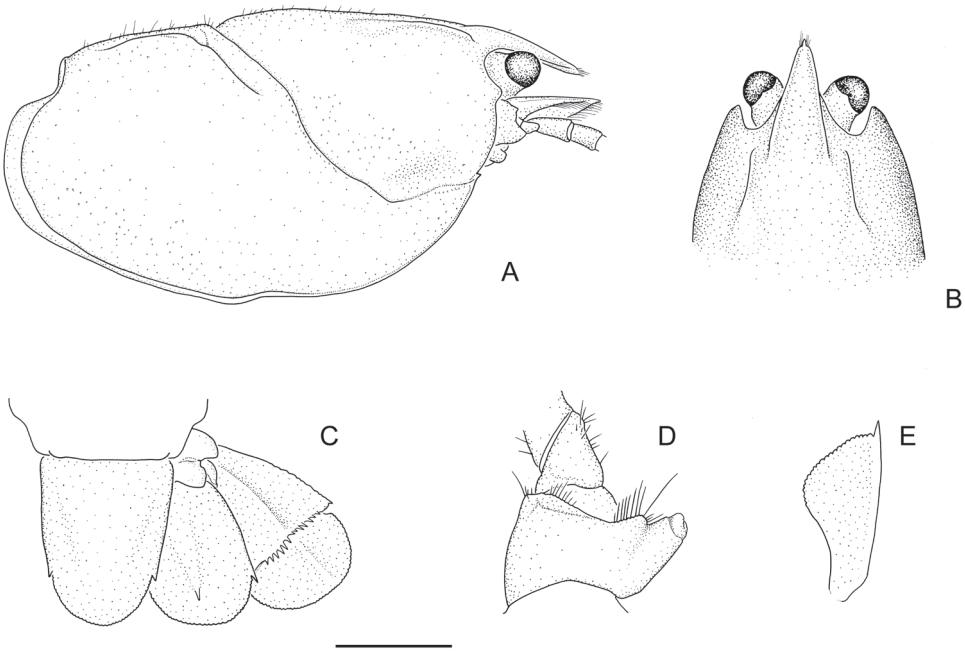

Figure 3. Holotype male: **A** carapace, right lateral view **B** anterior of carapace, dorsal view **C** telson and right uropod (setae omitted) **D** coxa of right pereopod 5 with gonopore, posterior view **E** right scaphocerite (antennal squame), dorsal view (setae omitted). Scale **A–C** = 5mm; **D–E** = 2.5 mm.

Antennae. Basipodite antennal spine small to very large and sharp. Coxopodite antennal spine absent. Interantennal spine wide, smooth raised margins, with blunt to pointed apex. Antennal flagellum twice OCL reaching posteriorly to 5th or 6th abdominal somites.

Third maxilliped. Third maxilliped with large raised spiniform process at mesioventral corner of coxopodite; ischium with sparse covering of short to long setae on ventrolateral surface, with carinate lateral edge and tuberculate laterodistal corner; exopodite multiarticulate and very long, 1.33–2.08 as long as ischium.

First cheliped. Distinctively elongated, proportionally larger in mature males than in reproductive females. General increase in setation along propodal and dactylar cutting edges in populations from the north to the south.

Propodus. Lightly punctated with mesial propodal margin starting as small rolled ridge at carpal articulation, progressing to small rounded mesial propodal spines scattered in twin lines giving a shadow effect to dactylar articulation. Cluster of small protuberances, some approaching small spines at perpendicular groove behind dactylar articulation. Dorsal and ventral propodal palm surfaces smooth. Shallow ventromesial and ventrolateral groves extending from apex along finger. Small granulations on lateral propodal edge usually (only distal 2/3 towards apex) but not on propodal finger. Light to medium setation along cutting edge with 2 prominent teeth.

Males (mean): PropL/OCL (1.07) 0.82-1.35, PropW/PropL (0.37) 0.33–0.44, PropD/PropL (0.255) 0.22–0.28.

Females (mean): PropL/OCL (0.87) 0.7–1.06, PropW/PropL (0.375) 0.30–42, PropD/PropL (0.245) 0.19–0.29.

Dactylus. Smooth, lightly punctated, with line of medium to large granulations along mesial edge increasing in size from dactylar articulation to apex between shallow dorsomesial and dorsolateral groves extending back from apex, fading out at approximately 40% dactylar length. One prominent tooth on cutting edge. Light to medium setation along cutting edges, more extensive in southern specimens. DactL/PropL: males (0.475) 0.41–0.54, females (0.51) 0.45–0.57.

Carpus. Smooth, with line of 6-9 small spines in irregular row along mesial edge, terminating in large sharp spine at articulation. Cluster of small protuberances in irregular line along ventromesial surface, several rounded, spiniform at articulation. Larger lateral carpal spine at articulation with group of smaller rounded spines along articulation edge. Setation absent.

Merus. Medium sharp outer distolateral meral spine, prominent dorsal meral spine. Setation absent.

Coloration. Colour varying with population. First chelipeds very dark, black to black-blue with bright blue highlights along propodal, carpal and meral lateral edges, light blue tint ventrally with articulations dull to bright red. Cephalon dark black-brown dorsally, lightening laterally, many with blue highlight on lateral surface. Thorax and abdomen light brown, green, tan or steel blue, usually with small, light cream or red speckles. Body clear to cream ventrally. Juveniles (Wallis Lake) light blue.

Sexes. No intersexed individuals were recorded, all being clearly either male or female. The male genital papilla is large, being a distinguishing character (Riek 1972) from other crayfish species in the region. They are opportunistic breeders, starting in early August being reliant on water availability in their ephemeral habitats, only breeding when flooded. Females mature at approximately 12 mm OCL and are very fecund for their size: ACP 2318, 12.3 mm OCL, September 2009, Myall Lakes, 44 eggs; ACP 2387, 13.08 mm OCL, September 2009, Myall Lakes, 29 eggs; ACP 2309, 15 mm OCL, August 2009, Lake Macquarie, 82 eggs; ACP 180, 15.93 mm OCL, August 2005, Myall Lakes, 79 eggs; ACP 2300, 16.74 mm OCL, August 2009, Lake Macquarie, 100 eggs; ACP 2307, 17.56 mm OCL, August 2009, Lake Macquarie, 143 eggs. Eggs are dark olive green colour (ACP 2318), to deep dark purple (ACP 2384). Berried females have previously been captured from water after rain events in August, September and October with approximately 30–150 eggs. Egg incubation is swift with a 6–8 week period between eggs laid and juveniles released. Females breeding in early September released juveniles in late October.

Gramastacus lacus shows sexual dimorphism. Reproductively active females typically have broader abdomens with an abdominal anterolateral flap on the pleuron of abdominal somite 2 and have a smaller/narrower major cheliped then males. Reproductively active females are recorded between 12 and 18 mm OCL, but more research is needed (berried females over 18 mm OCL have not yet been recorded).

Etymology. The Latin epithet *lacus* alludes to the coastal lake catchments that provide suitable habitat. Common name; previously referred to as "the lake yabby" (McCormack 2008), however, I recommend "the eastern swamp crayfish" as more appropriate, in parallel to the only other recognized *Gramastacus* species, *G. insolitus,* commonly known as "the western swamp crayfish"(Action Statement 2004).

Discussion

Gramastacus lacus is a small freshwater crayfish inhabiting lowland coastal environments between 3 and 48 m a.s.l. To date, it is known to occur from Wallis Lake in the north to Wamberal Lagoon in the south — a straight line distance of 165 km (Figure 4, Supplementary material 1). Further south, suitable habitat occurs around Avoca and Cockrone Lagoons (33.4917°S, 151.4201°E) but preliminary surveys have not yet identified the species presence. Further north, past Forster (32.0697°S, 152.5168°E), suitable habitat is also present but the species remains unrecorded. Future field surveys may increase the known species distribution.

Our research indicates that *G. lacus* has specific habitat requirements, preferring ephemeral habitats (smaller creeks, swamps, wet areas and stump holes) that offer conditions that enable their survival and presumably limit threats from other predators, such as eels. *Gramastacus lacus* populations were only found in ephemeral areas that reliably flood and then dry. Habitats in the same area that consist of permanent water invariably contain large numbers of freshwater eels (*Anguilla reinhardtii*) and native fish gudgeons (family Eleotridae) but not *G. lacus*. Many ephemeral swamps and creeks can revert from dry beds to water bodies several metres deep when flooded. Eels and fish may penetrate into and temporarily visit some habitat areas but *G. lacus* takes refuge in the periphery, protected within the thick reeds and flooded grasses along the shallow edges, well away from the deeper water.

Larger swamps, such as Boomeri Swamp, the type locality in the Ramsar Wetlands of the Myall Lakes National Park, will dry out completely and then flood with up to 1.5 m of water. Deep water over 1 m is usually devoid of *Gramastacus* except occasional moulted individuals. The only *Gramastacus* observed in >1 m of water are soft moulted animals, presumably they are seeking the deeper water away from the high density populations in the shallows to avoid cannibalism. We have not captured eels or fish from this swamp but giant water bugs (*Lethocerus insulanus*), turtles (*Chelodina longicollis*), lizards (*Physignathus lesueurii)* and birds all prey in deeper water making it a more dangerous habitat for the *Gramastacus* crayfish.

No quantitative data are available on abundance of *G. lacus*. From our observations and sampling , however, I estimate that at suitable sites they were abundant with densities up to 35/m^2 commonly in shallow heavily reeded areas within protected National Parks like the Myall Lakes. In altered habitats and areas impacted by human activity, the population numbers of *G. lacus* were low, at 1/10 m^2 around Budgewoi Lake.

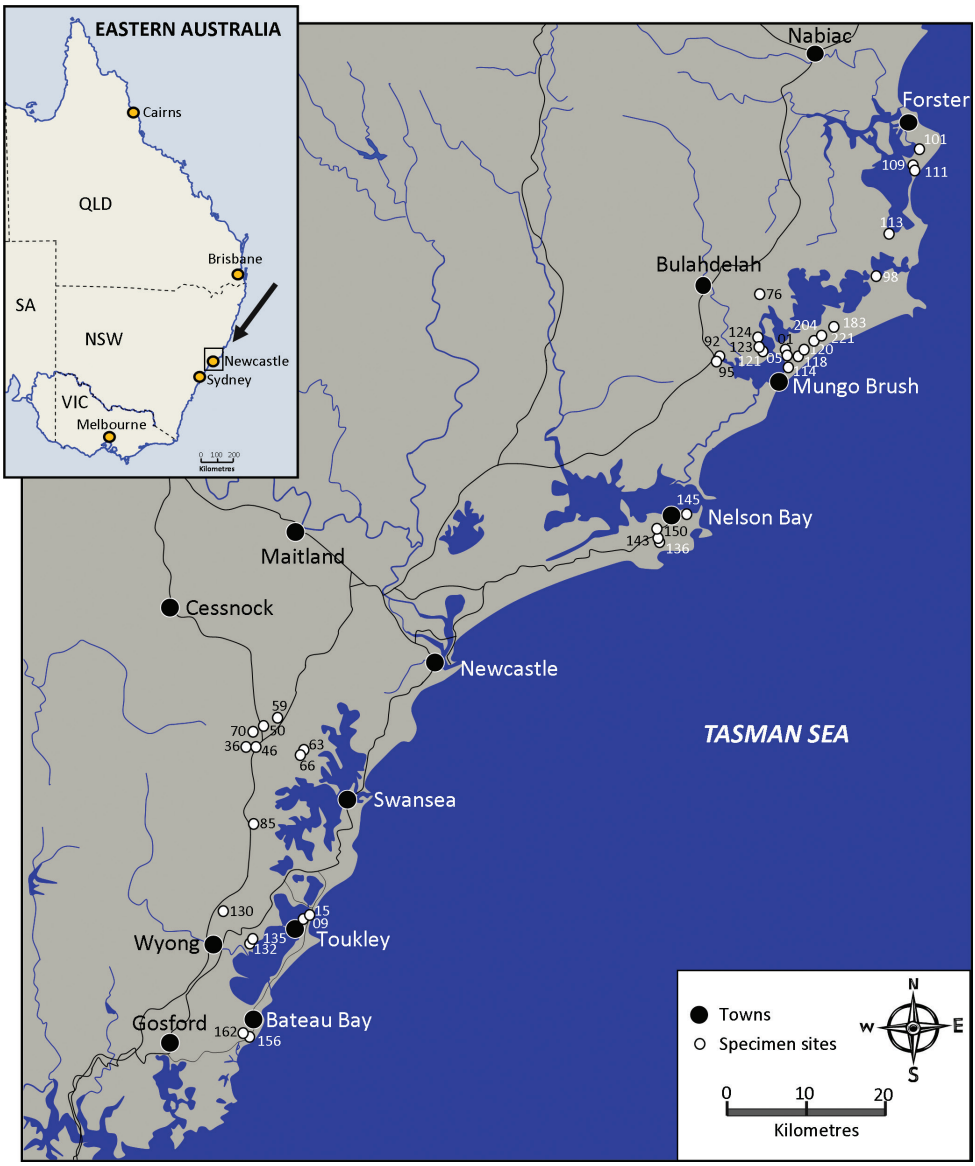

Figure 4. Distribution map. Records of population sites.

Gramastacus insolitus, unlike *G. lacus*, is a very small non-burrowing freshwater crayfish, being commensal with larger crayfish species, using their burrows to survive the seasonal drying of their habitat (Johnston and Robson 2009). *Gramastacus lacus* differs from its congener by being a robust, strong, burrowing species relying on its own burrow for extended survival during drying events. Burrows are relatively simple, with a single entrance and rounded cross-section, generally descending at a steep angle. Depth varies with soil types, but most have a single corridor, penetrating

450 to 750 mm, with older burrows having a small rounded base chamber. Deepest burrows observed extend down about one metre and generally reach the water table. The preferred lowland coastal areas generally have shallow water tables. Burrows are completely round and colloquially referred to as "bore holes"; they may be capped with mud but are generally open.

Only one adult crayfish per burrow was observed. On several occasions (at Myall and Wyee), however, one small juvenile crayfish was found with the adult. Unlike, *G. insolitus*, *G. lacus*, does not utilise the burrows of *Cherax* species, and generally other crayfish species are not found within the main habitat areas. In the northern distribution of *G. lacus*, the species was found in sympatry with *Cherax setosus*, occasionally with *Euastacus reductus* at the limits of its distribution. Typically in small intermittent streams, only *G. lacus* is found downstream in smaller/shallower burrows where the water tables are shallow, and only *C. setosus* is found upstream in much larger deeper burrows with a 20 m intermix zone between where both occur within burrows less than 200 mm apart. Unfortunately, translocated *C. destructor* exist amongst the Wamberal Lagoon population of *G. lacus* and I have grave fears for its future, with the outbreak of *C. destructor* being an ongoing significant threat to that population of native *Gramastacus* (McCormack in press).

Gramastacus lacus possesses large elongated chelae that are used quite effectively in water to defend themselves and ward off attack from other crayfish, small fish or macroinvertebrates. When submerged they will raise their open claws and wave them rapidly about in defence. However, they are seemingly rarely used for defence out of water. Crayfish are easily handled and will rarely nip when handled. Chelae are long and ungainly out of water, possibly being difficult to raise and hold extended. Individuals tend to rapidly lower their claws and retreat, preferring to avoid confrontation. In backwards retreat, the crayfish will drag the claws or use them in a series of claw over claw pushing motions to help them move more rapidly. If the crayfish moves forward, then it has two types of movement. Firstly, it will individually, alternatingly raise a claw, extend and lower it whilst moving forward till it reaches the claw tips then lift it up and extend it again. This coordinated claw over claw movement is usually only used at the start of the movement then the crayfish generally changes to a more unusual movement that may be easier or more efficient. Secondly, there is a unique forward movement via a series of rhythmic plunges. The crayfish raises the cephalothorax and both claws up with its legs and then moves forward overbalancing and plunges down and forward then repeats the movement. This up and forward movement is unusual, but the crayfish easily moves up, forward and down without "missing a beat".

Unlike many Australian freshwater crayfish, *G. lacus* is not known to be subject to temnocephalan infestation, with no records from any sampled populations. Notably, *Cherax destructor*, *Cherax setosus* and *Euastacus reductus* captured together with *Gramastacus lacus* have had healthy populations of temnocephalans. I feel that this is a significant facet of biological information that needs further research. Also noteworthy was one specimen ACP 4122 (in the vicinity of the type locality) with two eggs from an unidentified species attached to the lateral posterior of the carapace.

Further research into the genetics of each catchment population should be a priority. Other than minor setal variation on the first chelipeds, no consistent morphological differences could be identified between populations even though each population seems geographically separated, and some by large marine water barriers, such as the Hunter River and Port Stephens. The degree of genetic connectivity between populations is not yet known. I suspect that populations have been isolated through habitat fragmentation and may be highly divergent and genetically distinct, containing unique haplotypes. If so, these could represent different evolutionary significant units (ESUs) or conservation management units (MUs) that might require individual conservation attention, especially because an important goal of species conservation is to preserve genetic diversity.

As the species occurs along the coastal strip in some of the fastest developing areas of Australia, further field surveying to identify isolated populations should also be a priority, with an assessment of the conservation status of each population as currently nearly all are potentially endangered.

Acknowledgements

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Supplementary material 1

***Gramastacus lacus* specimen data.**

Authors: Robert B. McCormack

Data type: Specimen data.

Explanation note: Specimen record with locality details for known populations of the eastern swamp crayfish *Gramastacus lacus* sp. n., in coastal New South Wales.

Link: doi: 10.3897/zookeys.398.7544.app1

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Larval morphology of *Panorpodes kuandianensis* (Insecta, Mecoptera, Panorpodidae) and its evolutionary implications

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Abstract

Larval characters play a significant role in evolutionary and systematic studies of holometabolous insects. However, Panorpodidae, a derived family of Mecoptera, are largely unknown in their immature stages to date. Here, the first instar larva of the short-faced scorpionfly *Panorpodes kuandianensis* Zhong, Zhang & Hua, 2011 is described and illustrated using light and scanning electron microscopy. The larva of *Panorpodes* is remarkable for the absence of compound eyes on the head and the presence of seven small unpaired proleg-like processes along the midventral line on abdominal segments II–VIII. The homology of these unpaired appendage-like processes, their ecological adaptation, and the evolutionary implications of some larval characters of Panorpodidae are discussed.

Keywords

Chaetotaxy, evolution, homology, larva, mouthparts, proleg

Introduction

The larva is an important developmental stage of insects in Endopterygota (= Holometabola) (Grimaldi and Engel 2005; Van Emden 1957; Zacharuk and Shields 1991), the most successful lineage in terrestrial animals (Kristensen 1999). The larvae are dramatically divergent in external morphology and food habits, and frequently occupy

different ecological niches and habitats from their adults (Yang 2001). However, the evolutionary origin of insect larvae remains controversial (Hall and Wake 1999).

The Mecoptera are one of the primitive lineages in the Endopterygota, with the fossil record dated from lower Permian to Mesozoic (Byers and Thornhill 1983; Grimaldi and Engel 2005). The larvae of Panorpidae and Bittacidae are eruciform, bearing eight pairs of abdominal prolegs in addition to three pairs of thoracic legs. The prolegs are considered nonhomologous with the thoracic legs in Panorpidae, and different from other eruciform larvae in Lepidoptera and Hymenoptera (Du et al. 2009; Yue and Hua 2010). However, the larvae of Panorpodidae, the sister group of Panorpidae (Willmann 1987), have not been thoroughly investigated.

Panorpodidae consist of 13 described species distributed disjunctly in the Northern Hemisphere and are assigned to two genera (Zhong et al. 2011). *Panorpodes* MacLachlan, 1875 occurs in China, Korea, Japan, and western North America (Byers 2005; MacLachlan 1875; Tan and Hua 2008b; Zhong et al. 2011). *Brachypanorpa* Carpenter, 1931 is distributed exclusively in eastern North America (Byers 1997; Carpenter 1931b, 1953). The phylogenetic position of Panorpodidae in Mecoptera remains controversial between molecular and morphological evidence (Pollmann et al. 2008). The molecular evidence suggests that the sister group of Panorpodidae is Bittacidae (Whiting 2002), while morphological studies demonstrate a sister relationship between Panorpodidae and Panorpidae (Friedrich et al. 2013; Willmann 1987, 1989). Based on biological and morphological characters, Penny (1977) even concluded a close relationship between Panorpodidae and Boreidae. Detailed studies on larval morphology may provide additional or even crucial evidence for the phylogenetic analysis of Mecoptera (Beutel et al. 2009).

The knowledge of Panorpodidae larvae is far from satisfactory largely owing to the restricted species distribution and the mysterious larval diets (Byers 1997; Byers and Thornhill 1983; Carpenter 1931a, 1953; Zhong et al. 2011). The larvae of the North American *Brachypanorpa* are eyeless and lack prolegs on abdominal segments, and are regarded as scarabaeiform (Byers 1997), although a small cylindrical structure is present mid-ventrally on each abdominal segments III–VI of the larva. Suzuki (1985, 1990) successfully obtained the first instar larva of *Panorpodes paradoxa* in his embryological study, but provided no detailed description, such that the knowledge of larval *Panorpodes* still remains largely unknown.

In this study, we investigated the larvae of the short-faced scorpionfly *Panorpodes kuandianensis* Zhong, Zhang & Hua, 2011 through rearing, and illustrated the first instar larvae using light and scanning electron microscopy, in an attempt to acquire more evidence for the larval evolutionary study of Mecoptera.

Materials and methods

Adults of *P. kuandianensis* were captured from Huaboshan (41°06'N, 125°02'E, elev. 650–1100 m), Kuandian County, Liaoning Province of northeastern China from late June to July in 2011 and 2012. The adults were reared in pairs in plastic jars covered

with a piece of gauze. Humid soil (5 cm in depth) covered with moss was placed at the bottom of the jar for adults resting and oviposition. Fresh leaves, flowers and honey drops were daily provided as potential food items.

First instar larvae were fixed in Carnoy's fixative solution (95% ethanol: glacial acetic acid = 3:1, v/v) for 12 h before being preserved in 75% ethanol. After dehydration in a graded ethanol series (75%, 85%, 95%, 100%), the samples were transferred to isoamyl acetate twice for 30 min, critical-point dried with liquid carbon dioxide, sputter-coated with gold, and examined in a Hitachi S-3400N scanning electron microscope (Hitachi, Tokyo, Japan) at 15 kV.

To illustrate chaetotaxy, SEM photographs were taken for each segment of the first instar larva on dorsal, lateral and ventral surfaces, respectively. Draft drawings were improved with Adobe Illustrator CS4.

Results

General morphology of the larva

The first instar larva is white and 2.9 ± 0.31 mm in length (n = 10) (Fig. 1). The head is hypognathous and eyeless, with mandibulate mouthparts directed downward and a pair of three-segmented antennae lateroventrally. The trunk is cylindrical and furnished with numerous cuticular spinules and setiform setae. The thorax possesses three pairs of legs. The abdomen has eleven segments and possesses seven unpaired appendage-like processes mid-ventrally on each of abdominal segments II–VIII. The respiratory system is peripneustic, with one pair of spiracles on the prothorax and eight pairs of spiracles on the first eight abdominal segments. The telson bears a protrusile sucker.

Head capsule

The head is slightly flattened, 450 ± 15 μm in length and 315 ± 17 μm in width (n = 10) (Fig. 2A–C), lacking compound eyes, ocelli, or stemmata (Fig. 2C). The frons is subtriangular and is confined by two ecdysial cleavage lines and a frontoclypeal sulcus (Fig. 2B), bearing centrally a sharp egg burster, which aids in hatching of the larva (Fig. 2B, C). A pair of anterior tentorial pits is situated at the lateral corners of the frons (Fig. 2B). Thirteen pairs of setiform setae are present on the head capsule symmetrically (Fig. 2A–C). Additionally, four pairs of minute setae occur on the occiput (Fig. 2C).

Antennae

The antennae are three-segmented, each consisting of a basal scape, a pedicel, and a slender flagellum (Fig. 2D). The scape is very short and inserted into a prominent

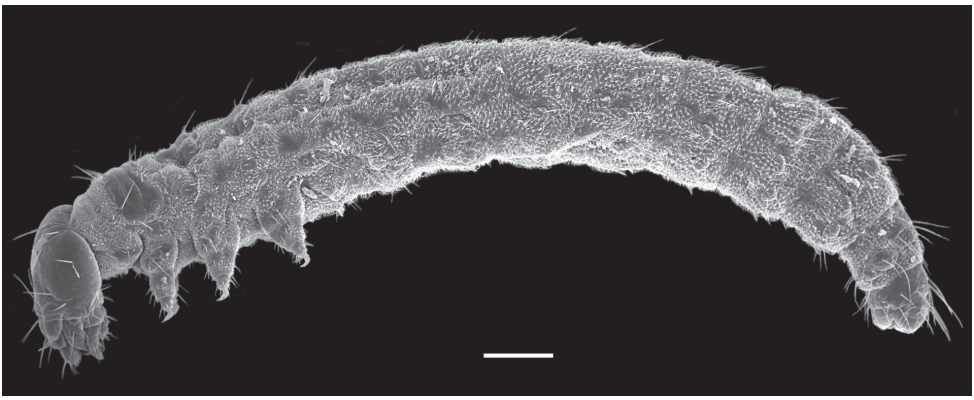

Figure 1. First instar larva of *Panorpodes kuandianensis*. Scale bar = 200 μm.

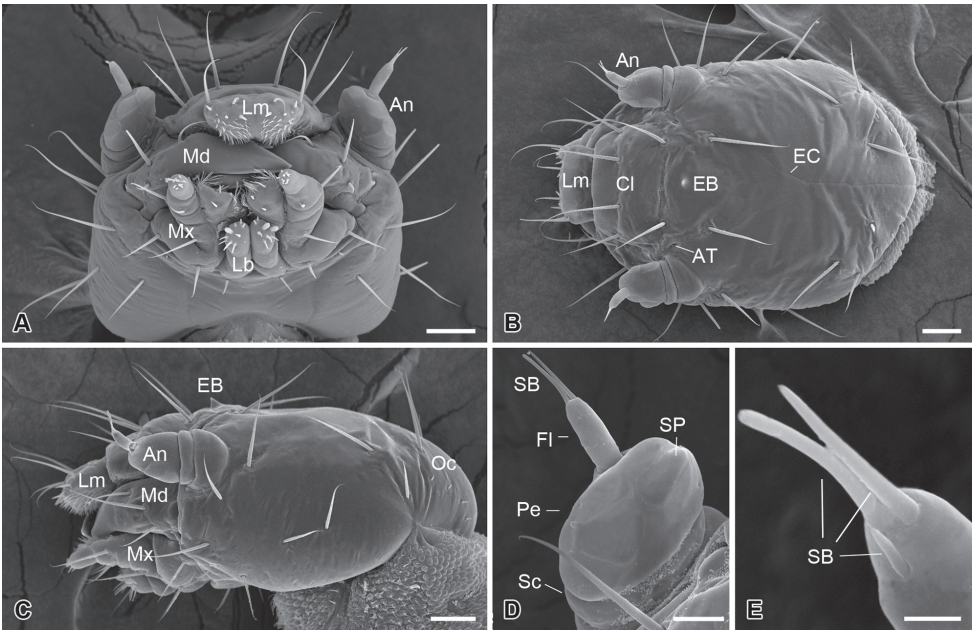

Figure 2. Larval head of *Panorpodes kuandianensis*. **A** Ventral view **B** Dorsal view **C** Lateral view **D** Antenna (ventral view) **E** Sensilla on flagellum (dorsal view). Abbreviations: **An =** antenna, **AT** = anterior tentorial pit, **Cl** = clypeus, **EB** = egg burster, **EC** = ecdysial cleavage, **Fl** = flagellum, **Lb** = labium, **Lm** = labrum, **Md** = mandible, **Mx** = maxilla, **Oc** = occiput, **Pe** = pedicel, **SB** = sensillum basiconicum, **Sc** = scape, **SP** = sensillum placodeum. Scale bars: (**A**)–(**C**) = 50 μm, (**D**) = 20 μm, (**E**) = 5 μm.

antennal socket. The pedicel is stout and slightly conical, five times longer than the scape, with ten sensilla placodea on the ventral surface. The distal flagellum is slender, inserted on the lateral apex of the pedicel, and bears apically one short and two long sensilla basiconica (Fig. 2E).

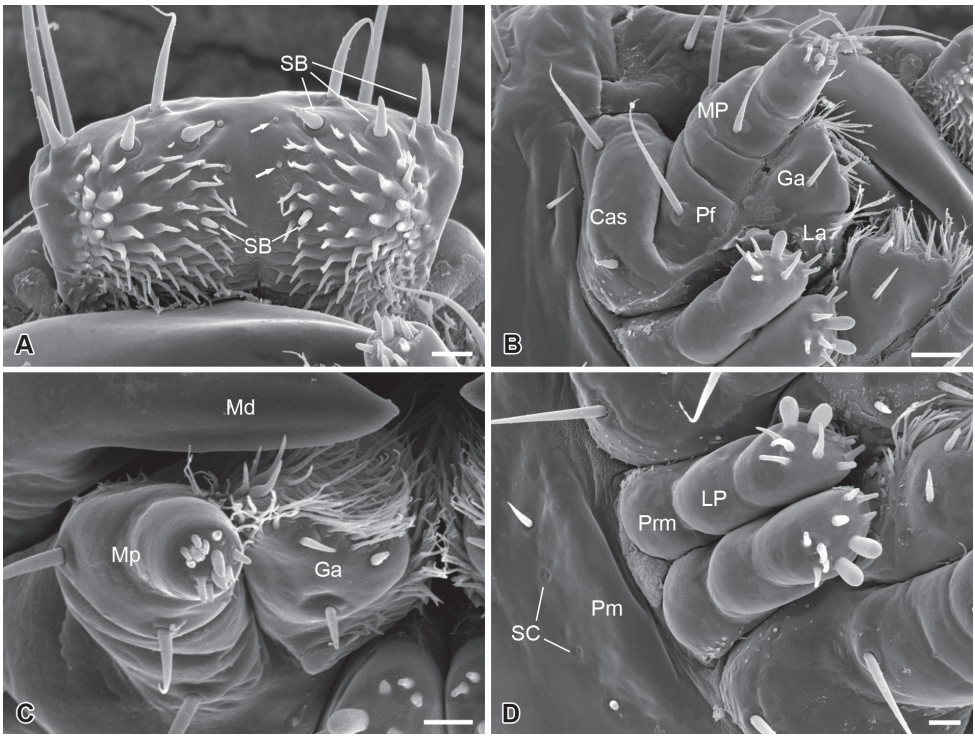

Figure 3. Larval mouthparts of *Panorpodes kuandianensis*. **A** Epipharyx, arrows show the inconspicuous sensilla basiconica **B** Maxilla (ventral view) **C** Maxilla (frontal view) **D** Labium. Abbreviations: **Cas** = cardo-stipes, **Ep** = epipharynx, **Ga** = galea, **La** = lacinia, **Lb** = labium, **LP** = labial palpus, **Md** = mandible, **MP** = maxillary palpus, **Pf** = palpifer, **Pm** = postmentum, **Prm** = prementum, **SB** = sensillum basiconicum, **SC** = sensillum campaniformium. Scale bars: (**A**), (**C**) and (**D**) = 10 μm, (**B**) = 20 μm.

Mouthparts

The mouthparts are of the mandibulate type (Fig. 2A), consisting of a labrum, a pair of mandibles, a pair of maxillae, and a labium.

The labrum is roughly trapezoid, articulated proximally with the anterior region of the clypeus (Fig. 2B). The labrum bears two pairs of apical setae, with the inner pair nearly half length of the outer pair (Fig. 2B).

The epipharynx is situated on the inner surface of the labrum (Fig. 3A), with three pairs of sensilla basiconica along the apical margin, a pair of short sensilla basiconica and two pairs of inconspicuous sensilla basiconica on the central part. The epipharynx is also furnished with sparse short microtrichia pointed inward at the lateral part, but lacks microtrichia along the middle axis.

The mandible is highly sclerotized, with the sharp incisor incurved apically; the mandibles cross each other apically. Each mandible possesses three sensilla chaetica on the outer surface (Fig. 2A–C).

The maxilla consists of a cardo-stipes, a galea, a lacinia, and a three-segmented palp (Fig. 3B). The original cardo and stipes are fused into a cardo-stipes, which bears two sensilla chaetica. The galea possesses three sensilla basiconica ventrally and numerous microtrichia distally (Fig. 3B, C). The lacinia is greatly reduced and bears a cluster of microtrichia distally. The palpifer carries a long sensillum chaeticum on the ventral surface. The maxillary palp is three-segmented and bears two short sensilla chaetica on the lateral surface of the second joint and 12 sensilla basiconica on the apical surface of the third joint (Fig. 3C).

The labium is highly vestigial, with the ligula absent (Fig. 3D). The postmentum is merged with the head capsule, bearing a pair of short sensilla chaetica and a pair of sensilla campaniformia. The prementum is mesally separated and bears distally a pair of two-segmented labial palps. The distal segment of the labial palp bears two large papillary and eight conical sensilla basiconica on the apex. These sensilla are slightly varied from specimen to specimen, even asymmetrical bilaterally between the left and the right palp.

Thoracic legs

The thoracic legs are four-segmented, each consisting of a coxa, a femur, a tibia, and a tarsus (Fig. 4A). The coxa and femur are sclerotized on the anterior surface but membranous on the posterior surface. The femur and tibia bear several microsetae. The tarsus is slender and curved cephalad, with a hirsute anterior surface and a wrinkled posterior surface (Fig. 4A).

Spiracles

Nine pairs of spiracles are located on the pleura of the larval trunk. The prothoracic spiracle is on the posterior corner of the prothoracic shield, with nine apertures surrounding the atrial orifice (Fig. 4C). Eight pairs of abdominal spiracles each are present on the pleura of the first eight abdominal segments, with 4–5 apertures (Fig. 4D).

Abdomen

The abdomen consists of 11 segments and is furnished with numerous setiform setae and prominent cuticular spinules (Fig 1). The larval abdomen bears seven inconspicuous unpaired mid-ventral processes on each A2–A8, with these smooth and unsegmented processes varying in length, greatly reduced on A2 (Fig. 4B, E, and F). The larval abdomen terminally bears a protrusile sucker, providing adhesive attachment during locomotion (Fig 4F).

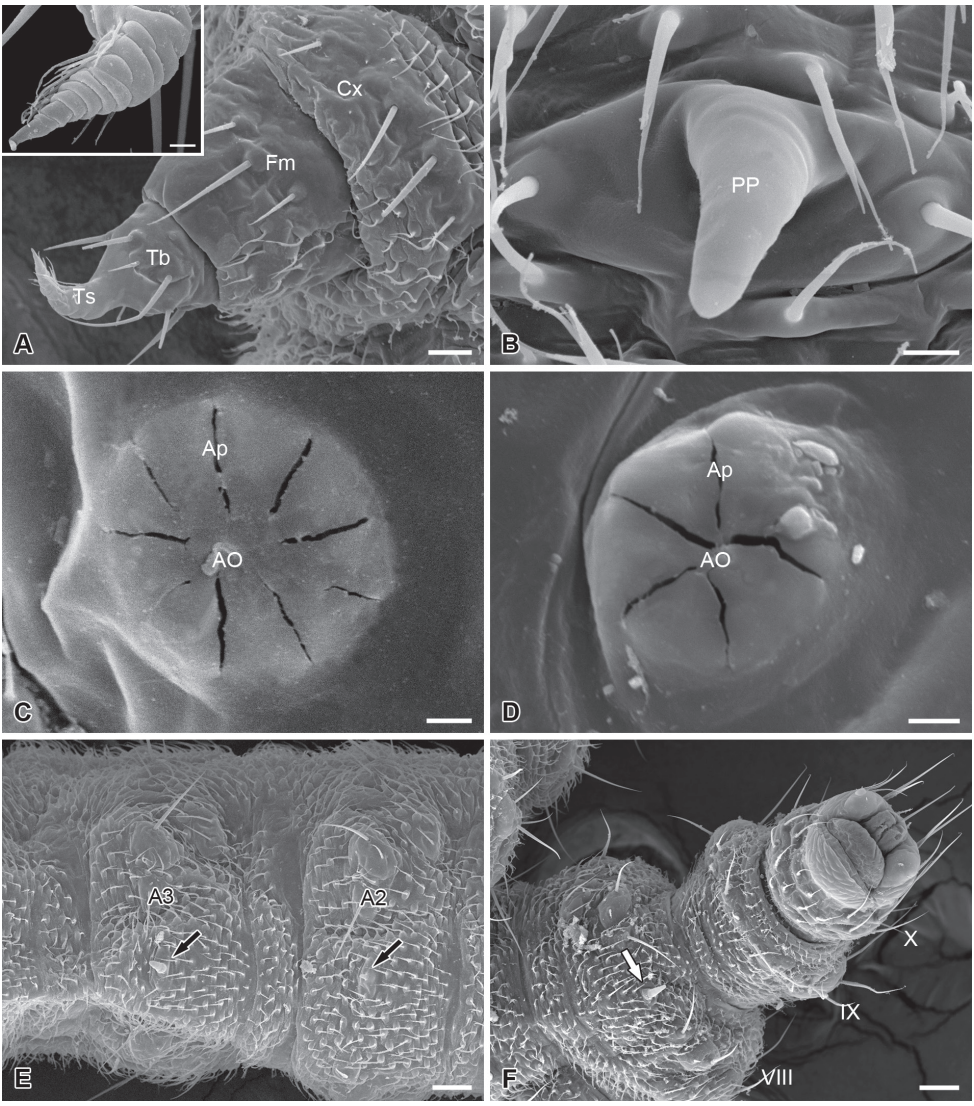

Figure 4. Thoracic leg, abdominal processes, spiracles and telson of the larva of *Panorpodes kuandianensis*. **A** Thoracic leg, inset shows magnification of the tarsus of thoracic leg **B** Proleg-like abdominal process **C** Prothoracic spiracle **D** Abdominal spiracle **E** Ventral view of abdominal segments II and III **F** Telson (ventral view). Abbreviations: **AO** = atrial orifice, **Ap** = aperture, **Cx** = coxa, **Fm** = femur, **PP** = proleg-like process, **Tb** = tibia, **Ts** = tarsus. Scale bars: (**A**) = 20 μm, (**B**) = 5 μm, (**C**) and (**D**) = 3 μm, (**E**) = 40 μm, (**F**) = 50 μm.

Chaetotaxy of the larval trunk

The meso- and metathorax are similar in chaetotaxy. Abdominal segments I–VII are similar in chaetotaxy (Fig. 5).

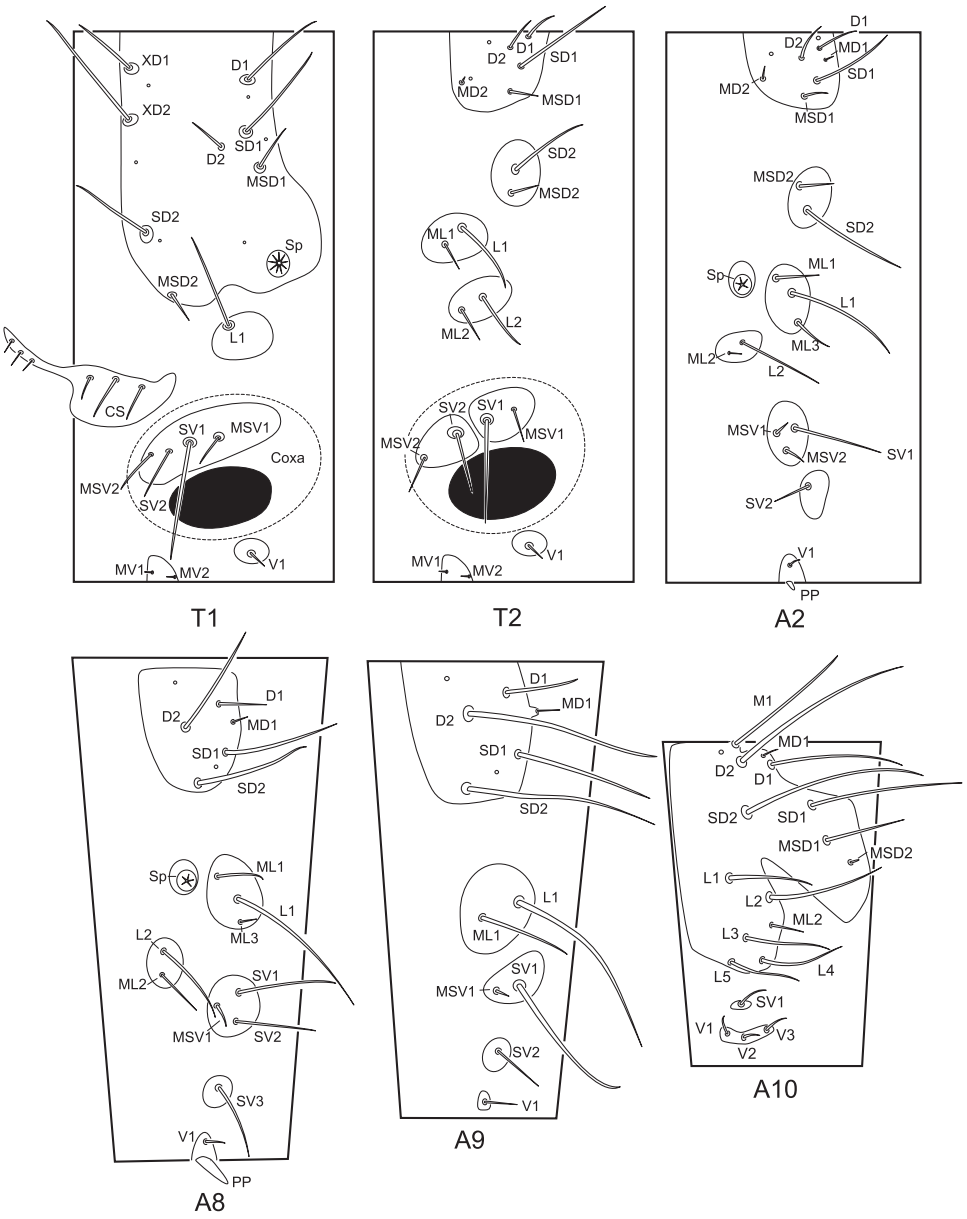

Figure 5. Chaetotaxy of the larval trunk of *Panorpodes kuandianensis*. Abbreviations: **CS** = cervical sclerite, **D** = dorsal seta, **L** = lateral seta, **M** = mid-dorsal seta, **MD** = minute dorsal seta, **MSD** = minute subdorsal seta, **MSV** = minute subventral seta, **MV** = minute ventral seta, **PP** = proleg-like process, **SD** = subdorsal seta, **Sp** = spiracle, **SV** = subventral seta, **V** = ventral seta, **XD** = prothoracic seta.

Prothorax (T1). The prothorax bears a prominent prothoracic shield, along the anterior margin of which are three long setae (XD1, XD2, and SD2) and one short seta (MSD2). Along the posterior edge of the shield are two long setae (D1 and SD1) and

one short seta (MSD1). Below the shield is a long lateral seta (L1) alone on the lateral pinaculum. Two long setae (SV1 and SV2) and two short setae (MSV1 and MSV2) are on the subventral pinaculum. Mesal to the coxal cavity are one short ventral seta (V1) on a small pinaculum and a pair of minute setae (MV1 and MV2) on a midventral pinaculum.

Meso- and metathorax (T2 and T3). On the dorsal pinaculum are one long seta (SD1), three short setae (D1, D2, and MSD1), and one minute seta (MD2). On the subdorsal pinaculum are one long seta (SD2) and one short seta (MSD2). Two lateral pinacula each bear a long and a short seta (L1 and ML1, L2 and ML2). Two subventral pinacula each bear a long seta and a short seta (SV1 and MSV1, SV2 and MSV2). The ventral setae (V1, MV1, and MV2) exhibit a similar pattern to prothorax.

Abdominal segments II–VII (A2–A7). On the dorsal pinaculum are three long setae (D1, D2, and SD1) and three short setae (MD1, MD2, and MSD2). On the subdorsal pinaculum are one long and one short seta (SD2 and MSD2). On the lateral pinaculum posterior to the spiracle are one long (L1) and two short setae (ML1 and ML3). Another small lateral pinaculum below the spiracle bears a long (L2) and a short seta (ML2). One long (SV1) and two short setae (MV1 and MV2) are arranged on a subventral pinaculum. A short seta (SV2) is situated alone on another subventral pinaculum. The midventral pinaculum bears a short ventral seta (V1).

Abdominal segment VIII (A8). The dorsal pinaculum bears three long setae (D2, SD1, and SD2), one short seta (D1), and one minute seta (MD1). One long (L1) and two short setae (ML1 and ML3) are situated on the lateral pinaculum posterior to the spiracle. Another lateral pinaculum below the spiracle bears two setae (L2 and ML2). Two long setae (SV1 and SV2) and one minute seta (MSV1) are arranged on a subventral pinaculum, whereas a long seta (SV3) alone is located on another pinaculum. One short seta (V1) is situated on the midventral pinaculum lateral to the mid-ventral abdominal process (AP).

Abdominal segment IX (A9). On the dorsal pinaculum are three long setae (D2, SD1, and SD2) and one short seta (D1). On the lateral pinaculum are one long (L1) and one short seta (ML1). One long seta (SV1) is located on one subventral pinaculum. One short seta (SV2) is on another subventral pinaculum. A ventral seta (V1) is situated alone on the ventral pinaculum.

Abdominal segment X (A10). The epiproct bears one mid-dorsal seta (M1). Four long setae (D1, D2, SD1, and SD2) and one short seta (MSD1) are situated on the dorsal part of the tergum. Five long (L1–L5) and one short setae (ML2) are inserted on the pleuron. On the subventral pinaculum is one short seta (SV1). Three short setae (V1, V2, and V3) are arranged on the elongated narrow ventral pinaculum.

Discussion

The larvae of Panorpodidae represented by *Panorpodes* are unique in Mecoptera for the absence of compound eyes on the head, presence of several unpaired midventral

processes on A2–A8, and absence of erect subdorsal annulated processes on stout basal protuberances as in Panorpidae and Bittacidae (Chen and Hua 2011; Jiang and Hua 2013; Ma et al. 2014; Tan and Hua 2008a).

In Mecoptera the larvae are eruciform in Panorpidae, Choristidae, Apteropanorpidae, and Bittacidae (Byers 1991; Jiang and Hua 2013; Tan and Hua 2008a); campodeiform in Nannochoristidae (Pilgrim 1972); and scarabaeiform in Boreidae (Cooper 1974; Penny 1977; Russell 1982). The larvae of *Brachypanorpa* in Panorpodidae were also described as scarabaeiform (Byers 1997). Considering the presence of the unpaired midventral abdominal processes on the larvae of *Panorpodes* and *Brachypanorpa*, however, it is difficult to regard them as true scarabaeiform larvae.

In general, the larvae of Mecoptera are remarkable for the presence of a pair of compound eyes (Chen et al. 2012; Gilbert 1994; Melzer et al. 1994; Pilgrim 1972; Tan and Hua 2008a). The larval compound eye is composed of ten or more ommatidia in Nannochoristidae (Melzer et al. 1994; Pilgrim 1972), three "stemmata" in *Boreus* (Cooper 1974) and seven in *Caurinus* of Boreidae (Russell 1982), seven ommatidia (or "stemmata") in Bittacidae (Gilbert 1994; Tan and Hua 2008a), and approximately 20–40 ommatidia in Panorpidae (Boese 1973; Chen et al. 2012; Melzer 1994; Paulus 1979), representing a true plesiomorphy of Mecoptera in Endopterygota (Beutel et al. 2009). A dorsal ocellus is also present on the larval head of Bittacidae (Tan and Hua 2008a, 2009). The larvae of *Panorpodes*, however, are completely eyeless, congruent with the larvae of *Brachypanorpa* (Byers 1997). In fact, the visual organs (optic lobe) of *Panorpodes paradoxa* are present in the early embryonic stage, but are degenerate in later stages, and finally disappear by the end of embryonic revolution (Suzuki 1985), indicating this eyelessness is a secondary degeneration and represents an autapomorphy of Panorpodidae.

The larval prolegs of Panorpidae are formed by an inner pair of proleg primordia near the midventral line mesal to the true appendage primordia, and are not homologous with the thoracic legs (Yue and Hua 2010), confirming the hypothesis that prolegs are secondary adaptive structures (Hinton 1955). The presence of unpaired midventral processes in Panorpodidae larvae is difficult to explain by a recent hypothesis of coxal endite on the evolutionary origin of larval prolegs (Bitsch 2012). Because of the shared similarities (each process is delimited by the paired ventral setae, and these processes are varied in length with anterior one great reduced but posterior one longest) of Panorpodidae and Panorpidae, the unpaired midventral processes are likely homologous with and degenerated from the prolegs of the eruciform larvae in Panorpidae. The degeneration of larval prolegs as a rule was considered an evolutionary tendency in most Diptera, leaf-mining Lepidoptera, Coleoptera, and parasite Hymenoptera and Strepsiptera (Chapman 2013). In this case, the unpaired midventral processes may represent an advanced evolutionary stage of larval abdominal prolegs, and Panorpodidae may occupy a derived position in the phylogeny of Mecoptera.

The larvae of Panorpodidae lack dorsal protuberances on the first ten abdominal segments, distinctly divergent from those of Bittacidae and Panorpidae. In Bittacidae, the furcated protuberances borne on the dorsal surface of the larval trunk may assist

adhering to soil particles as a camouflage (Tan and Hua 2008a). In Panorpidae, annulated protuberances are present on the larval trunk and are considered to keep the larval trunk from being injured in a subterresial life style (Ma et al. 2014). In Panorpodidae, the larvae of *Panorpodes kuandianensis* stay sedentary subterraneally with limited range of locomotion (L Jiang, unpublished data). We speculate that the absence of dorsal protuberances on the abdomen likely resulted from its inactive living habit in the soil, as in the soil-dwelling larvae in Scarabaeidae (Eilers et al. 2012).

The peculiar morphological characters of panorpodid larvae are likely related to their cryptic lifestyle. In the underground habitat, the larvae of Panorpodidae may reasonably use olfaction or gustation rather than vision as their sense organs. This situation is similar to the eyeless soil-dwelling larvae in Scarabaeidae (Eilers et al. 2012). Likewise, the larvae of Panorpodidae no longer need paired abdominal prolegs to support the abdomen and serve the locomotory function as in the larvae of Panorpidae (Yue and Hua 2010), thus their prolegs are reduced to vestigial unpaired mindventral processes. This reduction of prolegs may reduce the friction of the abdomen with the substrate, and facilitate the locomotion of the larvae in the soil.

During their evolution from the Mesozoic (Byers and Thornhill 1983; Grimaldi and Engel 2005), the Mecoptera have evolved diverse larvae to adapt to various living habits. In most primitive Nannochoristidae the larvae stay in the substrate of streams and prey on the larvae of Chironomidae (Fraulob et al. 2012). In Boreidae the larvae of *Boreus* creep over plants and feed on fresh leaves (Cooper 1974), whereas the larvae of *Caurinus* feed in stem-mines or galleries of leafy liverworts (Russell 1982) or perhaps on other materials in recently deforested clear cuts (Sikes and Stockbridge 2013). In Bittacidae and Choristidae the larvae live on the surface of soil and feed on dead arthropods (Byers 1991; Tan and Hua 2008a). In Panorpidae the larvae live mostly in the soil, burrowing and concealing themselves while feeding on dead arthropods (Mampe and Neunzig 1965). In Panorpodidae, however, the larvae of *Panorpodes* are peculiar for their sedentary living habits and potentially live a root-feeding lifestyle. This is similar to the soil-dwelling and root-feeding larvae in Scarabaeidae, which are mostly eyeless and lack abdominal prolegs (Lawrence 1991). The consistency may indicate that the eyeless and proleg-reduced larval morphology are secondary adaptive traits to the soil-dwelling lifestyle.

In our rearing trial, the first instar larvae of *Panorpodes* fed on neither dead arthropods nor fresh leaves, although a darkened line in the alimentary canal was observed through the translucent trunk (L Jiang, unpublished data). The larvae we reared died eventually without molting, resulting in a failure to obtain the following instar larvae and pupae. This situation is similar to the observation of the confamilial *Brachypanorpa* (Byers 1997). The larval morphology and biology of later instars remain unknown.

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Sawfly taxa (Hymenoptera, Symphyta) described by Edward Newman and Charles Healy

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Abstract

Type specimens of seven nominal species of sawfly described by Edward Newman and one by Charles Healy were studied. This material is housed in the Oxford University Museum of Natural History, United Kingdom. The following new synonymies are proposed (valid names in parentheses): *Hartigia* Schiødte, 1839 (*Phylloecus* Newman, 1838), *Cephus helleri* Taschenberg, 1871 (*Phylloecus faunus* Newman, 1838) and *Euura gallae* Newman, 1837 (*Euura mucronata* (Hartig, 1837)). The type species of *Euura* Newman, 1837 and *Euura* subgenus *Gemmura* E. L. Smith, 1968 belong to the same taxonomic species, *Euura mucronata* (Hartig, 1837), so that these genus group names become new synonyms. Lectotypes are designated for *Phyllotoma tormentillae* Healy, 1868, *Fenusa ianthe* Newman, 1837, *Fenusa parviceps* Newman, 1837, *Selandria pallida* Newman, 1837 and *Phylloecus faunus* Newman, 1838. 26 new combinations are proposed for species formerly placed in *Hartigia* and here transferred to *Phylloecus*, and 4 original combinations are re-instated as valid.

Keywords

Taxonomy, Tenthredinidae, Cephidae, *Euura*, *Phylloecus*, *Hartigia*, new synonyms, new combinations

Introduction

Edward Newman (1801–1876) described 24 species-group and six genus-group sawfly taxa as new to science. In many cases, the type material of these nominal taxa has apparently never been re-examined. During ongoing studies on West Palaearctic nematine sawflies (see STI Nematinae Group 2013), it became clear that clarification of the identity of *Euura gallae* Newman, 1837 is necessary. This being the type species of *Euura* Newman, 1837, the correct interpretation of the species name is required to ensure future nomenclatural stability. Through the kind assistance of the staff of the Oxford University Museum of Natural History (OUMNH), potential type specimens of several species described by Newman were located and sent to us for examination. Although only a few of these taxa belong to the Nematinae, it seems appropriate to deal here with the entire material, as well as the type series of a species described by Charles Healy (1826–1876). Newman undertook the identification of the Tenthredinidae, mostly leaf-mining species, on which Healy published several papers describing their biology.

In his introduction, Newman (1837) stated that the material referred to in that article was "in the possession of the Entomological Club". The statement applies also to the sawflies discussed by Newman (1838), which despite its different title, is effectively a continuation of the same work. The Hymenoptera in the collection of the Entomological Club were donated in to the OUMNH in 1927 (Smith AZ 1986; J. Hogan personal communication).

Material and methods

All specimens mentioned in this paper are deposited in the Hope Collections, Oxford University Museum of Natural History, Oxford, United Kingdom. They are all mounted in a similar way (Figs 1, 7): pinned along the dorso-ventral axis through the thorax with a short, headless pin which is carried on a small cardboard stage supported by a longer pin with a head. When the specimens were received for examination, nearly all had only a single label, with an identical printed, lower part (Figs 6, 12). At the top of this label appears the handwritten name under which the specimens stood in the collection of the Entomological Club. Although these labels are not original, they are interpreted here as representing determinations made by Newman. None of the specimens bears any data on collection locality or date on the labels or cardboard stage.

Taxa are listed in alphabetical order, under their current valid names. Complete lists of all the synonyms of species mentioned below and references to their original descriptions may be found in Taeger et al. (2010). Below are cited only the names and descriptions of taxa described by Newman or Healy, and of taxa considered here to be conspecific with the former, when the latter names are in general current use as valid.

Results and discussion

***Euura mucronata* (Hartig, 1837) (Tenthredinidae)**
http://species-id.net/wiki/Euura_mucronata

= *Euura gallae* Newman, January 1837: 260; sex not stated; type locality: Scotland. **syn. n.**
= *Nematus* (*Cryptocampus*) *mucronatus* Hartig, March 1837: 223; ♀♂; type locality: not stated.

Type material examined. *Euura gallae*. Holotype ♀, figs 1–6: "[handwritten] Euura gallae Newm. [printed] Det. in Coll. Ent. Club , Inst.'d 1826. Pres'd 1927 by Club to Hope Coll.", "[red] Holotype (teste A. Liston, 2013) Euura gallae Newman, 1837", "Euura mucronata (Hartig, 1837) det. Liston 2013", "DEI-GISHym 19993". Condition: apical three flagellomeres of both antennae and right rear tarsus missing.

Discussion. Newman's very short original description of *Euura gallae*, based on a single specimen [holotype], is impossible to identify as belonging to one of the currently recognised species. The description reads: "Euura gallae. *Nigra : antennis nigris, apice ferrugineis: pedibus pallidis.* Black: mouth yellow; antennæ rust-coloured at the tip; the legs *entirely* pale. The insect is the size of *Nematus pallipes:* the only specimen I have observed was taken by Mr. Walker, in Scotland."

Five specimens bearing the name *Euura gallae*, all females, were found in the Hope Collections. Four of these belong to the *Euura atra* species group. They have nearly completely dark mouthparts, except for the labrum, and the femora are conspicuously black basally. They therefore do not agree with the description of the holotype. The fifth specimen (Figs 1–6) has more extensively pale mouthparts and antennae, the malar area is conspicuously pale, and the legs are almost completely pale. This specimen is identified as the holotype of *E. gallae*.

Newman's description of *E. gallae* pre-dates Hartig's of *N. mucronatus* by a couple of months. Article 23.9 of the International Code of Zoological Nomenclature is here applied, to reverse the precedence of the species names, because the name *E. gallae* (nomen oblitum) has not been used as valid after 1899, and *E. mucronata* (*Nematus mucronatus*: nomen protectum) has been used as a valid species name in [very many] more than 25 works published by more than 10 authors in the last 50 years. A list of these references is available from us on request.

The type species of *Euura* Newman, 1837 by subsequent designation of Rohwer (1911: 80) is *Euura gallae* Newman, 1837. Dalla Torre (1894: 276) listed *E. gallae* as a valid species of *Cryptocampus* Hartig, 1837, but with a footnote "= ? *C. saliceti* (Fall.)." At this time, *C. saliceti* (Fallén, 1808) was in use as the name of the species called *Euura mucronata* (Hartig, 1837) by most recent authors. Konow (1905b) placed *E. gallae* as a synonym of *Cryptocampus medullarius* (Hartig, 1837). The latter is a junior subjective synonym of the species currently known as *E. amerinae* (Linnaeus, 1758). Rohwer (1911: 94) and all subsequent authors followed Konow's opinion, in that *gallae* was regarded as a synonym of *E. amerinae*, or one of the subjective junior synonyms of that

Figures 1–6. *Euura gallae* Newman, 1837; holotype **1** dorsal **2** head, dorsal **3** head, lateral **4** abdomen, dorsoapical **5** abdomen, lateral **6** labels.

taxon. It is unlikely that any specialist, apart from Newman himself, has examined the holotype of *E. gallae*. Clearly, the identity of *E. gallae* has until now been widely misinterpreted. As a result of the new identification, the genus group name *Gemmura* E. L. Smith, 1968 (type species *Nematus mucronatus* Hartig, 1837), proposed as a subgenus of *Euura*, becomes a junior objective synonym of *Euura* Newman, sensu stricto. If in future it should be considered that recognition of subgenera within *Euura* is necessary, then a new name for the stem-galling groups would be needed. However, in our opinion there is at present neither sufficient phylogenetic support, nor a practical justification (because the genus includes too few species) for such an act. Distinction of species

groups, if considered necessary, should be achieved by employing "informal" group names whose use is not regulated by the International Code of Zoological Nomenclature. Such names might, for example, be the "mucronata group" for the bud-gallers and the "atra group" for the stem-gallers.

***Fenella nigrita* Westwood, 1839 (Tenthredinidae)**
http://species-id.net/wiki/Fenella_nigrita

= *Fenella nigrita* Westwood, 1839: 54; sex not stated; type locality: not stated.
= *Phyllotoma tormentillae* Healy, 1868: 140–141; larvae; adults reared [sex not stated] but not described; type locality: Highgate, Hornsey, Hampstead, Norwood and Croydon [parts of London].

Type material examined. *Phyllotoma tormentillae*. Lectotype (**hereby designated**) ♀ [adult]: "[handwritten] Phyllotoma tormentillae, N. [printed] Det. in Coll. Ent. Club, Inst.'d 1826. Pres'd 1927 by Club to Hope Coll."; "[red] Lectotype Phyllotoma tormentillae Healy, 1868 des. A. Liston 2013"; "Fenella nigrita Westwood, 1839 det. A. Liston 2013", "DEI-GISHym 19994". Condition: fair. Paralectotypes: 11♀ with same original labels, [blue] paralectotype labels, and "Fenella nigrita Westwood, 1839 det. A. Liston 2013". One paralectotype has a large handwritten label reading "Phyllotoma Tormentillae N A complete life history of this species by Mr Healy appears in the Entomologist Vol iv p140".

Discussion. It is considered that the lectotype of *Phyllotoma tormentillae* [adult] was reared from the larvae described by Healy. The synonymy of *P. tormentillae* with *F. nigrita* as given by Kirby (1882) and in various subsequent works is confirmed.

***Harpiphorus lepidus* (Klug, 1818) (Tenthredinidae)**
http://species-id.net/wiki/Harpiphorus_lepidus

= *Tenthredo* (*Emphytus*) *lepida* Klug, 1818: 277–278; ♀♂; type locality: Germany.
= *Fenusa ianthe* Newman, 1837: 261; sex not stated; type locality: "[..]woods of the metropolitan district[..]" (= around London).
= *Asticta ianthe*: Newman (1838: 484), comb. n.

Type material examined. *Fenusa ianthe*. Lectotype (**hereby designated**) ♀:"[handwritten] Phyllotoma"; "[handwritten] P. ianthe, Newm [printed] Det. in Coll. Ent. Club , Inst.'d 1826. Pres'd 1927 by Club to Hope Coll."; "[red] Lectotype Fenusa ianthe Newman, 1837 des. A. Liston 2013"; "Harpiphorus lepidus (Klug, 1818) det. A. Liston 2013", "DEI-GISHym 19995". Condition: fair, but apical tarsomeres of all legs missing.

Discussion. Although the sex of the type specimen[s] is not explicitly mentioned by Newman, the described colour pattern is found only in the female of this species.

The comment "This insect appears generally distributed[..]" leads one to suppose that the description is based on more than one specimen. However, Westwood (1839: 53) wrote "This description is drawn from Mr. Newman's typical specimen, which he has been so kind as to lend me; and of which the fore wings are unlike, the transverse nerve separating the first two submarginal cells being obliterated in one of them[..]". The specimen here designated as lectotype possesses this abnormality (vein Rs+M is missing in the left forewing), and therefore is probably the same specimen as examined by Westwood. The synonymy of *F. ianthe* with *H. lepidus*, already adopted by Kirby (1882), is confirmed.

***Heterarthrus nemoratus* (Fallén, 1808) (Tenthredinidae)**
http://species-id.net/wiki/Heterarthrus_nemoratus

= *Hylotoma nemorata* Fallén, 1808: 47; ♀; type locality: Sweden [according to title of work].
= *Fenusa parviceps* Newman, 1837: 261–262; sex not stated; type locality not stated.
= *Druida parviceps* (Newman, 1837); Newman 1838: 484.

Type material examined. *Fenusa parviceps*. Lectotype **(hereby designated)** ♀:"[handwritten] Druida parviceps, Newm [printed] Det. in Coll. Ent. Club , Inst.'d 1826. Pres'd 1927 by Club to Hope Coll."; "[red] Lectotype Fenusa parviceps Newman, 1837 des. A. Liston 2013"; "Heterarthrus nemoratus (Fallén, 1808) det. A. Liston 2013", "DEI-GISHym 19996". Condition: fair. Paralectotypes: 2♀ and 1 cocoon-disc with same original labels, [blue] paralectotype labels, and "Heterarthrus nemoratus (Fallén, 1808) det. A. Liston 2013".

Discussion. The synonymy of *F. parviceps* with *H. nemoratus* as proposed by Cameron (1876) and adopted in numerous subsequent works, is confirmed.

***Hoplocampa alpina* (Zetterstedt, 1838) (Tenthredinidae)**
http://species-id.net/wiki/Hoplocampa_alpina

= *Tenthredo alpina* Zetterstedt, 1838: 339; ♀♂; type locality: "Raschstind in insula Schiervoe Nordlandiae; Gamstenstind ad Alteidet" [in northern Norway: see clarification by Greve (1986)].
= *Selandria pallida* Newman, 1837: 262; sex not stated; type locality: not stated.

Type material examined. *Selandria pallida*. Lectotype (**hereby designated**) ♀:"[handwritten] Hoplocampa pallida, Steph. [printed] Det. in Coll. Ent. Club , Inst.'d 1826. Pres'd 1927 by Club to Hope Coll."; "[red] Lectotype Selandria pallida Newman, 1837 des. A. Liston 2013"; "Hoplocampa alpina (Zetterstedt, 1838) det. A. Liston 2013", "DEI-GISHym 19997". Condition: fair. Paralectotype: 1♂ with same original label, [blue] paralectotype label, and "Hoplocampa alpina (Zetterstedt, 1838) det. A. Liston 2013".

Discussion. Within *Hoplocampa*, *Selandria pallida* Newman is a junior secondary homonym of *Tenthredo pallida* Serville, 1823 (= *Hoplocampa flava* (Linnaeus, 1760): Lacourt 2000). The synonymy of *S. pallida* with *H. alpina*, which has long been recognised (e.g. Kirby 1882), is confirmed.

***Phylloecus* Newman, 1838 (Cephidae)**
http://species-id.net/wiki/Phylloecus

= *Phylloecus* Newman, 1838: 485–486.
= *Phylloecus*: Rohwer 1911; type species designated as *Phylloecus faunus* Newman, 1838; placed as synonym of *Janus* Stephens, 1829.
= *Hartigia* Schiødte, 1839: 331–332, 347, 370. Boie 1855; type species designated as *Astatus satyrus* Panzer, 1801 [= *Phylloecus niger* (Harris, [1779])]. **syn. n.**

Discussion. *P. faunus* was stated by Abe and Smith (1991) to have been designated by monotypy as the type species of *Phylloecus* Newman, 1838. This is not so, because Newman (1838, p. 486) ends his discussion on his new genus with the words "[..] but it seemed to me that the division containing *Faunus,* &c. is equally distinct, and therefore I would submit the propriety of raising these also, to the rank of a genus, under the name *Phylloecus*". His foregoing text makes it clear that at least *Cephus satyrus* (Panzer, 1801) (a junior synonym of *Hartigia nigra* (M. Harris, [1779]) was thus considered also to belong to *Phylloecus*. Rohwer (1911) interpreted this correctly and accordingly designated *P. faunus* as type species. However, Rohwer (1911, p. 94 [index], under the names *cynosbati* and *faunus*) makes it clear that he regarded *P. faunus* as conspecific with *Janus cynosbati* (Linnaeus, 1758) (= *J. femoratus* (Curtis, 1830): see Blank et al. (2009) on nomenclature). From Newman's description and subsequent discussion it is evident that his concept of *Phylloecus* corresponds closely with that of what in recent years has been called *Hartigia*, and this correct interpretation was followed by various authors during the 19th Century. The lectotype of *Phylloecus faunus* belongs to the species recently known as *Hartigia helleri* (Taschenberg, 1871) (see below, under *P. faunus*). Benson (1951) and Pagliano and Scaramozzino (1990) treated *Hartigia* and *Phylloecus* as synonymous, but did not use the latter as the valid name. On the other hand, the misinterpretation of *Phylloecus* as *Janus* also has a long history, which can be traced back at least to Kirby (1882), and in recent years this wrong synonymy has become universally accepted. The International Code of Zoological Nomenclature (ICZN 1999) unfortunately provides no opportunity of maintaining the name *Hartigia* in precedence over *Phylloecus*, because the use of *Phylloecus* as a valid name after 1899, by for example Marchand (1902) and Richter von Binnenthal (1903), precludes the application of Article 23.9. (reversal of precedence). Neither are the species of *Phylloecus* of such economic, scientific or cultural importance that an application to the Commission to conserve the name *Hartigia* seems likely to achieve

success, although some species are of rather minor significance to growers of soft fruit and ornamental roses in North America (Smith DR 1986), and *Phylloecus faunus* has been considered for use in the biological control of *Rubus* in Australia (e.g. Bruzzese 1982; as *Hartigia albomaculatus*). As a result of the new synonymy, the following species names are either newly transferred to *Phylloecus* (comb. n.) or the original name combinations are re-instated as valid (comb. rev.). New combinations are followed in parentheses by the original combination of the species group name. Only the nominal species which were considered to be valid by Taeger et al. (2010) are listed:

Phylloecus agilis (F. Smith, 1874), comb. n. (*Cephus agilis*)
Phylloecus albotegularis (Wei & Nie, 1996), comb. n. (*Hartigia albotegularis*)
Phylloecus algiricus André, 1881 comb. rev.
Phylloecus bicinctus Provancher, 1875 comb. rev.
Phylloecus cheni (Wei & Nie, 1999), comb. n. (*Hartigia cheni*)
Phylloecus coreanus (Takeuchi, 1938), comb. n. (*Hartigia coreana*)
Phylloecus cowichanus (Ries, 1937), comb. n. (*Hartigia cowichana*)
Phylloecus elevatus (Maa, 1944), comb. n. (*Hartigia elevata*)
Phylloecus epigonus (Zhelochovtsev, 1961), comb. n. (*Hartigia epigona*)
Phylloecus etorofensis (Takeuchi, 1955), comb. n. (*Hartigia etorofensis*)
Phylloecus fasciatus (Cresson, 1880), comb. n. (*Cephus fasciatus*)
Phylloecus faunus Newman, 1838 comb. rev.
Phylloecus kamijoi (Shinohara, 1999), comb. n. (*Hartigia kamijoi*)
Phylloecus linearis (Schrank, 1781), comb. n. (*Tenthredo linearis*)
Phylloecus mexicanus (Guerin, [1844]), comb. n. (*Cephus mexicanus*)
Phylloecus minutus (Wei & Nie, 1997), comb. n. (*Hartigia minuta*)
Phylloecus niger (M. Harris, [1779]), comb. n. (*Sirex niger*)
Phylloecus nigratus (Dovnar-Zapolskij, 1931), comb. n. (*Pachycephus nigratus*)
Phylloecus nigritus (Forsius, 1918), comb. n. (*Macrocephus nigritus*)
Phylloecus nigrotibialis (Wei & Nie, *1977),* comb. n. (*Hartigia nigrotibialis)*
Phylloecus pyrrha (Zhelochovtsev, 1968), comb. n. (*Hartigia pyrrha*) [Zhelochovtsev gives no etymology for this species name. It is here considered to be a noun, the name of a figure in Greek mythology]
Phylloecus riesi (D. R. Smith, 1986), comb. n. (*Hartigia riesi*)
Phylloecus sibiricola Jakovlev, 1891 comb. rev.
Phylloecus simulator (Kokujev, 1910), comb. n. (*Macrocephus simulator*)
Phylloecus stackelbergi (Gussakovskij, 1945), comb. n. (*Hissarocephus stackelbergi*)
Phylloecus stigmaticalis (Wei & Nie, 1996), comb. n. (*Hartigia stigmaticalis*)
Phylloecus trimaculatus (Say, 1824), comb. n. (*Cephus trimaculatus*)
Phylloecus viator (F. Smith, 1874), comb. n. (*Cephus viator*)
Phylloecus xanthostoma (Eversmann, 1847), comb. n. (*Cephus xanthostoma*)
Phylloecus zhengi (Wei & Nie, 1996), comb. n. (*Hartigia zhengi*)

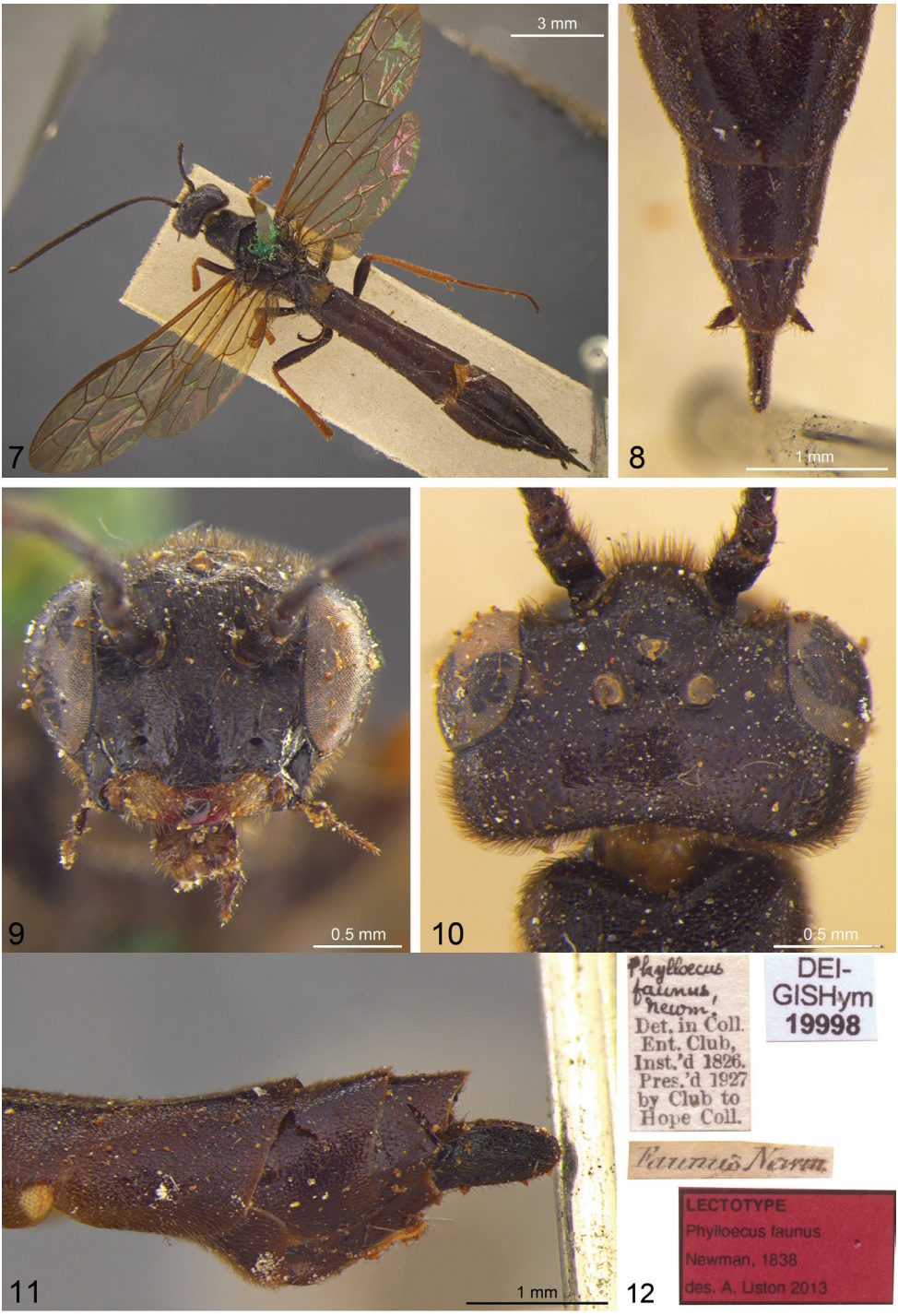
***Phylloecus faunus* Newman, 1838, spec. rev. (Cephidae)**
http://species-id.net/wiki/Phylloecus_faunus

= *Phylloecus faunus* Newman, 1838: 485–486; ♀♂; type locality: "in the vicinity of London". Note: *faunus* is a noun; the name of a Roman deity.
= *Cephus helleri* Taschenberg, 1871: 305–306; ♀; type locality: Insula Lesina [Island of Hvar, Croatia]. **syn. n.**

Type material examined. *Phylloecus faunus*. Lectotype (**hereby designated)** ♀, Figs 7–12. "[handwritten] Phylloecus faunus, Newm. [printed] Det. in Coll. Ent. Club , Inst.'d 1826. Pres'd 1927 by Club to Hope Coll."; "[handwritten] Faunus Newm."; "[red] Lectotype Phylloecus faunus Newman, 1838 des. A. Liston 2013"; "Hartigia faunus (Newman, 1838) det. A. Liston 2013". Condition: missing most of right antennal flagellum, most tarsi except right middle and rear; abdomen after tergum 5 glued to specimen.

Discussion. [see also under *Phylloecus*, above]. Newman refers to a syntype series of three specimens of *P. faunus*: "Two specimens of this insect have been taken by Mr. Ingall, and one by Mr. Stephens". The single specimen examined agrees well with the brief description. Most taxonomic works and catalogues (e.g. Konow 1905a; Taeger et al. 2010) have until now placed *P. faunus* as a synonym of *Janus cynosbati* (Linnaeus, 1758), although it should have been apparent from several characters described or discussed by Newman (1838), that these are not conspecific. The mistaken synonymy was possibly first published by Kirby (1882).

Although the name *faunus* has not to the best of our knowledge been used as valid after 1899, neither has the name *helleri* been sufficiently used (in 21 publications by 27 authors including co-authors) as valid in the last fifty years to satisfy the conditions of Article 23.9 (reversal of precedence) of the International Code of Zoological Nomenclature (ICZN 1999). A list of these references is available from us on request. The lectotype of *P. faunus* agrees in all important points with the characterisation of *Hartigia helleri* by Jansen (1998). Quinlan (1970) identified a second female specimen in the Natural History Museum, London, which should be regarded as a paralectotype of *P. faunus*, as *H. albomaculatus* [sic!], noted that it bore a label "faunas" [presumably in reality *faunus*] and mentioned that no reliable information is available on where it was caught. One might doubt the reliability of Newman's statement that the types of *Phylloecus faunus* were collected around London, because under its synonyms *Hartigia albomaculata* and *H. helleri* no evidence for the presence of this species in the British Isles has been published, and because neither of the two type specimens still in existence bears any explicit label data referring to the collection locality. However, an occurrence in the London area, at least historically, seems not unlikely. Chevin (1993) presented several records from northern France, under the name *H. albomaculata*, and later (Chevin and Chevin 2007) recorded *H. helleri* from the Département de la Manche, not far from the Channel coast. It is concluded that *Phylloecus faunus* should



Figures 7–12. *Phylloecus faunus* Newman, 1838; lectotype. **7** dorsal **8** abdomen, dorsoapical **9** head, frontal **10** head, dorsal **11** abdomen, lateroapical **12** labels.

be used as the valid name of the species referred to in recent years first as *Hartigia albomaculata* (or *H. albomaculatus*, misspelling) and latterly as *H. helleri*, and that after weighing up the evidence, the type locality of *P. faunus* can be accepted as being in the area of London.

Specimens without type status

Amongst the specimens borrowed for examination were also the following Tenthredinidae, apparently identified by Newman. None of these specimens is considered to be a type.

1♂ *Euura atra* (Jurine, 1807), det. A. Liston, with handwritten superscript on the printed label "Euura cynips, Newm." and the following additional labels: "[printed] 1 Cynips Newm.", "[handwritten / blue paper] Euura roboris Newman". Remarks: The colouration of this specimen (completely black antennae, femora basally black) does not fit Newman's (1837: 260) very short original description of the male of *Euura cynips*. The specimen therefore cannot be considered to belong to the type series of *E. cynips*. Newman (1869: 319) wrote that "*Euura cynips* produces the familiar gall to be found almost everywhere on the leaves of the crack willow (*Salix fragilis*): this gall is of an oblong form, and protrudes equally from both surfaces of the leaf; it is usually of a red tint on the upper surface [..]". This statement clearly refers to the gall of *Pontania proxima* (Serville, 1823), but having been published more than thirty years after the description of *E. cynips*, it cannot be used as an argument for interpreting the name as a synonym of *P. proxima*. Based on the inadequate original description, Liston et al. (2006) treated *E. cynips* as a synonym of *E. testaceipes* (Brischke, 1883) and as a nomen oblitum. This treatment should be maintained.

1♀ *Heterarthrus ochropoda* (Klug, 1818), det. A. Liston, with handwritten superscript on the printed label "Druida populi". Remarks: No publication has been located in which the name "Druida populi" is used.

2♀ *Pontania proxima* (Serville, 1823), det. A. Liston, with handwritten superscript on the printed label "Euura roboris, Newm." Remarks: No publication has been located in which the name "Euura roboris" is used. Newman (1869) did make a name *Euura quercus* available (Taeger et al. 2010), by publishing a seven-word description of a gall on oak that he supposed to have been caused by a sawfly. Whether this has anything to do with "E. roboris", a name possibly indicating a relationship with *Quercus robur*, cannot at present be answered.

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