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The natural history of *Calyptraea aurita* (Reeve, 1859) from Southern Chile (Gastropoda, Calyptraeidae)

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Abstract

Hard bottom communities of the Reloncaví Estuary and adjacent areas, Region de los Lagos, Chile (42°S), were studied between 2008 and 2011. All hard substrates between the lower intertidal and 25 m depth were dominated by the calyptraeid gastropods, *Crepipatella dilatata* and *C. fecunda*. Epibenthic coverage of the hard bottoms markedly decreased further down with the exception of vertical cliffs. In a depth range between 26 to 48 m repeatedly dense patches of another calyptraeid species, *Calyptraea aurita* (Reeve, 1859), were observed. Densities reached up to 1475 individuals m^{-2} and covered up to 50 % of the rock surfaces. In shallower depths *C. aurita* was not present. However, despite its huge abundance, *C. aurita* has not been documented for more than 150 years in the southeastern Pacific, being described superficially by Reeve, through only shell characteristics. Here, we redescribe and compare it with other members of the family Calyptraeidae through characteristics of shell, radula, and soft parts, including also details of the egg mass and intracapsular development of their embryos. Males were mobile and females sessile. Shell size ranged from 6.6 to 12.4 mm for immature individuals, from 10.6 to 24.9 mm for males, 15.1 to 25.9 mm for intersex individuals, and from 21.0 to 39.6 mm for females. Up to three individuals stacked together were found, always presenting a female at the base with up to a maximum of two male individuals above. Laboratory studies demonstrated that *C. aurita* has an indirect larval development, liberating planktotrophic larvae with a bilobed ciliated velum into the water column. A transplantation experiment demonstrated that survival, growth, and reproduction of *C. aurita* is also possible in depths shallower than its normal distribution. The geographic distribution of *C. aurita*, was previously only known as being from Valparaíso (33°S) and is now extended down to the Reloncaví Sound (41°S).

Keywords

Calyptraeid gastropod, external morphology, growth, radula, reproduction, shell, transplantation

Resumen

Comunidades de fondos duros del estuario de Reloncaví y áreas adyacentes, Región de los Lagos, Chile (42 ° S), fueron estudiados entre el 2008–2011. Todos los fondos duros entre la profundidad intermareal inferior y 25 m fueron dominados por los gasterópodos caliptreidos, *Crepipatella dilatata* y *C. fecunda*. La cobertura epibentónica de los fondos duros disminuyó notablemente a mayor profundidad, con la excepción de los acantilados verticales. En un rango de profundidades entre 26 y 48 m fueron observados parches repetidamente densos de una tercera especie de Calyptraeidae, *Calyptraea aurita* (Reeve, 1859). La densidad alcanzó hasta 1475 ind. m^{-2} y una cobertura de hasta un 50 % de la superficie de las rocas. En profundidades menores *C. aurita* no estuvo presente. A pesar de su gran abundancia, *C. aurita* no se ha documentado desde hace más de 150 años en el Pacífico Suroriental, siendo descrita superficialmente por Reeve, solamente a través de las características de la concha. En este trabajo, nosotros redescribimos y comparamos esta especie con otros miembros de la familia Calyptraeidae a través de características de la concha, rádula y partes blandas, incluyendo algunos detalles de la masa de huevos y desarrollo intracapsular de sus embriones. Los machos fueron móviles y las hembras sésiles. El tamaño de la concha varía entre 6,6 y 12,4 mm para los individuos inmaduros, 10,6 a 24,9 mm para los machos, 15,1 a 25,9 mm para los individuos intersexo y desde 21,0 a 39,6 mm para las hembras. Fueron encontrados hasta tres individuos apilados juntos, presentando siempre una hembra en la base y hasta un máximo de dos individuos machos sobre ella. Los estudios de laboratorio demostraron que *C. aurita* tiene un desarrollo larval indirecto liberando una larva planctotrófica con un velo ciliado bilobulado en la columna de agua. Un experimento de trasplante demostró que la supervivencia, el crecimiento y la reproducción de *C. aurita* es posible en profundidades menores que su distribución normal. La distribución geográfica de *C. aurita*, anteriormente sólo se conocía desde Valparaíso (33°S) extendiéndose hasta el Seno de Reloncaví.

Palabras clave

Crecimiento, concha, Gasterópodos caliptreidos, morfología externa, rádula, reproducción, trasplante

Introduction

Gastropods with external shells belong to the most studied groups of all the marine fauna of Chile due to their economic importance (Valdovinos 1999, Castilla and Defeo 2001, Letelier et al. 2003, Aldea and Valdovinos 2005, Cárdenas et al. 2008). Nevertheless, the total number of species is still unknown, because many coastal regions are hard to access due to their remoteness or their extreme exposure to waves, which makes sampling difficult. Therefore new species records or redescriptions of species not found for decades are not surprising for the Chilean marine fauna (Sanamyan and Schories 2003, Lee et al. 2008, Sanamyan et al. 2010). Less attention has been paid to those species that are not used for human consumption. In this sense, only three calyptraeid gastropods have been studied intensively during the last decade (Brown and Olivares 1996, Chaparro et al. 1999, Chaparro et al. 2001a, Chaparro et al. 2001b, Chaparro and Flores 2002, Navarro and Chaparro 2002, Chaparro et al. 2005, Daguin et al. 2007, Chaparro et al. 2008, Brante et al, 2011). Two of these calyptraeid species, *Crepipatella dilatata* (Lamarck, 1822) and *Crepipatella peruviana* (Lamarck, 1822) [named as *C. fecunda* (Gallardo 1979b) before Véliz et al. (2012)], are among the most common species in the Northern Chilean fjord and channels ecoregion of southern Chile and adjacent estuaries, often covering rocks, boulders and mussels up to a depth of 25 m. Despite their abundance in the subtidal zone of the northern fjords of southern Chile, the presence of a third species (*Calyptraea aurita*) with a vertical distribution below the other ones was unknown up until now and is really surprising. (1) The Reloncaví Sound has been studied intensively on several previous expeditions, including the Swedish Lund expedition (1948–1949) which produced several monographs of different taxa (Brattström and Dahl 1951, Brattström and Johannsen 1983) and (2) the species was found in high abundance in an area easy to access by SCUBA divers.

Among the 18 known species of Calyptraeidae of the continental coast of Chile (Valdovinos 1999), the case of the species *C. aurita* is unique and no documented in that report. The species was only described by shell morphology of individuals dredged in the vicinity of Valparaíso. Besides the short species description provided by Reeve (1859) only two small illustrations showing both sides of a shell were available. Various authors made an inventory of the Chilean malacofauna, however no one mentioned or found this species again along the Chilean coast or included it in databases or check–lists (Guzmán et al. 1998, Gutt et al. 2003, Aldea and Valdovinos 2005, Osorio et al. 2006, Ramajo and Osorio 2010). Due to its stable population presence during 2008–2012 we were able to add new biological and ecological data to the species.

A major part of taxonomic studies of mollusks is still based on shell characteristics of type specimens (Guzmán et al. 1998). Often no associated additional biological data or information about their geographical distribution exists. Consequently, taxonomic classification underlies continuous modifications due to the apparition of new characteristics. This, at the same time, produces continuous modifications in the taxonomy of many taxa due to the apparition of new characteristics and the evaluation of the plasticity of different nominal species within a specific group (Ponder and Lindberg 1997, Letelier et al. 2003). A good example is sibling species, which may be indistinguishable by appearance, but nevertheless are reproductively isolated from one another (Gallardo 1979b).

The family Calyptraeidae counts approximately 139 living species (123 valid) in total (MolluscaBase 2018), of which nearly 40 have been reported from the Eastern Pacific (Paredes and Cardoso 2007). All species of the family are protandrous hermaphrodites (Brown 1989) and sedentary filter–feeders (Collin 2003a, Collin et al. 2007). All species of the genus *Calyptraea* have a patelliform shell that retains some remains of spiral coiling (Simone 2002). The septum is modified into a curved plate. The presence of the genus *Calyptraea* has been reported since the Paleocene and Oligocene for the Pacific Ocean and since the Eocene for the Northern Atlantic (Saul and Squires 2008). Morphological plasticity, in response to environmental factors and a relatively simple morphology within the Calyptraeidae, resulted in a huge variation of the shell form. Therefore the relatively high number of synonyms for each species is not surprising. Today, additional characteristics like internal anatomy and DNA sequencing (Simone 2002, Daguin et al. 2007) are often used, where shell characteristics and larval development are not sufficient. In calyptraeid gastropods embryonic and larval development present an additional tool to distinguish morphologically very similar species like *Crepipatella dilatata* and *C. peruviana* (Coe 1938, Coe 1949, Gallardo 1977, Gallardo 1979b). Our study provides information on the encapsulated development of the embryos of *C. aurita* as well as information on its subtidal distribution.

Materials and methods

The study area

In May 2008 we noticed for the first time the presence of *Calyptraea aurita* in depths below 30 m near Caleta Yerbas Buenas (41°40'20"S, 72°39'24"W), Reloncaví Sound, Chile (Figure 1). Yerbas Buenas is situated 32 km southeast of the city of Puerto Montt (Region de los Lagos) (Figure 2). Large diurnal temperature fluctuations of the upper water column, measured during summer time, are not detectable below 20 m depth, where water temperature varies only between 10 °C and 13 °C, salinity is constant at approx. 35 PSU. Mean tidal range is approximately 4 m with 2 m at neap and more than 6 m at spring tides. All animals collected for laboratory studies were taken from Caleta Yerbas Buenas.

Three additional sites were chosen to study the possible presence of *C. aurita* in the vicinity of the first place: (1) Reloncaví Estuary: 41°42'33.13"S, 72°37'30.95"W, (2) Caleta Gutiérrez, 41°39'15.48"S, 72°40'1.20"W, and (3) Caleta Chaicas: 41°38'17.78"S, 72°40'10.94"W (Figure 2).

Coverage, depth distribution, size, and abundance

The distribution and coverage of *C. aurita* were studied using transect photographs along a depth gradient and analyzed with the Windows–based software CPCe 3.6 (Kohler and Gill 2006). Five images, taken with a Nikon D70s, were taken and analyzed for each depth of 5 m, 10 m, 15 m, 20 m, 25 m and 30 m in a total horizontal distance of 15 m. A total of 100 specified numbers of points were distributed uniformly on each transect image and the individuals underlying the points were counted. Each photo frame had a size of 26 × 30 cm. The camera was fixed on a rack to guarantee that all images were taken from the same distance to the rock and to avoid inclination of the camera in relation to the rock. The abundance of the *C. aurita* within each frame was counted with the software ImageJ 1.41. Additional images were taken from patches of *C. aurita* in depths between 25 m to 48 m to estimate its maximum abundance and density.

In October 2009 a total of 190 individuals were sampled from four quadrants in order to estimate shell length distribution, sex ratio and the relationship between dry tissue biomass and shell length. The samples were fixed in 4% formalin–seawater after sampling. Shell sizes were measured with a digital caliper and sexual maturity status was registered for each individual. Afterwards, tissue biomass was separated from the shell, rinsed with freshwater and dried for 48 h at 60 °C.

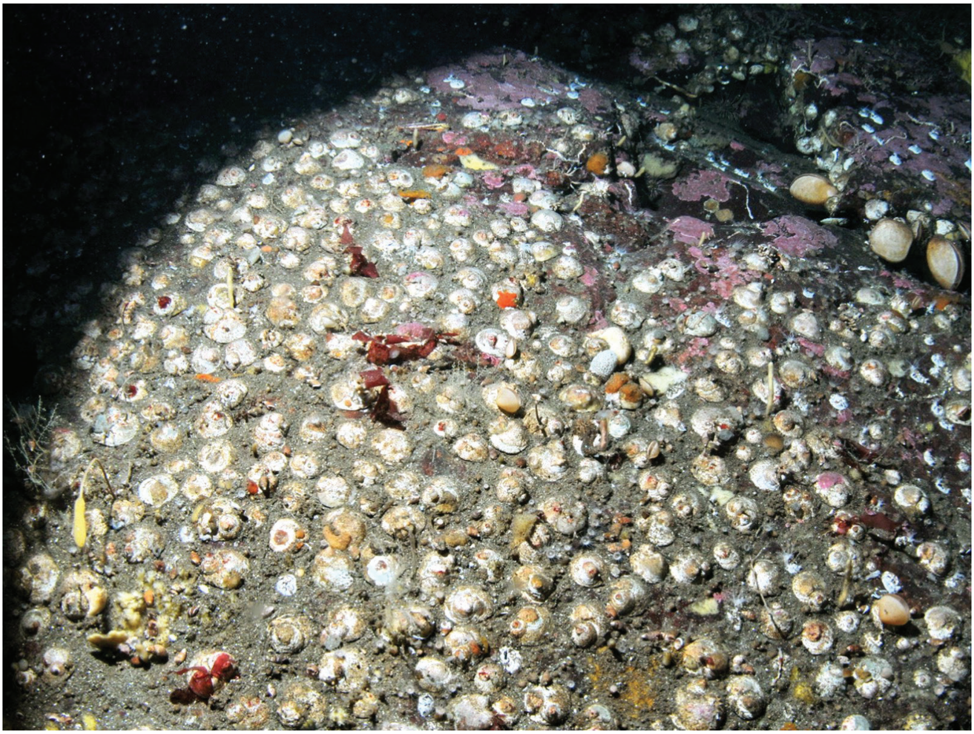

Figure 1. High densities of *Calyptraea aurita* in a depth of 34 m mean tide level at Caleta la Arena (Reloncaví Sound).

Taxonomy

Ten specimens were used for the species description. These specimens are deposited in the collections of mollusks, Laboratory of Malacology, Zoology of Invertebrates of the National Museum of Natural History (**MNHNCL**), Santiago de Chile, and the Instituto de Zoología, Universidad Austral de Chile (**IZUA-UACH**), Valdivia, Chile (Table 1). Additionally, images of four syntypes have been examined from the collection of the Natural History Museum of London (**NHMUK**). Radulae from three individuals (approx. 20 mm) were obtained by dissection under stereomicroscope and washed in sodium hypochlorite (5 %) in distilled water for five min to remove extraneous tissue.

Reproduction

Scuba divers in the field collected a total of 38 females with egg capsules in their mantle cavity, removing them carefully from the rock. Females without egg capsules were reattached by the divers to the rock. Back at the beach, the collected individuals reattached quickly to acrylic plates stored in coolers with 80 L saltwater and were transferred to

Table 1. Shell morphometrics of *Calyptraea aurita*. All measurements in mm.

Specimen	Total length	Width	Height	Sex
MNHNCL 7570	32.92	31.89	11.64	female
MNHNCL 7571	28.5	28.05	10.41	female
MNHNCL 7572	16.2	17.04	6.64	male
MNHNCL 7573	17.14	16.44	7.66	male
MNHNCL 7574	37.29	34.66	13.15	female
IZUA-UACH Mg 501	21.51	21.48	9.25	male
IZUA-UACH Mg 502	20.88	19.69	7.83	male
IZUA-UACH Mg 503	29.59	26.36	12.65	female
IZUA-UACH Mg 504	25.78	25.41	11.45	female
IZUA-UACH Mg 505	33.84	33.65	12.27	female

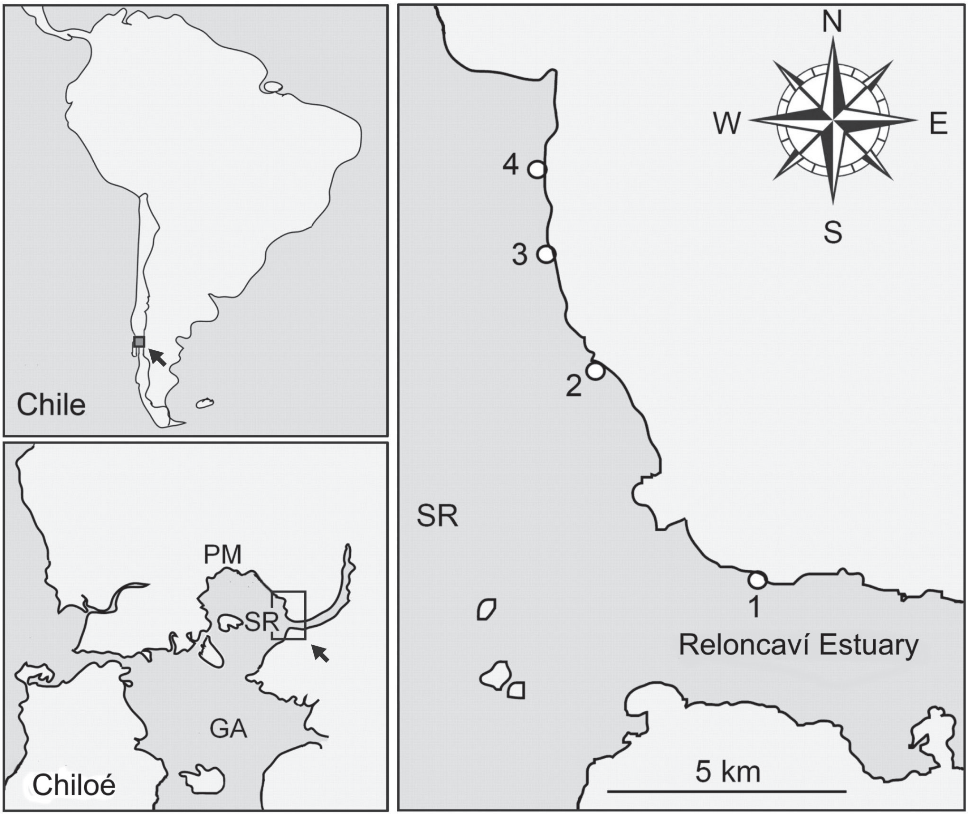

Figure 2. The study was carried out in Southern Chile (upper left) in the Reloncaví Sound (lower left) in its eastern part (right). **1** Reloncaví Estuary **2** Caleta Yerbas Buenas **3** Caleta Gutiérrez **4** Caleta Chaicas; PM = Puerto Montt; GA = Golfo de Ancud; SR = Reloncaví Sound.

the laboratory. Up to ten individuals were allowed to fix on a single acrylic plate, 40 × 45 cm in size. In the laboratory the plates with the attached individuals were transferred to 250 L tanks with permanent water flow and exchange. We used unfiltered seawater for the experiment and did not supply additional food. Intracapsular development was observed every three days with inverted light microscopy.

The different embryonic stages up to the liberation of the larvae are described using the criteria of Wyatt (1960), Zaixso (1973), Gallardo (1977), Penchaszadeh (1976), and Véliz et al. (2003).

Transplantation experiment

An experiment was conducted to determine whether growth and reproduction is possible in shallower depths than those found in the field. Specimens collected at 30 m depth at Yerbas Buenas were marked with numbers on the shore and allowed to reattach onto transparent acrylic plates (25 × 40 cm, 20 individuals per plate). Each animal was measured and the plates with the animals on them were placed vertically at 10 and 20 m depth (four plates in each depth). After 165 and 326 days each animal's length was measured to monitor its growth. Growth percentage was estimated based on the initial size compared with the final size at the end of the experiment. A t–test was used to compare growth after 165 days in 10 m and 20 m depth. The heavy loss of individuals during the course of time did not allow for the data measured at the end of the experiment to be included in the statistics.

Systematics

Phylum Mollusca Linnaeus, 1758
Class Gastropoda Cuvier, 1795
Order Littorinimorpha Golikov & Starobogatov, 1975
Family Calyptraeidae Lamarck, 1809
Genus *Calyptraea* Lamarck, 1799

Calyptraea aurita (Reeve, 1859)

Calyptraea striata (non Say, 1826): Broderip 1834: 38; Broderip 1835: 202, pl. 28, fig. 6.
Crucibulum auritum Reeve, 1859: sp. 17, pl. 6, fig. 17a, b; Tryon 1886: 118 (in part), pl. 32, figs 32, 33.

Type material. [*Crucibulum auritum*] is housed at NHMUK 197798.

Material examined. MNHNCL 7570 (female), MNHNCL 7571–7574 and MZUA–UACH 501–505, all specimens from Caleta Yerbas Buenas, 41°40'20"S, 72°39'24"W. All coll. Jorge Holtheuer and Dirk Schories.

Description. *Shell* (Figures 3a–h, 4b–d, 5a–h): Limpet–like, circular, conic, with spiral septum in center and right of ventral surface. The shell externally is usually opaque white and internally dark brown porcelaneous. The apex is small, sub–central;

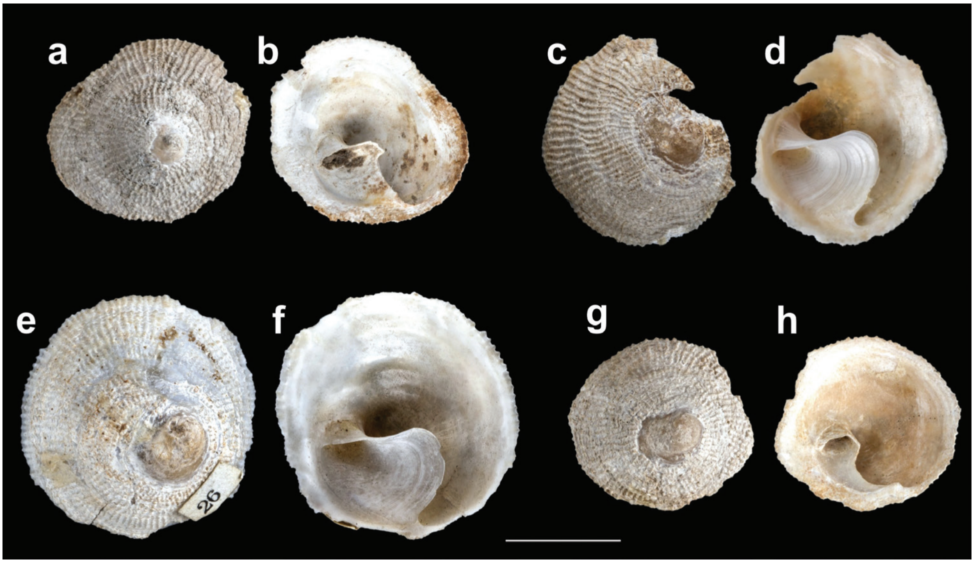

Figure 3. *Calyptraea aurita*. NHMUK 197798, syntypes of *Crucibulum auritum* Reeve, 1859. **a, c, e, g** dorsal view **b, d, f, h** ventral view. Scale bar: 1 cm.

protoconch apparently smooth, with a total diameter difficult to measure because the protoconch–teleoconch boundary is not evident, but may have ~500 μm (Figure 6a). Sculpture has ~ 70 to 80 fine radial ribs, of uniform size, aligned concentrically. Inner surface without visible muscle scars. Septum incompletely conical (opened anteriorly), situated obliquely, from shell apex to posterior. Shell size ranged according to the sexual phases, being from 6.6 to 12.4 mm for immature individuals, from 10.6 to 24.9 mm for males, 15.1 to 25.9 mm for intersex individuals and from 21.0 to 39.6 mm for females (Table 2). Septum (Figures 3d, f, 4c, 5b, d, f, h) white brilliant color, beginning in a spiral conic curve and ending in a wide platform curved to the left side of the specimen (seen from below). Fine growth lines are visible on the septum.

Radula (Figure 6b, c, d): Radula with ca. 30 rows. Rachidian tooth broad, approx. 15 cusps, central cusp more elongated than secondary cusps (Figure 6b, d); lateral teeth curved inwards, with approx. 16 sharp cusps, two cusp on inside and ca. 13 gradually decreasing towards lateral on outside of main cusp (Figure 6c, d); marginal teeth long, tall, slender, with approx. seven sharp, sub–terminal cusps in inner edge (Figure 6b, d); outer marginal weakly narrower than inner marginal teeth.

Head–foot (Figures 4a, 7a, b): Head and neck regions somewhat similar to the other *Calyptraea* species, including neck ventral surface and flaps, penis present in all specimens behind right tentacle, but it is reduced or missing in females. Snout–proboscis very well developed, cephalic tentacles simple, eyes dark and small, located on the tentacle basis at lateral outward position. Foot similar to that of other *Calyptraea* species, with planar, dorso–ventrally flattened sole compressed by shell septum. Mantle, as in *Calyptraea*, attached to dorsal surface of foot sole and extending beyond its posterior and lateral borders.

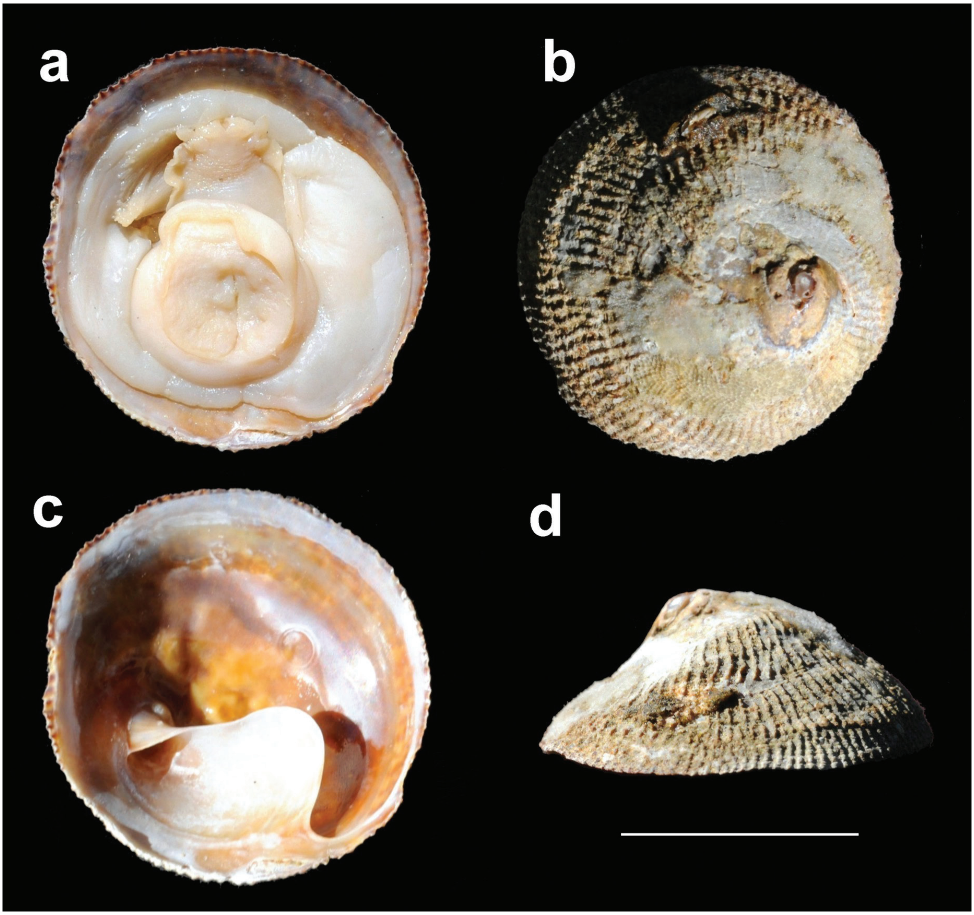

Figure 4. *Calyptraea aurita*. MNHNCL 7570, female specimen. **a** ventral view of shell and head–foot **b** dorsal view **c** ventral view **d** lateral view. Scale bar: 2 cm.

Table 2. Shell length in mm of *Calyptrea aurita* sampled randomly at Yerbas Buenas. N = number of individuals sampled; SD = Standard Deviation.

	N	Min	Max	mean	SD
immature	9	6.6	12.5	9.1	1.7
Male	82	10.6	24.9	17.6	3.7
intersex	6	15.1	25.9	19.2	4.2
Female	83	21.0	39.6	31.7	3.5

Mantle (Figures 4a, 7a, b): Mantle border very broad, including region surrounding foot, occupying 90% of pallial cavity. Pallial cavity conical and curved, begins just inside shell septum. Pallial aperture proportionally small, if animal compared with a clock, this aperture begins at 10 and finishes at 6 o'clock.

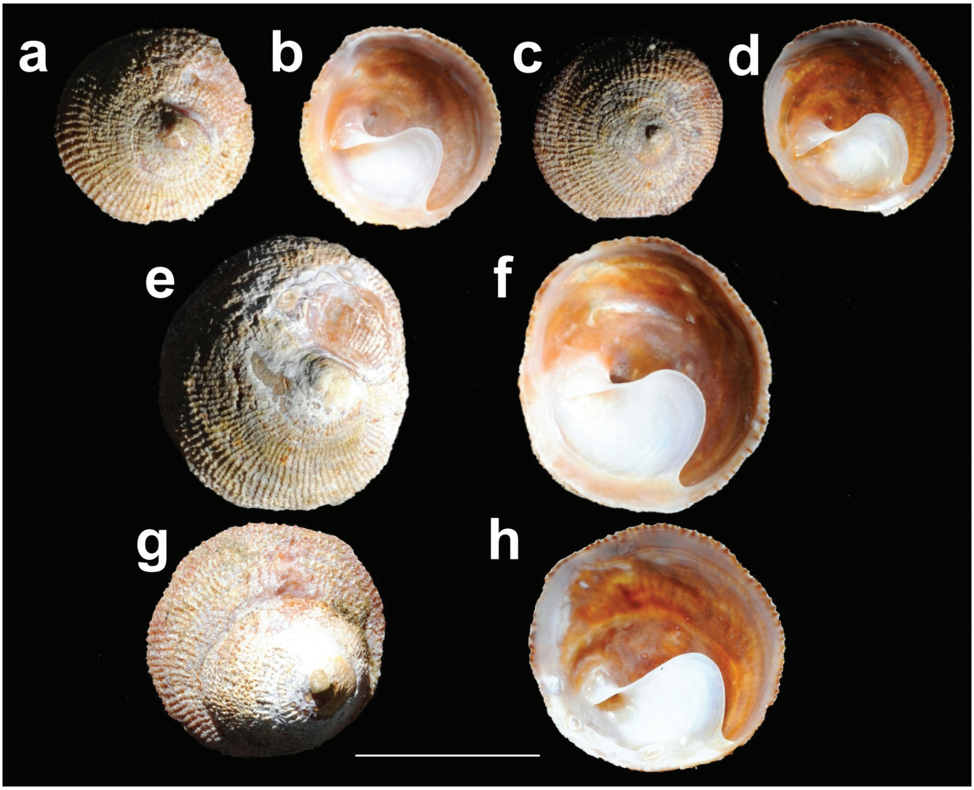

Figure 5. Shells of *Calyptraea aurita*. **a** IZUA-UACH Mg 501 dorsal view **b**, ventral view **c** IZUA-UACH Mg 505 dorsal view **d** ventral view **e** IZUA-UACH Mg 502 dorsal view **f** ventral view **g** IZUA-UACH Mg 504 dorsal view **h** ventral view. Scale bar: 2 cm.

Gill: typical to those of *Calyptraea*, occupying most of inner pallial space, inserts all along left and anterior pallial margins. Gill filaments also similar to those of *Calyptraea*, with very long (Figure 7b), rigid rod, mainly of the apical region. Gill posterior end just in posterior end of cavity; gill anterior end in central region of pallial aperture.

Male (Figure 7c): Only small specimens (up to 10.58 mm) are functional males, all mobile. Penis is very long (approx. three times head length), originating dorsally and extending to right tentacle. Papilla on penis tip, very long, approx. 1/3 of penis length. The penis sperm groove runs along middle region of the ventral surface of penis. The male of *Calyptrea aurita* is always attached onto a female, and is never found directly attached on primary substrate.

Female (Figures 4a–d, 7a–c): Very similar to other *Calyptraea* species, the female is sessile. Only specimens larger than 20.98 mm were present in our material. During field work the presence of egg capsules in the pallial cavity of the females could be seen. The females always settle on the rock surface where they attach and protect their egg capsules up to larvae release (Figure 8).

Reproduction and development. *Calyptraea aurita* (Reeve, 1859) is a protandric hermaphrodite producing a maximum of 16 egg capsules per female which contain an average

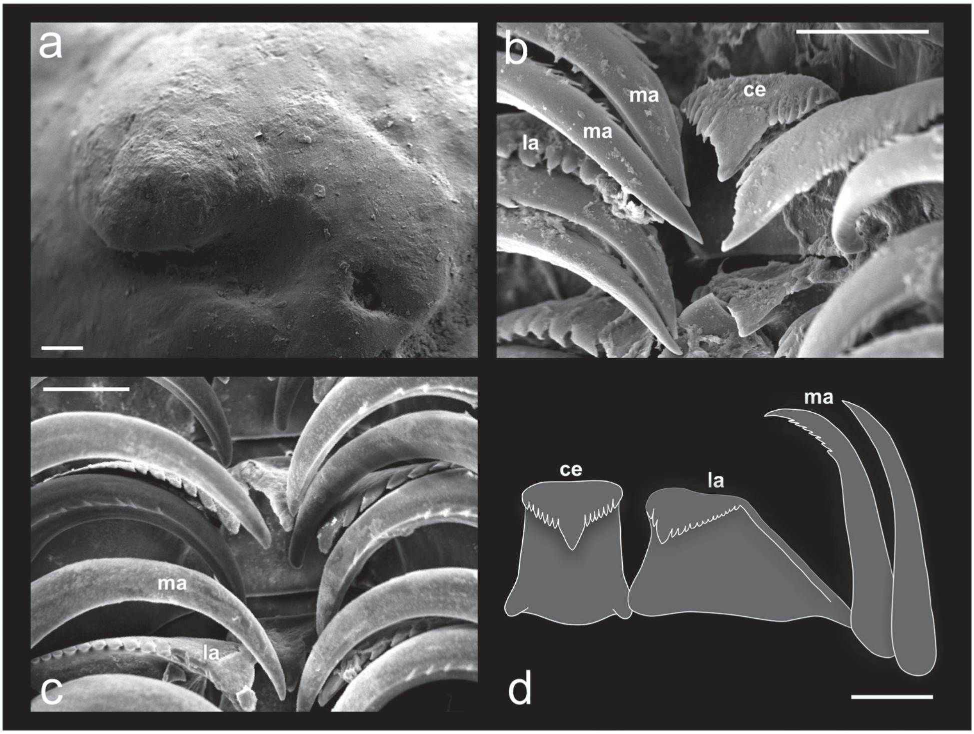

Figure 6. Protoconch and radula of *Calyptraea aurita*. **a** protoconch in apical view **b** SEM showing teeth in folded position **c** SEM showing teeth in folded position **d** radular structure. Abbreviations: ce: central tooth; la: lateral tooth; ma: marginal tooth. Scale bar: 100 µm (**a**), 50 µm (**b, c, d**).

of 119 eggs each (Figure 8). Up to three individuals were observed stacked together, always with a female at the base and up to a maximum of two male individuals on her shell. Brooding was observed during the months March (38 of 100 females studied below water), April (38 of 100), August (44 of 55), September (150 of 199), October (36 of 50) and December (10 of 19). In May 2009 none of 100 observed females were brooding. Weather conditions did not allow for the verification of brooding during the other months.

The females of *Calyptraea aurita* deposit their eggs in thin–walled brooded capsules directly attached to hard substrates. These capsules have a triangular, flattened morphology and are fixed with a fine stem to the substrate. The eggs are concentrated at the distal end of the sac embedded in an uncoloured liquid. All eggs are able to develop into planktonic veliger stages which are liberated into the water column. The veliger has a bilobed ciliated and pigmented velum and two small black–coloured eyes between the velar lobes, a circular mouth, and a transparent protoconch. The mean initial egg size is ca. 150 µm and the size of the veliger, when liberated into the water column is ca. 300 µm. The intracapsular development up to the larval release took ca. 42 days in the laboratory.

Size: A total of 180 individuals were collected in October 2010 in 30 m depth. Shell length, height, and width were measured. Shell length distribution was two peaked, the first peak corresponded to males and intersex individuals and the second peak to females (Figure 9). Mean shell length was 23.9 ± 8.3 mm, mean width 23.6 ±

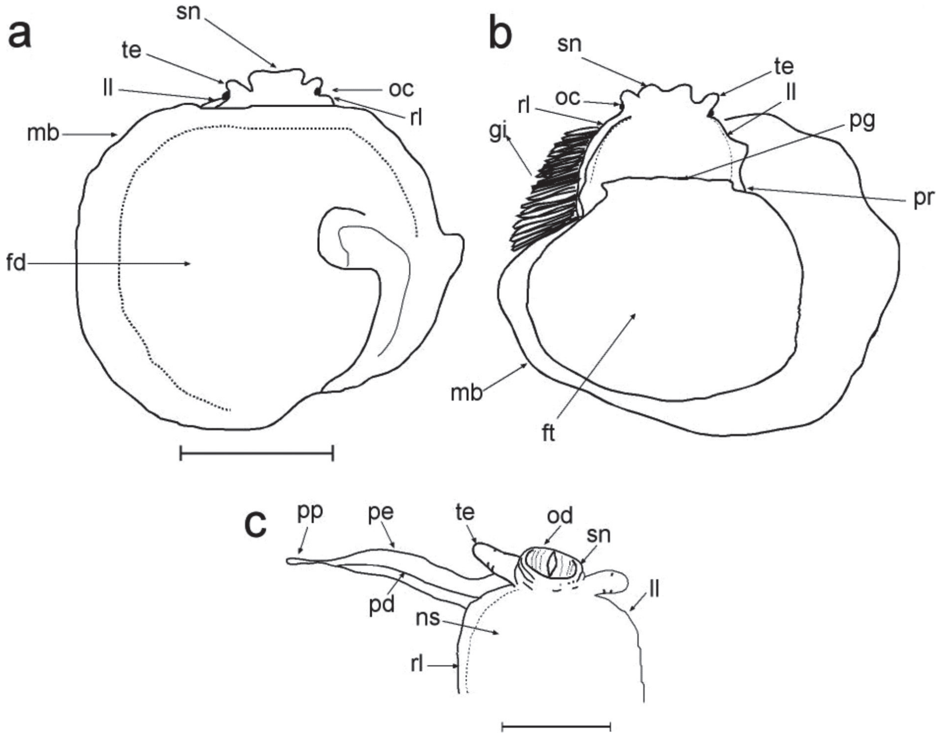

Figure 7. External morphology of *Calyptraea aurita*. **a** female, without shell and whole, dorsal view **b** same animal, whole, ventral view **c** male, foot removed, ventral view. Abbreviations: fd: dorsal surface of foot; ft: foot; gi: gill; ll: left lateral expansion (flan); mb: mantle border; ns: neck "sole"; pd: penis sperm groove; pe: penis; pg: pedal gland anterior furrow; pp: penis papilla; pr: propodium; rl: right lateral expansion (flan); sn: snout–proboscis; te: cephalic tentacle. Scale bar: 10 mm (**a, b**), 5 mm (**c**).

8.5 mm and mean height 9.1 ± 2.7 mm. Shell width to length relation was close to 1 (0.99 ± 0.10) and shell height to length relation was 0.39 ± 0.05.

Symbionts. Four samples with 38 to 77 females were taken in October 2010 and were checked underwater for the presence of the pinnotherid decapod *Calyptraeotheres politus* (Smith, 1870). A total of 4.5 ± 1.3 % of females were infested by *C. politus*. None of the infested females deposited eggs.

Distribution and habitat. *Calyptraea aurita* occurs at Valparaíso at depth of 82–110 m (Broderip 1834). In this study it was found exclusively on hard substrates in the Reloncaví Sound between 26 to 48 m depth showing a patchy distribution. The species was present in three of four study sites using vertical transects down to 30 m depth. 37.6 ± 14.4 % of the rocks were covered by *C. aurita* at the location Caleta Yerbas Buenas at 30 m depth. This represented a density of 743 ± 307 ind. m^{-2}. The highest observed density was 1475 ind m^{-2} covering 50 % of the primary substrate. Coverage at the locations Caleta Gutiérrez and the Reloncaví Estuary were low with 1.8 ± 2.7 and 0.8 ± 1.8 % at the same depth. In none of the locations *C. aurita* was present along transects in shallower depths (5 to 25 m).

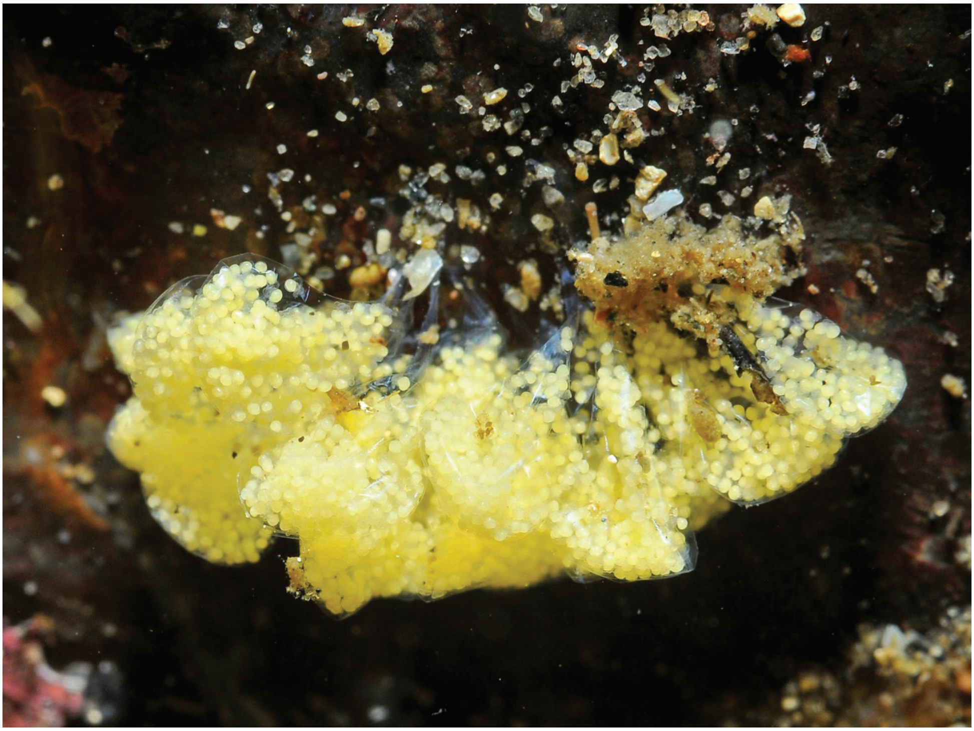

Figure 8. Egg capsules of *Calyptraea aurita*. Female was removed by the diver, 31 January 2009.

Transplantation experiment. The experiment was realized for a total time span of 326 days. Several individuals got lost during transport from the experimental depth to the shore, died during course of time, or did not reattach once unintentionally detached from the acrylic plate. Nevertheless all remaining individuals grew several mm in both depths (Figure 10). Additionally some individuals deposited egg capsules, which were visible from the reverse of the acrylic plate. A t–test did not show differences between the growth rates in 10 and 20 m depth after 165 days, t(32) = t–1.555, p = 0.13. Mean growth rate in 10 m depth was 1.11 mm (n = 20) and 1.68 mm (n = 14) in 20 m depth, respectively.

Discussion

Taxonomic remarks

Broderip (1834, p. 38) described *Calyptraea striata*, from Valparaíso, briefly indicating the same characteristics as the currently valid species *C. aurita*. However, *C. striata* Say, 1826 was previously described for the northwestern Atlantic. This last species is currently valid as *Crucibulum striatum* (Say, 1826). Then the same author again describes and figures the species (Broderip 1835, p. 202, pl. 28, fig. 6). Later Reeve

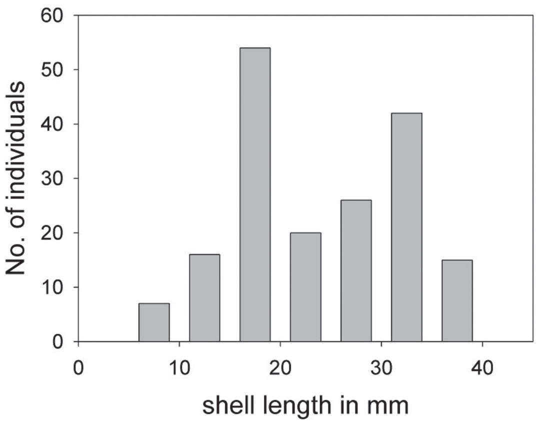

Figure 9. Size distribution of *Calyptraea aurita* (n = 180).

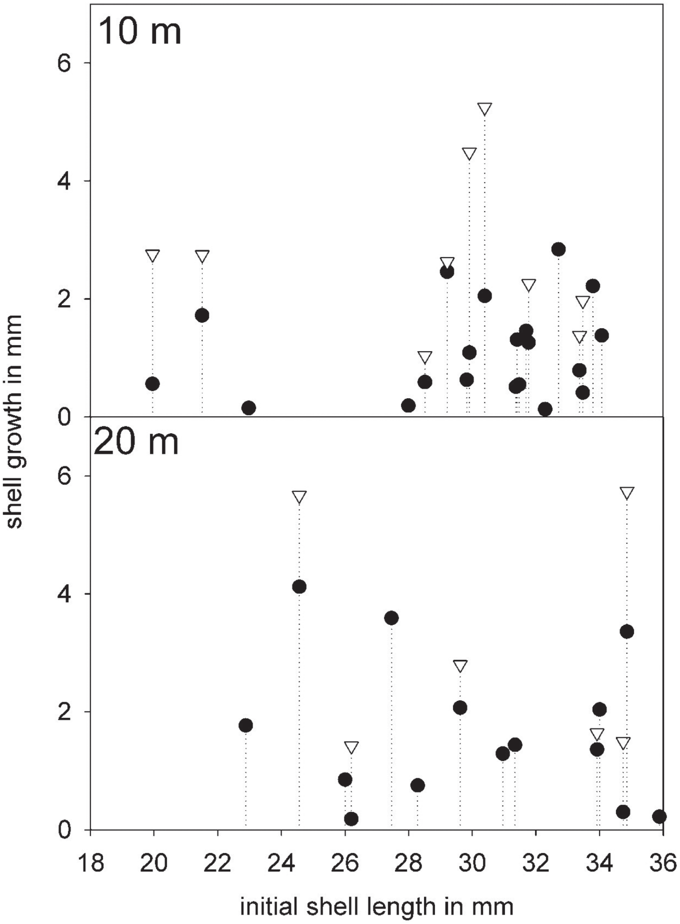

Figure 10. Individual growth rates of *Calyptraea aurita* after 165 (black circles) and 326 days (triangles) in 10 m and 20 m depth.

(1859) described *Calyptraea aurita*, to report *Calyptraea striata* Broderip (not of Say) as a junior synonym, noting that this species is less conoid than the valid *Crucibulum striatum* (Say, 1826) and differently striated; the internal septum reaches nearly to the margin. The original description and the illustrations made by *Calyptraea aurita* fit best with the material collected in the Reloncaví Sound: Shell dirty white, approximately circular, subconic, subturbinate, numerous corrugated longitudinal stripes, brownish–yellow color inside. Diameter 2.12 cm, height 0.76 cm.

Only two valid species of *Calyptraea* (s. s.) are considered along the coasts of the southeastern Pacific: *C. aurita* (Reeve, 1859), distributed in central and south Chile, and *C. mamillaris* Broderip, 1834, from Baja California to Peru. *Calyptraea mamillaris* differs externally from *C. aurita* in having only growth striations and external and internal white coloration and the apex located in the central part of the shell (see Collin 2003a, Paredes and Cardoso 2007).

Calyptraea aurita was reported by Barnard (1963) for the South African coasts, providing the description of the shell and radula of the species. Nevertheless, Kilburn (1980) re–established Barnard's (1963) record as a new species, *C. barnardi* Kilburn, 1980, arguing that having examined four syntypes of *C. aurita* (British Museum Natural History, No. 197798), the apex is markedly more eccentric and the sculpture consists of wavy, granular radial ribs, instead of concentric rings of fine scales as in the new South African taxon. However, Rolan (2005) in his discussion of another South African species, kept *C. aurita* valid for South Africa, without mentioning *C. barnardi*. Considering the abovementioned, there are obvious differences between *C. barnardi* and *C. aurita*. Apart from the characteristics of the shell mentioned by Kilburn (1980), the radula of *C. barnardi* drawn by Barnard (1963) has a rachidian tooth with five cusps and laterals teeth with ca. 13 cusps, instead of the rachidian with 15 cusps and laterals with 16 cusps of *C. aurita*.

According MolluscaBase (2018), other species of *Calyptraea* described from the Southeastern Pacific and Southwestern Atlantic are:

- *Calyptraea araucana* Lesson, 1830 (taxon inquirendum), from Concepción Bay, a species with marked radial ribs, that was established as junior synonym of *Trochita trochiformis* (Born, 1778) by Tryon (1886) and Rehder (1943).
- *Calyptraea pallida* Broderip, 1834 (taxon inquirendum), from Falkland Islands, is a junior synonym of *Crepipatella dilatata* (Lamarck, 1822) according Tryon (1886) and Hoagland (1977), although Veliz et al. (2012) cited it as species with "status unclear". However, according description and figures provided by Broderip (1835: 204, pl. 29, fig. 3), the generic position of this species certainly should be in *Crepidula* or *Crepipatella*.
- *Calyptraea strigata* Broderip, 1834 (taxon inquirendum), from Valparaíso, was cited first as a junior synonym of *Crepipatella dorsata* (Broderip, 1834) by Tryon (1886) and was cited later as a junior synonym of *Crepipatella dilatata* (Lamarck, 1822) by Hoagland (1977), but Veliz et al. (2012) cited it as species with "status unclear, possibly a valid species". In the same way that *C. pallida*, this species

should belong to the genus *Crepidula* or *Crepipatella* (see Broderip 1835: 203, pl. 28, fig. 12, Veliz et al. 2012: fig. 6A).

- *Calyptraea chiliensis* Lesson, 1830 (nomen dubium), from Concepción, is an unfigured and undetermined species with types probably lost (see Tryon 1886, Hoagland 1977, Veliz et al. 2012).
- *Calyptraea depressa* Lesson, 1830 (nomen dubium, invalid), from Concepción Bay, is a junior synonym of *Crepipatella dilatata* (Lamarck, 1822), but some specimens of the lot may be belong to *Crepipatella peruviana* (Lamarck, 1822) (see Veliz et al. 2012).

On the other hand, the species *Calyptraea intermedia* d'Orbigny, 1839, was described from the coasts of Peru, and still awaits confirmation of validation. Until today it has not been re-recorded, except for the reports of Tryon (1886) and Keen (1966). According to the description by its author, this species is white, conical, thin, diaphanous, and very depressed, with marked radial coasts very distant and not very prominent. According to Tryon (1886) it could be an aberrant juvenile form of *Trochita trochiformis* (Born, 1778).

The geographical distribution of other calyptraeid species, *Trochita pileus* (Lamarck, 1822) and *T. pileolus* (d'Orbigny, 1841), overlaps with *C. aurita* (Forcelli 2000, Collin 2003a), but shell characteristics are distinct (see Pastorino and Urteaga 2012).

Biological and ecological aspects

Reproduction of *Calyptraea aurita* occurs during most of the year with the exception of the winter months. In June (austral winter) no brooding was observed, but data for July and August are lacking. In general, calyptraeid species are known to vary in brooding season, some species like *Crepidula adunca* brood throughout the year whereas others like *C. lingulata* brood only throughout the summer months (Collin 2000, Henry et al. 2010). *C. aurita* liberates approx. 2000 veliger larvae, because virtually all embryos hatch. This number is low compared to *Crepidula fornicata* but comparable to *C. lingulata* (Collin 2000, Richard et al. 2006, Brante et al. 2009). The size of the veliger of *C. aurita*, when liberated in the water column, is ca. 300 µm and comparable to other calyptraeid planktotrophic species (Collin 2003b), but approx. one third smaller than those of *C. fornicata* (Pechenik et al. 1996a, Pechenik et al. 1996b).

4.5 ± 1.3 % of females were infested by the pinnotherid crab *Calyptraeotheres politus*, which inhabits the mantle cavity of the limpets. This obligatory symbiont to slipper snails (Campos 1999) is also present in *Crepipatella* spp. in the same area. Chaparro et al. (2001b) showed that *Crepipatella peruviana* (named as *C. fecunda*) females that hosted a pinnotherid crab, which was most probably the same species as the one found in our study, in the incubation space did not deposit eggs during a 12–month study period. However, infestation rate of *C. aurita* was low and most probably did not influence population reproduction.

The transplantation experiment demonstrated that *Calyptraea aurita* can survive, grow, and reproduce successfully in the shallow subtidal zone, although it was never found at that depth in the field. The vertical distribution of *C. aurita* is discrete and marked by its complete absence in depths of less than 25 m, whereas another calyptraeid species, *Crepipatella peruviana* (named as *C. fecunda*), dominates the sessile fauna (del Moral and Schories, pers. comm.). Although abundance of *C. peruviana* diminishes with increasing depth, the free space is not used immediately by *C. aurita*. Additional factors than competition for space must explain its absence in shallower water. *C. aurita* does not form continuous belts but shows a patchy distribution. This explains the huge differences in abundance and coverage between the four sample sites, because in the immediate vicinity of the transect lines dense patches of *C. aurita* were always found. Broderip (1834) and Reeve (1859) reported that this species was found at depth between 45–60 fathoms (82–110 m) on shells in sandy mud. As a suspension feeder, filtering phytoplankton and particulate organic matter, *C. aurita* may find sufficient food to develop permanent populations in depths where other grazing gastropods are limited. Its depth distribution is deeper than reported for *Crepidula fornicata* which is known to settle down to 64 m (Orton 1950, Blanchard 1997), *Crepidula argentina*, 35–50 m (Simone et al. 2000) and *Crepidula unguiformis*, 70 m (Osorio et al. 2006); however, Cárdenas et al. (2008) reported the presence of *Crepipatella dilatata* and *Crepidula philippiana* in 70 to 160 m and 252 m depth, respectively, in the northern Patagonia of Chile.

Dense aggregations of calyptraeid species covering up to 100% of the substrate are common. Blanchard (2009) reported that 85% of an analyzed area from 0 to 15 m depth was inhabited by *Crepidula fornicata* making up the highest benthic biomass. In southern Chile *Crepipatella* spp. shows comparable densities. *Calyptraea aurita* was found in patches covering up to 50 % of the substrate. This value is still high, because phytoplankton availability is markedly reduced (Gieseke, pers. comm.) and resuspended particulate material might be the main food supply. We found up to 1475 ind. m^{-2} with a mean of 743 ± 307 ind. m^{-2} at Caleta Yerbas Buenas. Population levels reported in the Bay of Brest in France for *Crepidula fornicata* are similar although peak concentrations of 4000 to 5000 individuals can be much higher (Dupont 2004, Guerin 2004, Richard 2005). Abundances of C. *fornicata* reported for the German Wadden Sea are, in general, lower (Thieltges et al. 2003), although maximum density is in the same order as that of *C. aurita*.

Conclusions

Using traditional shell characteristics, *Calyptraea aurita* bears no resemblance to suggested synonyms among other species. Most probably the species has not been redescribed before due to its depth distribution, which is below the main diving activities in the region. *Calyptraea aurita* can be easily identified by the spiral channel present in the umbilicus at the inner surface of the shell and by the sculpture with numerous fine radial ribs. In addition the edges of the shell have a regular circular shape. However, we never found empty shells of this species on the beaches or in the intertidal zone, which might be explained by the pronounced depth gradient along the rocky coast of the eastern part of the Reloncaví Sound, also the material type of the species was dredged at 82–110 m. In contrast to the Atlantic coast of South America no detailed revision of the genus *Calyptraea* (s. s.) has been undertaken.

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Abstract

During an environmental impact assessment survey of a proposed tailings storage facility for a platinum mine in Sekhukhuneland, South Africa, five adult and five larval specimens of a new species of *Boloponera* were found while excavating soil to a depth of 10–15 cm at the base of a tree in riparian woodland. These specimens represent a 3400 km range extension and the first reported record of the genus since its description in 2006, which was based on a single specimen collected in the Central African Republic in 2001. A description of the worker and ergatoid queen of *Boloponera ikemkha* **sp. n.** is presented, with a description of the mature larva and a key to distinguish workers of the two currently known species of the genus. The taxonomic relationships of *Boloponera* are discussed with respect to several confirmed and newly identified autapomorphies that support its retention as a distinct genus, although closely related to *Plectroctena* and *Loboponera*. A preliminary assessment of the conservation status and discussion of potential threats to the survival of *B. ikemkha* is also provided. Evaluation of current data under the IUCN Red List criteria would result in *B. ikemkha* being assessed as Critically Endangered, but further investigation is required to test the validity of placing it in this category.

Keywords

Afrotropical, *Boloponera*, conservation, range extension, taxonomy

Introduction

Boloponera was described by Fisher (2006) on the basis of a single worker specimen collected in the Central African Republic in 2001 and, with no further specimens apparently having been recorded since (Fisher and Bolton 2016), it is one of the most rarely encountered African ant genera known. During an environmental impact assessment (EIA) of a proposed tailings storage facility (TSF) for the Two Rivers Platinum Mine (TRPM) in Limpopo Province, South Africa, five adult specimens and five larvae of a previously unknown species of *Boloponera* were collected, representing a 3400 km range extension for the genus.

The single known specimen of the previously described *B. vicans* Fisher lacks eyes, but while three of the newly collected specimens also lack eyes, two have moderately well-developed compound eyes with *circa* 15 ommatidia each; these specimens are also larger and more stoutly built and in particular have larger gasters and relatively longer legs. While the possibility that the specimens with eyes represent a distinct major worker caste cannot entirely be excluded, it seems most likely that they are ergatoid queens. An eyeless specimen is thus designated as the holotype worker and a list of the characters distinguishing the putative ergatoid queens is presented.

A description of the new species is provided and includes a description of larval morphology, an update of some of the diagnostic characters of *Boloponera* and a key to distinguish the two currently known species of the genus. The taxonomic relationships between *Boloponera*, *Loboponera* and *Plectroctena* are discussed with respect to several confirmed and newly identified *Boloponera* autapomorphies. An assessment of the conservation status of *B. ikemkha* sp. n. and potential threats to the survival of the species is also presented.

Materials and methods

Measurements were taken using a Leica MZ16 stereomicroscope equipped with an axial shift carrier and an ocular graticule calibrated against a stage micrometer; specimens were photographed using a Leica DFC 425 digital camera connected to the same microscope. Multifocus images were captured using Leica Application Systems (LAS); montage images were generated using Helicon Focus V6.2.0 and edited with Adobe Photoshop CS3. Scanning electron microscope (SEM) images of larvae (carbon coated using an Emitech K950 X Turbo Evaporator) were taken with a Zeiss Gemini Crossbeam 540 field emission SEM.

Terminology

Terminology relating to adult morphology follows Fisher and Bolton (2016) and Keller (2011), descriptions of surface sculpture follow Harris (1979) and larval morphology follows Wheeler and Wheeler (1976a, b). Of particular significance are the lobes covering the antennal insertions, which were termed frontal lobes in Fisher's (2006) description of *Boloponera vicans* and in previous treatments of members of the *Plectroctena* genus-group. Keller (2011) coined the term *torular lobe* to describe these structures, which are formed from a laterad expansion of the highest parts of the median arches of the toruli and are not homologous to the frontal lobes of other ants. Also important is the *ventral flap on metapleural gland opening*, a term used by Keller (2011) for a thin cuticular extension of the ventral margin of the orifice of the metapleural gland.

Measurements

Gt1L	Maximum length of first gastral tergite (A3) in dorsal view
Gt1W	Maximum width of first gastral tergite (A3) in dorsal view
Gt2L	Maximum length of second gastral tergite (A4) in dorsal view
Gt2W	Maximum width of second gastral tergite (A4) in dorsal view
HH	Head height: maximum height of head in profile view, measured perpendicular to the longitudinal axis of the head capsule
HL	Head Length: length of head measured in full-face view, from the midpoint of the clypeal margin to the midpoint of the posterior margin; where either of these margins is concave the measurement is taken from the midpoint of a line joining the anterior-most portions of the clypeus or the posterior-most portions of the posterior margin
HW	Head Width: maximum width of head, measured in full-face view
ML	Maximum length of mandible from apex to intersection with the outer clypeal margin
MesTL	Maximum length of dorsal surface of the mesotibia measured from the proximal constriction to the apex
MetTL	Maximum length of dorsal surface of the metatibia measured from the proximal constriction to the apex
OD	Ocular Diameter: maximum diameter of the eye, measured with head in profile view
PeNH	Maximum height of petiole node in profile view, excluding the subpetiolar process
PeH	Maximum height of petiole in profile view, including the subpetiolar process
PeW	Maximum width of petiole in dorsal view
PeNL	Maximum length of petiole node in profile view
PeL	Maximum length of petiole in profile view, including anterior and posterior articulations
ProTL	Maximum length of dorsal surface of the protibia measured from the proximal constriction to the apex
PW	Pronotal Width: maximum width of pronotum measured in dorsal view
SL	Scape Length: maximum straight-line length of the scape, excluding the basal constriction

TL	Total Length: sum of HL + WL + PeL + gaster length (sum of gastral segment 1 + combined lengths of gastral segments 2–5 measured in lateral view at the height of the 1st and 2nd gastral spiracles respectively); this measure excludes the mandibles and is an approximation due mainly to variable extension and flexion of gastral segments
TLW	Torular lobe width: maximum width across torular lobes in full-face view
WL	Weber's Length: diagonal length of mesosoma in profile, from the junction of the pronotum and the cervical shield, to the posterior basal angle of the metapleuron

Indices and estimates

CI	Cephalic Index: (HW*100)/HL
Gt1LI	Gastral Tergite 1 Length Index: (Gt1L*100/WL)
Gt1WI	Gastral Tergite 1 Width Index: (Gt1W*100/WL)
Gt2LI	Gastral Tergite 2 Length Index: (Gt2L*100/WL)
Gt2WI	Gastral Tergite 2 Width Index: (Gt2W*100/WL)
HVe	Head Volume estimate: (HL*HW*HH)*(4π/3); while not an exact measure of cephalic volume, this estimate is directly proportional to actual volume and for a given head shape the proportionality remains constant and so allows comparison of relative cephalic volumes between individuals with similar head shapes; estimates presented in mm^3
OI	Ocular Index: (OD × 100)/HW
SI	Scape Index: (SL × 100)/HW

Abbreviations of depositories

AFRC	AfriBugs collection, Pretoria, South Africa
CASC	California Academy of Sciences Collection, San Francisco, USA
SAMC	Iziko South African Museum Collection, Cape Town, South Africa

Taxonomy

Diagnosis of *Boloponera* worker

As in Fisher (2006), with the following additions and modifications:

1. Glandular structure present on metafemur, visible for approximately 2/3 of the length of the femur as a thinning of the cuticle, without any impression or groove. No such structure visible on mesofemur.

2. Anterior disc of petiole with a broadly C-shaped to horseshoe-shaped strip of thicker cuticle surrounding a roughly semicircular patch of thinner cuticle.
3. Anterior margin of clypeus with a strongly convergent pair of long setae that cross at approximately their mid-length.
4. Posterior margin of clypeus projecting anterad of the anterior clypeal margin and overhanging the closed mandibles.
5. Mandible with an apical tooth plus three pre-apical teeth; the sub-apical tooth is close to the apical and is followed by a large diastema before the third tooth, which is located near the fourth (basal) tooth at the basal angle, near the mid-length of the mandible.
6. Metapleural gland orifice opening dorsally oriented, clearly visible in dorsal view but largely obscured in lateral view; posteriorly it is completely hidden by the upwardly extended ventral flap.

Key to workers of *Boloponera*

1 Torular lobes distinctly longitudinally striate; first gastral tergite with standing hairs and appressed pubescence; subpetiolar process with a strongly developed posteriorly projecting acute tooth; mesosoma dorsum, scapes and femora longitudinally striate between scattered piligerous foveolae (Central African Republic)..***vicans* Fisher, 2006**

– Torular lobes smooth and highly polished without striations; first gastral tergite with appressed pubescence only; subpetiolar process terminating in a blunt slightly recurved posteroventral point, but without a strong posteriorly projecting acute tooth; mesosoma dorsum, scapes and femora smooth and shining between scattered piligerous foveolae (South Africa) ....***ikemkha* sp. n.**

***Boloponera ikemkha* sp. n.**

http://zoobank.org/8849C864-5D24-4BD6-A772-A09F2D8E9C7F
Adult, Fig. 1A–F, Fig. 2A–F, Fig. 3A–B, Larva, Fig. 4A–E

Worker measurements (3 measured, holotype in parentheses): TL 3.35–3.42 (3.42), HL 0.76–0.77 (0.77), HW 0.61–0.62 (0.62), HH 0.48–0.49 (0.49), SL 0.43–0.44 (0.44), TLW 0.28–0.29 (0.28), ML 0.46 (0.46), PW 0.45–0.46 (0.45), WL 1.06–1.08 (1.07), PeNH 0.38–0.39 (0.39), PeH 0.43–0.44 (0.43), PeW 0.34–0.35 (0.34), PeNL 0.36–0.37 (0.36), ProTL 0.36–0.38 (0.37), MesTL 0.30–0.32 (0.31), MetTL 0.38–0.39 (0.38), Gt1L 0.56–0.57 (0.57), Gt1W 0.57–0.58 (0.58), Gt2L 0.66–0.68 (0.68), Gt2W 0.57–0.58 (0.58). **Indices and estimates**: CI 80–82 (81), SI 70–71 (71), HVe 0.117–0.124 (0.124), Gt1WI 53–55 (55), Gt2WI 53–55 (55) (all measurements in mm except HVe, which is presented in mm^3).

Description. *Head* subrectangular, moderately longer than wide (CI 80–82), posterior margin shallowly indented medially, sides almost straight but slightly divergent in anterior half, rounding posteriorly into the broadly convex vertices. Torular lobes extremely large and protruding anteriorly over the clypeus, forcing the medial portion of the posterior margin of the clypeus anterad of, and overhanging, the medially concave anterior clypeal margin. Torular lobes translucent and highly polished, without any trace of striate sculpture except in the median strip between the lobes, a few piligerous foveolae present adjacent to this strip and in the posterior portion of the lobes. A pair of short, weakly diverging setae arise medially at the upper (= posterior) margin of the clypeus, a second similar but more strongly divergent and longer pair arise below these from about the midlength of the clypeus and a third pair of strongly convergent setae arise from the lower (= anterior) clypeal margin. The latter setae cross each other at about their midlength. Lateral portion of clypeus divided into sloping anterior and flat posterior sections by a transverse carina. Clypeus smooth and shining, weakly sculptured posterolaterally and with several weak and incomplete diagonal carinae anteriorly. Frontal carinae are very short, fading out immediately behind the torular lobes and failing to reach the mid-length of the head. Eyes and ocelli absent. Mandibles smooth and shining with scattered piligerous punctures, elongate, curved inward apically and each with an apical and a preapical tooth, with an additional blunt tooth near and another at the base of the masticatory margin. A fine but distinct groove arises dorsally at the base of the basal margin, running diagonally across the outer surface of the mandible, reaching the lateroventral margin at about one third of the length of the mandible and continuing along this margin to the apex, but no dorsal groove parallel to the masticatory margin is present. Antennal scapes short, stout, basally curved and distally thickened; when laid back, scapes fall short of the posterior margin of the head by about half their length. Antennal segment 2 slightly longer than broad, segments 3 to 10 distinctly broader than long. Two-segmented club formed by segment 11, which is slightly broader than long, and the apical (12th) segment, which is twice as long as broad. Scapes with strong sub-appressed pubescence only, lacking erect setae, remaining segments with appressed pubescence and short suberect setae, all segments smooth and shining, unsculptured except for piligerous punctures. Head smooth and shining with scattered piligerous foveolae everywhere apart from a small posterodorsal patch medially, the foveolae irregularly spaced but on average separated by more than their diameter. Hairs arising from the foveolae appressed and medially oriented on dorsum of head. Foveolae weakly longitudinally aligned, spaces between them smooth and shining dorsally and posteriorly but laterally, anteriorly and ventrally undose. *Mesosoma*: laterally striate, becoming undose dorsolaterally, the sculpture stronger on the pronotum and metapleura, weaker on the mesopleura. All dorsal surfaces smooth and shining medially, weakly undose laterally. Entire dorsal mesosoma with scattered piligerous foveolae which are more widely spaced medially. Promesonotal suture well-defined and flexible, metanotal groove entirely absent dorsally and only faintly discernible laterally. Katepisternum well-defined and isolated by a sharply incised suture, anepisternum also sharply defined dorsally, but not posteriorly, where it is contiguous with the metapleuron. Propodeal spiracles round, situated at about the mid-height of the sides of the propodeum. Propodeal declivity flanked by strongly developed translucent lamellae running from the posterolateral corners of the propodeum to the metapleural lobes, with which they are confluent. In profile the propodeal dorsum meets the declivity in an obtuse angle, the surfaces separated by a weakly defined arched edge that is confluent with the lateral lamellae. Declivity shallowly concave in dorsal view, mostly smooth, but weakly shagreenate in upper half. Metapleural lobes broadly rounded, incurved ventrally. Metapleural gland bulla expanded and protruding posterolaterally, the orifice opening dorsally and obscured posteriorly in lateral view by the upwardly extended ventral flap. Pretarsal claws without preapical teeth. Metafemur dorsally with a strip of thin cuticle slightly more than half its length through which an apparently glandular structure can be seen (see Figure 2D), the surface neither flattened nor indented; mesofemur lacking any such feature. *Petiole*: node in dorsal view distinctly wider behind than in front, in profile with anterior and posterior faces convergent, the node tapering to the very broadly rounded summit. Subpetiolar process keel-like, slightly recurved and produced posteriorly as a blunt point. Anterior face of node with weakly reticulate sculpture; posterior face smooth, with a few very faint short striae radiating from the posterior petiolar peduncle. Sides of node smooth posterodorsally and with undose sculpture anteroventrally restricted; dorsal surface of node smooth. Lateral and dorsal surfaces of node with scattered piligerous foveolae, which are absent from anterior and posterior faces. Anterior disc of petiole ventrally with a broad C-shaped strip of cuticle around a thinner semicircular patch (see Figure 2F). *Gaster* with tergites smooth and shining, very weakly undose between scattered piligerous foveolae, pubescence appressed to sub-appressed. Sting present, weakly curved. *Pilosity*: standing hairs absent from all dorsal surfaces of head and body apart from posterior margin of gastral tergite 2 (A4) and dorsum of subsequent tergites. Meso- and metathoracic tibiae each with a single subapical pectinate spur and a pair of elongate setae located more proximally on the ventral surface. Outer surfaces of femora as well as pro- and metathoracic tibiae with short appressed pubescence, mesothoracic tibiae with longer suberect setae. *Colour*: medium reddish-brown, legs and apical two antennal segments slightly paler.

Material. Holotype worker. SOUTH AFRICA, Limpopo, Sekhukhune, De Grooteboom 373 KT portion 1, 1025 ±10m, -24.93625, 30.14494 ±5m, P. Hawkes, J. Fisher, S. Pillay, 08.xii.2016, TRP2016b-TSF-131, Riverine fringe forest (in Sekhukhune Mountain Bushveld), hand collected 10–15 cm deep in soil at base of tree, CASENT0254322 (SAMC).

Paratype workers. 2 specimens, same data as holotype, CASENT0254323 (CASC), CASENT0254324 (AFRC)

Ergatoid queen measurements (2 measured): TL 3.82–3.84, HL 0.84, HW 0.70, HH 0.54–0.55, SL 0.51, OD 0.06, TLW 0.31, ML 0.48–0.49, PW 0.51, WL 1.17–1.18, PeNH 0.43, PeH 0.48, PeW 0.39–0.40, PeNL 0.40–0.41, ProTL 0.43, MesTL 0.36, MetTL 0.47, Gt1L 0.66, Gt1W 0.70, Gt2L 0.82, Gt2W 0.71–0.72. **Indices and estimates**: CI 83, SI 72–73, HVe 0.166–0.167, Gt1WI 60, Gt2WI 61, OI 9 (all measurements in mm except HVe, which is presented in mm^3).

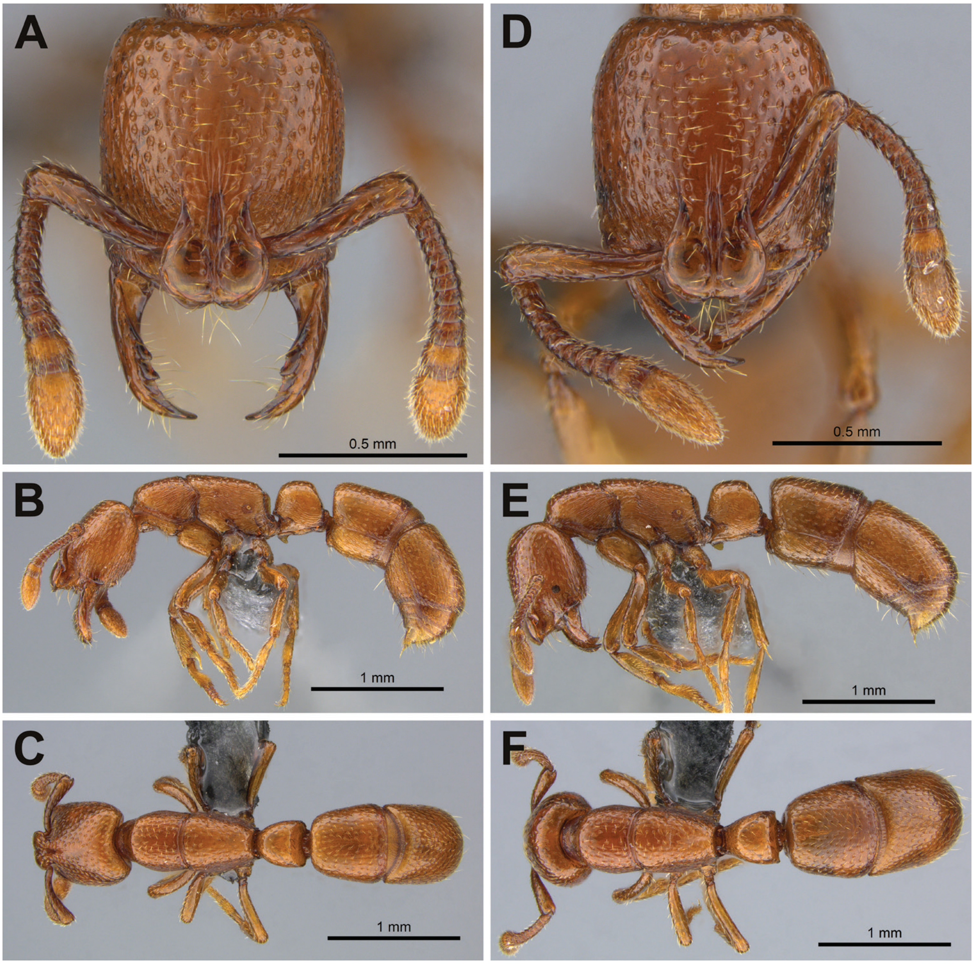

Figure 1. *Boloponera ikemkha*. **A–C** holotype worker, CASENT0254322 **A** full-face view **B** lateral view **C** dorsal view **D–F** paratype ergatoid queen, CASENT0254320, same magnifications as worker images **D** full-face view **E** lateral view **F** dorsal view (photographs by Peter Hawkes, from www.AntWeb.org).

Matching the description of the worker but differing in the following respects:

1. Larger overall, with head relatively slightly broader and scapes relatively slightly longer (see Table 1);
2. Gastral segments 1 & 2 absolutely and relatively broader and longer (see Table 1, Figures 1B–F);
3. Mesosoma relatively very slightly broader (PW/WL 0.44 vs 0.43), metanotal suture faintly visible in dorsal view in one specimen;
4. Compound eyes present, with 12–17 rather poorly defined ommatidia of varying size and shape, making precise counts difficult;
5. Head somewhat more rounded posterolaterally (compare Figures 1A and 1D).

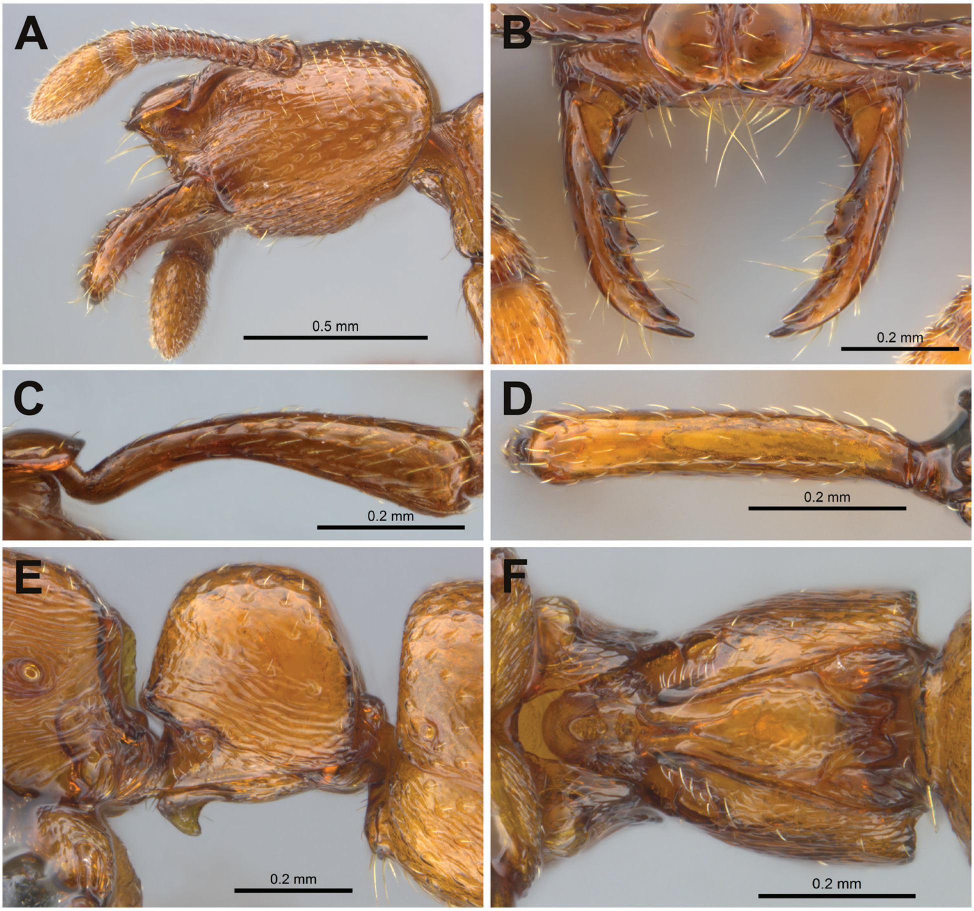

Figure 2. *Boloponera ikemkha*. **A, B, D, E** holotype worker CASENT0254322 **C, F** paratype ergatoid queen CASENT0254320 **A** head, lateral view **B** clypeus and mandibles **C** right scape, posterior view showing inflection from which length is measured **D** left metafemur, dorsal view **E** petiole, lateral view **F** petiole, ventral view (photographs by Peter Hawkes, from www.AntWeb.org).

6. Subpetiolar process more bluntly rounded apically, not posteriorly recurved (compare Figures 1B and E).
7. Legs longer, the difference being more pronounced in the middle and hind legs (compare Figures 1C and 1F and see Table 1); in absolute terms the pro-, meso- and metathoracic tibiae of the ergatoid specimens are 14%, 17% and 21% longer respectively than those of the workers and relative to Weber's length are 4%, 7% and 10% longer.

Paratype ergatoid queens. 2 specimens, same data as holotype worker, CASENT0254320 (AFRC), CASENT0254321 (CASC).

Male. Unknown.

Etymology. *ikemkha* is derived from Ancient Egyptian (*ikem* = shield; *kha* = shining) and refers to the very large, highly polished torular lobes. The specific epithet is a noun in apposition and is thus invariant.

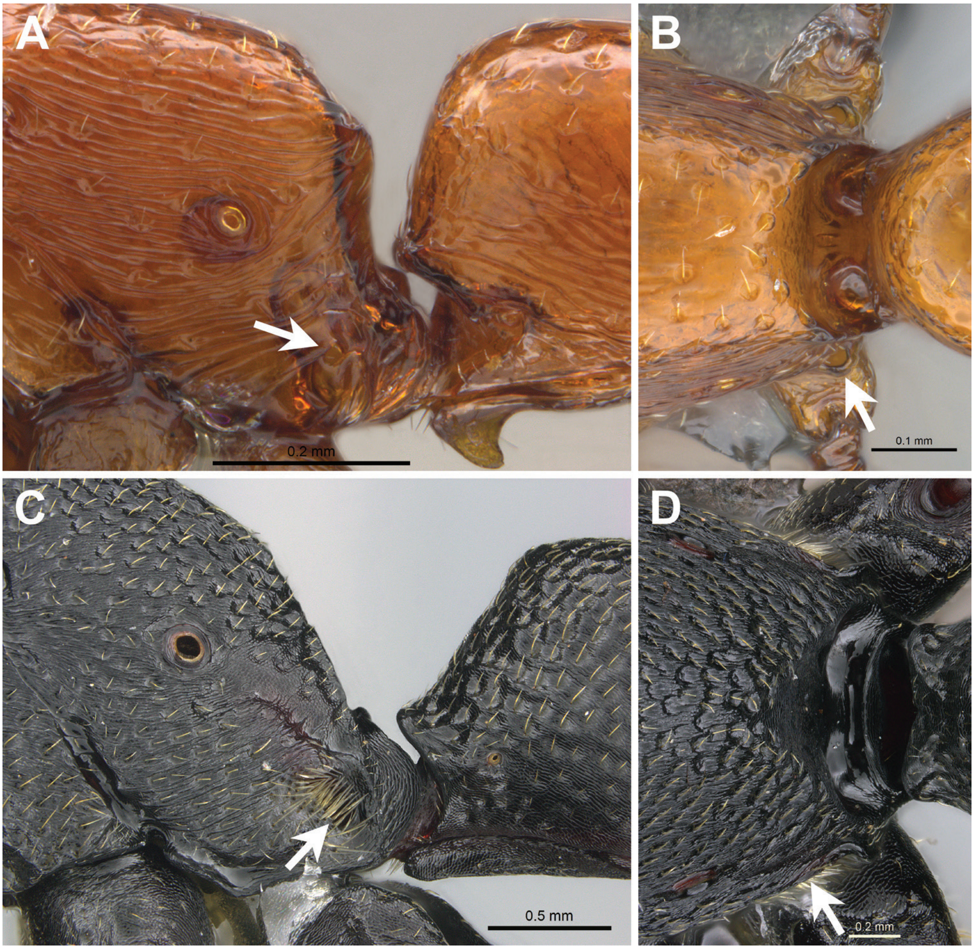

Figure 3. Metapleural gland openings. **A, B** *Boloponera ikemkha* holotype worker, CASENT0254322 **A** profile view **B** dorsal view **C, D** *Plectroctena strigosa* CASENT0235672 **C** profile view **D** dorsal view. Metapleural gland openings indicated by arrows (photographs by Peter Hawkes, from www.AntWeb.org).

Table 1. Comparison of worker and ergatoid queen measurements (in mm) and indices of *B. ikhemka*; values presented as mean ± standard deviation.

Caste	TL	CI	SI	Gt1WI	Gt1LI	Gt2WI	Gt2LI	ProTL/WL	MesTL/WL	MetTL/WL
Worker	**3.39** ± 0.04	**81** ± 0.70	**70** ± 0.65	**54** ± 0.64	**54** ± 0.75	**53** ± 0.73	**63** ± 0.53	**0.35** ± 0.006	**0.29** ± 0.004	**0.36** ± 0.003
Ergatoid queen	**3.83** ± 0.01	**83** ± 0.30	**73** ± 0.20	**60** ± 0.02	**61** ± 0.02	**56** ± 0.27	**70** ± 0.43	**0.36** ± 0.001	**0.31** ± 0.001	**0.40** ± 0.002

Larval morphology. Mature (assumed, based on size) larva: white, length through spiracles 4.1 ± 0.7 mm (three measured), elongate pogonomyrmecoid form, weakly curved but distinctly differentiated into head, neck (T1–3 + AI–II) and body (AIII–X).

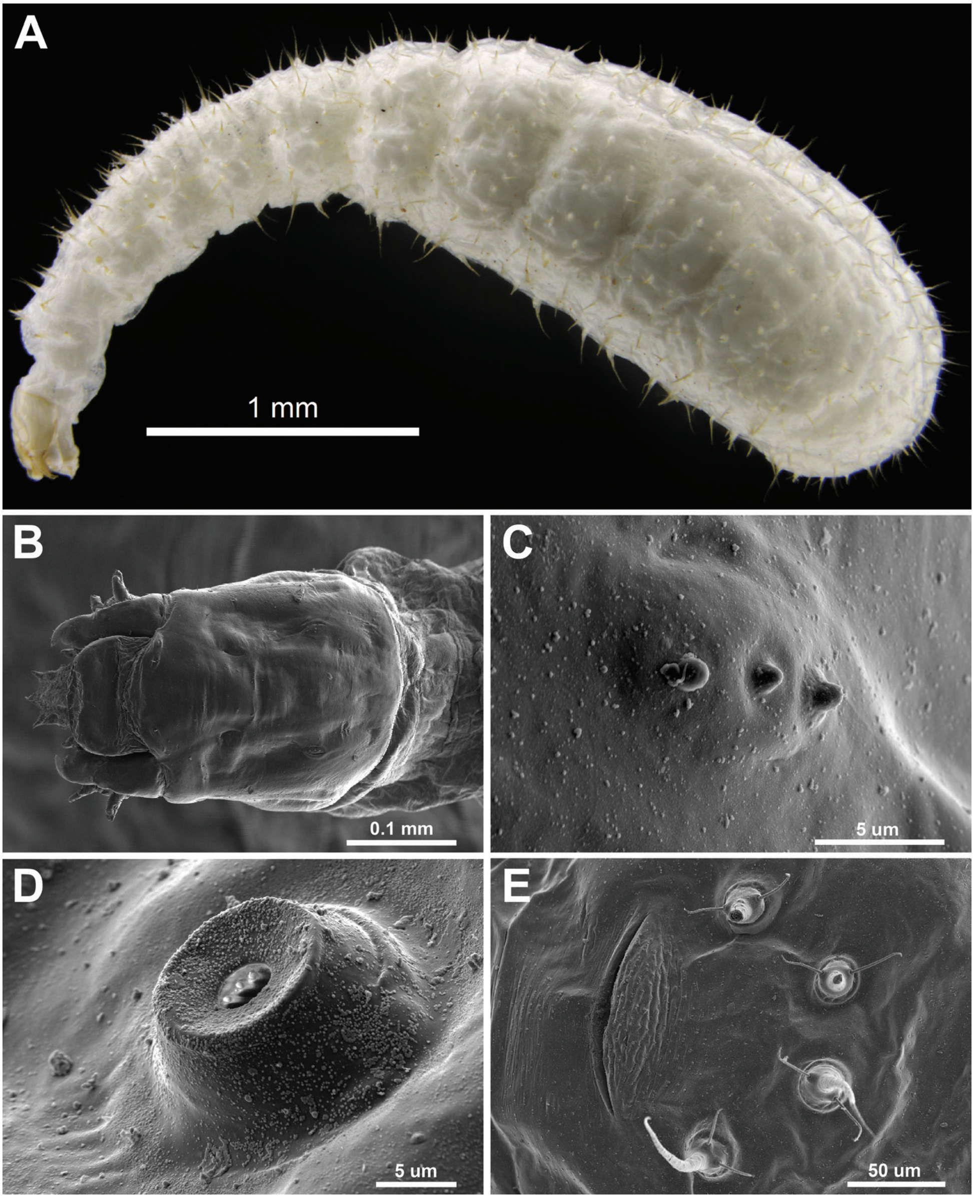

Figure 4. *Boloponera ikemkha* larva. **A–B** CASENT0257321 **A** lateral view before coating for SEM. (Photo by Peter Hawkes, from www.AntWeb.org) **B** head, dorsal view **C–E** CASENT0257322 **C** left antenna **D** left spiracle on abdominal segment 2 **E** anus with associated tubercles (SEM images by Peter Hawkes & Jonathan Fisher, from www.AntWeb.org).

Tubercles yellowish-white, very numerous (708 on CASENT0257322), conoid with 0–2 simple hairs (0–2 on T1–T3, 0–1 on AI–AX) and surmounted by an elongate slender cone with spinulose integument (= conoid with spine *sensu* Wheeler and Wheeler 1976a). Tubercles absent ventrally on T1–T3.

Anus ventral, a weakly recurved transverse slit approximately 0.1 mm across, with a very fine anterior and much larger posterior lip (Figure 4E), four tubercles (conoid with a spine and two setae) arranged in a semicircle just behind the anus. Spiracles visible on T2–T3 and A1–A8, all ten of similar diameter (3.5–4.0µm); each set in a short cone-like peg set in a slight depression and with spinules on the inner surface of the atrium (Figure 4D). Thoracic segments and first eight abdominal segments distinct; abdominal segments nine and ten difficult to distinguish. Spinules abundant, in scattered short transverse rows of 2–7 posteriorly, longer rows ventrally on thoracic segments.

Head small (0.32 mm, ca. 8% of body length) subquadrate, clypeus arcuate, antennae high on head, at about the upper third, each an elongate oval with stronger lateral but weak anterior and posterior demarcation, the three weakly defined sensilla each with a small blunt subglobular spinule. Hairs on head very sparse (ca. 16–20) and short (approximately 5 µm): two on each side near the anterolateral margin of median portion of clypeus, two on each side near posterolateral margin of clypeus, one on the side of head behind the mandibular insertion, a short longitudinal row of 2–3 hairs on sides near posterolateral corners of head and a single hair on each side between these rows, behind the level of the antennae. Labrum subrectangular, slightly wider than long, with a row of four hairs on the anterior margin, a few rows of elongate spinules posteriorly on the ventral border and numerous rows on the posterior margin. Mandible pogonomyrmecoid, with a sharp-edged, strongly inwardly curved apical tooth and two very blunt preapical teeth. Maxillae paraboloidal, anterior and interior surfaces of the lacinia with rows of spinules, stipes without spinules, but with 3–4 hairs on the outer surface; the paxilliform maxillary palp stouter and sub-equal in length to the digitiform galea, both with apical and subapical sensilla. Anterior surface of labium with short rows of elongate spinules, labial palps paxilliform and ventrolaterally situated, with one subapical and three apical sensilla. Hypopharynx densely spinulose, the spinules arranged similarly to those on the posterior margin of the labrum.

Larval morphology is similar to that described for *Plectroctena cryptica* Bolton (Wheeler and Wheeler 1976b) and *P. mandibularis* Smith (listed as *P. conjugata* Santschi in Wheeler and Wheeler 1989). The number of tubercles in *B. ikhemka* (ca. 700) is intermediate between that reported for *P. mandibularis* (ca. 1600) and *P. cryptica* (ca. 350), the latter being similar to the 300 reported for *Streblognathus aethiopicus* (Smith) by Wheeler and Wheeler (1989). The spiracle structure in *Boloponera* most closely matches that described by Wheeler & Wheeler (1976a) for *Paraponera* and *Thaumatomyrmex*. Although the spiracle form in *Plectroctena* was not explicitly described for either *P. mandibularis* or *P. cryptica*, inspection of a *P. mandibularis* larva at 230× magnification suggests that the structure is similar in this genus; SEM examination would be required to confirm this.

Discussion

Related species and phylogenetic affinities

Boloponera ikemkha is readily distinguished from its only known congener by the highly polished torular lobes and lack of standing hairs on the scapes, mesosoma, petiole and first gastral tergite. The discovery of a second species in the genus has confirmed the consistency of several characteristics, as well as enabling the recognition of new autapomorphies, that are of significance in elucidating relationships with other closely related genera, as discussed below.

It is pertinent first to correct some errors in previous discussions of the relationship of *Boloponera* with other members of the *Plectroctena* genus-group. Firstly, Schmidt and Shattuck (2014) state that *Boloponera* has abundant short pilosity but no pubescence, but both *B. vicans* and *B. ikhemka* do in fact possess pubescence; *B. ikhemka*, however, almost entirely lacks standing pilosity. Secondly, Schmidt and Shattuck (2014), as well as Fisher (2006), described the propodeum of both *Boloponera* and *Plectroctena* as having posterolateral margins expanded into lamellae. However, reference to the descriptions in Bolton (1974), and inspection of images on AntWeb as well as specimens of three species represented in the AFRC, indicate that the majority of *Plectroctena* species have what can at most be described as a distinctly marginate propodeum (e.g. *P. strigosa*, Emery, illustrated here in Figure 3C, D). Some species have propodeal ridges that could be described as laminae but only a few could be considered to have lamellae (which are thin and generally translucent) developed to a significant degree. In contrast, both *B. vicans* and *B. ikhemka* have well-developed, thin and translucent lamellae that run the entire length of the margin between the lateral propodeum and the declivity. The presence of lamellae thus cannot be considered a synapomorphy linking *Plectroctena* with *Boloponera*. Thirdly, Schmidt and Shattuck (2014) state that, as in *Loboponera*, the anepisternum in *Plectroctena* appears fused to the mesonotum and metapleuron. While this may be the case for a few of the 16 *Plectroctena* species illustrated on AntWeb, in the majority of these, and in all three species represented in the AFRC, the anepisternum is moderately to well-defined by impressed sutures and is more distinct from the mesonotum and metapleuron than it is from the katepisternum. This character therefore cannot be considered a synapomorphy linking *Plectroctena* and *Loboponera*. In *B. vicans* and *B. ikemkha*, the anepisternum is contiguous with the metapleuron, but remains separated from the mesonotum by a weak but distinct suture.

Fisher (2006) noted similarities between *Boloponera* and *Plectroctena*, but also highlighted several differences and concluded that *Boloponera* should be excluded from the *Plectroctena* genus-group (*Loboponera* + *Plectroctena* + *Psalidomyrmex*) as defined by Bolton and Brown (2002). However, based on molecular and morphological evidence, Schmidt and Shattuck (2014) subsequently placed *Boloponera* within their expanded *Plectroctena* genus-group (*Boloponera* + *Centromyrmex* + *Dolioponera* + *Feroponera* + *Loboponera* + *Plectroctena* + *Psalidomyrmex*), as sister to *Plectroctena* and/or *Loboponera*, and suggested that *Boloponera* might even be nested within *Plectroctena*. The discovery of *B. ikhemka* has however confirmed several of the previously recognised differences between *Boloponera* and *Plectroctena* including:

1. the lack in *Boloponera* of a highly modified mandibular articulation (incorporating differences in the structure of the mandibular articulation itself as well as of the clypeus and genae);

2. the presence of both an apical and a preapical tooth on the mandible in *Boloponera*, as well as an additional tooth on the masticatory margin close to the basal tooth. *Plectroctena* have a blunt or truncated mandible with neither an apical nor a preapical tooth and, when present, the masticatory margin tooth is widely separated from the basal angle or tooth;
3. the absence in *Boloponera* of a longitudinal groove on the inner half of the dorsal mandibular surface running parallel to the masticatory margin;
4. the absence of a mesofemoral gland in *Boloponera*, and
5. the differing form of the anterior disc of the petiole in *Boloponera*.

Schmidt & Shattuck (2014: 162) downplayed the significance of the last character and, based on the rather indistinct published image of this feature in *B. vicans*, suggested that the shape of the median depression in the petiolar articulatory surface in *Boloponera* is more similar to that of *Plectroctena* than is its shape in *Loboponera*. However, this cannot be said of *B. ikhemka*, in which the median depression is very distinct from that of *Plectroctena* (compare Figure 2F with figures 28–29 of Fisher, 2006) and is more like a foreshortened version of that found in *Loboponera* (see figures 25, 26 in Fisher, 2006). The median depression in *Boloponera* is broadly rounded anteriorly and approximately equal in width to the horseshoe-shaped articulatory strip, which perfectly matches Bolton & Brown's (2002) description of the ancestral structure. In strong contrast, in *Plectroctena* (and *Psalidomyrmex*), the median depression is reduced in width anteriorly to a very narrow groove by inward expansion of the articulatory surfaces (as described in Bolton & Brown, 2002), with an overall arrowhead-like appearance in most species. *Loboponera* appears intermediate, with the median depression significantly narrower than the lateral strips, but approximately uniform in width and by no means as compressed anteriorly as in *Plectroctena*. On current evidence I therefore disagree with Schmidt & Shattuck's (2014) assessment of this character and consider *Boloponera* to display a more ancestral form, with *Loboponera* showing slight modification and *Plectroctena* + *Psalidomyrmex* with the most derived state.

Boloponera, *Loboponera* and *Plectroctena* share a longitudinal metafemoral gland as a synapomorphy, but *Boloponera* lacks the similar mesofemoral gland found in both *Loboponera* and *Plectroctena*. There is no visible groove in *Boloponera*, in which the gland is indicated merely by a thinning of the cuticle. However, the development of the grooves appears variable in the *Plectroctena* material I have inspected, and thus not much weight can be placed on this character; only the presence/absence of the mesofemoral glandular structure is of significance here and again *Boloponera* appears to display the less-derived state.

While Bolton and Brown (2002) considered the strongly arched A4 tergite autapomorphic in *Loboponera*, several *Plectroctena* (e.g. *P. dentata* Santschi, *P. strigosa* and *P. thaui* Fisher) have the A4 moderately arched (tergite approximately three times the length of the sternite) and in at least one (*P. laevior* Santschi), the A4 is even more strongly arched (tergite more than four times the length of the sternite) than that of some *Loboponera*, while most other *Plectroctena* species have the A4 slightly arched (tergite roughly twice the length of the sternite). The variability of this character within *Plectroctena* means that in addition to not being autapomorphic for *Loboponera*, it also cannot be considered a synapomorphy for *Plectroctena* + *Loboponera*. In *Boloponera* the A4 tergite (Figure 1B, C) is straight or weakly arched anteriorly and only weakly arched posteriorly, the tergite being approximately 2.5 times the length of the sternite. Although I believe that relatively little weight can be afforded to the character, *Boloponera* does again appear to have the least derived form, with *Plectroctena* and *Loboponera* showing increasing modification.

In contrast, the extreme protrusion of the torular lobes beyond the posterior clypeal margin, which itself is extended anterad of the anterior clypeal margin and overhanging the mouthparts, appears unique to *Boloponera* and hence more derived. While the anterior clypeal margin also overhangs the mouthparts in *Loboponera*, the torular lobes do not extend anterad of the clypeus as they do in *Boloponera*. In *Plectroctena* the torular lobes are less developed and the clypeus is approximately vertical, not overhanging the mouthparts. Movement of the antennal scapes in *Boloponera* (as well as *Loboponera*) is highly constrained by the enlarged torular lobes. Despite the complex shape of the basal portion of the scape (see Figure 2C), which allows some additional dorsal movement, the entire scape is prevented from rising above a plane at approximately the level of the top of the lobes. The adaptive significance of this is unknown.

The arrangement of clypeal setae described here for *B. ikemkha* appears identical in *B. vicans* and, at least within the *Plectroctena* genus-group, seems to be unique to the genus. Inspection of AntWeb images of all other Afrotropical members of the *Plectroctena* genus-group *sensu* Schmidt and Shattuck (2014), including 10 *Centromyrmex*, one *Dolioponera*, one *Feroponera*, nine *Loboponera*, 16 *Plectroctena* and six *Psalidomyrmex* species, did not reveal the pattern repeated. Although *Feroponera ferox* Bolton & Fisher, *Plectroctena anops* Bolton, *Loboponera nobiliae* Fisher, *Centromyrmex bequaerti* (Forel), *C. praedator* Bolton & Fisher and *C. sellaris* Mayr each have a moderately convergent pair of clypeal setae, which in two cases meet at the tips, none have setae which cross substantially proximal of the tips as in *Boloponera* and in no case are there two divergent pairs in addition to the convergent pair. This also appears to be a character for which *Boloponera* displays the most modified state.

In both *Loboponera* and *Plectroctena*, as in all other members (except *Feroponera*) of Schmidt & Shattuck's (2014) expanded *Plectroctena* genus-group, the metapleural gland opens laterally and is clearly visible in profile view, while both *Boloponera* species have the metapleural gland bulla strongly laterally expanded ventrally (visible in ventral view in Figure 2F and in dorsal view in Figure 3B), resulting in a dorsally oriented orifice (Figure 3B). This is obscured in profile view (Figure 3A), especially posteriorly, by the dorsad extension of the ventral flap on the metapleural gland opening; in this character *Boloponera* again shows an apparently more derived state than either *Loboponera* or *Plectroctena*.

The prora in *Boloponera* is fairly inconspicuous, while in *Plectroctena* the prora is evident as a much more prominent projection on the anterior face of the first gastral sternite; in *Loboponera* the prora is often exceptionally well developed and in several species forms a very prominent antero-ventral projection.

In summary, *Boloponera* and *Loboponera* are similar with respect to petiole articulation, the expansion of the torular lobes, orientation of the clypeal surface, mandibular articulation and absence of a dorsal groove on the mandible. *Loboponera* and *Plectroctena* are similar in having the torular lobes not overhanging the mouthparts, configuration of clypeal setae, presence of a mesofemoral gland, position of the metapleural gland opening, development of the prora and degree of arching of A4. *Plectroctena* and *Boloponera* are similar in the absence of posterolateral head flanges and shape of the mandibles, although the latter may not be equivalent in view of the extreme development of other mandibular characters in *Plectroctena*.

Thus there appear to be fewer characters suggesting a close relationship between *Boloponera* and *Plectroctena* than there are linking *Boloponera* with *Loboponera* or *Loboponera* with *Plectroctena*; this assessment implies that *Boloponera* and *Plectroctena* are less similar to each other than either is to *Loboponera*, suggesting an intermediate position for the latter.

The characters discussed above strongly support the retention of *Boloponera* as distinct from *Plectroctena* and suggest that *Boloponera* is sister to *Loboponera* and *Plectroctena* rather than being closer to or even nested within *Plectroctena*. Nesting of *Boloponera* within *Plectroctena* would imply either that, in addition to the appearance of several new unique characteristics in *Boloponera*, there had been secondary loss (with reversion to the ancestral state) of three *Plectroctena* autapomorphies relating to the mandibles and head capsule (mandible dentition, dorsal groove and articulation), or alternatively that *Plectroctena* is paraphyletic and that these same adaptations had arisen independently in two separate lineages. The latter is highly improbable, but even the former seems unlikely and the hypothesis that *Boloponera* split from an ancestral line, before the unique mandibular configuration of *Plectroctena* had arisen, is a far more parsimonious explanation.

Finally, were *Boloponera* to be included within *Plectroctena*, which ranges in total length from 7 mm to more than 18 mm, they would be by far the smallest representatives of the genus, both species being barely more than half the length of the smallest *Plectroctena* currently known, and hence resulting in a very disjunct size range. *Loboponera* includes species intermediate in size between *Boloponera* and *Plectroctena*, with lengths ranging from about 3–7 mm, which may provide further (if weak) support for *Loboponera* being intermediate between *Plectroctena* and *Boloponera*.

The question remains, which of the three genera is closest to the ancestral form and which is the most derived? The characters discussed here are equivocal, with each genus displaying some that appear most derived and others that appear least derived, suggesting that *Boloponera*, *Loboponera* and *Plectroctena* have all undergone substantial modification since separation of their respective lineages; more comprehensive DNA phylogenetic analysis will most likely be required to resolve this question.

After the above reassessment of characters linking and separating *Boloponera*, *Loboponera* and *Plectroctena*, only the presence of a longitudinal metafemoral gland remains as a synapomorphy linking all three genera, other synapomorphies each being shared by only two of the three genera.

Ergatoid queens

Although there is no direct evidence that the *B. ikemkha* specimens with eyes are ergatoid queens, this seems the most likely explanation for the differences between the two distinct forms collected. In addition to having eyes, the larger specimens also have relatively larger gasters, with the width and length of gastral tergites 1 and 2 averaging 6–13% larger relative to the size of the mesosoma in specimens with eyes than in eyeless specimens. The relatively larger gasters would allow for development of ovaries. The lack of clear differences in the structures of the pronotum and mesonotum, where ergatoid queens normally exhibit distinguishing characters, may be due to the extreme fusion of segments in *Boloponera* (Christian Peeters, pers. comm.). Additional arguments in favour of the larger specimens being ergatoids rather than large workers are 1) it is very probable that *Boloponera* forage entirely underground, so there would be no apparent function for eyes in workers of any size, 2) it is also very likely that colony size in *Boloponera* is small and this would suggest that they could not afford the cost of producing size-variable workers (Christian Peeters, pers. comm.) and 3) analysis of morphological measurements indicates that there are discrete differences in relative proportions of body parts, including leg lengths (the specimens with eyes have relatively longer legs overall as well as differing relative leg lengths), which is different from what would be expected from a simple allometric relationship between leg length and body size within the worker caste. Ideally, dissection of colony samples or observation of live colonies in the laboratory should be carried out to confirm or refute the ergatoid hypothesis. However, given the extreme rarity of *Boloponera*, the opportunity to do this may not arise for a considerable time. One of the eyeless specimens has thus been designated as the holotype worker and the larger specimens with eyes treated as ergatoid queens, pending further investigation.

Habitat and distribution

Boloponera ikemkha was found within the Sekhukhuneland Centre of Plant Endemism (SCPE), an area recognised for its unique plant diversity (Siebert et al. 2002, 2010) but as yet very poorly known from an invertebrate perspective. The type locality of *B. ikemkha* is approximately 3400 km SSE of that of *B. vicans* (see Figure 5) and in a markedly different habitat with very different climatic conditions. While *B. vicans* was collected well within the Central African rainforest belt, *B. ikemkha* was found in a narrow strip of riparian woodland (canopy less than 10 m) in the much drier Sekhukhune Mountain Bushveld (*sensu* Mucina and Rutherford 2006). The mean annual rainfall is more than double in the *B. vicans* (1621 mm) than in the *B. ikemkha* (718 mm) type locality and seasonality of both precipitation (45.8 vs 79.6) and temperature (57.8 vs 387.9) also differ substantially (WorldClim dataset, Hijmans et al. 2005). While the subterranean conditions to which these ants are presumably mainly subjected are probably significantly buffered in comparison to the above-ground measured climate,

Figure 5. Map of Africa (inset, with Central African Republic and South Africa shaded dark grey) and detail of South Africa (main image), showing known distribution of *Boloponera*. Key: white circle - *B. vicans*; white square - *B. ikemkha*; white outline - Sekhukhuneland Centre of Plant Endemism.

it is remarkable that the two species inhabit such strongly contrasting habitats. Several other ant genera previously recorded in Africa only from tropical regions have recently been recorded in southern Africa; these include *Apomyrma stygia* Brown, Gotwald & Lévieux from the Kruger National Park, South Africa and *Aenictogiton* from northern Namibia and southern Angola (Parr et al 2003), as well as unpublished South African records in the AFRC of *Centromyrmex fugator* Bolton & Fisher from Gauteng and an undescribed *Anillomyrma* from Limpopo. In the case of *Apomyrma* and *Centromyrmex* the southern African material represents species that also occur in West and Central Africa respectively, but whether or not this is also the case cannot yet be determined for the *Aenictogiton* and *Anillomyrma* records. All of these genera are generally subterranean in their habits and thus, like *Boloponera*, are presumably buffered from climatic extremes when they occur outside their apparently normal range.

Further surveys may reveal the existence of additional *Boloponera* species and yield a less disjunct genus-level distribution, but unfortunately the currently available information does not provide any indication of the types of habitat that should be surveyed in order to maximise the chance of such discoveries being made. The *B. ikemkha* specimens were found during a brief *ad hoc* active sampling survey of riverine fringe vegetation within the predicted zone of influence of the proposed tailings storage facility at Two Rivers Platinum. No *Boloponera* specimens were located during far more intensive surveys, equivalent to the ALL-Protocol (Agosti et al. 2000), of three areas of other more open habitat types within the footprint of the proposed TSF, as well as at least 20 other ALL-Protocol equivalent surveys within 6 km and a further six within 11–27 km of this site, from 2007–2016. It therefore seems likely that *B. ikemkha* prefers the moister riverine fringe which has denser tree cover; such habitat forms only a small proportion of the total area of the SCPE.

The ultramafic soils (of igneous origin, high in magnesium and iron, with very low silica content) of the SCPE vary considerably in mineral composition and structure and this contributes to the complex pattern of very varied and unique plant communities, with many endemic species of limited distribution linked to particular soil forms (Siebert et al. 2001). Recent extensive sampling of ants in the region suggests that the same holds true for ant communities and species; for example two undescribed *Cardiocondyla* species are each apparently limited to particular soil types within the SCPE (unpublished data). It is possible that *B. ikemkha* is both rare and restricted to a particular habitat and soil type within the SCPE, but nothing is known about its ecology or behaviour.

Conservation and threats

Boloponera is one of the most rarely encountered ant genera in Africa, with each of the two known species having been recorded only once to date. Whether this is an indication of true rarity, or an artefact of an extremely cryptic lifestyle, may be difficult to determine with certainty. However, *Hypoponera*, a related genus of cryptic, largely subterranean ants, with workers similar in size to or, more commonly, smaller than *Boloponera*, has 51 described species endemic to the Afrotropical region (Bolton and Fisher 2011). While some of these are also known from only a single collection, many are widespread and are frequently encountered in soil at depths similar to that at which *B. ikemkha* was found. During a survey of leaf litter ants in Ghana, Belshaw and Bolton (1994) collected 2410 specimens of *Hypoponera* representing six species, with a range from six to 1828 individuals per species. Unless *Boloponera* are very significantly more cryptic and deeply subterranean, this suggests that they are far rarer than *Hypoponera*, probably by at least 2–3 orders of magnitude.

The type locality of *B. ikemkha*, ca. 5 km east of the confluence of the Groot and Klein Dwars Rivers, lies approximately 400 m from the nearest boundary of the proposed new tailings storage facility for TRPM. Very little similar habitat lies within the proposed TSF site. However, approximately 2 km of this riverine fringe vegetation lies within 350–900 m down-slope of the proposed TSF. A further 1 km (within which the type locality lies) falls within 350–700 m of the proposed TSF but is separated from it by a low (10–30 m high) ridge. Dust clouds emanating from existing tailings dams in the region are frequently observed rising well over 500 m into the air and dispersing over distances of at least 8 km. It is thus clear that significant dust pollution can be expected close to the proposed new TSF unless far more effective dust control is implemented in the new facility. In addition, while possible seepage of contaminated water could not directly affect the *B. ikemkha* type locality due to the intervening ridge, such pollution could impact on the 2 km section of likely habitat down-slope of the proposed TSF, which thus poses a potential threat to the survival at least of part of the local population of the species; it is thus imperative that effective water management and dust control measures be implemented.

The Groot and Klein Dwarsrivier valleys and surrounding areas are subject to intense mining and prospecting pressure, with at least eight operational mines within an eight km radius of the *B. ikemkha* type locality and more mines further afield. Many more mines are planned for this region, which forms part of the Bushveld Igneous Complex, recognised to contain the richest deposits of platinum-group elements (PGE) in the world, representing 80% of known PGE reserves (Cawthorn 2010). There is also considerable habitat transformation due to increasing human populations (in part caused by an influx of job-seekers attracted by the mines) with concomitant increases in subsistence agriculture and livestock grazing. The cumulative effect of anthropogenic impacts and mining operations on natural habitats in the region may become highly significant and could pose a serious threat to the survival of *B. ikemkha*, as well as that of other species endemic to Sekhukhuneland. Habitat transformation and fragmentation would be especially significant for *B. ikemkha* if the species does, as seems most likely, have ergatoid queens and hence very limited dispersal abilities.

Application of the IUCN Red List Criteria (IUCN 2012) to the currently available data would indicate that *B. ikemkha* should be classified as Critically Endangered (CR B1ab(iii)+2ab(iii)), since it has been recorded from only a single locality which is currently under threat and other potential habitat in the region is also under threat. However, the species could alternatively be considered as Data Deficient and in urgent need of investigation. Additional surveys could either confirm a CR status or result in a lower threatened category such as Endangered (EN) or Vulnerable (VU) being assigned, but it is highly unlikely that a category lower than VU will result. Formal Red List assessments of the conservation status of species such as *B. ikemkha* may enable action to be taken to protect sufficient areas of natural habitat to ensure their continued survival and such assessments should be undertaken as a matter of urgency.

Conclusions

The discovery of a second species of *Boloponera* confirms the consistency of several previously recognised as well as some newly identified autapomorphies for the genus and provides support for its retention as distinct from *Plectroctena*, although full relationships between *Boloponera*, *Loboponera* and *Plectroctena* remain unresolved. *Boloponera ikemkha* is considered to be extremely rare and is believed to be threatened by current and planned mining activities in the Sekhukhuneland Centre of Plant Endemism.

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The morphology of the preimaginal stages of *Cleopomiarus micros* (Germar, 1821) (Curculionidae, Coleoptera) and notes on its biology

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Abstract

As yet little is known of the bionomics of weevils of the genus *Cleopomiarus* Pierce, 1919; current knowledge is limited to data on the morphology and biology of the preimaginal stages of certain species. This paper includes original information on the life cycle of *Cleopomiarus micros* (Germar, 1821). It presents the morphology of the egg, last larva (L_3) and pupa. Data on the host plant (*Jasione montana* L.) and breeding plant (*Campanula patula* L.) and on the oviposition and phenology of the species are updated. The anatomy of the third-stage larva of *C. micros* shares certain traits with other species of the tribe Mecinini Gistel, 1848. Comparison of the morphology of preimaginal stages of *C. micros* with those previously described for other species of the genera *Cleopomiarus* and *Miarus* Schönherr, 1826 – previously considered the same genus – reveals species differences in larval body length, colour of the body and epicranium, and chaetotaxy of head and body.

Keywords

Central Europe, developmental stage, host plant, mature larva, Mecinini, oligophagy, pupal stage

Introduction

The tribe Mecinini Gistel, 1848 is currently represented by six genera worldwide, of which five (*Cleopomiarus* Pierce, 1919, *Gymnetron* Schoenherr, 1825, *Mecinus* Germar, 1821, *Miarus* Schoenherr, 1826 and *Rhinusa* Stephens, 1829) are known from the Palearctic fauna and one, *Rhinumiarus* Caldara, 2001, was discovered in the Neotropical realm (Caldara 2001, 2013, Caldara et al. 2014). This last genus was established based on analysis of the species *Rhinumiarus lyali* Caldara, 2001, found in Argentina, a taxon in which the external anatomy of the adult looks intermediate between species of the genera *Miarus* and *Cleopomiarus*.

The analysis of previously known adult forms and their preimaginal stages reveals a number of morphological characters common to the tribe Mecinini. In the larvae, these are as follows: head usually with 3 distinct pairs of setae (*des*) and with long, unbranched endocarina, which together with the epicranial suture extends 2/3 the length of the head; labial palpus usually with 1 segment – if there are 2 segments the basal segment is not distinctly separated; hypopharynx with usually 4, less often 2 or 6 epithelial anteromedial setae; premental rounded, less often pointed; sclerites of the prosternum joined or free, numbering 0, 2, 4 or 6; thoracic and abdominal spiracles uni- or bicameral depending on the genus, abdominal spiracles located laterally on the intersegmental membrane, tubes well defined from atrium (Emden 1938, Scherf 1964, Anderson 1973, May 1993, Marvaldi 2003, 2005, Gosik et al. 2010, Jiang and Zhang 2015). Species belonging to the Mecinini tribe are poly-, oligo- or monophages of plants of the families Campanulaceae, Scrophulariaceae and Plantaginaceae (Scherf 1964, Smreczyński 1976, May 1993, Burakowski et al. 1997, Caldara 2001, Caldara et al. 2014).

The genus *Cleopomiarus* includes 40 species dispersed throughout the world. Palearctic species (19 spp) are associated with plants of the genera *Adenophora* Fisch., *Campanula* L., *Jasione* L. and *Phyteuma* L. (Campanulaceae, Campanuloideae), whereas beetles from southern Africa and Mexico live on plants of the genera *Codonopsis* Wall., *Lightfootia* L'Hér., *Roella* L. and *Wahlenbergia* Schrad. ex Roth from the same subfamily (Campanuloideae), as well as on representatives of the genus *Lobelia* L. from the subfamily Lobelioideae (Caldara 2001, 2007, Caldara et al. 2014, Caldara and Legalov 2016). In Poland, the species thus far recorded are *Cleopomiarus distinctus* (Boheman, 1845), *C. graminis* (Gyllenhal, 1813), *C. plantarum* (Germar, 1823) and *C. micros* (Germar, 1821). In the case of *C. plantarum*, the data come from the late 19th and early 20th centuries and require confirmation (Smreczyński 1976, Burakowski et al. 1997, Petryszak 2004, Wanat and Mokrzycki 2005).

As yet little is known of the bionomics of insects of the genus *Cleopomiarus*. Certain data on the morphology and biology of preimaginal stages can be found in Scherf (1964), pertaining to *C. graminis* and *C. micros*, and in Emden (1938) on *C. graminis* and *M. campanulae* (Linnaeus, 1767) and Anderson (1973) on *Cleopomiarus hispidulus* (LeConte, 1876). While species identification of adult individuals poses little difficulty, systematic identification on the basis of preimaginal stages has not been possible.

In the present study the immature stages of *Cleopomiarus micros* are described. This is a rare, psammophilous species, found only in certain areas. It inhabits sand, dunes, ruderal communities, forest clear-cuts, and thickets. It has previously been recorded in southern, western and central Europe, as far north as northern Denmark and southern Sweden. It has also been recorded in North Africa. In the literature it has been described as a monophage living exclusively in the flowers of sheep's bit scabious, *Jasione montana* L. (Burakowski et al. 1997, Kubisz et al. 1998, Kuśka 1999, 2001, Łętowski and Gosik 2002, Wanat 2005, Gosik 2006, Caldara et al. 2014).

The objective of the study is to describe the morphology and biology of the preimaginal stages of *Cleopomiarus micros*, which is one of the rarest representatives of its genus in Poland.

Material and methods

The material for the study consisted of developmental stages (egg, three larval instars and adult) of *Cleopomiarus micros*, isolated in the laboratory from plants of the genus *Campanula* L.: *C. bononiensis* L., *C. glomerata* L., *C. patula* L., *C. persicifolia* L., *C. rapunculoides* L., *C. sibirica* L., and *C. trachelium* L., as well as from *Jasione montana*. The plants were collected in the field in the Lublin region: Łysaków 50°45'40.96"N, 22°11'17.31"E, Podzamcze Reserve near Bychawa 51°01'24.85"N, 22°31'59.04"E, Nasutów 51°22'31.29"N, 22°31'07.32"E, Spiczyn 51°19'59.47"N, 22°44'29.63"E, Jakubowice Murowane 51°16'21.26"N, 22°38'15.52"E, Łęczna 51°18'09.7"N, 22°51'47.8"E, Ciechanki Łańcuchowskie 51°16'37.00"N, 22°55'28"E, Niedzieliska 50°41'57.07"N, 23°05'00.75"E, Kąty 50°42'21.98"N, 23°06'58.73"E, Gródek 50°46'58.18"N, 23°56'47.04"E and Czumów 50°46'28.79"N, 23°58'05.68"E. The lack of knowledge of the host plants and breeding plants of the species was the reason for the wide range of species examined from these plant genera. The selection and distribution of habitats with potential host plants were based on the results of faunistic studies in which insects of the genera *Miarus* and *Cleopomiarus* have been caught (Cmoluch 1962, 1971, Minda-Lechowska and Cmoluch 1984, Cmoluch et al. 1994, Gosik and Łętowski 2008).

Adult insects were collected by hand directly from host plants and isolated from samples collected with an entomological net in 8 × 25 series at the sampling sites over the entire growing period of the plants, from May to September, on sunny days without wind, between 10:00–16:00 local time, once a month for a period of three years (2009–2011).

Part of the individual developmental stages of these insects were fixed in 70% ethyl alcohol and a part incubated (in a ratio 1:4 – ¼ to fixation, ¾ to incubation). Breeding of preimaginal stages was carried out in Petri dishes lined with filter paper, placed in a breeding chamber with constant temperature parameters (daytime minimum 25 °C, daytime maximum 35 °C, minimum at night 15 °C, maximum at night 20 °C), humidity (60%), light intensity and duration (day 16 h, night 8 h), in order to obtain all developmental stages of the beetles (egg, three larval instars, pupa and adult) and describe their biology.

Observations of the ecology of adult beetles were also conducted in the laboratory on individuals bred together with their host plants in glass insulators at 25 °C with a 14:10 photoperiod.

Two methods were used to prepare microscope slides, as described in Łętowski (1991) and Gosik et al. (2010). Photographs of the habitat and breeding plant were taken with a NIKON Coolpix B500 camera. To prepare the drawings we used an OLYMPUS SZX12 microscope with a DP72 camera at magnifications from 200× to 400× and a TESCAN VEGA3LMU scanning microscope at magnifications from 500× to 2000×. The figures were made based on the biological preparations using COREL DRAW 18 software. The terminology of Scherf (1964), Marvaldi (1998, 1999, 2003), Skuhrovec (2004), Skuhrovec and Bogusch (2016), Wang et al. (2013) and Oberprieler et al. (2014) was used in the morphological descriptions of larva and pupa, and for chaetotaxy. The distribution and number of setae are given for half of the larval body, while the chaetotaxy of the larval head and the body of the pupa is given with respect to the whole body. Measurements of the head (following decapitation) were made on the head capsule, isolated from the body, with the mandibles closed. The dimensions of the preimaginal stages are determined from two measurements of the ova, three measurements of larval instar L_1, 4 of L_2, 7 of L_3 and eight of the pupae.

Results

Egg

Egg white, transparent, teardrop-shaped, ca. 0.45 mm long, ca. 0.21 mm wide. Chorion surface smooth and shiny (120× magnification).

Larva

First larval instar (L_1) – white body, slightly transparent. Body length ca. 1 mm (0.96–1.07 mm), width ca. 0.46 mm (0.42–0.51 mm). Clearly visible pale brown head ca. 0.23 mm long (0.21–0.27 mm) and ca. 0.16 mm wide (0.13–0.20 mm). Larva with sparse setae, pedal tubercles nearly imperceptible. Anterior stemmata present.

Second larval instar (L_2) – creamy white body, also with sparse setae. Length body ca. 2.15 mm (2.02–2.41 mm), width ca. 1.01 mm (0.90–1.09 mm). Intersegmental grooves clearly visible, pedal lobes lightly outlined. Head pale grey, length ca. 0.4 mm (0.38–0.42 mm), width ca. 0.27 mm (0.26–0.28 mm). Anterior stemmata clearly visible.

Third larval instar (L_3) – body massive, strongly curved, rounded in cross-section, creamy-white, ca. 3.41 mm long (3.12–3.73 mm) and ca. 1.51 mm wide (1.38–1.80 mm) (Figures 1, 10). Head chitinized, dark brown. Prothorax ca. 0.28 mm wide, much narrower than other two thoracic segments, ca. 0.34 mm wide. Abdominal seg-

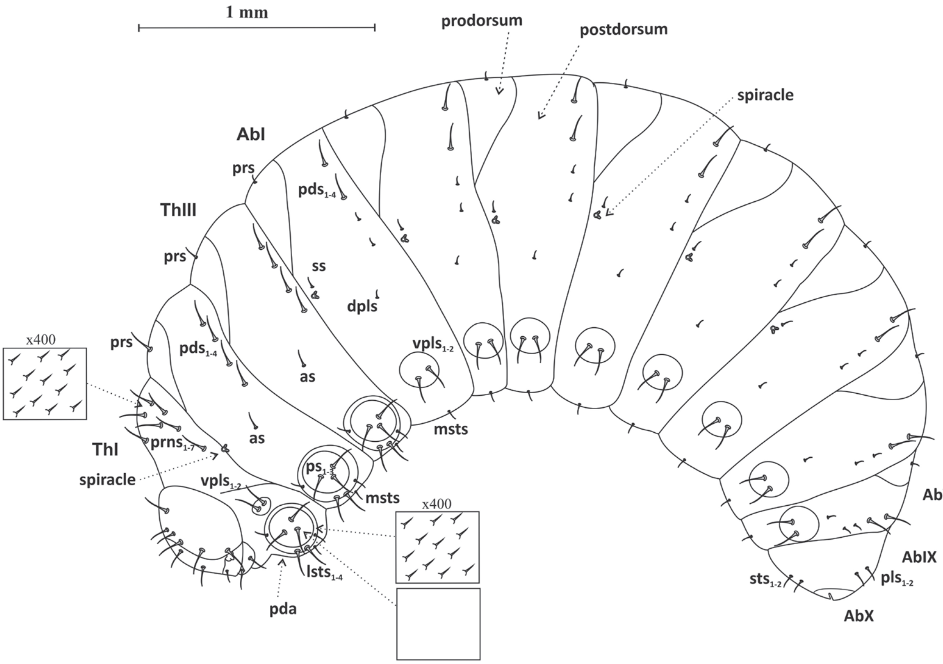


Figure 1. Mature larva (L_3), lateral view: ***prns*** pronotal setae, ***vpls*** ventropleurolateral setae, ***pda*** pedal tubercle (area), ***ps*** pedal setae, ***lsts*** laterosternal setae, ***prs*** prodorsal seta, ***pds*** postdorsal setae, ***dpls*** dorsopleural setae, ***ss*** spicular setae, ***vpls*** ventropleurolateral setae, ***msts*** mesosternal seta, ***sts*** sternal setae, ***pls*** pleural setae.

ment I wider than others (ca. 0.49 mm), II–VII of similar width (ca. 0.38 mm). Segment VIII much narrower than others (ca. 0.23 mm), but IX markedly wider than VII – ca. 0.29 mm. Segment X reduced. Anus x-shaped. Cuticle microstructure of entire body with many small, sharply pointed cuticular structures (Figure 1). Chaetotaxy – setae of varied length, pale yellow, visible.

Head: Oval, dark brown, ca. 0.62 mm long (0.59–0.65 mm), ca. 0.42 mm wide (0.40–0.44 mm). Frontal suture distinct, Y-shaped, touching antennae. Endocarina distinct, long, unbranched, together with epicranial suture extends 2/3 length of head (Figure 2). On head capsule: 4 pairs of setae of varying length – the shortest *des1* and *3*, the longest *des4* and *5*, and 2 pairs of lateral setae (*les 1,2*). Seta *des2* closer to lateral edge of epicranium, *des3* and *des5* closer to frontal suture – especially *des3*. Also, 3 pairs of setae *pes1-3*. On frons 4 pairs of long hair-like setae (*fs1,2,4,5*), 1 pair of spine-like microsetae (*fs3*). Setae *fs1,2* near frontal suture, *fs4,5* near epistomal suture and two pairs of sensillae (Figure 2).

Clypeus wider than long (ca. 0.11 mm × 0.03 mm), trapezium-shaped. Anterior margin concave, 2 pairs of short, sharp, thorn-shaped microsetae (*cls1,2*) along posterior margin, between them sensilla (Figures 3a, 14). Antennae (*at*) at end of frontal suture, clearly visible, sensorium slightly elongated, finger-shaped, with 4 sensilla at base (Figure 16). Anterior stemmata visible. Tentorial bridge present.

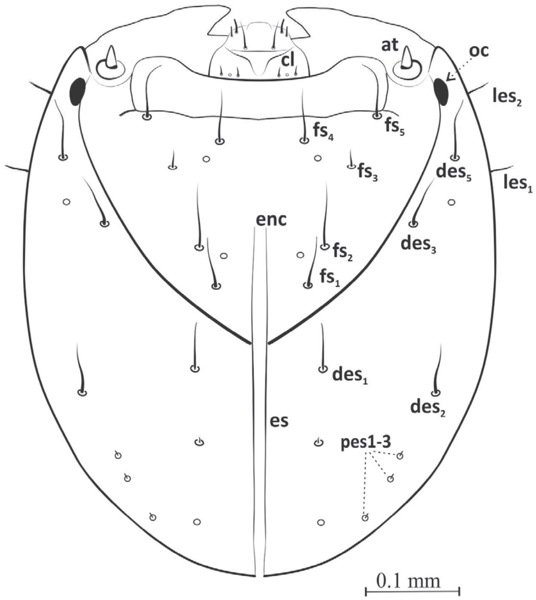


Figure 2. Epicranium (L_3), dorsal view: ***les*** lateral epicranial setae, ***fs*** frontal s., ***des*** dorsal epicranial s., ***pes*** posterior epicranial s., ***cl*** clypeus, ***at*** antenna, ***oc*** ocellus, **enc** endocarina, **es** endocarina suture.

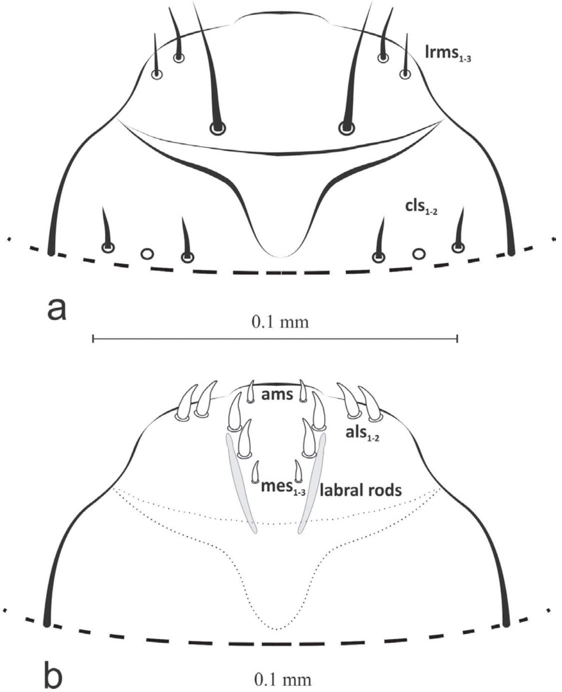


Figure 3. Labrum and clypeus (L_3): dorsal view (**a**) ***lrms*** labral setae, ***cls*** clypeus s., ventral view (**b**) ***mes*** median s., ***ams*** anteromedial s., ***als*** anterolateral s..

Mouthparts: Labrum more then 2× wider than long (ca. 0.06 mm × 0.02 mm), with 3 pairs of setae (*lrms1-3*) of varying length – *lrms3* the shortest and *lrm1* the longest (Figures 3a, 14). Epipharynx with 1 pair of finger-shaped setae (*als1*) and 3 pairs of *mes*, 2 pairs of *ams* and visible long labral rods (Figure 3b). Mandible large, highly sclerotized, dark brown, apically bidentate (Figure 4); teeth of equal size, with apices usually worn down in mature larvae; inner margin with triangular tooth at half-height from base. Mandibular setae *mds1*,*2* of similar length. Maxilla – stipes (*st*) quite wide, with 3 distinct, hair-like setae (Figure 5). Setae *pfs1* and *stps* long, of approx. equal length, *pfs2* half their length and located at base of palpus. Malar part

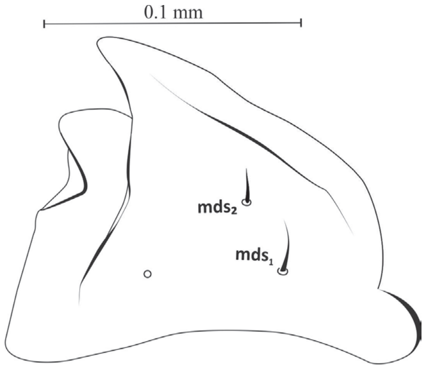


Figure 4. Mandible (L_3), right: ***mds*** – dorsal malae setae.

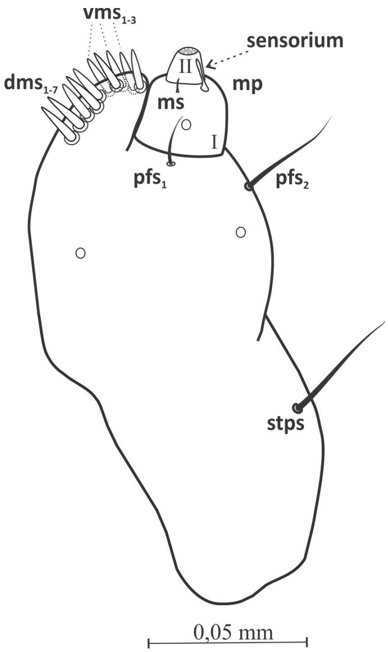


Figure 5. Maxillae (L_3), dorsal (a) and ventral (b) view: ***dms*** dorsal maxillary setae, **vms** ventral maxillary s., ***pfs*** palpiferal s., ***sts*** stipal s., ***mps*** maxillary palpus s., ***mp*** maxillary palpus, ***ms*** microseta.

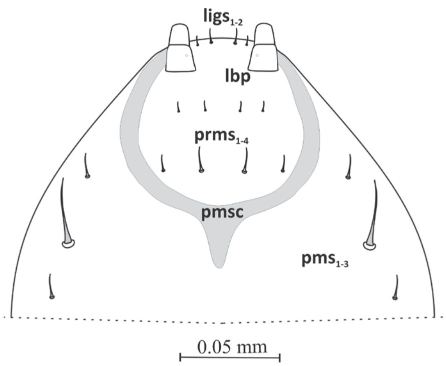


Figure. 6. Labium (L_3): ***prms*** prelabium setae, ***pms*** postlabium s., ***pmsc*** premental sclerite, ***ligs*** ligular s., ***lbp*** labial palpus.

of maxilla with clearly visible, finger-shaped setae of equal length: 7 on dorsal side (*dms1-7*) and 3 on ventral side (*vms1-3*) (Figure 15). Maxillary palpus (*mp*) with 2 segments, distal segment markedly smaller than basal segment, with 10 nodular cuticular tubercles situated apically (Figure 15). On basal segment sensorium and 1 microseta (*ms*) (Figure 5).

Labium – prementum (prms) nearly oval, with 2 pairs thorn-shaped microsetae (*ligs1,2*) near anterior margin and 4 pairs of hair-like micro- and macrosetae arranged in 2 rows in middle and at base (*prlbs1-4*). Prementum base (*prmsc*) rounded, weakly sclerotized. Labial palpus (*lba*) longer than wide with 2 segments of similar length, basal segment weakly separated from distal (Figure 17). Postmentum (*pms*) with 3 pairs of setae along the sides – middle setae (*pslbs2*) at least 3 times longer than others (Figure 6).

Thorax: Prothorax with 7 long setae (*prns*) on dorsal side, 2 long ventropleurolateral setae (*vpls*), 7 setae on pedal tubercle (*pda*), including 2 micro- and 5 macrosetae – *ps1-3*, *lsts1-4*, and 1 ventral seta (*msts*) (Figure 1). Mesothorax with 1 long prodorsal seta (*prs*), 4 long postdorsal setae (*pds1-4*), 1 microseta *dpls*, 7 setae on pedal tubercle (*pda*), including 2 micro- and 5 macrosetae, and 1 ventral seta (*msts*). Distribution and shape of metathoracic setae as on mesothorax, except for much shorter dorsal seta (*prs*). On all thoracic segments pedal tubercles visible, with distinctive cuticle structure. Centre of tubercles with smooth cuticle and 3 setae, edge zone of tubercles with thorn-like structure and remaining setae (*lsts1-4*) (Figure 1). All thoracic macrosetae about 2 times longer than microsetae. Thoracic spiracle bicameral, 6-ringed, located intersegmentally, between ThI and ThII.

Abdomen: Abdominal segments I–VII with 1 prodorsal microseta (*prs*), 4 (2 macro- and 2 micro-) postdorsal setae (*pds*), 1 spicular seta (*ss*), 1 dorsopleurolateral microseta (*dpls*), 2 ventropleurolateral setae (*vpls*) and 1 mesosternal seta (*msts*). Macrosetae clearly extend beyond outline of body and are several times longer than microsetae. Segment VIII with similar chaetotaxy as previous segment. Abdominal segment IX with 2 dorsal microsetae (*pls*) and 2 ventral microsetae (*sts*) of similar length (Figure 1). All spiracles (6) bicameral, 6-ringed, positioned size.

Pupa

The female and male morphology is externally very similar at the pupal stage, with a significant difference only in the length of the rostrum, which is longer in the female and its apex extends to the middle of the second tarsal segment of the prolegs; in the male the rostrum is short and its apex extends to the end of the first segment.

Body: Length ca. 3.33 mm (2.95–3.53 mm), width ca. 1.41 mm (1.15–1.67), colour dark brown (Figures 7–9, 11).

Head: Head capsule with 2 pairs of *sos*. Female rostrum longer (ca. 0.68 mm), broader in middle with 1 pair *brs*, 2 pairs *drs* and 1 pair *es*, and extends to end of segment II of first pair of legs. All setae on rostrum short, spinescent (Figures 7, 8).

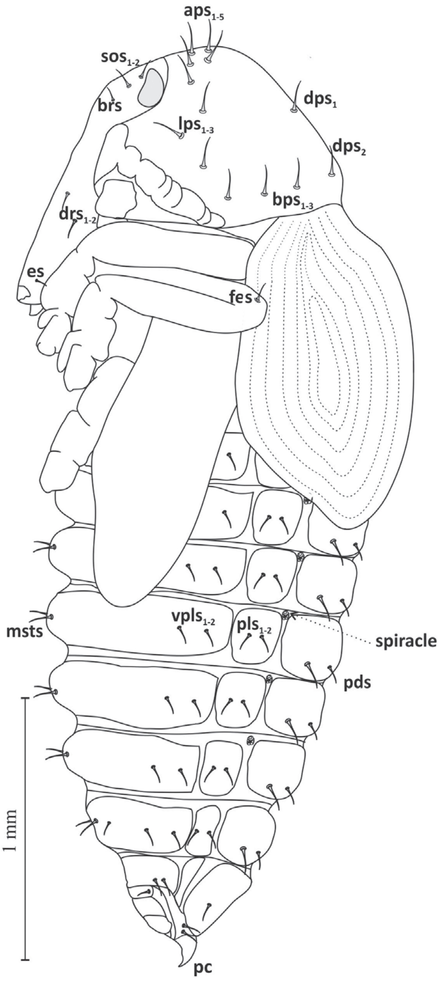


Figure 7. Pupa, lateral view: ***aps*** apical pronotal setae, ***lps*** lateral pronotal s., ***dps*** discal pronotal s., **bps** basal pronotal s., ***sos*** supraorbital s., ***brs*** basirostral s., ***drs*** distriostral s., **es** epistomal s., ***fes*** femoral s., **msts** mediosternal s., **pls** pleural s., **vpls** ventropleural s., **pds** postdorsal s., ***pc*** urogomphi (pseudocerci).

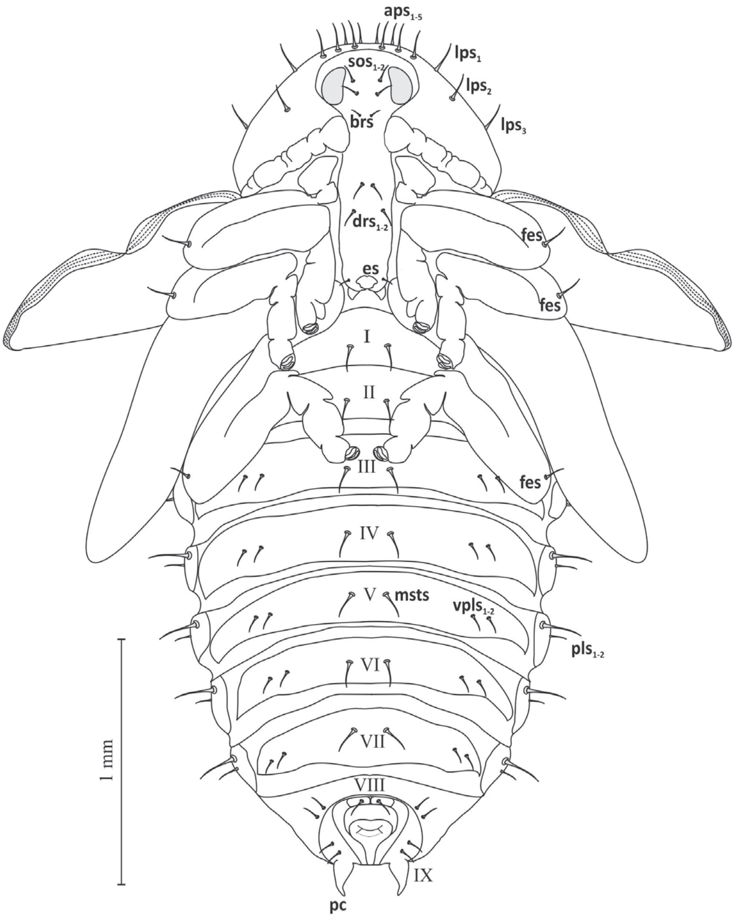


Figure 8. Pupa, ventral view.

Thorax: Pronotum broad (width ca. 1.12 mm, length ca. 0.65 mm) with 5 pairs of apical pronotal setae (*aps1-5*), 3 pairs of lateral pronotal setae (*lps1-3*), 2 pairs of discal pronotal setae *dps* and 3 pairs of basal pronotal setae (*bps1-3*) (Figures 7–9). Mesonotum shorter than metanotum (length ca. 0.26 and ca. 0.36 mm) (Figure 9). On dorsal part of meso- and metanotum 3 pairs each of spinescent setae, *msns* and *mtns*. All setae of pro-, meso- and metanotum of equal length.

Abdomen: Tergites of abdominal segments I–VII of similar width, gradually narrowing slightly towards rear, with 4 pairs of setae *pds* (2 macro- and 2 micro-) arranged alternately, parallel to posterior margin of segment (Figure 9). On tergite VIII 1 pair of short microsetae. Macrosetae of tergites longer than microsetae by more than half. Sternites I–VII with 1 pair of larger *msts* and 2 pairs of smaller *vpls*, on sternite VIII 2 pairs of microsetae (Figures 7, 8). Macro- and microsetae of sternites somewhat longer than macro- and microsetae of tergites, but in similar proportions. Segment IX with short, pointed, slightly curved urogomphi (*pseudocerci*), and 3 pairs of setae (Figure 8). Pleu-

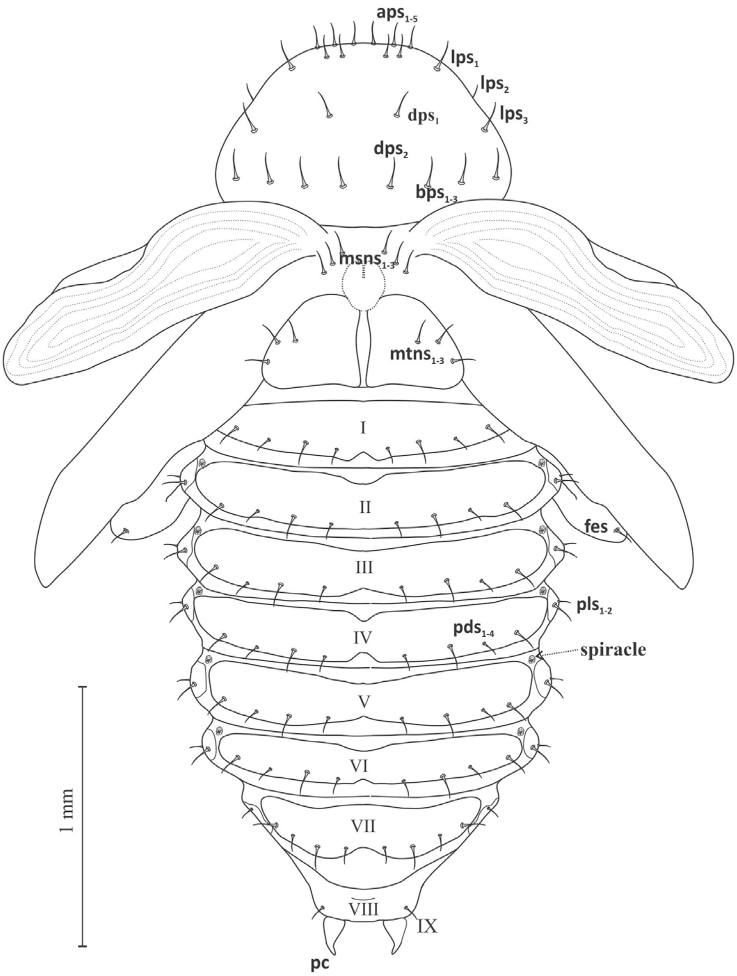
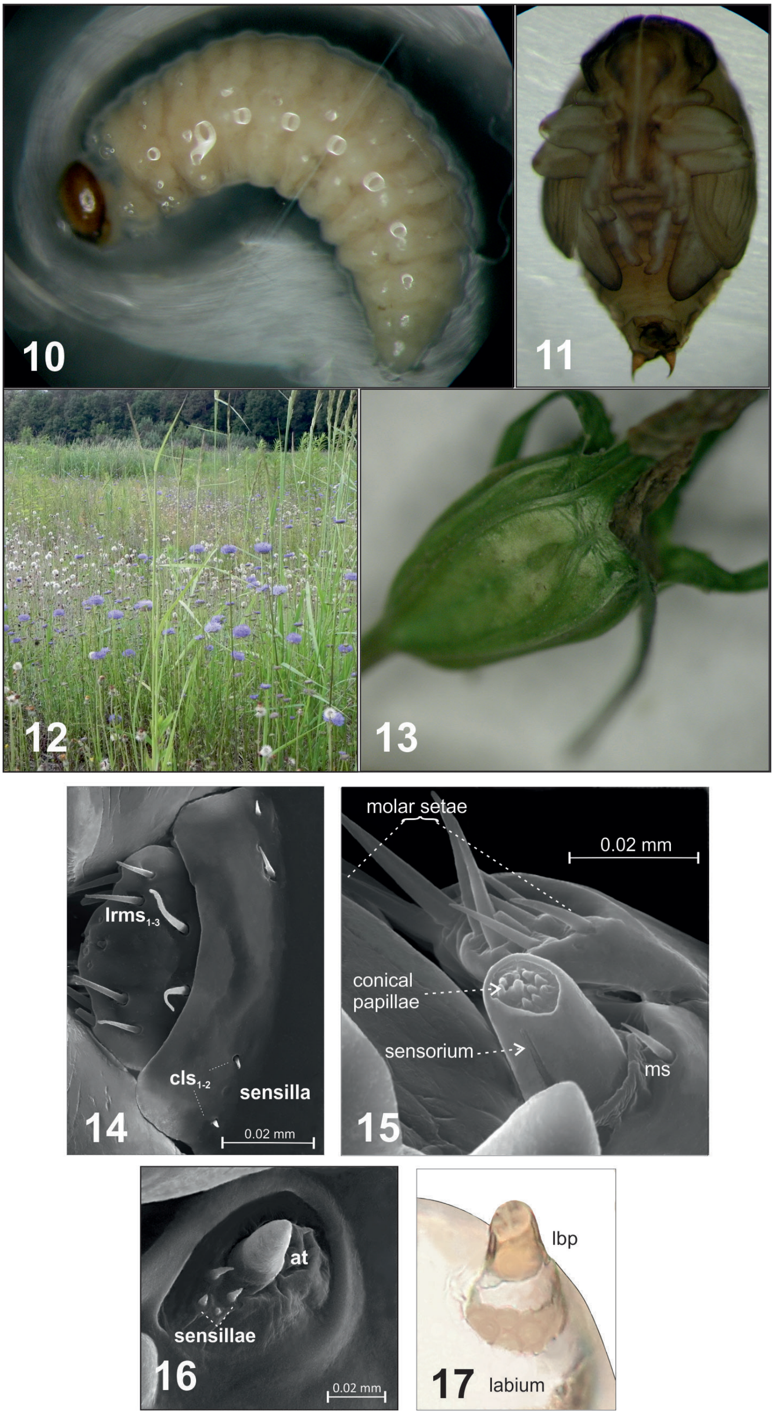


Figure 9. Pupa, dorsal view: **msns** mesonotal setae, **mtns** metanotal s.

rites of segments I–VII with 2 setae *dpls* (Figure 7). Spiracles (*spiracle*) located between tergite and pleurite, in upper part of border, clearly visible on segments I–VI, on others absent (Figures 7, 9).

Biological information

Analysis of eight plant species of the family Campanulaceae revealed that *Cleopomiarus micros* uses *Jasione montana* as a food source for the imago and *Campanula patula* for breeding larvae. The presence of these two plants at the same habitat provides ideal conditions for the occurrence of the species (Figure 12). Only at the study site in Nasutów were these plants present at the same time. Adults of *Cleopomiarus micros* hibernate in the soil and come out in late spring. The first adults were caught in the



Figures 10-17. *Cleopomiarus micros* (Germar, 1821): **10** mature larva **11** pupa **12** the environment of the species **13** place lay eggs on the plant breeding **14** labrum and clypeus (L_3) (dorsal view): ***lrms*** labral setae, ***cls*** clypeus s. **15** apical part of the maxilla: ***ms*** microseta **16** antennae (***at***) **17** labial palpus (***lbp***).

second half of May. A total of 35 specimens were caught, but even this small number allowed for important conclusions to be formulated:

- Adult insects feed on sheep's bit scabious (*Jasione montana*).
- Following copulation at the beginning of June, females choose a different plant species of the same family – spreading bellflower (*Campanula patula*) – to lay their eggs.
- Single eggs are laid on the wall of the ovary (seed chamber). Unlike in other beetle species of this genus, delicate traces of oviposition appear on the plant's epiderm. To lay an egg the female uses a natural hollow on the exterior of the seed chamber, which she gently gnaws and enlarges (Figure 13).
- The L_1 larva bores a tunnel to the inside of the chamber. Subsequent larval instars (L_2 and L_3) eat the contents of the seed chamber. After seven days of growth the L_3 larva transforms into a pale brown pupa, and after another four days into an imago. As observed, the adult leaves the seed through cracks formed during its drying prior to germination.
- One generation per year was noted in the species.
- The insect does not cause cecidia in the breeding plant.

Discussion and conclusions

The results of the study, the first morphological description of the preimaginal stages of the species *Cleopomiarus micros*, serve to verify and supplement previous scant data on the biology of the species. Previous data on the morphology of the preimaginal stages of representatives of the genus *Cleopomiarus* concern only *C. graminis*, *C. hispidulus*, and *Miarus campanulae*, which in the old taxonomic system belonged to the genus *Miarus* (Emden 1938, Scherf 1964, Anderson 1973, Caldara and Legalov 2016).

The morphology of the L_3 larvae and pupa of *C. micros* does not differ from the typical characters of preimaginal stages of weevils of the tribe Mecinini (Emden 1938, Scherf 1964, Anderson 1973, Marvaldi 2003, 2005, Marvaldi and Lanteri 2005, Gosik 2010, Jiang and Zhang 2015). The morphology of mature *C. micros* larva is similar to the description of the characters of the third-stage larvae of the tribe presented by Emden (1938), including pronounced convexity of the body, a long, unbranched endocarina, which together with the epicranial suture extends two-thirds the length of the head, and an intersegmental thoracic spiracle.

Certain anatomical traits of *C. micros* are common to species of Mecinini tribe, but less frequent. As mentioned in the Introduction, the head of species of this tribe usually has 3 pairs of *des*. In *C. micros* there are 4 pairs, as in *Rhinusa bipustulata* (Rossi, 1792) or *Gymnetron miyoshii* Miyoshi, 1922 (Gosik 2010, Jiang and Zhang 2015). In Mecinini, the labial palpus usually has a single segment; if there are two segments the basal segment is not distinctly separated. This is the case with *C. micros*, which has a two-segmented palpus. The presence of a two-segmented labial palpus seems to be a character common to species of the genus *Cleopomiarus*. This is confirmed by species such as *Cleopomiarus distinctus* and *C. graminis* (the authors' unpublished data). A two-segmented labial palpus is also present in species of the genus *Miarus* (*M. campanulae* and *M. ajugae*) (Scherf 1964, the authors' unpublished data). Another character present in *C. micros*, which is common to species of the Mecinini tribe but occurs less frequently, is the presence of two anteromedial setae (*ams*) on the epipharynx. The same number of *ams* is found in *Gymnetron antirrhini*, *G. lineariae* and *G. villosulum* (Emden 1938, Scherf 1964). Analysis of the anatomical characters of *C. micros* in comparison with other species of the Mecinini tribe suggests that it is most similar to species of the genus *Miarus* (Emden 1938, Scherf 1964, Anderson 1973).

Comparison of the morphology of *C. micros* with previously described preimaginal stages (L_3 and pupa) of species of the genera *Cleopomiarus* and *Miarus* (previously the same genus) on the basis of previously described features reveals species differences in larval body length, the colour of the body and epicranium, and the chaetotaxy of the head and body (Emden 1938, Scherf 1964). Among the species compared, the larva of *C. micros* has the shortest body (on average 3.41 mm), and *C. graminis* has the longest (up to 5 mm). In *M. campanulae*, as distinct from *M. ajugae* (Herbst, 1795), the average larval length is 4.75 mm (our own data). The colour of the larvae ranges from white in *M. campanulae* to cream-coloured in *C. micros* to yellowish-white in *C. graminis*. The head in individual species is of various shades of brown: yellowish-brown in *C. graminis*, dark brown in *C. micros* and black-brown in *M. campanulae* (Scherf 1964). The differences in the chaetotaxy of the body and the form of the mouthparts are presented in Table 1. The present research made it possible to discover, supplement and describe the chaetotaxy of many parts of the body of *C. micros*.

Differences between the species analysed were also noted in the size and colour of the pupal body. In *C. micros* the body is 2.95–3.53 mm long and dark brown, whereas the yellow-white pupa of *C. graminis* reaches a length of 3.4–5.0 mm and the body of the *M. campanulae* pupa is 2.5–3.0 mm long and white (Scherf 1964, Anderson 1973). The differences in the chaetotaxy of the body and the shape of the pseudocerci are presented in Table 2.

The development of the Mecinini species is correlated with the phenology of the breeding and host plants and is strongly dependent on the environmental conditions prevailing at a given site. In the case of *C. micros*, a necessary condition for the presence of the beetle is the co-occurrence of plants of the species *Campanula patula*, in which it develops, and *Jasione montana*, which constitutes the food base for adults. This was confirmed by the observations in the field and experiments in the laboratory. The species was not found when only one of the listed plant species was present in the environment. Among the species from genus of *Miarus* and *Cleopomiarus* so far studied, only *C. micros* develops in such a way – development in a one species of plant and feeding on another. This indicates that this species is oligophagous. Until now, it would have been described as a monophag (Scherf 1964). Probably, the development in *Campanula montana* is conditioned by the appropriate size of the ovary and the abundance of seeds.

Understanding of the morphology of the adult larva of the described species allows for the recognition of potential generic features for the labial palp of *Cleopomiarus* spe-

Table 1. Diagnostic features of the mature larvae of *Cleopomiarus micros*, *C. graminis*, *C. hispidulus* and *Miarus campanulae* ('-' indicates lack of descriptive data).

Traits	Character	*Cleopomiarus micros*	*Cleopomiarus graminis* (Emden 1938, Scherf 1964)	*Cleopomiarus hispidulus* (Anderson 1973)	*Miarus campanulae* (Emden 1938, Scherf 1964)
Head	Epicranium	*pes1-3*; *des1-3,5; des4* absent; *les1-2*; *fs1-6*; *oc* present; *cls1-2* and 1 *sa*	–	–	–
	Antennal sensorium	slightly elongated, finger-shaped, with 4 *sa*	short, conical	elongated	finger-shaped
Mouthparts	Mandible	2 apical teeth, incisive margin with tooth, *mds1-2*, 1 *sa*	2 apical teeth, incisive molar edge with tooth	–	2 apical teeth, *mds1-2*
	Labrum	*lrms1-3*	*lrms1-3*	–	*lrms1-3*
	Epipharynx	1 pair *ams*, *als1*, 3 pair *mes*, long epipharyngeal rods present	*ams1-3*, *als1-3*, 2 pair *eps* (*mes*) short epipharyngeal rods present	–	–
	Maxilla	1 *stps*, *pfs1-2*, 2 *sa*, *mxp2* segments, basal with 1 *sa* and accessory process, apical segment with 1 *sa*; *dms1-7* and *vms1-3*	*mxp2* segments, 8 maxilla setae (*dms* and *vms*)	–	*mxp2* segments, maxilla setae present
	Labium	*pms1-3*; premental sclerite „Y" shaped; *prms1-4*; *ligs1-2*; *lbp2* segments – *lbp* longer then wide, basal segment with 1 sa, segments of similar length, basal segment fairly well defined	premental sclerite rounded, *lbp* 2 segments – *lbp* longer then wide, 2nd segments as long as wide, basal segment rather well defined	–	premental sclerite rounded, *lbp2* segments – *lbp* as long as wide, 2nd segments distinctly longer then wide, basal segment not well defined
Thorax	Th1	*prns1-7*, *vpls1-2*, *ps1-3*, *lsts1-4*, 1 *msts*	*pda* with 3 setae (*ps1-3*)	–	a few setae on dorsal plate, *pda* with 3 setae (*ps1-3*), *vpls* with 1 setae, 1 *msts*
	Th2	1 *prs*, *pds1-4*, 1 *as*, *ps1-3*, *lsts1-4*, 1*msts*, 1 *as*	*pda* with 3 setae (*ps1-3*)	–	1 pair *prs*, *pds1-4*, *ps1-3*, *as1-2*
	Th3	same as Th2	*pda* with 3 setae (*ps1-3*)	–	same as Th2
Abdomen	Abd I–VII	1 *prs*, *pds1-4*, 1 *dpls*, *vpls1-2*, 1 *msts*	–	–	–
	Abd VIII	same as AbI–VII	–	–	–
	Abd IX	*ds1-2*, *sts1-2*	–	–	–
	Abd X	anal x-shaped, without setae	anal x-shaped	anal x-shaped	anal x-shaped
Spiracles	Thorax	bicameral	bicameral	bicameral	bicameral
	Abdomen	bicameral	bicameral	bicameral	bicameral

Table 2. Diagnostic features of pupae of *Cleopomiarus micros*, *C. graminis* and *Miarus campanulae* ('-' indicates lack of descriptive data).

Traits		*Cleopomiarus micros*	*C. graminis* (Scherf 1964)	*Miarus campanulae* (Scherf 1964)
Head		*sos1-2*, *brs1*, *drs1-2*, *es1*	–	*os1-2*, *sos1*, *vs1*, *rs1* (*drs*)
Thorax (one side)	Pronotum	*aps1-5*, *lps1-3*, *dps1-2*, *bps1-3*	–	as1-3(*aps*), *ls1-2* (*lps*), *ds1*(*dps*), *pls1* (*bps*)
	Mesonotum	*msns1-3*	–	3 *sas* (*msns*)
	Metanotum	*mtns1-3*	–	3 *sas* (*mtns*)
Abdomen (one side)		*pds1-4*, *dpls1-2*,	–	4 pairs of setae
Legs		1 *fes*	–	1 *fes*
Pseudocerci		pointed, slightly curved	pointed, curved	pointed, massive, arched

cies. They are: the ratio of length to its width – the labial palp is longer than wider, the apex is clearly longer than wider, and the basic segment is pronounced. Morphological descriptions of the next species of the genera *Miarus* and *Cleopomiarus* will probably allow other generic features to be distinguished, and, in turn, for the production of a key to identify mature larvae.

The data presented herein provide new information on the biology and ecology of the species. Previous data covered only its living environment, host plant and the number of generations per year (Scherf 1964, Burakowski et al. 1997).

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Revision of the genus *Lichtwardtia* Enderlein in Southeast Asia, a tale of highly diverse male terminalia (Diptera, Dolichopodidae)

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Abstract

In the present paper the Oriental species of the genus *Lichtwardtia* Enderlein, 1912 are revised based on the type material of known species and new material from Singapore and Cambodia. A re-description and illustration of the holotype female of *Lichtwardtia ziczac* (Wiedemann, 1824) is given but since it has been described on the basis of a female only and its provenance India Orientalis is only a vague indication of its type locality, it is considered as a nomen dubium. All the species put as junior synonyms by Becker (1922) of *L. ziczac* are re-established to their original status with diagnosis: *Lichtwardtia polychroma* (Loew, 1864) and *Lichtwardtia formosana* Enderlein, 1912. However, *L. coxalis* is now also considered as a nomen dubium since the original description is too short to distinguish it from other species and the holotype female is lost. In addition a re-description and illustrations of *L. hirsutiseta* (de Meijere, 1916) are provided. Eight new species for science are described and illustrated: *Lichtwardtia cambodiensis* Tang & Grootaert, **sp. n.** (Cambodia), *Lichtwardtia conspicabilis* Tang & Grootaert, **sp. n.** (Cambodia), *Lichtwardtia infuscata* Tang & Grootaert, **sp. n.** (Cambodia), *Lichtwardtia monstruosa* Tang & Grootaert, **sp. n.** (Cambodia), *Lichtwardtia nodulata* Grootaert & Tang, **sp. n.** (Singapore), *Lichtwardtia semakau* Grootaert & Tang, **sp. n.** (Singapore) and *Lichtwardtia singaporensis* Grootaert & Tang, **sp. n.** (Singapore). *Lichtwardtia zhangae* Tang & Grootaert, **sp. n.** (Bali, Indonesia) is a new name for the species described by Zhang, Masunaga & Yang, 2009, as *Lichtwardtia ziczac* (Wiedemann, 1824). There are only a few good diagnostic non-genitalic characters for the species, but the male terminalia are distinctive, from simple to very complicated and armed structures. A key is given to the species of the Oriental region. Barcodes are provided for the Singaporean species.

Although *Lichtwardtia* is a common genus in Southeast Asia it is generally not abundant locally. It is often found in anthropogenic disturbed habitats only. Four species are recorded from Singapore while eight species are sympatric and very abundant at the locality of Siem Reap in Cambodia.

Keywords

Lichtwardtia, new species, Oriental, revision

Introduction

With its bayonet-like vein M and long soft hairs on the apical segment of the arista-like stylus, the genus *Lichtwardtia* Enderlein is easily recognised as such, but assigning specimens to the correct species is another story. Even the validity of the genus *Lichtwardtia* itself has been uncertain since its establishment (Becker 1922, Brooks 2005, Grichanov and Brooks 2017). Brooks (2005) recognised *Lichtwardtia* as a subgroup of *Dolichopus* with very delicate morphological analyses. However, we have some concerns about the study: Only *Lichtwardtia angularis* (Macquart, 1842) and a species not confirmed to species level was used for building the character matrix. Meanwhile, *Lichtwardtia angularis* was a species assigned by a female holotype, which might have brought some potential risk. Besides, only three *Dolichopus* species were used while there were four *Hercostomus*, four *Paraclius*, five *Gymnopternus* and six *Tachytrechus* included. The sampling size has obvious bias, which might have caused the paraphyletic status of *Dolichopus*. Last but not least, a unique specialisation of male terminalia is firstly reported, with various denticles on the hypandrium and the phallus. This character was not shown in the matrix while it is rather unusual. Though *Lichtwardtia* was suggested to be paraphyletic with *Dolichopus* in Brooks 2005, its monophyly was confirmed as a group based on three homoplasious character states, including the possession of an S-shaped bend in vein M, the T-shaped ejaculatory apodeme, and the feather-like stylus. Notably, these features are quite rare in not only Dolichopodinae, but also Dolichopodidae. Apparently the other species of *Dolichopus*, if we consider *Lichtwardtia* a group of *Dolichopus*, do not possess these features, which could help distinguishing the other genera. Therefore we restore the genus *Lichtwardtia*, as was by done by Selivanova et al. 2010 and Grichanov and Brooks 2017. Beyond morphological data, some molecular studies that could prove the monophyly of *Lichtwardtia* are in preparation for publication.

There are 22 species known in the world: 16 from the Afrotropical Region (Grichanov 1998, 2004), four species are present in the Oriental Region (Yang et al. 2011; Zhang et al. 2009), and two species are known from Australia (Yang et al. 2006; Bickel 2008; Grichanov 2011).

The taxonomy of *Lichtwardtia* suffered from the description of species based on females only. Pairing males to these females became almost impossible as females of *Lichtwardtia* barely differ without careful observation and dissection, which also raises the concern of synonyms. Typically, many species were set to be the synonym of the iconic species *L. ziczac* (Wiedemann) that itself was described on the basis of a female. Its provenance “India orientalis” is vague since in the past this area extended from Pakistan in the West to New Guinea in the East. In addition, the single type of *L. coxalis* Kertész, 1901 is probably destroyed (Foldvary and Papp 2007). Zhang et al. (2009) were the first to illustrate detailed male terminalia of Oriental *Lichtwardtia*. They assumed that a male according to the re-description by Becker (1922) belonged to the iconic *L. ziczac* or *L. zickzack* as Becker (1922) spelled it. The problematic previous studies caused the subsequent prudence in describing new species. Male characters, which are used to define the species here, are very distinctive, especially the terminalia. In addition, COI barcoding and NGS barcoding (Meier et al. 2016) are used to support morpho-species concepts and to associate females with males in difficult cases, at least for the Singaporean species.

Techniques to study ancient DNA might one day elucidate its status and that of related species. Wing interference patterns (WIPs) might be a tool which would help pairing the specimens. It has been proved useful on the taxonomy of *Campsicnemus*, which is another genus of Dolichopodidae. However, this could not fully support the identification independently. Often the pattern arrangements between females and males are still at 'similar' level, not exactly the same. Here we do not consider this a reliable method for identification because there is no standard at this stage. Meanwhile, the WIPs of *Lichtwardtia* between different species seem not as distinguishably different as those of *Campsicnemus*. The level of similarity that could confirm the pairing to the level of species should not only be determined by the study of one single genus but extended to other genera.

In the present paper we revise the known Oriental fauna and add eight species new for science. With illustrations of the male terminalia we hope to provide a framework to recognise well-defined morpho-species. In addition, some (COI) barcodes (600 bp) and NGS barcodes (313 bp) are provided for all the species with fresh specimens available (uploaded to GenBank, with accession number MH536852–MH536856). In contrast to what we originally thought, the genus *Lichtwardtia* is as diverse in the Oriental region as it is in the Afrotropical region (Grichanov and Brooks 2017). A high diversity and complexity of the morphology of the phallus and hypandrium of the genus are noticed for the first time, from very simple structures, over spiny, saw-toothed to heavily armed intromitted (phallus) organs as well as guiding organs (hypandrium) bearing hooks and thorns.

Materials and methods

In addition to the type material of the previously described species, new *Lichtwardtia* specimens were collected during a two-year survey with Malaise traps at the temple site of Preah Khan, a temple at the Angkor site (Siem Reap, Cambodia) and in the garden of the Sam Vesna Centre in Siem Reap. The site of Preah Khan was situated along a path bordering a secondary forest (Figure 1), with elevation ca. 40–45 m. The material from Singapore and Thailand were collected with Malaise traps and by sweep netting as part of a survey of the mangroves and forests of Singapore (Grootaert 2018). The sites from Singapore are all with elevation ca. 0–5 m. All collected specimens were preserved and described in 75% ethanol unless specifically noted.

Figure 1. Malaise trap at the site of the Preah Khan Temple (Angkor, Siem Reap, Cambodia).

Morphological terminology for adult structures mainly follows McAlpine (1981), and the structures of the male genitalia follow Cumming and Wood (2009). Photos were taken with a Canon EOS 600D camera at the Royal Belgian Institute of Natural Sciences and then stacked by Helicon Focus 6.0 or with the Visionary Digital BK Plus Lab System at the Laboratory for Evolutionary Biology (**NUS**).The type material is deposited at the Royal Belgian Institute of Natural Sciences (**RBINS**, Brussels) and at the Lee Kong Chian Natural History Museum (**LKCNHM**) in Singapore.

COI barcoding was done following the techniques described in Lim et al. (2010) and by Meier et al. (2016). The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. A discrete Gamma distribution was used to model evolutionary rate differences among sites (six categories (+G, parameter = 0.2535)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 5.69% sites). The analysis involved nine nucleotide sequences. There were a total of 649 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kuma et al.

2016). Additional barcodes were downloaded from GenBank with the accession number in front of the species name on the phylogeny, are mostly from Sikes et al. (2017).

As for the other old type material seen in this study, we preferred not to dissect the specimens for examination as the features on the body are enough for identification and the specimens might be examined in the near future with less destructive techniques. Abbreviations in the text are as follows:

a	anterior;
acr	acrostichal bristle(s);
ad	anterodorsal bristle(s);
av	anteroventral bristle(s);
C	costal vein;
d	dorsal bristle(s);
dc	dorsocentral bristle(s);
hm	postpronotal bristle(s);
npl	notopleural bristle(s);
p	posterior bristle(s);
pa	postalar bristle(s);
pd	posterodorsal bristle(s);
pm	presutural supraalar bristle(s);
pv	posteroventral bristle(s);
sa	postsutural supraalar bristle(s);
sc	scutellar bristle(s);
sr	presutural intraalar bristle(s);
v	ventral bristle(s).

Abbreviations on the figures are as follows:

ap	apical bristle(s);
bvl	basoventral epandrial lobe;
dsur	dorsal surstylus;
eb	epandrial bristles;
hy	hypandrium;
L	left hand side;
ph	phallus;
pg	postgonite;
R	right hand side;
vsur	ventral surstylus;
sur	surstylus.

Taxonomy

Family DOLICHOPODIDAE Latreille, 1809
Subfamily Dolichopodinae Latreille, 1809

Genus *Lichtwardtia* Enderlein, 1912

Lichtwardtia Enderlein, 1912: 406. Type species: *Lichtwardtia formosana* Enderlein, 1912 (original designation).

Vaalimyia Curran, 1926: 398. Type species: *Vaalimyia violacea* Curran, 1926 [= *Dolichopus angularis* Macquart, 1842] (original designation).

Notes. The generic synonymy list is as given by Yang et al. (2005) except that *Lichtwardtia formosana* Enderlein, 1912 was considered to be a junior synonym of *L. ziczac* (Wiedemann 1824); it is confirmed a valid species in this work.

Diagnosis. Small to medium-sized species (3.5–5.0 mm). Head: overall dark metallic green including vertex, with thick pale pollinosity; face wide, slightly narrow at middle, slightly raised from lateral view, frons and face both with thick pale pollinosity that hiding the ground colour, face parallel sided, but slightly narrowed near mid-length. Hairs and bristles on head black but lower postocular bristles pale. Vertex flat. Ocellar tubercle distinct. With pairs of strong vertical and diverging ocellar bristles present, with weak postvertical bristles which are approx. a half-length of vertical bristles. Antenna wholly or mostly yellow; scape with short dorsal bristles, longer than pedicel; arista-like stylus dorsal, two-segmented, with feather-like long hairs on apical segment. Eyes dichoptic, with hairs between facets. Clypeus long and wide.

Thorax: dark metallic blue-black. Metapleuron with one narrow black stripe. Hairs and bristles on thorax black. Acr biseriate, hair-like. Five strong pairs of dc. With one pa, two sa, one sr, one hm, one pm, two npl. Propleuron with long curved bristle just above base of fore coxa. With hairs anterior to spiracle. Scutellum with two pairs sc, apical pair long and strong, basal pair short and weak. Mid and hind coxae with outer bristle(s). Fore femur without distinct bristle. Mid and hind femora each with preapical bristle. Hind femur thick, 5.0 times longer than wide. Hind tarsomere I with strong dorsal bristles, shorter than hind tarsomere II.

Wing: usually clear, sometimes with faint brown clouding around distal vein M and dm-cu crossvein, occasionally partly smoky. Vein costa sometimes widened at the joint with R_1, with various callus. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. Vein M joining margin just before apex. CuAx around 1.0.

Abdomen: metallic green (in Oriental), nearly 1.5 times longer than wide, with pale pollinosity. Hairs and bristles on abdomen black.

Male terminalia: Epandrium distinctly longer than wides. Epandrial lobe with three long pale bristles Cercus nearly triangular, margin rounded, usually pale with broad black outer margin, with weak digitations around outer margin, with black simple or specialised marginal bristles on digitations. Hypandrium and phallus various, often with various denticles.

Lichtwardtia nodulata group

Diagnosis. The cercus is more or less triangular, bordered by some strong marginal bristles that are strongly flattened or truncate. All cercus have one or two large inside bristles near the dorsal border. The postgonite is broad and the tip is bifid. The tip of the phallus is ventrally denticulate and the hypandrium is a simple tube.

Lichtwardtia cambodiensis Tang & Grootaert, sp. n.

http://zoobank.org/B81C8619-7CE6-4E98-B533-FE53867568EC
Figs 2–3

Material. Holotype male (coll. RBINS): CAMBODIA: Siem Reap prov., Angkhor, Preah Khan Temple, 24 January–21 February 2006, Malaise trap in secondary forest (leg. Oul Yothin). **Paratypes** (all coll. RBINS): CAMBODIA: Same provenance as

Figure 2. *Lichtwardtia cambodiensis* Tang & Grootaert, sp. n. habitus male.

holotype, 13 males, 2 females, 24 January–21 February 2006; 3 males, 4–11 April 2006; 6 males, 8 February–7 March 2006; 8 males, 28 November–7 December 2005; 9 males, 8 March–5 April 2005; 46 males, 17–24 February 2005; 1 male, 1 female, Siem Reap prov., Bakheng, 23–31 October 2005, Malaise trap in secondary forest (leg. Oul Yothin).

Diagnosis. Wing entirely hyaline; with a slight swelling of the costa where R_1 merges with costa. Postpedicel mainly dark yellow but blackish on apically half, nearly as long as wide. Mid coxa with a dark brown stripe anteriorly, a paler brown band posteriorly. Hind coxa entirely yellow. Cercus rounded, marginal bristles black, not flattened nor on tubercles. Hypandrium simple and smooth, with no denticle; phallus with double rows of spinules on ventral half.

Description. Male. *Body* length 3.4–3.7 mm, wing 3.4–3.5 × 1.0 mm.

Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black but lower postocular bristles pale. Antenna dark yellow; postpedicel blackish on apical half, blunt apically, nearly as long as wide arista-like stylus dark brown, nearly as long as width of head, feather-like, with long pubescence, basal portion 0.2 times as long as apical portion. Proboscis dark yellow, with black hairs; palpus dark yellow, with1 short black apical bristle.

Thorax dark green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of long acr. Scutellum with two pairs sc, apical pair long and strong, basal pair short and weak. Legs mainly yellow; but mid coxa with two large brown square spots laterally: a dark brown stripe anteriorly, a paler brown band posteriorly; mid and hind tarsomeres I–II blackish on tip, mid and hind tarsomere III blackish at apical half, mid and hind tarsomeres IV–V wholly black. Fore and mid coxae anteriorly with rows of bristle-like hairs, fore coxa anteriorly with three long strong marginal bristles, mid coxa with two outer bristles and two marginal bristles, hind coxa with two outer bristles, basal one strong, apical one smaller. Mid trochanter with three short ap, all dorsal. Hind trochanter with two weak ap, one dorsal, one posteroventer. Fore femur with one short weak av. Mid femur with one strong pd and one preapical pv in normal strength. Hind femur with one strong pd at apical quarter. Fore tibia with four ad (of which one strong and preapical), one av and three ap. Mid tibia with two ad, three pd, one pv, and four ap. Hind tibia with four ad, four pd, two pv and three ap. Fore tarsomere I with one short av at base. Hind tarsomere I with one strong ad and two short av. Relative lengths of tibia and five tarsomeres of legs LI : 7.0 : 5.0 : 2.5 : 2.0 : 1.2 : 1.0; LII : 15.0 : 7.5 : 3.8 : 3.0 : 2.0 : 1.5; LIII : 16.2 : 7.0 : 7.5 : 5.0 : 2.2 : 1.5. Wing nearly hyaline, veins brown. Costa slightly widened at the joint with R_1. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. CuAx ratio 1.0. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

Male terminalia (Figure 3): Epandrium 1.6 times longer than wide; epandrial lobe with three long pale bristles. Ventral surstylus and dorsal surstylus both with five short nail-like ap (Figure 3A). Cercus nearly triangular, margin rounded, pale with broad black outer margin, with weak digitations around outer margin, with black simple marginal bristles on digitations, with two relatively strong bristles on lateral digitations at apical half. Hypandrium simple and smooth, with no denticle. Phallus with double rows of spinules on ventral half.

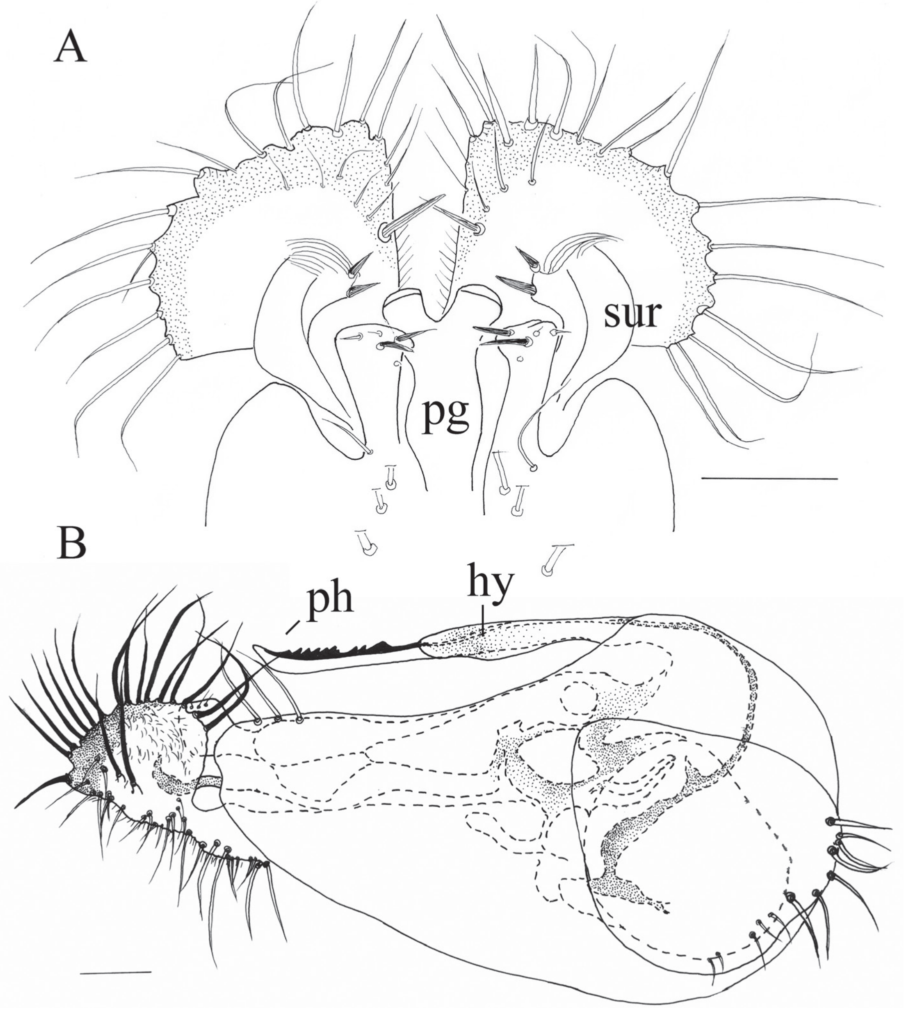

Figure 3. *Lichtwardtia cambodiensis* Tang & Grootaert, sp. n. male terminalia: **A** Cerci, ventral view on inside **B** lateral view male terminalia. Scale bars 0.1 mm.

Female. Similar to male in size and morphology except for the male terminalia.

Etymology. This new species is named after the country (Cambodia) where it was found.

Comments. *Lichtwardtia cambodiensis* sp. n. belongs to the *L. nodulata* group and thus is related to *L. dentalis* and *L. semakau* sp. n. The latter two species both have distinctly darkened and flattened marginal bristles on the cercus while *L. cambodiensis* sp. n. only has thin, and weak marginal bristles on the cercus. Further, *L. cambodiensis* has a weak swelling on the joint point of wing vein R_1 and costa, whereas the swelling is distinct in *L. dentalis*, and absent in *L. semakau*.

Distribution. Cambodia.

Lichtwardtia dentalis Zhang, Masunaga & Yang, 2009

Figs 4–5

Lichtwardtia dentalis Zhang, Masunaga & Yang, 2009: 198, figs 1–4.

Material examined. CAMBODIA (all coll. RBINS): 4 males, Siem Reap prov., Angkhor, Preah Khan Temple, 24 January–21 February 2006, Malaise trap in secondary forest (leg. Oul Yothin); 1 male, Siem Reap prov., Angkhor, Preah Khan Temple, 12 May 2006, sweep netting (leg. Oul Yothin).

THAILAND: 1 male (coll. RBINS); Loei prov., Na Haeo Field Research Station (17°29'27.1"N, 101°03'34.6"E), 769 m, 16 May 2003, sweep netting along stream and water fall in secondary forest (leg. P Grootaert).

Diagnosis. Costa swollen before and at the level where R_1 joins the costa (Figure 4). Wing clear. Hind coxa yellow. Tip of phallus ventrally with a number of denticles. Hypandrium unarmed. Cercus (Figure 5) with weakly digitated margin. Marginal bristles on cercus brown. Apical marginal bristle longest, blunt-tipped inserted on a long tubercle; subsequent two marginals flattened and blunt-tipped, subsequent bristles with simple tip; 6th bristle fine, pale; subsequent bristles long, brown. A long blunt-tipped interior, longer than the apical marginal.

Description. We refer to the detailed description of *L. dentalis* in Zhang et al. 2009.

Comments. The specimens from northern Thailand and central Cambodia correspond entirely to the description and figures given by Zhang et al. (2009). Especially the widening of the costa where the R_1 joins the costa as is drawn by Zhang et al. (2009) but not mentioned in the description. The new records are not unlikely since the locality in the Loei province (Thailand) and the one in Cambodia are not so far from the southern Yunnan province (China) that is the type locality of this species.

L. dentalis is very closely related to *L. semakau* sp. n. from Singapore and we refer to the comments under the latter species.

Distribution. China, Cambodia, Thailand.

Lichtwardtia nodulata Grootaert & Tang, sp. n.

http://zoobank.org/4E1274BE-F088-4E95-B57A-DC815816C2C3

Figs 6–7

Material examined. Holotype male (coll. LKCNHM): SINGAPORE: Semakau, 30 August 2012, sweep netting (leg. Jayanthi Puniamoorthy & P Grootaert). **Paratypes** (all coll. LKCNHM): SINGAPORE: 1 male, Pulau Ubin, 14 July 2012, sweep netting in mangrove (leg. Jayanthi Puniamoorthy & P Grootaert); 9 males, 14 females, Kranji nature trail, 27 July 2005, sweep netting in park (leg. P Grootaert); 1 male, Semakau, 13 December 2012, sweep netting in halophilous vegetation on sandy beach (leg. P Grootaert).

Figure 4. *Lichtwardtia dentalis* Zhang, Masunaga & Yang, 2009 male habitus (Cambodia).

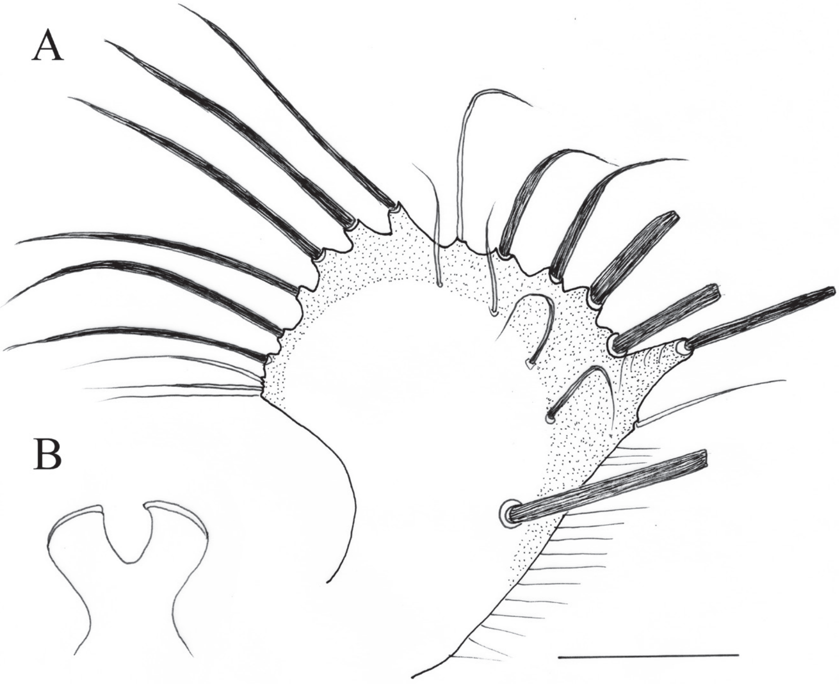

Figure 5. *Lichtwardtia dentalis* Zhang, Masunaga & Yang, 2009 (Cambodia). **A** male cercus **B** tip postgonite. Scale bar: 0.1 mm.

Figure 6. *Lichtwardtia nodulata* Grootaert & Tang, sp. n. Holotype male habitus.

Diagnosis. Basal 7/8 of mid coxa and basal half of hind coxa brown, mid coxa with one black band at middle. Vein R_1 with one oval thickness at where connect to C. Phallus with two rows of black denticles on ventral half, anterior row only with four small sparse denticles, posterior row with eight dense denticles.

Description. Male. *Body* length 3.6–3.8 mm, wing 3.2–3.3 × 1.0 mm.

Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black except posteroventral hairs pale. Antenna dark yellow; postpedicel nearly triangular, blunt apically, nearly as long as wide, with short black pubescence; arista-like stylus dorsal, inserted at basal half of postpedicel, nearly as long as head width, black, feather-like, with long black pubescence, basal segment 0.3 times as long as apical portion of arista-like stylus. Proboscis brown, with black hairs; palpus dark brown, with one short black apical bristle.

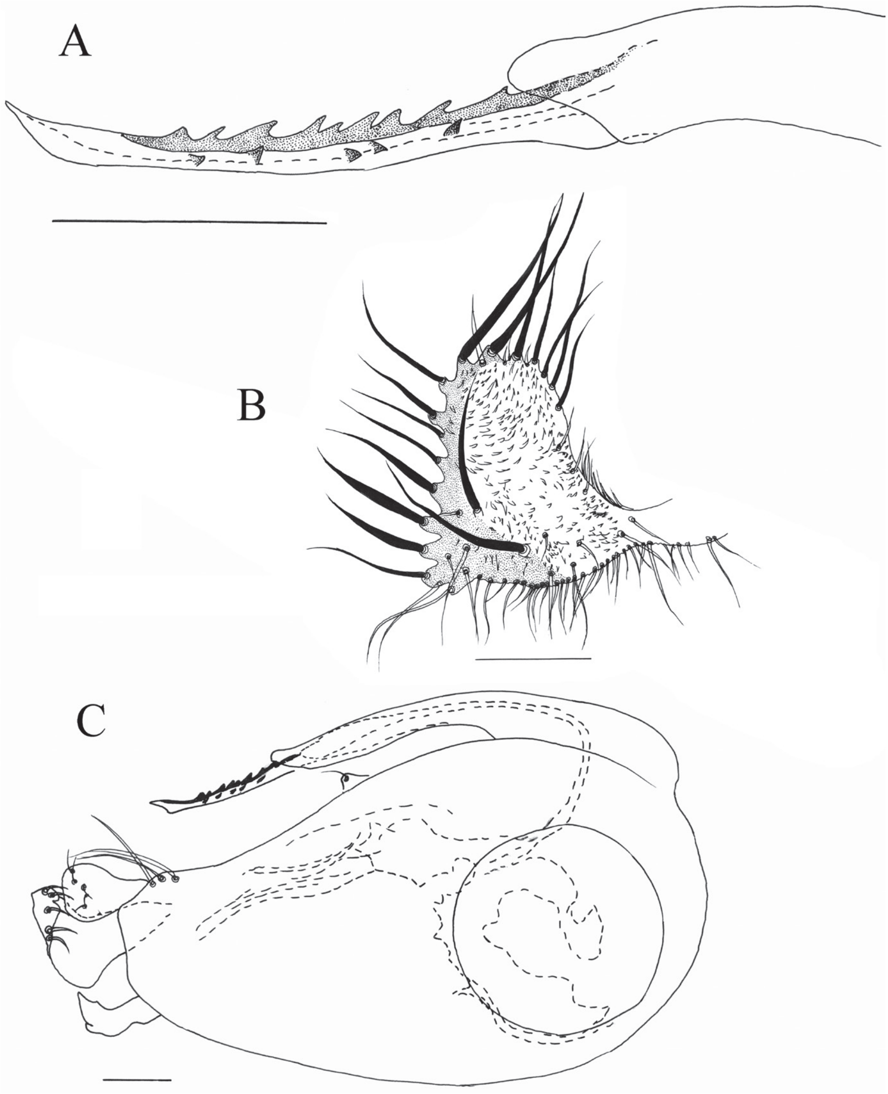

Figure 7. *Lichtwardtia nodulata* Grootaert & Tang, sp. n. Male terminalia: **A** tip of phallus **B** epandrium lateral **C** cercus inside view. Scale bars 0.1 mm.

Thorax dark metallic green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of acr. Scutellum with two pairs sc, apical pair long strong, basal pair short and weak. Legs mainly dark yellow, but base of fore coxa, basal 7/8 of mid coxa and basal half of hind coxa brown, mid coxa with one black band at middle. Fore coxa anteriorly with three strong bristle at apical half, mid coxa with cluster of anterior hairs three marginal bristles anteriorly and one long strong outer bristle, hind coxa with one long strong outer bristle. Mid trochanter with two weak apical bristle dorsally. Fore femur without distinct bristle. Mid and hind femora each with one preapical pv. Fore tibia with one row of two ad, two pd, one pv and three ap. Mid tibia with three ad, four pd, two pv and four ap. Hind tibia with four ad and three ap. Mid tarsomere I with one short strong pd. Hind tarsomere I with one short strong ad at middle and one av at basal quarter. Relative lengths of tibia and five tarsomeres of legs LI : 6.6 : 3.3 : 1.6 : 1.3 : 1.0 : 1.0; LII : 10.6 : 5.0 : 2.0 : ? : ? : ? (mid tarsus partly lost); LIII : 10.0 : 4.0 : 5.0 : 3.3 : 2.0 : 1.3. Wing nearly hyaline, tinged brown; veins brown. R_1 with one oval thickness at where connect to C. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu somewhat arched. CuAx ratio 1.4. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

Male genitalia: Epandrium 1.7 times longer than wide; epandrial lobe with three pale ap. Ventral surstylus and dorsal surstylus both with five short nail-like ap. Cercus nearly triangular, pale except the black ring, with weak digitations around outer margin, with long strong black marginal bristle on digitations, with one long strong bristles on digitation at apical half. Hypandrium simple. Phallus with two rows of black denticles on ventral half, anterior row only with four small sparse denticles, posterior row with eight dense denticles.

Female. Similar to male in size and morphology except for the male terminalia.

Etymology. The name refers to the swelling of the costa.

Comments. The new species should be compared to *L. hirsutiseta* since there is a node on the costa that is however distinctly before R_1 joins the costa (Figure 22) in the latter.

Distribution. Singapore.

Lichtwardtia semakau Grootaert & Tang, sp. n.

http://zoobank.org/36C23F6C-8675-45A1-8860-8271B32C4088

Figs 8–9

Material examined. Holotype male (coll. LKCNHM): SINGAPORE: Semakau, back mangrove in the old mangrove (1°12'19.9"N, 103°45'34.1"E), 3 April 2012, sweep netting along path (leg. P Grootaert). **Paratype** (coll. LKCNHM): 1 female, same provenance as holotype.

Diagnosis. Antenna entirely dark yellow. Arista-like stylus feathered. Wing clear, faintly brownish tinged. No thickening of the costa where R_1 joins the costa. Fore and hind coxae entirely yellow. Mid coxa with a rectangular brown spot anteriorly, posteriorly pale brownish. Cercus yellow, brownish seamed with margin distinctly digitated and bearing five thickened marginal bristles near the tip. Hypandrium simple, lacking protuberances. Phallus ventrally with at least twelve black denticles.

Description. Male. *Body* length 3.9 mm, wing 3.5 × 1.2 mm. Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black but lower postocular hairs pale. Antenna dark yellow (Figure 21); postpedicel nearly trian-

Figure 8. *Lichtwardtia semakau* Grootaert & Tang, sp. n. (Singapore).

gular, blunt apically, 1.2 times as long as wide; arista-like stylus black, nearly as long as width of head, feather-like, with long pubescence, basal portion 0.3 times as long as apical portion. Proboscis dark yellow, with black hairs; palpus dark yellow, with a short black apical bristle.

Thorax dark green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of long acr. Scutellum with two pairs sc, apical pair long strong, basal pair short and weak. Legs mainly yellow, but mid coxa with a dark brown rectangular spot anteriorly and a pale brown posteriorly. Fore and mid coxae anteriorly with rows of bristle-like hairs, fore and mid coxae anteriorly with three long strong preapical bristles and rows of anterior bristles, mid coxa with one outer

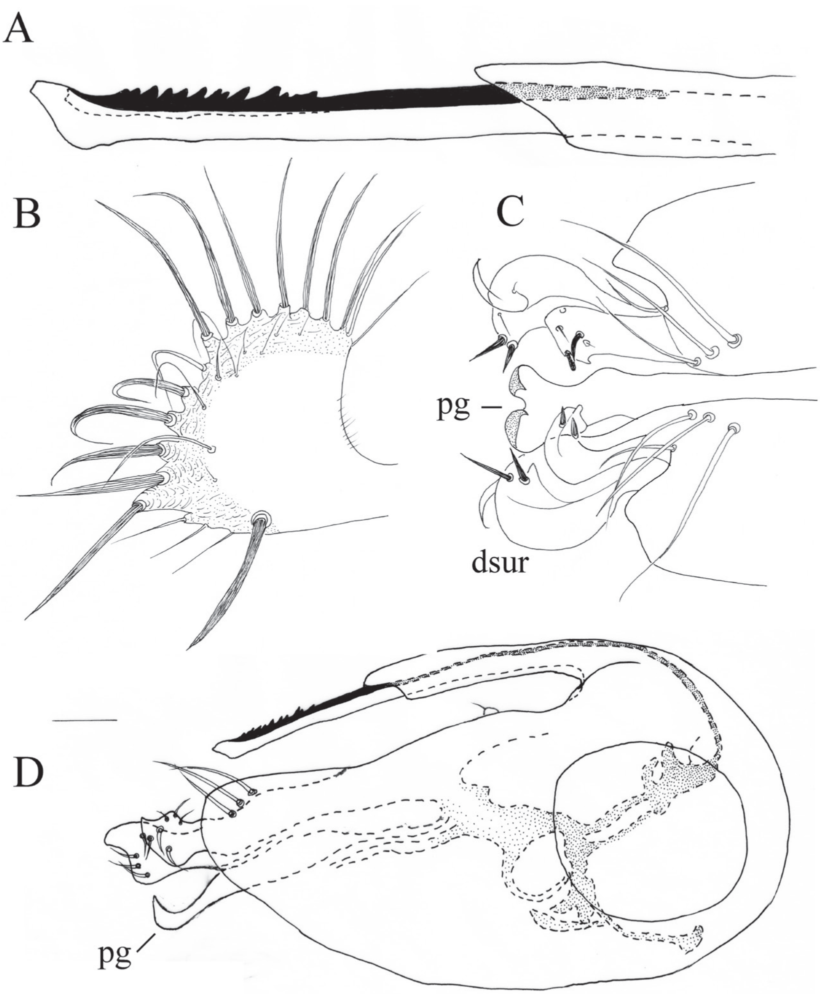

Figure 9. *Lichtwardtia semakau* Grootaert & Tang, sp. n. male terminalia (Singapore): **A** tip phallus **B** cercus **C** surstyli ventral view **D** epandrium lateral. Scale bar: 0.1 mm.

bristles at apical third, hind coxa with two outer bristles, basal one strong, apical one relatively weak. Mid trochanter with two ap dorsally. Hind trochanter with one outer bristle at middle. Fore femur with one strong pv at middle. Mid femur with one strong pd and one preapical pv. Hind femur with one strong pd at apical quarter. Fore tibia with one ad, three pd, one av and three ap. Mid tibia with two ad, three pd, one pv and four ap. Hind tibia with three ad, three pd (one preapical), two weak pv and three ap. Fore tarsomere I with one short av at base. Hind tarsomere I with one strong ad at middle and two short apical bristle. Relative lengths of tibia and five tarsomeres of legs LI : 7.0 : 3.2 : 1.2 : 1.2 : 1.0 : 1.0; LII : 6.0 : 4.0 : ? : ? : ? : ? (mid tarsus partly lost); LIII : 10.4 : 4.0 : ? : ? : ? : ?. Wing nearly hyaline, nearly pale; veins brown. No thickening of the costa where R_1 joins the costa. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. CuAx ratio 1.2. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

Male terminalia (Figure 9): Epandrium 1.8 times longer than wide; ventral epandrial margin at the level of the reduced epandrial lobe with three long pale bristles (Figure 9D), and with a small basoventral bristle. Ventral surstylus and dorsal surstylus both with five short nail-like ap. Cercus nearly triangular, pale except the black margin, with distinct digitations around outer margin, bearing strong black marginal bristles. On the inside a single strong bristle near the dorsal margin, with two relatively strong blade-like bristles on digitations at apical half. Hypandrium simple. Phallus with black small dense irregular denticles on ventral half (Figure 9A).

Female. Body length 4.3 mm, wing 3.4 × 1.3 mm. Resembling the male except for the terminalia and face wider.

Etymology. The name refers to the type locality Semakau, an island on the southern coast of Singapore.

Comments. *L. semakau* sp. n. resembles very much *L. dentalis* Zhang et al. 2009 described from the Yunnan province (China). It differs in that *L. dentalis* has a broadening of the costa at the level where the R_1 joins the costa (Zhang et al. 2009, Figure 1). *L. semakau* sp. n. has no swelling at all of the costa. The outer margin of the cercus seems more weakly digitated with only a strong marginal bristle dorsally and one on the tip. In *L. semakau* sp. n., the margin of the cercus is more deeply indented and there are five broad black marginal bristles at the tip of the cercus.

L. semakau sp. n. should be compared also to *L. cambodiensis* sp. n. that differs in the male also by a thickening of a costa and the marginal bristles on the cercus that are all thin and paler.

Distribution. Singapore.

Lichtwardtia singaporensis Grootaert & Tang, sp. n.

http://zoobank.org/7D25B148-FAC6-4EB7-B1B0-5162751376EF
Figs 10–11

Material examined. Holotype male (coll. LKCNHM): SINGAPORE: West Coast Park, 7 December 2003, shrubs along sandy beach (leg. P Grootaert), sweeping. **Paratype** (coll. LKCNHM): 1 female; collecting information same to the holotype

Diagnosis. Wings clear, but having the cross veins brownish seamed. Mid coxa brown with one anterior dark brown stripe and one lateral band, both dark brown. Hind coxa yellow. Hypandrium simple. Phallus with one row of five big clear regular black denticles ventrally, with one blunt denticle hidden in hypandrium.

Description. Male. *Body* length 3.7 mm, wing 3.4 × 1.1 mm.

Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black except posteroventral hairs pale. Antenna dark yellow; postpedicel

Figure 10. *Lichtwardtia singaporensis* Grootaert & Tang, sp. n.: Habitus male.

nearly triangular, blunt apically, 1.2 times as long as wide, with pale pubescence; arista-like stylus dorsal, inserted at basal half of postpedicel, nearly as long as head width, dark yellow, feather-like, with long black pubescence, basal segment 0.3 times as long as apical portion of arista-like stylus. Proboscis dark yellow, with black hairs; palpus dark yellow, with one black apical bristle.

Thorax dark green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of strong acr. Scutellum with two pairs sc, apical pair long strong, basal pair short and weak. Legs mainly yellow, but fore coxa somewhat brownish at base, mid coxa brown with one anterior dark brown stripe and one lateral band, both dark brown, hind coxae dark yellow, lightly brownish at basal half; mid and hind tarsi brown from tip of tarsomere II to tarsomere V. Fore and mid coxae anteriorly with row of thin bristles and four strong bristles at apical half, hind coxa with two short outer bristles. Fore trochanter with three weak outer bristle at middle. Mid trochanter with two short weak outer bristles. Fore femur with one short weak av. Mid femur with one strong pd at apical 1/6 and one preapical pv. Hind femur with one strong pd at apical quarter. Fore tibia with two pd, one av, and one pv and three short ap. Mid tibia with two ad, four pd (basal 1st relatively weak), one pv at middle and four ap. Hind

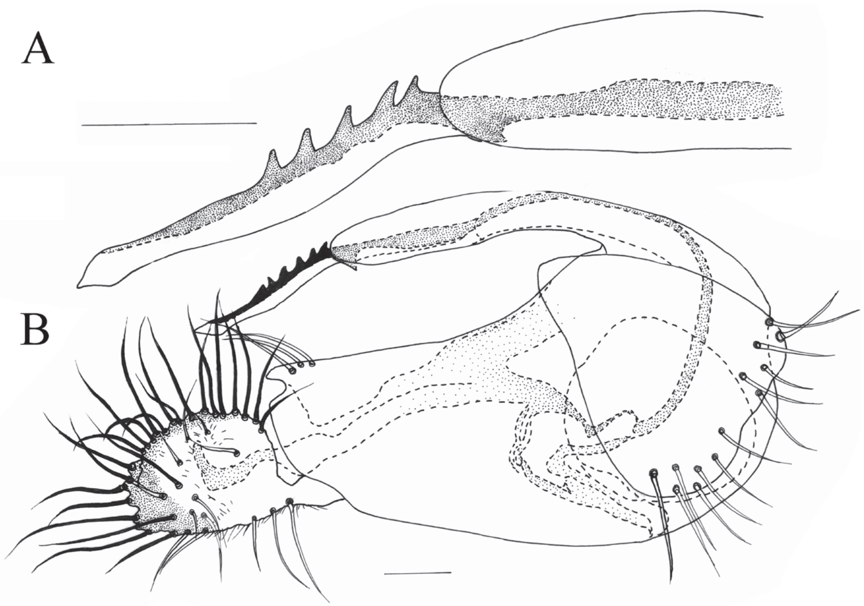

Figure 11. *Lichtwardtia singaporensis* Grootaert & Tang, sp. n. male terminalia (Singapore):). Male terminalia: **A** Tip phallus **B** epandrium lateral. Scale bars: 0.1 mm.

tibia with four ad, four pd, two pv and three ap. Fore tarsomere I with one short av at base. Hind tarsomere I with one short strong ad at middle, one pv at basal fifth and two short apical bristle. Relative lengths of tibia and five tarsomeres of legs LI : 6.0 : 3.0 : 1.6 : 1.2 : 1.0 : 1.0; LII : 8.0 : 5.0 : 2.0 : 1.6 : 1.2 : 1.0; LIII : 10.0 : 4.0 : 4.0 : 3.0 : 2.0 : 1.4. Wing nearly hyaline, cross veins seamed brownish; veins brown. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. CuAx ratio 1.0. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

Male terminalia: Epandrium 1.8 times longer than wide; epandrial lobe with three pale ap. Surstylus with five short nail-like ap. Cercus nearly triangular, pale, covered by thin pale bristles, with weak digitations around outer margin, with long strong pale marginal bristle on digitations. Hypandrium simple. Phallus with one row of five big clear regular black denticles ventrally, with one blunt denticle hidden in hypandrium.

Female. Body length 3.6–4.0 mm, wing length 3.1–3.2 × 1.1 mm. Very similar to male, but postpedicel as long as wide. Wing hyaline with anterior border faintly brownish and cross veins brownish seamed. No swelling of the costa before or at the point where R_1 joins the costa. The ratio of the proximal section of M_1, and the distal section is 0.4/0.6 (Figure 12). Thus the distal section is much longer than the proximal section. Fore coxa yellow, mid coxa brown and hind coxa yellowish.

Comments. This species is comparable to *L. infuscata* sp. n. but the latter has quite brownish wings also with the cross veins seamed brown. Both species have also the hind coxa entirely yellow and the phallus bears ventrally rather strong spines. In

L. singaporensis sp. n. there are five strong spines while in *L. infuscata* there is a double row of ten denticles that are smaller than in *L. singaporensis* sp. n. The epandrium has a pointed tip while its tip in *L. infuscata* sp. n. is truncated. The size of the cercus is larger in *L. infuscata* sp. n. *L. infuscata* has a pale ventral protuberance with a black seam on the epandrium on which the phallus-hypandrial complex is resting. This protuberance is absent in *L. singaporensis* sp. n. The brownish seams along the cross veins suggest that *L. singaporensis* sp. n. represents the enigmatic *L. ziczac*. However we refrain from given the description of the male of *L. singaporensis* sp. n. as the true *L. ziczac*. See comments under *L. ziczac*.

Distribution. Singapore.

Lichtwardtia conspicabilis group

Diagnosis. The hypandrium has a bifurcated tip and a curved appendage. The phallus has a preapical ventral process.

Lichtwardtia conspicabilis Tang & Grootaert, sp. n.

http://zoobank.org/EB34AB9B-242C-4698-80AA-DD536C6B70D1
Figs 12–13

Material examined. Holotype male (coll. RBINS): CAMBODIA: Siem Reap prov., Angkhor, Preah Khan Temple, 6 June 2006; sweep netting (leg. Oul Yothin). **Paratypes** (coll. RBINS): CAMBODIA: 2 males, locality same to the holotype, 4–14 April 2006, Malaise trap (leg. Oul Yothin).

Diagnosis. Antenna nearly entirely dark yellow. Wing brownish tinged, a distinct swelling of the costa present just before R_1 joins the costa. Mid coxa with two light brown bands laterally. Hind coxa entirely yellow. Cercus with elongate tip bearing a longer bristle than the other marginals; hypandrium with bifurcate tip and a black curved appendage on the right-hand side. Phallus wide, with preapical ventral process.

Description. Male. *Body* length 3.7 mm, wing 3.5 × 1.1 mm.

Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black; upper four postocular bristles black but all lower postoculars pale. Antenna dark yellow except portion at level of arista-like stylus slightly brownish. Postpedicel nearly triangular, blunt apically, with brown pubescence, nearly as long as wide; arista-like stylus dark brown, nearly as long as width of head, feather-like, with long pubescence, basal portion 0.2 times as long as apical portion. Proboscis dark yellow, with black hairs; palpus dark yellow, with one short weak black apical bristle.

Thorax dark green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of long acr. Scutellum with two pairs sc, apical pair long and strong, basal pair very short and weak. Legs mainly yellow, but mid coxa with two light brown bands laterally, hind coxa entirely yellow. Fore and mid coxae anteriorly

Figure 12. *Lichtwardtia conspicabilis* Tang & Grootaert, sp. n. male habitus. *Lichtwardtia infuscata* Tang & Grootaert, sp. n. Male terminalia: **A** Phallus **B** epandrium lateral view. Scale bar: 0.1 mm.

to laterally with rows of bristle-like hairs and six marginal bristles; mid coxa with one outer bristles at apical third, hind coxa with two outer bristles, basal one strong, apical one weak. Mid trochanter with two ap. Hind trochanter with one outer hair at middle. Fore femur without distinct bristles. Mid femur with one strong ad and one preapical pv. Hind femur with one strong anterior on apical fourth. Fore tibia with three ad, one av and three ap (of which one posterodorsal very long). Mid tibia with two ad, five pd, two pv, and four ap. Hind tibia with three ad, two pd, one pv and four ap. Fore tarsomere I with one short av at base. Hind tarsomere I with one strong dorsal beyond middle, two short av close to base and two short apical bristle. Relative lengths of tibia and five tarsomeres of legs LI : 10.0 : 4.0 : 2.5 : 2.0 : 1.5 : 1.5; LII : 16.0 : 7.5 : 4.0 : 3.0 : 1.5 : 1.0; LIII : 18.0 : 7.5 : 7.5 : 6.0 : 4.0 : 2.5. Wing brownish tinged, veins brown. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. CuAx ratio 1.3. Lower calypter pale with long black hairs. Haltere white.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

Male terminalia (Figure 13): Epandrium 1.6 times longer than wide. Surstylus with several digitations. Cercus ovoid with short apical process, pale except the black seam around the apical margin, with weak digitations around outer margin only, ventral marginal bristles black; with elongate tip bearing a longer bristle than the other marginal, a long black inner bristle below the tip. Hypandrium thick, with bifurcate tip and a black curved appendage on the right-hand side. Phallus wide, with preapical ventral process.

Female. Unknown.

Comments. This species resembles superficially *L. polychroma* in having a swelling of the costa and by the similar elongate cercus and the complicated structure of the tip of the hypandrium. However, *L. polychroma* possesses a large brown tooth at the right

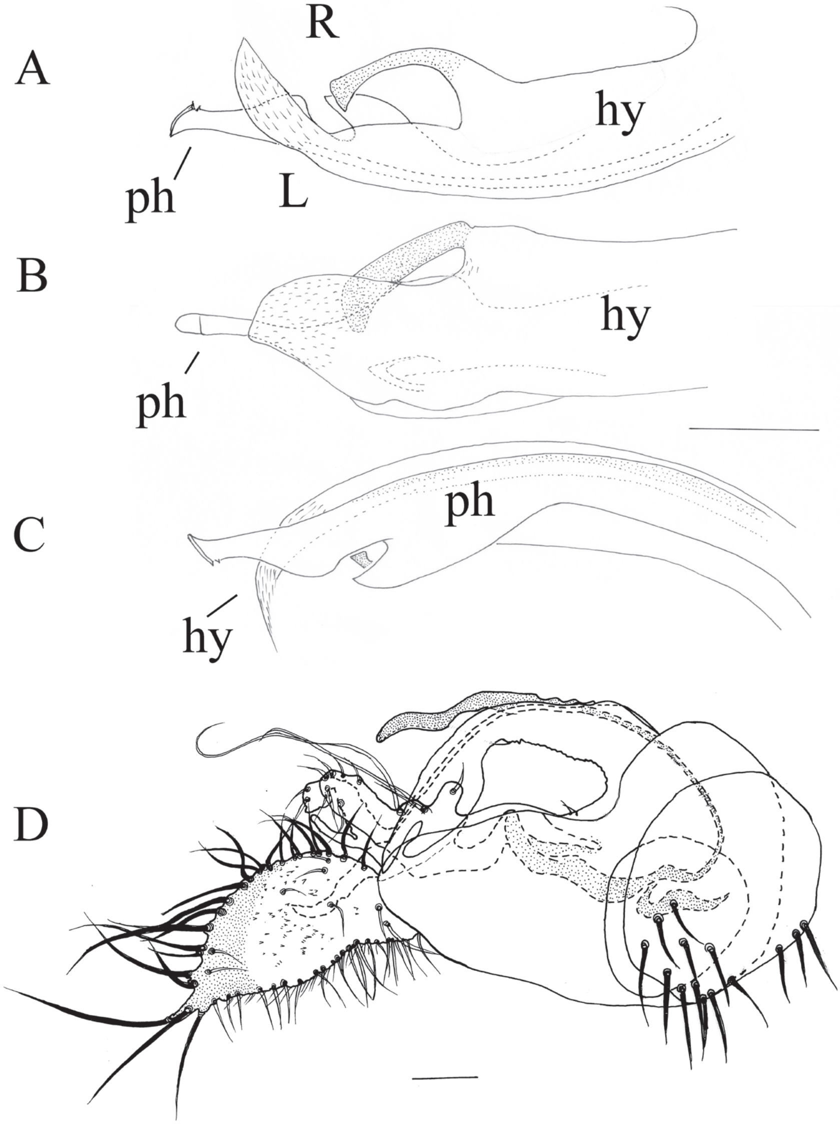

Figure 13. *Lichtwardtia conspicabilis* Tang & Grootaert, sp. n. male terminalia: **A** right view on hypandrium and phallus **B** ventral view of hypandrium and phallus **C** view on left side of phallus and hypandrium **D** lateral view of hypopygium. Scale bars: 0.1 mm.

side. *L. conspicabilis* sp. n. has a curved black extension of the left side and the tip of the phallus is deeply indented.

Etymology. The name *conspicabilis* refers to the remarkable structure of the hypandrium.

Distribution. Cambodia.

Lichtwardtia monstruosa **Tang & Grootaert, sp. n.**

http://zoobank.org/D6EBAA51-43A7-4803-9CB9-84CF23DEA3CA

Figs 14–15

Material examined. Holotype male (coll. RBINS): CAMBODIA: Siem Reap prov., Angkhor, Preah Khan Temple, 18–25 April 2006, Malaise trap in secondary forest (leg. Oul Yothin). **Paratype** (coll. RBINS): 1 male, same provenance as holotype.

Diagnosis. Postpedicel of antenna mostly brownish yellow. Wing faintly tinged brownish, no swelling of the costa present. Mid coxa with two brown bands laterally, one brown, one light brown. Hind coxa entirely yellow. Tip of hypandrium bifurcate with a black narrow ventral arm that is strongly toothed and a broad arm dorsally yellowish at base and black at tip; apex rounded, bordered with teeth. Phallus yellowish with a very strong yellowish brown dorsal hook near middle resting in the ventral cavity of the epandrium; apical half of phallus, with a ventral curved followed by a strong dorsal curved; tip pointed.

Description. Male. *Body* length 4.0 mm, wing 3.8 × 1.3 mm. Head with frons shining metallic green; face slightly raised, as wide as postpedicel, frons and face covered with a thick white dusting. Hairs and bristles on head black; upper five postoculars black, lower postocular bristles pale. Antenna mainly yellow, postpedicel largely brownish yellow; postpedicel nearly triangular, blunt apically, with yellow pubescence, 1.2 times as long as wide; arista-like stylus dark yellow, nearly as long as width of head, feather-like, basal portion 0.5 times as long as apical portion. Proboscis dark yellow, with black hairs; palpus dark yellow, with one short weak black apical bristle.

Thorax dark metallic green, with a fine pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of acr half as long as dc. Scutellum with two pairs sc, apical pair long strong, basal pair short and weak. Legs mainly yellow, but mid coxa with two brown bands laterally, one brown, one light brown. Fore and mid coxae anteriorly to laterally with rows of bristle-like hairs and eight preapical bristles; mid coxa with one strong outer bristles at apical third, hind coxa with two outer bristles, basal one strong, apical one relatively weak. Mid trochanter with two dorsal ap. Hind trochanter with one outer bristle at middle. Fore femur without distinct bristles. Mid femur with one strong ad and a weak pd. Hind femur with one strong ad at apical quarter. Fore tibia with two ad, two pd, one av and four ap. Mid tibia with two ad, three pd, one pv and four ap. Hind tibia with four ad, four d and four ap; ventrally in basal third with a row of small erect hairs. Fore and mid tarsomere I without distinct bristles. Hind tarsomere I with one strong d at apical third. Relative lengths of tibia and five tarsomeres of legs LI : 8.2 : 5.0 : 2.0 : 1.6 : 1.0 : 1.0; LII : 13.2 : 6.6 : 3.3 : 2.6 : 2.0 : 1.3; LIII : 13.2 : 6.0 : 6.0 : 4.3 : 3.3 : 1.6. Wing nearly hyaline, tinged brown; veins brown. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. CuAx ratio 1.0. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with fine pale pollinosity. Hairs and bristles on abdomen black.

Figure 14. *Lichtwardtia monstruosa* Tang & Grootaert, sp. n. (Cambodia).

Male terminalia (Figure 15): Epandrium 2.1 times longer than wide, narrowing towards tip; epandrial lobe with three long pale bristles. Ventral surstylus with three long pale bristles. Cercus nearly quadrate, as long as wide, pale except the black marginal seam, with weak digitations around outer margin, with black marginal bristles on digitations. Tip of hypandrium bifurcate with a black narrow ventral arm that is strongly toothed and a broad arm dorsally yellowish at base and black at tip. Apex rounded bordered with teeth. Phallus yellowish with a very strong yellowish brown dorsal hook

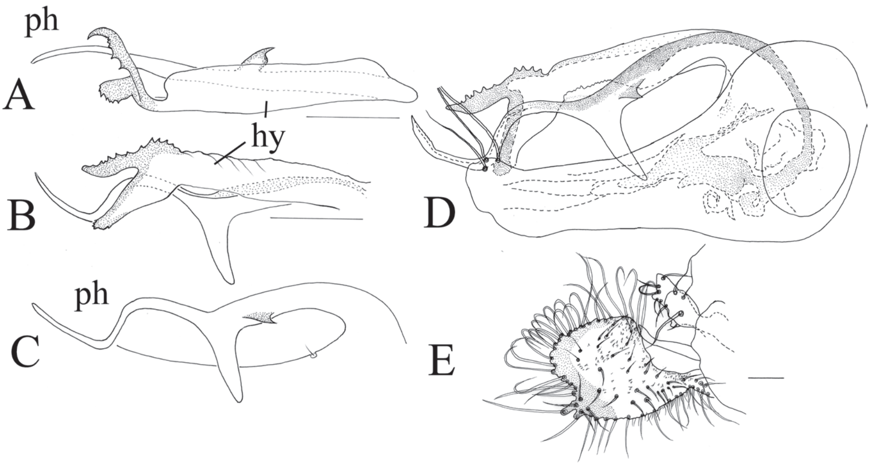

Figure 15. *Lichtwardtia monstruosa* Tang & Grootaert, sp. n. Male terminalia (Cambodia): **A** hypandrium ventrally **B** hypandrium lateral **C** phallus lateral **D** epandrium lateral **E** cercus lateral. Scale bars: 0.1 mm.

near middle resting in the ventral cavity of the epandrium (Figure 14); a short black pointed tooth at the left side and at the base of the large tooth; apical half of phallus, with a ventral curved one followed by a strong dorsal curved; tip pointed.

Female. Unknown.

Etymology. The species name alludes to the monstrous appendages on the male terminalia.

Comments. The male is easily recognised having these huge extensions on the terminalia. The very strong dorsal tooth on the phallus resembles superficially the strong tooth present on the tip of the hypandrium of *L. polychroma* and *L. zhangae*. However the origin of these structure is different: in *L. monstruosa* sp. n., it is on the phallus, while in the others it is on the hypandrium.

Distribution. Cambodia.

Lichtwardtia polychroma-group

Diagnosis. The cercus is more or less triangular, with some strong flattened marginals and a dorsal marginal that shifted to the inside. The postgonite is tubiform. The tip of the phallus has no denticle. The hypandrium has a strong subapical dorsal hook.

Lichtwardtia polychroma (Loew, 1864)

Figs 16–18

Rhagoneurus polychromus Loew, 1864: 346, Fig 3, a, b, c. Male & female. Type locality: Sri Lanka.

Material examined. There is a single male conserved in the collection of Becker (MfN, Berlin) bearing the label "*Rhaconeurus polydromus* m" in the handwriting of Loew (Figure 16). We think that the "m" stands for mihi (mine or my species) or for manuscript name. It was this specimen that Becker cited in his 1922 book commenting on the writing error *Rhaconeurus* by Loew which should have been *Rhagoneurus*. Becker labelled the specimen *zickzak* Wied. det. Becker though he published it as *zickzack*. It bears a third yellow label with "Ceylon Nietner S." in print and in handwriting Rambodda. Below we see in handwriting Loew (Figure 16). Nietner S. means Nietner sammelt.

The information on the locality is new because Loew did not give a precise locality in his description. Rambodda (nowadays cited as Ramboda) is a small village in Sri Lanka known for its famous waterfalls. Johannes (John) Nietner (died 1874) was a German naturalist chiefly interested in botany and entomology. Born in Potsdam, he was a plantation owner in Rambodda, Ceylon and described many new insect species from the island. Having a special interest in insects, he made large collections and sent specimens for study by experts abroad. Collections from him are in the Deutschen Entomologischen Institut, the Museum für Naturkunde in Berlin, in the Naturhistorischen Museum in Vienna and the Natural History Museum in London.

We designate the male as lectotype since Loew did not designate a holotype. A female was included in the description but we failed to find it. Stacked images of the lectotype male were provided by the courtesy of Mr. Bernhard Schurian and Sven Marotske (MfN, Berlin).

Comments. Zhang et al. 2009 re-described and illustrated a similar species as *Lichtwardtia ziczac* (Wiedemann). Thanks to her detailed drawings and re-description we could see that her species does not correspond to the female holotype of *L. ziczac*. The latter has the cross veins brownish seamed (Figure 25). The male that she studied was found on Bali (Indonesia) and we are not sure if it is really conspecific with our *L. polychroma* from Cambodia although the huge dorsal hook near the tip suggests so. Temporarily we consider the specimens from Cambodia as *L. polychroma* both having a swelling of the costa, while the species from Bali without swelling of the costa as a different new species.

Additional material examined for the descriptions. CAMBODIA (all coll. RBINS): 1 male, Siem Reap prov., Angkhor, Preah Khan Temple; 17–24 February 2005, Malaise trap in secondary forest (leg. Oul Yothin). 4 males; same provenance, 24 January–21 February 2006. 1 male, same provenance, 28 March–7 April 2006. 1 male; Siem Reap prov., Angkhor, Bakheng; 23–31 October 2005; Malaise trap in secondary forest (leg. Oul Yothin).

Diagnosis. Antenna largely yellow, legs yellow (Figs 16, 17). Postpedicel 1.5 times as long as wide. Arista-like stylus with rather short hairs. Wing entirely hyaline with a short widening of the costa just before R_1 joins the costa. Mid coxa anteriorly with a blackish brown band, posteriorly with a brown band. Hind coxa entirely yellow. Hypandrium (Figure 18) with a strong brown subapical spine. Phallus smooth. Cercus pointed with broadened bristles.

Description. Male. *Body* length 4.2 mm, wing 3.8 × 1.3 mm.

Figure 16. *Lichtwardtia polychroma* (Loew, 1864) lectotype male habitus, Sri Lanka (photograph by Bernhard Schurian).

Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black except postocular bristles yellow. Antenna yellow; postpedicel with extreme tip and dorsal margin brownish; elongate triangular, blunt at tip, nearly as long as wide; arista-like stylus with long densely set hairs. Proboscis dark yellow, with short black hairs; palpus, dark yellow with one black apical bristle.

Thorax dark green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of strong acr, with dense short strong bristles at anterior portion. Scutellum with two pairs sc, apical pair long strong, basal pair short and weak. Legs mainly yellow. Fore and hind coxa entirely yellow, but mid coxa with a black band anteriorly and a broad band posteriorly. Fore coxa anteriorly at base with a few short erect bristles, anteriorly densely covered with short black bristle-like hairs, four very long ap and a few shorter bristles. Mid coxa anteriorly densely covered with short black hairs, with a long outer bristle at the tip of the blackish band; hind coxa with two outer bristles, basal one strong, apical one short and weak. Mid and hind trochanters both with several short weak hairs. Fore femur lacking ventral bristles. Mid femur with one preapical pv. Hind femur with one strong ad at apical quarter. Fore tibia with two ad, two pd, one av, and three ap. Mid tibia with two ad, three pd, one av, and four ap; all long strong. Hind tibia with two ad, four pd, one pv, and three ap; all long. Hind tarsomere I with

Figure 17. *Lichtwardtia polychroma* (Loew, 1864) male habitus Cambodia (photograph Maimon Hussin).

one strong ad at middle, one short strong ad at basal third and two short apical bristle. Relative lengths of tibia and five tarsomeres of legs LI : 9.0 : 6.0 : 2.4 : 1.2 : 1.0 : 1.0; LII : 16.0 : 8.0 : 5.0 : 4.0 : 2.4 : 1.6; LIII : 18.0 : 6.0 : 4.0 : 4.0 : ? : ?. Wing nearly hyaline, tinged brownish, veins brown. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. CuAx ratio 1.1. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

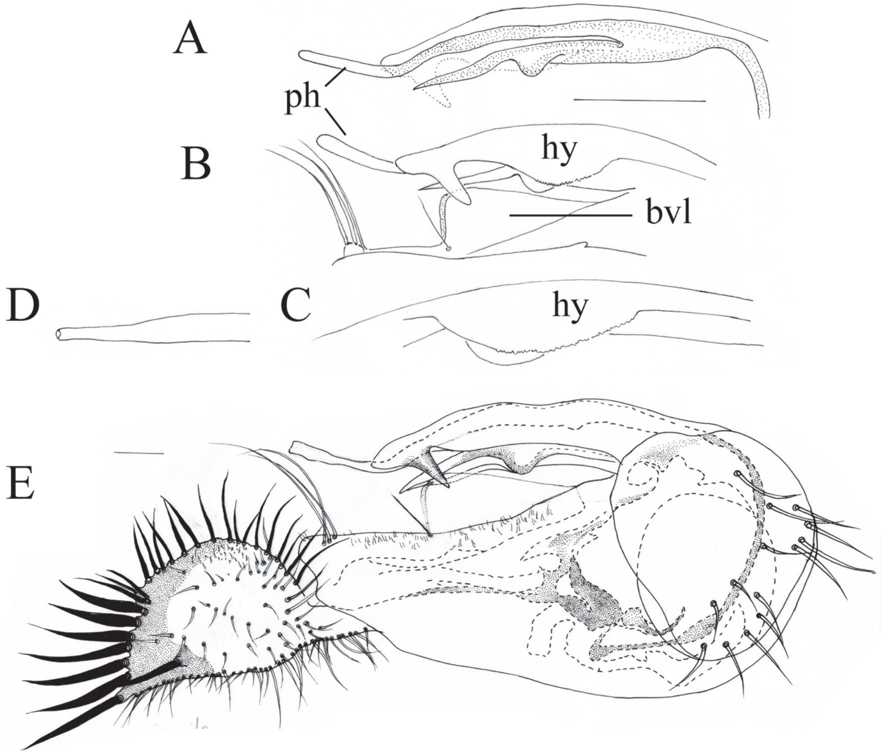

Figure 18. *Lichtwardtia polychroma* (Loew, 1864) male terminalia (Cambodia): **A** phallus **B** hypandrium covering phallus **C** hypandrium with toothed dorsal border **D** tip postgonite **E** epandrium lateral view, but cercus inner view. Scale bars: 0.1 mm.

Male terminalia. Epandrium 1.9 times longer than wide (Figure 18E); epandrial lobe with three long pale ap. Surstylus thin and long with three thin ap and three bristles at middle. Cercus nearly triangular, pale except the thick black seam, with weak digitations around outer margin, with black blade-like marginal bristles on digitations. The tip is elongated with the apical bristle on a papilla. A strong black bristle present on the inner margin of the cercus near the tip. Hypandrium with one large brown hook-like tooth at tip and some tiny denticles on the dorsal margin (Figure 18C). The large brown tooth is resting on the brownish tip of a large pale membranous projections on both sides of the ventral margin of the epandrium (Figure 18B). Phallus bifurcate with a dorsal rounded swelling on the dorsal fork (Figure 18A). Tip of the ventral fork somewhat truncate (Figure 18E).

Female. Unknown.

Comments. Loew (1864) gave a very detailed description of the male and although he mentioned the small swelling of the costa before the R_1 reaches the costa, he did not indicate it on his drawings of the wing (Loew 1864: figure 3 and 3C). That caused more confusion. Having this characteristic *L. polychroma* resembles our *L. nodulata* that has however a larger swelling of the costa on the point where R_1 joins the costa (Figs 16, 17) and it lacks the large brown tooth a the tip of the hypandrium. *L. zhangae* sp. n. from Bali has no broadening before the R_1 joins the costa but identical armed hypandrium. *Lichtwardtia hirsutiseta* has a broad swelling much more in advance of the point where R_1 meets the costa; its antenna is also much darker while entirely yellow in *L. polychroma*. Here again we did not dissect the specimen waiting for appropriate techniques to study the ancient DNA. Nevertheless we think that *L. polychroma* is conspecific with specimens from Cambodia that we describe above in more detail.

Distribution. Cambodia, Sri Lanka.

Lichtwardtia zhangae Tang & Grootaert, sp. n.

http://zoobank.org/7C6ADA9C-E5C2-457A-900D-74822F90DFBD

Lichtwardtia ziczac (Wiedemann, 1824) sensu Zhang, Masunaga & Yang, 2009: 199, figs 11–14. Re-description.

Etymology. This species is dedicated to Dr. Lili Zhang of the IOZ Museum in Beijing who re-described and illustrated *Lichtwardtia ziczac* (Wiedemann) for the first time with detailed drawings.

Diagnosis. Costa without swelling. Hypandrium with a large brown dorsal preapical tooth. Phallus smooth.

Description. For a full description we refer to Zhang et al. 2009.

Comments. In having the cross veins clouded, *Lichtwardtia ziczac* (Wiedemann) is distinctly different from *L. ziczac* sensu Zhang et al. 2009 and therefore we give a new name *Lichtwardtia zhangae* sp. n. to the species that she (re-) described from Bali. It is very closely related to *L. polychroma* from Sri Lanka and differs in the lacking of a swelling of the costa.

Distribution. Bali (Indonesia).

Unplaced species

Lichtwardtia formosana Enderlein, 1912

Figs 19–20

Lichtwardtia formosana Enderlein, 1912: 407. Type locality: Taiwan (China).

Lichtwardtia taiwanensis Zhang, Masunaga & Yang, 2009: 199, figs 6–10. Type locality: Taiwan (China).

Lichtwardtia formosana Enderlein, 1912, in Selivanova, Negrobov & Yang, 2010: 144, re-description, figs 1–5.

Material examined. CAMBODIA (all coll. RBINS): 10 males, Siem Reap prov., Angkhor, Preah Khan Temple, 24 January–21 February 2005, malaise trap in secondary forest (leg. Oul Yothin). 1 male; same provenance, 12 November–7 December 2005. 4 males, same provenance, 28 March–4 April 2006. 2 males, same provenance, 4–11

Figure 19. *Lichtwardtia formosana* Enderlein male habitus (Singapore)

April 2006. 1 male; same provenance, 12 May 2006. 1 female, Siem Reap prov., garden Sam Vesna Centre, 6 June 2005, malaise trap (leg. Oul Yothin).

SINGAPORE (all coll. LKCNHM): 1 male, Semakau, 3 April 2012, sweeping along path in back mangrove (leg. P Grootaert). 1 male, Semakau, 12 July 2012, sweeping along path in mangrove (leg. Jayanthi Puniamoorthy & P Grootaert). 1 male, 2 females, West Coast Park, 7 December 2003; sweeping nets along shrubs along sandy beach (leg. P Grootaert). 1 male, 5 female, Sungei Buloh, 1–6 June 2005, malaise trap in mangrove (leg. P Grootaert). 5 female, same provenance the previous, 20–27 July 2005. 1 male, 2 females, Clementi Woods, sweeping nets along drains in park (leg. P Grootaert). 1 male, 1 female, Labrador Park, 3 April 2005, sweeping along drains (leg. P Grootaert).

Diagnosis. Antenna entirely dark yellow. Mid coxa entirely darkened, hind coxa anteriorly with a rectangular brown spot. No thickening of the costa present. Wing nearly hyaline, lightly tinged brown. Apex of phallus looking smooth though microscopic small denticles are present.

Description. Male. *Body* length 3.8 mm, wing 3.5 × 1.2 mm.

Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black except postocular bristles pale. Antenna dark yellow; postpedicel nearly triangular, blunt apically, 1.4 times as long as wide, covered by short brown pubescence; arista-like stylus dorsal, black to dark yellow onwards, inserted at basal half of postpedicel, nearly as long as head width, black, feather-like, with long black pubescence, basal segment 0.5 times as long as apical portion of arista-like stylus. Proboscis brown, with black hairs; palpus yellow, with one short black apical bristle.

Thorax dark metallic green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of strong acr. Scutellum with two pairs sc, apical pair long strong, basal pair short and weak. Legs mainly yellows, except basal 2/3 of mid coxa brown, hind coxa with one brown spot, tip of hind tibia brown, tarsi yellow to brown onwards. Fore coxa anteriorly with five strong bristles at apical half, mid coxa anteriorly with four strong bristles, hind coxa with two short outer bristles. Fore and hind trochanters with one weak outer bristle at middle. Mid trochanter with two short weak outer bristles. Fore femur without distinct bristle. Mid and hind femora each with one preapical pv. Fore tibia with two short ad, two pd, one av, and three ap. Mid tibia with two ad, three pd and four ap. Hind tibia with six ad, four pd, two pv, and three ap. Hind tarsomere I with one short strong ad at apical third, one av at basal fifth and one short apical bristle. Relative lengths of tibia and five tarsomeres of legs LI : 2.5 : 1.1 : 0.5 : 0.4 : 0.3 : 0.3; LII : 3.0 : 2.0 : 1.0 : 0.8 : 0.5 : 0.3; LIII : 4.0 : 1.5 : 1.5 : 1.0 : 0.8 : 0.5. Wing nearly hyaline, lightly tinged brown; veins brown. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu almost straight. CuAx ratio 1.0. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

Male terminalia: Epandrium 2.0 times longer than wide (Figure 20); epandrial lobe three pale ap. Ventral surstylus with five long ap, dorsal surstylus with three ap and two digitations each with one apical bristle, of which one thin spinous, one rod-like; all pale except rod-like bristle brown. Cercus nearly triangular, pale except for the black marginal seam, with weak digitations around outer margin and with thin pale marginal bristle on digitations. Hypandrium simple. Phallus with a patch of minute spinules near apex (these spinules are visible like a darkened patch and can only be distinguished well under a light microscope).

Female. Has the same characteristics as the male: large rectangular black spot on the hind coxa and the tip of the hind tibia brown.

Comments. *Lichtwardtia formosana* is the only Oriental species known at the moment with a rectangular black (dark brown) patch on the hind coxa. In all other species the hind coxa is entirely yellow. *Lichtwardtia formosana* looks different but has a double row of microscopic denticles ventrally on the tip of the phallus. Otherwise the shape of the postgonite is identical to the *L. nodulata* group. The cercus lacks a strong dorsal bristle at the inside.

Distribution. China (Taiwan), Cambodia, Singapore.

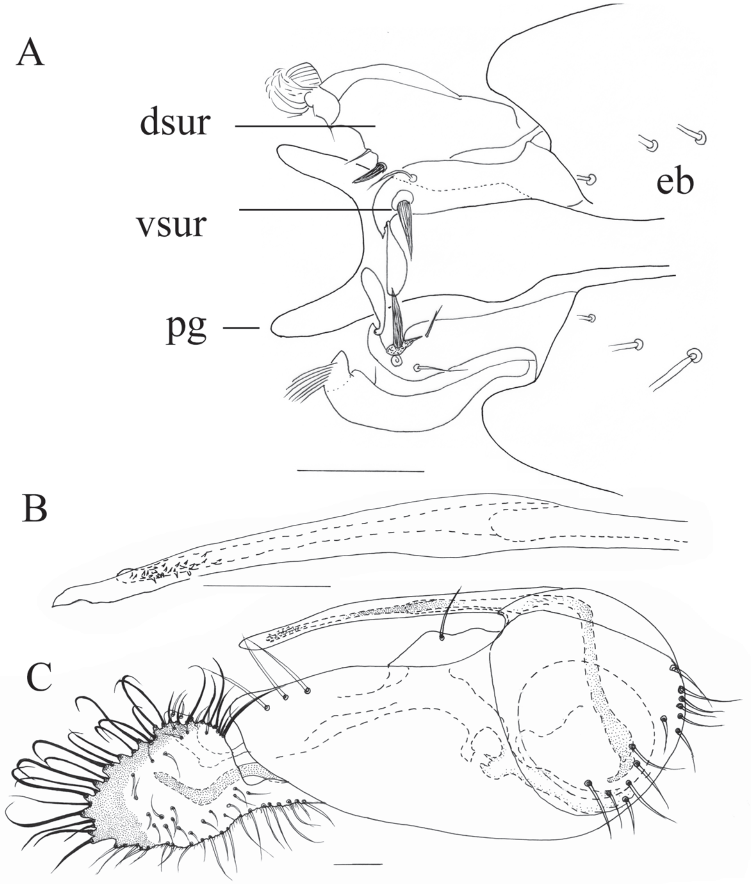

Figure 20. *Lichtwardtia formosana* Enderlein male terminalia (Singapore): **A** ventral view on surstyli and cerci **B** tip phallus bearing microscopic denticles **C** lateral view epandrium. Scale bars: 0.1 mm.

Lichtwardtia hirsutiseta (de Meijere, 1916)
Figure 21

Rhagoneurus hirsutisetus de Meijere, 1916: 229. Male. Type locality Batavia (= Jakarta, Indonesia).

Material examined. Holotype male Batavia, August, 1907, leg. Jacobson (Figure 21) Naturalis (Leiden, Netherlands). The male was not physically examined by us, only the photographs (courtesy of Ben Brugge).

Diagnosis. A larger species (body length 4.5 mm; wing length 4.0 mm). Postpedicel mainly dark yellow, but blackish on dorsum and tip. Wing brown with cross veins not brownish seamed and costa with a distinct swelling well before R_1 reaches the costa (Figure 21D). Hind coxa yellow (Figure 21B). Male terminalia with complicated twisted hypandrium and phallus (Figure 21B).

Figure 21. *Lichtwardtia hirsutiseta* (de Meijere, 1916) holotype male (Jakarta, Indonesia) (photograph by Mr Ben Brugge): **A** head lateral **B** thorax and abdomen lateral **C** labels **D** wing. Arrow indicates the swelling before R_1 reaches the costa.

Comments. As marked in the diagnosis *L. hirsutiseta* is quite unique in having a largely darkened postpedicel while other *Lichtwardtia* have generally an entirely yellow postpedicel. The swelling of the costa is also unique. It is well separated and before R_1 reaches the costa. The complex male terminalia resemble *L. monstruosa* sp. n. but not *L. polychroma*.

Lichtwardtia infuscata Tang & Grootaert, sp. n.

http://zoobank.org/D34616A1-6260-4145-894B-5EDEA83DABFA
Figs 22–24

Material examined. Holotype male (coll. RBINS): CAMBODIA: Siem Reap prov., Angkhor, Preah Kahn Temple, 24 January–21 February 2006, Malaise trap in secondary forest (leg. Yothin Oul). **Paratypes**: 2 males, same provenance as holotype, 28 March–5 April 2005.

Diagnosis. Wing anteriorly brown, grey below Cu and M_1. Costa lacking a swelling. Postpedicel about as long as wide with rounded tip, apex brownish yellow, ventrally

Figure 22. *Lichtwardtia infuscata* Tang & Grootaert, sp. n. Holotype male (terminalia removed). Cambodia (photograph by Ms Maimon Hussin).

yellowish. Mid coxa anteriorly with a black stripe, posteriorly brown. Hind coxa entirely yellow. Phallus with strong black spines, phallus and hypandrium resting on a ventral protuberance of the epandrium. Cercus seamed with long, fine black and pale bristles.

Description. Male. *Body* length 4.1 mm, wing 3.2 × 1.1 mm. Head dark metallic green, with thick pale pollinosity; face slightly raised, frons and face both with thick pale pollinosity, gradually narrowed downward. Hairs and bristles on head black but lower postocular bristles yellowish. Antenna yellow except but apical half of postpedicel brown; postpedicel nearly triangular, 1.2 times as long as wide; arista-like stylus black, almost as long as head width, feather-like, with long regular pubescence. Proboscis dark yellow, with black hairs; palpus very small, dark yellow, with one short black apical bristle.

Figure 23. *Lichtwardtia infuscata* Tang & Grootaert, sp. n. Paratype male. **A** Wing **B** Male terminalia (photograph by Ms Maimon Hussin).

Thorax dark green, with pale grey pollinosity. Hairs and bristles on thorax black; five strong dc, ten pairs of strong acr. Scutellum with two pairs sc, apical pair long strong, basal pair short and weak. Legs mainly yellow. Mid coxa anteriorly with a black stripe, posteriorly brown. Hind coxa entirely yellow. Fore tarsomere I brown, apical fifth of each hind tarsomere brownish. Fore and mid coxae anteriorly with rows of bristle-like hairs, and four strong ap, hind coxa with two outer bristles, basal one strong, apical one relatively weak. Mid and hind trochanters both with four short weak ap. Fore femur with one weak preapical av. Mid femur with one strong pd and one preapical av. Hind femur with one strong pd at apical quarter. Fore tibia with two ad, three pd, one av and three ap. Mid tibia with two ad, four pd and four ap; all long strong. Hind tibia with four ad, four pd (of which one preapical), one d, two pv and three ap. Fore tarsomere I with one short av at base. Hind tarsomere I with one strong ad at apical third, one short ad at base and two short apical bristle. Relative lengths of tibia and five tarsomeres of legs LI : 7.5 : 4.5 : 1.8 : 1.3 : 1.0 : 1.0; LII : 11.2 : 6.3 : 3.0 : 2.5 : 1.5 : 1.3; LIII : 12.5 : 5.0 : 5.2 : 3.8 : 2.5 : 1.5. Wing anteriorly brownish infuscate (Figure 22A) including the cross veins; veins brown. M with fading M_2, M_1 with one short subvein. Crossvein dm-cu straight. CuAx ratio 1.0. Lower calypter pale with black hairs. Haltere pale.

Abdomen metallic green, with pale pollinosity. Hairs and bristles on abdomen black.

Male terminalia: Epandrium 2.0 times longer than wide; epandrial lobe with three long pale bristles. Ventral surstylus with five ap, of which ventral most apical bristle rod-like and others spinous; dorsal surstylus both with five short nail-like ap and one long digitation close to dorsal margin which with one long spinous bristle. Phallus with strong black spines, phallus, and hypandrium resting on a ventral protuberance of the epandrium. Cercus nearly triangular, pale except the thick black ring, with weak digitations around outer margin, with long pale marginal bristles on digitations. Hypandrium simple. Phallus strong with strong black denticles (Figure 24A).

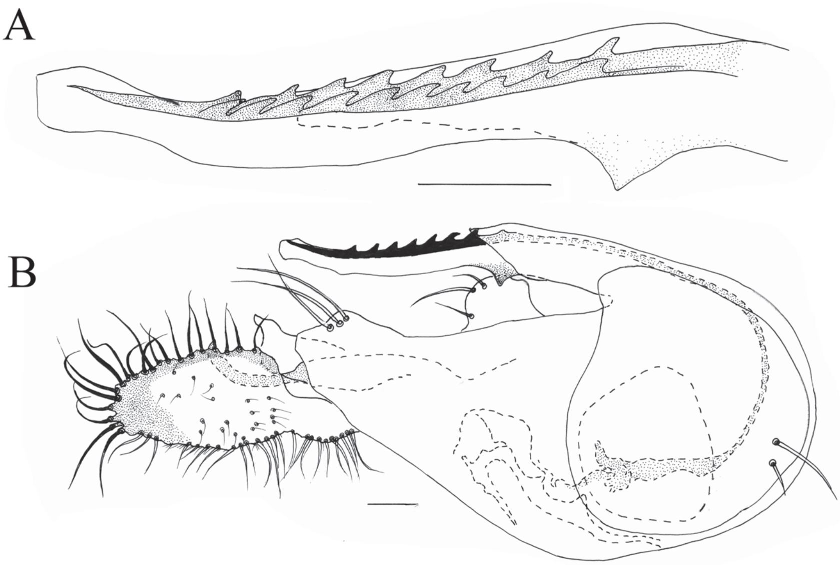

Figure 24. *Lichtwardtia infuscata* Tang & Grootaert, sp. n. Male terminalia: **A** Tip phallus **B** epandrium lateral. Scale bars: 0.1 mm.

Female. Unknown.

Etymology. From Latin infuscāre to darken.

Comments. It is not clear if *L. infuscata* sp. n. belongs to the *L. dentalis* –group though it has the tip of the phallus ventrally denticulate and a simple hypandrium. The cercus is more elongate and lacks a dorsal bristle at the inside. Others see comments under *L. ziczac*.

Distribution. Cambodia.

The nomina dubia

Lichtwardtia coxalis Kertész, 1901

Rhagoneurus coxalis Kertész, 1901: 411. Female. Type locality: Singapore.

Material. The type was probably conserved at the Hungarian Museum for Natural History. It is no longer in their collections and probably destroyed (Foldvary and Papp 2007).

Diagnosis from Kertész. Hind coxa largely blackish brown. Hind femur without preapical bristle and hind basal tarsomere lacking long bristles. Cross veins not brownish seamed.

Comments. It is likely that this species is one of the four species that we actually recorded in Singapore. It is not *L. singaporensis* sp. n. since it has the cross veins brownish seamed and the hind coxa yellowish. *L. nodulata* sp. n. has a broad swelling where R_1 joins the costa and the hind coxa is yellowish (Figure 6). *L. semakau* sp. n. has also a yellow hind coxa (Figure 8). *L. formosana* Enderlein is the most plausible candidate because it is particular in having only the anterior half of the hind coxa dark brown (rectangular sclerotisation) while the posterior part is yellowish to yellowish brown (Figure 19). Maybe Kertész meant this in stating that *L. coxalis* has "auch die hinterhüften in grosser ausdehnung schwarzbraun". However the shape of the black sclerotisation is so remarkable. In his key, Kertész (1901) described the similar shape of the mid coxa of *L. polychroma* but not for the hind coxa. *L. formosana* from Singapore possesses an anterior preapical bristle on the hind femur (lost on Figure 19) that is according to Kertész not present in *L. coxalis*.

Since it was described on the basis of a female and that the holotype seems to be lost, we think it is not appropriate to sink *L. formosana* as a junior synonym having its holotype conserved. The confusion is bigger since de Meijere (1912) quotes *L. coxalis* (as *Rhagoneurus*) from "Neu Guinea" but this is a misreading because Kertész clearly says that he obtained a female from Singapore by the courtesy of his friend Biro. Meanwhile, the description by Kertész is too simple to acquire any further comparison. Therefore, at this moment, *L. coxalis* is considered as a nomen dubium.

***Lichtwardtia ziczac* (Wiedemann, 1824)**

Figure 25

Dolichopus ziczac Wiedemann, 1824: 40. Female. Type locality: India Orientalis
Dolichopus ziczac Wiedemann, 1830: 232
Dolichopus zickzack Wiedemann, 1824. Male in Becker 1922: 8, figure 1. Non *ziczac* sensu Wiedemann 1824
Lichtwardtia ziczac (Wiedemann, 1824) sensu Zhang, Masunaga & Yang, 2009: 199, figs 11–14.

Material examined. Holotype female, India Orientalis, on pin in collection ZMUC (Copenhagen).

Diagnosis. Female. A medium-sized species (body: 4 mm; wing: 3.2 mm). Wing hyaline with anterior border faintly brownish and cross veins brownish seamed. No swelling of the costa before or at the point where R_1 joins the costa. The ratio of the proximal section of M_1, and the distal section is 0.4/0.6 (Figure 25). Thus the distal section is much longer than the proximal section. Fore coxa yellow, mid coxa brown and hind coxa yellowish.

Comments. *Dolichopus ziczac* was described by Wiedemann (1824) on the basis of a female collected in India Orientalis. No precision is given about the locality so that the type locality could be everywhere in the Oriental region ranging from Pakistan to New Guinea at that time. The description of the species in the work of Wiedemann (1824) is very short, but fortunately the holotype female is still well preserved in the collections of the Zoological Museum in Copenhagen.

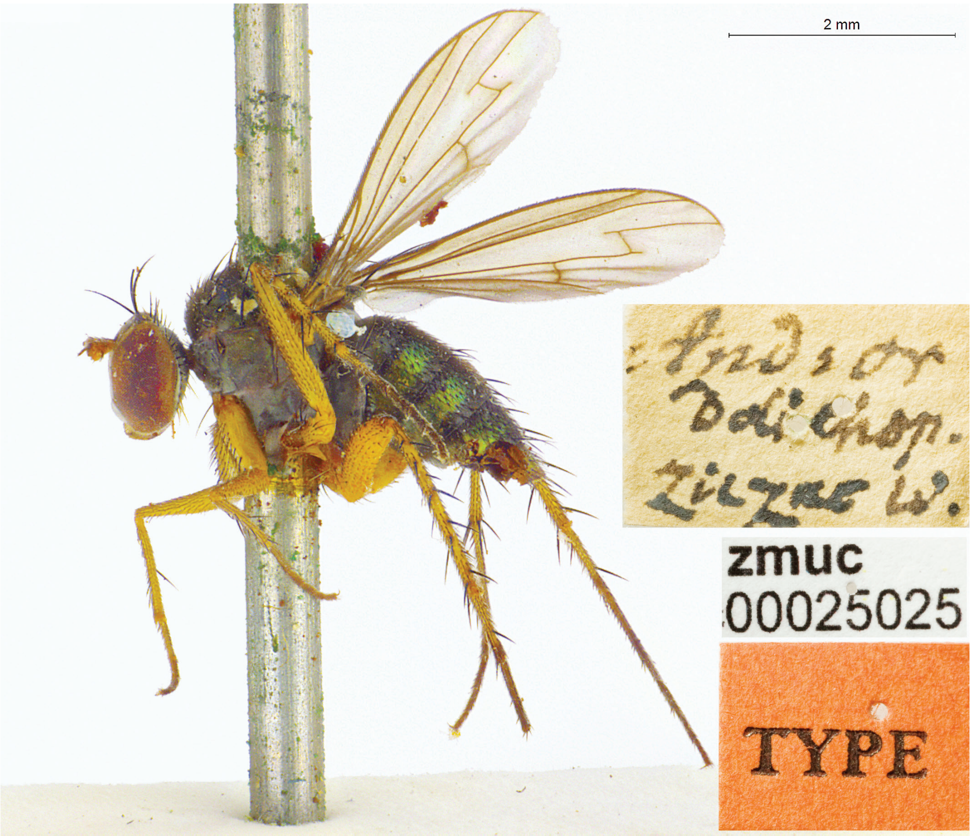

Figure 25. *Lichtwardtia ziczac* (Wiedemann, 1824) holotype female (photograph by Dr Thomas Pape).

Becker (1922) knew about this specimen but did not see it. A colleague described it to him in a letter and Becker was sure that the specimens that he had in his own collection or had seen at the Hungarian Museum from Taiwan (China), India, Bangladesh, Rangoon (Myanmar), Ceylon (Sri Lanka), and the Bismarck Archipelago (Papua New Guinea) were all the same species. The range is thus also very wide according to Becker. He gave a description of a male but did not mention the origin of the male. Hence we cannot rely on his re-description that fits to quite a number of species. At the same time he put *Rhagoneurus coxalis* Kertész, 1901, *Lichtwardtia formosana* Enderlein, 1912 and *Rhagoneurus polychromus* Loew, 1864 all *Lichtwardtia* species as junior synonyms of *L. ziczac*. Now we see that all are good species and we re-establish here their names as valid species. The previous holotype female is examined (Figure 25). It is not clear why Becker (1922) changed the name *ziczac* to *zickzack*.

Brownish seams along the cross veins are not very common in *Lichtwardtia* and actually only known in *L. ziczac* and *L. singaporensis* sp. n. The ratio of the proximal section of M_1, and the distal section is however 0.435/0.564. Thus the distal section is not as long as in *L. ziczac*. We do not consider both species as conspecific for the moment because in the near future it might be possible to extract ancient DNA from the holotype without using destructive techniques. This can be decisive about the status of both species. We propose to consider *L. ziczac* (Wiedemann) from *terra incognita* as a *nomen dubium* and not to complicate again the taxonomy of *Lichtwardtia* by applying the name *ziczac* to the male of *singaporensis* without genetic information. Remarkable is that among the more than 200 *Lichtwardtia* specimens belonging to six species that we found in Seam Reap, no specimens with brownish seamed cross veins were found.

Key to males of Oriental *Lichtwardtia*

1 Hind coxa with anteriorly half or basal half brownish **2**

– Hind coxa entirely yellow **3**

2 Anterior half of hind coxa with a dark brown rectangular area that bears the two black exterior bristles; cross veins not seamed brownish. Apex of phallus looking smooth though microscopic small denticles are present (Figure 20) [Taiwan (China), Cambodia, Singapore]..................... ***formosana* Enderlein**

– Hind coxa with basal half brownish and cross veins brownish seamed. Phallus ventrally with strong denticles, hypandrium unarmed. (Figs 10–11) (Singapore) .... ***singaporensis* sp. n.**

3 Wing with a colour pattern (Figure 23). Phallus with strong black spines, phallus, and hypandrium resting on a ventral protuberance of the epandrium (Figure 24). (Cambodia) .... ***infuscata* sp. n.**

– Wing uniformly hyaline, greyish or brownish tinged but without pattern. Male terminalia various.. **4**

4 Costa with a swelling before or at the point where R_1 joins the costa **5**

– Costa without swelling.. **10**

5 A distinct brown swelling of the costa where R_1 joins the costa (Figure 6). Phallus with two rows of black denticles on ventral half, anterior row only with four small sparse denticles, posterior row with eight dense denticles (Figure 7). (Singapore) .... ***nodulata* sp. n.**

– Costa widened before R_1 reached costa (Figure 21 D) **6**

6 Costa widened distinctly before R_1 joins costa (Figure 21D); postpedicel black except for yellowish base. Male terminalia with complicated twisted hypandrium and phallus (Figure 21 B) (Java, Indonesia) .... ***hirsutiseta* de Meijere**

– Costa widened just before tip of R_1 (Figure 4); postpedicel yellowish, sometimes dorsally darker **7**

7 Tip of phallus with black ventral denticles (Figure 4). Tip of hypandrium without a preapical tooth or not bifurcate (Yunnan China, Thailand, Cambodia, Singapore) **8**

– Tip of phallus lacking ventral denticles; tip of hypandrium with a large brown tooth (Figure 18) or tip hypandrium bifurcate (Figure 13).......................... **9**

8 Marginal bristles on cercus strong, black (Figure 5). A distinct swelling on costa at level of R_1. Tip of phallus ventrally with a number of denticles. Hypandrium unarmed (Figs 4, 5) .... ***dentalis* Zhang et al.**

– Marginal bristles on cercus weak, pale. Swelling of costa weak. Hypandrium simple and smooth, with no denticle; phallus with double rows of spinules on ventral half (Figure 3) .... ***cambodiensis* sp. n.**

9 Tip of hypandrium with a single large brown preapical tooth (Figs 18, 19) (Sri Lanka, Cambodia, China) .... ***polychroma* (Loew)**

– Tip of hypandrium dorsally curved, bifurcate; with a long black appendage near middle of hypandrium (Cambodia)......................... ***conspicabilis* sp. n.**

10 Hypandrium simple (Figure 9) .... ***semakau* sp. n.**

– Hypandrium with tooth-like extensions.. **11**

11 Hypandrium with a large preapical brown tooth (Bali) .... ***zhangae* sp. n.**

– Hypandrium with ventral and dorsal black saw-toothed extensions (Figure 15) (Cambodia) .... ***monstruosa* sp. n.**

General discussion

Diversity

With twelve species known now in the Oriental Realm, the genus *Lichtwardtia* is apparently quite species rich especially in view of the poor local sampling. Here we added eight species new for science. Interestingly, the external non-genitalic morphology is not very diverse but the male terminalia are distinctly different. It is perhaps too early to place all species into species groups but some closely related species can be distinguished.

A first large species group is the *L. nodulata* group with *cambodiensis* sp. n., *L. dentalis* Zhang et al., *L. nodulata* sp. n., *L. semakau* sp. n., and *L. singaporensis* sp. n.

The sister-group *L. polychroma* and *L. zhangae* are probably related to the *nodulata*-group in having a similarly shaped cercus with some strong flattened marginals and a dorsal marginal that shifted to the inside. However the postgonite is tubiform while it is broad with bifid tip in the *L. nodulata* group. Moreover the tip of the phallus is not ventrally denticulate and the hypandrium has a strong subapical dorsal hook. Provisionally we put both species in the *polychroma*-group.

Lichtwardtia conspicabilis sp. n. and *L. monstruosa* sp. n. seem to be related in having a forked hypandrium and a forked phallus, thus lacking the ventral denticles on the tip of the phallus in the *nodulata* group. Provisionally we place both species in the *conspicabilis*-group.

It is not clear if *L. infuscata* sp. n. belongs to the *L. nodulata* group though it has the tip of the phallus ventrally denticulate and a simple hypandrium. The cercus is more elongate and lacks a dorsal bristle at the inside. *L. formosana* looks different but has a double row of microscopic denticles ventrally on the tip of the phallus (Figure 26A). Otherwise the shape of the postgonite is identical to the *L. nodulata*-group. The cercus lacks a strong dorsal bristle at the inside. *L. hirsutiseta* is quite unique as its callus of the

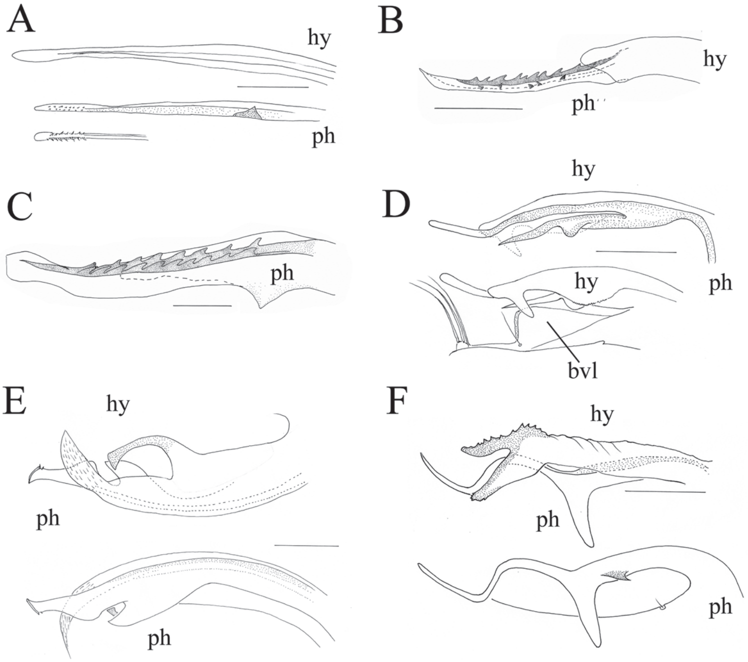

Figure 26. Variation in morphology of hypandrium and phallus in Oriental *Lichtwardtia* species. Scale bars: 0.1 mm. **A** *formosana*-group: *L. formosana*: phallus slender, hidden in hypandrium. Tip of phallus ventrally with a double row of microscopic denticles **B** *nodulata*-group: *L. nodulata*: tip of phallus ventrally with strong denticles. Tip of phallus never retracted in the hypandrium **C** *nodulata*-group: *L. infuscata*: dorsal spine present at base of exposed part of phallus **D** *polychroma*-group: *L. polychroma* hypandrium with a large dorsal preapical tooth resting on a large basoventral epandrial lobe. Phallus robust, forked **E** *conspicabilis*-group: *L. conspicabilis:* tip of hypandrium membranous and with a right extension. Phallus robust with a forked tip **F** *conspicabilis*-group: *L. monstruosa* tip of forked hypandrium with denticles. Tip of phallus slender but with a large dorsal tooth near middle of phallus.

costa is well separated and before R_1 reaching the costa. The complex male terminalia resemble *L. monstruosa* sp. n., but not *L. polychroma*. Thus we leave it unplaced.

At the moment we could only provide barcodes of the species from Singapore (uploaded to GenBank, with accession number MH536852-MH536856). The material from Cambodia was collected in ethanol of poor quality and therefore not suitable for sequencing. The optimal tree with the sum of branch length = 0.27350170 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. As can be seen in Figure 27 the species *L. nodulata* sp. n. and *L. singaporensis* sp. n. cluster well while *L. formosana* is more distant, as what it inferred from morphological study. The species with simple phallus (*L. formosana*) is shown to have a more primitive placement, which clearly demonstrates that the species with more complex male genitals are the derived species, the characters are tending to be more complicated by the evolution. The phylogeny also support the monophyly of *Lichtwardtia*.

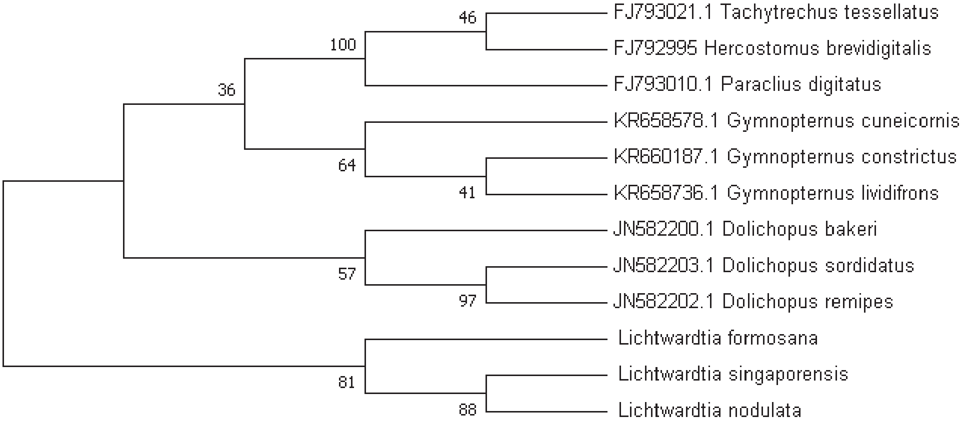

Figure 27. Phylogeny of Singaporean *Lichtwardtia* species and some other species of Dolichopodinae.

From bare to heavily armed male terminalia

As can be seen on Figure 26 it seems as if a various diversification in armament of the phallus and hypandrium took place in Oriental *Lichtwardtia* species. There have been such finding in beetles and bugs, but never had such a phenomenon reported from Diptera (Crudgington and Siva-Jothy 2000; Matsumura et al. 2017; Rōnn et al. 2007; Stutt and Siva-Jothy 2001). It has been proved that males with longer genital spines were more successful in gaining fertilisations, providing experimental evidence that male genital morphology influences success in post-copulatory reproductive competition (Hotzy et al. 2012). Normally, three potential benefits are speculated for such specialisation: last-male sperm precedence, suboptimal re-mating frequencies for the maintenance of female fertility, and reduced longevity and reproductive success in females (Stutt and Siva-Jothy 2001). These are all plausible explanations in our case. On the other hand no distinct male secondary sexual characters so typical for dolichopodids are present such as coloured whiskers, flag-like tarsi usually used in display to the females.

Acknowledgements

We thank Dr Zoltán Soltész (Hungarian Natural History Museum, Budapest) who checked the collection in Budapest for the type of *Rhagoneurus coxalis* Kertész and for communicating the report about the loss of the Diptera collection in Budapest. Dr Thomas Pape (Copenhagen) helped us with the photos of the holotype of *Lichtwardtia ziczac* (Wiedemann). Mr Ben Brugge (Naturalis, the Netherlands) sent us the photos of the holotype of *L. hirsutiseta* (de Meijere). Sven Marotzke (MfN, Berlin) recovered the lectotype of *L. polychroma* and provided us with photos made by Bernhard Schurian. Prof Rudolf Meier helped with the sequencing of the specimens of Singapore.

Finally Dr Lili Zhang commented on the species that she described. Ms Maimon Hussin (LKCNHM, NUS) Singapore made a number of stacked photos.

Thanks for the help of Dr Torsten Dikow and Dr Norman Woodley (Smithsonian Institution, Washington DC, USA) we could trace the holotype of *L. polychroma* (Loew).

The sampling in Siem Reap (Cambodia) coordinated by Mr Jérôme Constant (RBINS) was part of a Global Taxonomy Project with financial support from the Belgian Directorate-General for Development Cooperation, partim GTI.

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Checklist of known moth flies and sand flies (Diptera, Psychodidae) from Armenia and Azerbaijan

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Abstract

All credible and available published records for 17 species of moth flies known so far from Armenia (Phlebotominae 11 species, Psychodinae 6 species) and Azerbaijan (Phlebotominae 18 species) are summarized. The first records of 18 species of Psychodinae (tribes Mormiini, Paramormiini, Psychodini, Pericomaini) from Armenia and 6 new faunistic records (tribes Psychodini, Pericomaini) for the fauna of Azerbaijan are listed. The checklist of recent moth flies from Armenia includes now 35 species, and from Azerbaijan, 24 species.

Keywords

Caucasus, checklist, Diptera, faunistics, first records, Phlebotominae, Psychodidae, Psychodinae

Introduction

As mentioned by Oboňa et al. (2017b), the Caucasus is among the top 25 biologically richest and most endangered hotspots in the world, with an exceptional concentration of endemic species and species at risk. It is also one of the ecoregions of WWF's Global 200 project, which were identified as having outstanding terrestrial, freshwater, and marine habitats (Myers et al. 2000; Krever et al. 2001). The remarkable richness of the flora and fauna is determined by complex orography, geology, and climate and results in a variety of habitats, landscapes, and microclimates in this mountain range at the border of Europe and Asia and at the junction of temperate and subtropical zones. This area is affected by both Atlantic air masses and the dry continental climate of continental Eurasia (Price 2000).

The location of this area creates favourable conditions for entomological research. However, several families of flies have not been well studied in the Caucasus, and in particular, in Armenia and Azerbaijan (Oboňa et al. 2016b, 2017b).

Moth flies (Psychodidae) are represented only by 17 species previously recorded in Armenia (mainly Perfil'ev 1966; Lewis 1982; Artemiev and Neronov 1984; Vaillant 1972; Wagner 1981, 1990, 2013; Wagner and Joost 1986; Wagner et al. 2002; Melaun et al. 2014; Oboňa et al. 2017a). These species belong to subfamilies Phlebotominae [11 species: *Phlebotomus* (*P.*) *papatasi* (Scopoli, 1786); *P.* (*Paraphlebotomus*) *alexandri* Sinton, 1928; *P.* (*P.*) *cucasicus* Marzinovsky, 1917; *P.* (*P.*) *sergenti* Parrot, 1917; *P.* (*P.*) *similis* Perfil'ev, 1963; *P.* (*Larroussius*) *kandelakii* Shurenkova, 1929; *P.* (*L.*) *tobbi* Adler & Theodor in Adler, Theodor & Lourie, 1930; *P.* (*Adlerius*) *halepensis* Theodor, 1958; *P.* (*A.*) *simici* Nitzulescu, 1931; *Sergentomyia* (*Neophlebotomus*) *pawlowskyi* (Perfiliev, 1933); and *S.* (*Parrotomyia*) *palestinensis* (Adler & Theodor, 1927)] and Psychodinae [6 species: *Parajungiella monikae* (Wagner & Joost, 1986); *Paramormia* (*P.*) *fratercula* (Eaton, 1893); *Joostiella caucasica* Vaillant, 1983; *Pericoma* (*P.*) *exquisita* Eaton, 1893; *Pneumia joosti* (Wagner, 1981); and *Thornburghiella veve* Oboňa & Ježek, 2017].

From Azerbaijan, 18 species of Psychodidae have been recorded, all belonging to subfamily Phlebotominae [*Phlebotomus* (*P.*) *papatasi* (Scopoli, 1786); *P.* (*Paraphlebotomus*) *alexandri* Sinton, 1928; *P.* (*P.*) *cucasicus* Marzinovsky, 1917; *P.* (*P.*) *jacusieli* Theodor, 1947; *P.* (*P.*) *mongolensis* Sinton, 1928; *P.*(*P.*) *sergenti* Parrot, 1917; *P.* (*P.*) *similis* Perfil'ev, 1963; *P.* (*Larroussius*) *kandelakii* Shurenkova, 1929; *P.* (*L.*) *perfiliewi transcaucasicus* Perfiliew, 1937; *P.* (*L.*) *perniciosus* Newstead, 1911; *P.* (*L.*) *tobbi* Adler & Theodor in Adler, Theodor & Lourie, 1930; *P.* (*Adlerius*) *balcanicus* Theodor, 1958; *P.* (*A.*) *brevis* Theodor & Mesghali, 1964; *P.* (*A.*) *halepensis* Theodor, 1958; *P.* (*A.*) *simici* Nitzulescu, 1931, *Sergentomyia* (*S.*) *dentata* Sinton, 1933; *S.* (*Neophlebotomus*) *pawlowskyi* (Perfiliev, 1933); and *S.* (*Parrotomyia*) *palestinensis* (Adler & Theodor, 1927)].

Material and methods

The material presented here comes from two different sampling campaigns. The first campaign collected material by sweep-netting vegetation along streams and lakes in Armenia from August 26 to September 4, 2015 by J. Oboňa, P. Manko and Ľ. Hrivniak; it is preserved in 75% ethanol. A list of 28 sampling sites, with coordinates and altitudes, is given in Table 1. Captured moth flies were mounted in Canada balsam on 85 slides in the laboratory. The second collection campaign by Ľ. Hrivniak in Azerbaijan from May 26 to June 4, 2017 used the same collecting methods. Samples were collected at various sites by sweep-netting from vegetation along streams and lakes and

Table 1. List of sampling sites in Armenia.

Site no.	site name (province, short description of locality)	Latitude (N)	Longitude (E)	Altitude (m) a.s.l.
Arm 1	Yerevan, Tigran Mets Avenue	40°10'19.6"	44°30'49.1"	976
Arm 2	Kotayk Province, Marmarik district, Marmarik Secondary School	40°35'04.0"	44°40'10.1"	1750
Arm 3	Kotayk Province, Marmarik district, Marmarik near roud H28	40°35'04.0"	44°40'10.1"	1750
Arm 4	Kotayk Province, Marmarik River, below Hankavan	40°38'04.7"	44°29'19.4"	1974
Arm 5	Kotayk Province, Hrazdan River, above Solak town	40°28'19.7"	44°42'42.2"	1567
Arm 6	Kotayk Province, Hrazdan River, below Hrazdan Reservoir	40°29'12.8"	44°43'55.9"	1705
Arm 7	Kotayk Province, tributary of Marmarik River, above Meghradzor, behind railway	40°37'12.7"	44°40'18.3"	1825
Arm 8	Kotayk Province, tributary of the Marmarik River, above recreation center	40°33'52.0"	44°40'09.1"	1872
Arm 9	Tavush Province, Lake Parz Lich and its tributary, lake Parz Lich	40°44'57.7"	44°57'33.3"	1376
Arm 10	Tavush Province, Bldan River, above the Dilijan City	40°44'49.1"	44°49'03.5"	1354
Arm 11	Tavush Province, Bldan River, below Jukhtakvank monastery and the mineral water factory/plant	40°45'11.8"	44°48'25.7"	1411
Arm 12	Tavush Province, tributary of Aghstev River, above Teghut town	40°48'09.3"	44°53'43.7"	1382
Arm 13	Gegharkunik Province, Dzknaget River, at Sevan Lake and road M14	40°37'01.8"	44°57'44.2"	1930
Arm 14	Ararat Province, Gekhard River, at Gerghard monastery (parking place)	40°11'03.2"	44°31'18.6"	1770
Arm 15	Ararat Province, Gekhard River, below Garni Temple	40°08'24.7"	44°49'04.2"	1240
Arm 16	Ararat Province, small tributary of Azat River, waterfall at road	40°06'33.2"	44°43'49.3"	1249
Arm 17	Ararat Province, above the confluence of Azat and Gekhard rivers	40°06'39.4"	44°43'45.3"	1273
Arm 18	Ararat Province, small tributary of Gekhard River, crossroad at the factory	40°07'00.4"	44°44'35.7"	1340
Arm 19	Ararat Province, Azat River, at Lanjazat village	40°03'27.0"	44°34'38.3"	976
Arm 20	Lori Province, tributary of the Aghstev River, above M8 road at Lermontov village	40°45'24.6"	44°38'42.0"	1853
Arm 21	Lori Province, Zamanlu River, a tributary of Pambak River, at Vahagnadzor town	40°53'07.0"	44°34'39.0"	1092
Arm 22	Lori Province, tributary of Pambak River, at the H24 road switch-backs	40°56'52.7"	44°37'37.2"	1030
Arm 23	Lori Province, small steppe brook, tributary of Dzoraget River	41°03'59.9"	44°05'44.2"	1949
Arm 24	Shirak Province, tributary of Akhurian River, in valley below road from above Amasia town	40°58'20.5"	43°46'06.9"	1987
Arm 25	Shirak Province, tributary of Akhurian River, at Torosgyugh village	40°55'55.0"	43°52'45.3"	1885
Arm 26	Lori Province, small brook, in valley at the road H23 to Pushkin Pass	40°54'22.9"	44°25'33.3"	1839
Arm 27	Lori Province, tributary of the Dzoraget River, above Pushkin village	40°58'04.8"	44°24'49.7"	1485
Arm 28	Tavush Province, tributary of the Gosh River, spring area at parking place and cafeteria	40°44'15.9"	45°01'01.2"	1039

Table 2. List of sampling sites in Azerbaijan.

Site no.	Site name (province, short description of locality)	Latitude (N)	Longitude (E)	Altitude (m) a.s.l.
Aze 1	Khizi district, S of Sitalçay, wetland/pasture near Sumgayit bypass highway	40°40'38.4"	49°29'08.9"	169
Aze 2	Quba district, Xinaliq village, mountain stream	41°11'00.3"	48°07'42.9"	2170
Aze 3	Lankaran district, SW of Lankaran, stream with woody vegetation, tributary of Lankaran River	38°42'59.2"	48°44'17.8"	75
Aze 4	Qabala district, S of Durca, light trap near stream, tributary of Qaraschay River	41°02'11.2"	47°53'13.6"	1236

light trapping by H. A list of 4 sampling sites, with coordinates and altitudes, is given in Table 2. The collected material was preserved in 96% ethanol in the field. Captured moth flies were mounted in Canada balsam on 7 slides in laboratory.

All material, determined by the first author, is deposited in the National Museum, Natural History Museum, Department of Entomology, Prague, Czech Republic. Slides are numbered by inventory slide number of the family Psychodidae, and catalogue number (cat. no.) of the slide to be included in the NMPC Diptera collection (Tkoč et al. 2014). Nomenclature is according to Perfil'ev (1966), Lewis (1982), and Artemiev and Neronov (1984) for Phlebotominae; for Psychodinae the nomenclature is modified from Vaillant (1972) and Wagner (1990, 2013) using the classifications of e.g. Ježek and van Harten 2005, Oboňa and Ježek (2014), and Kroča and Ježek (2015). The following abbreviations are used in the paper: H = Ľ. Hrivniak leg., J = J. Ježek det., Ma = P. Manko leg., O = J. Oboňa leg., NMPC = collections of the National Museum Prague, HC = hand collecting, SW = sweep netting, LT = light traps.

Results

Psychodidae
Phlebotominae
Phlebotomus Rondani, 1840 (sensu Sabrosky, 1999)
Subgenus *Phlebotomus* s. str.

1. *Phlebotomus* (*Phlebotomus*) *papatasi* (Scopoli, 1786)

Selected published records. Perfil'ev (1966: 252–254); Lewis (1982: 138–140); Artemiev and Neronov (1984: 39, 40); Wagner (1990: 13); Wagner et al. (2002: 323); Melaun et al. (2014: 2299).

Distribution. Afghanistan, Albania, Algeria, Armenia, Azerbaijan, Baleares, Bosnia and Herzegovina, Bulgaria, Crete, Crimea, Croatia, Cyprus, Egypt, Ethiopia, France, Georgia, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Jordan, Kazakhstan (southern), Kuwait, Libya, Macedonia, Malta, Moldova, Montenegro, Morocco, Oman, Pakistan, Portugal, Romania, Sardinia, Saudi Arabia, Serbia, Slovenia, Spain, Sudan, Syria, Tunisia, Turkey, Ukraine (southern), Yemen.

Subgenus *Paraphlebotomus* Theodor, 1948

2. *Phlebotomus* (*Paraphlebotomus*) *alexandri* Sinton, 1928

Selected published records. Perfil'ev (1966: 264–267); Lewis (1982: 143); Artemiev and Neronov (1984: 45); Wagner (1990: 14); Melaun et al. (2014: 2298).

Distribution. Afghanistan, Albania, Algeria, Armenia, Azerbaijan, Bulgaria, China (western), Crimea, Cyprus, Djibouti, Ethiopia, Georgia, Greece, India, Iran, Iraq, Israel, Kazakhstan (southern), Moldova, Mongolia, Morocco, Pakistan, Romania, Saudi Arabia, Spain, Sudan, Tunisia, Turkey, Ukraine, United Arab Emirates, Yemen; northern Sahara, Caucasus (southern), Near and Middle East, Central and Eastern Asia; Afrotropical and Oriental regions.

3. *Phlebotomus* (*Paraphlebotomus*) *cucasicus* Marzinovsky, 1917

Selected published records. Perfil'ev (1966: 257–261, as *grimmi* Porchinskyi, 1876); Lewis (1982: 144–145, as *caucasicus*); Artemiev and Neronov (1984: 49, as *caucasicus*); Wagner (1990: 14, as *caucasicus*); Melaun et al. (2014: 2298, as *caucasicus*).

Distribution. Afghanistan, Armenia, Azerbaijan, Bulgaria, China (not verified), Georgia, Greece, Iran, Kazakhstan, Macedonia, Turkmenistan, Uzbekistan.

4. *Phlebotomus* (*Paraphlebotomus*) *jacusieli* Theodor, 1947

Selected published records. Perfil'ev (1966: 110, distribution only); Lewis (1982: 145); Artemiev and Neronov (1984: 50); Wagner (1990: 14); Melaun et al. (2014: 2298).

Distribution. Albania, Azerbaijan, Cyprus, Greece, Israel, Jordan, Iran, northern Palestine, Turkey.

5. *Phlebotomus* (*Paraphlebotomus*) *mongolensis* Sinton, 1928

Syn. *Phlebotomus imitabilis* Artemiev, 1974 (see Artemiev 1978: 18).

Selected published records. Perfil'ev (1966: 267–271); Lewis (1982: 146); Artemiev and Neronov (1984: 55–56); Wagner (1990: 14); Melaun et al. (2014: 2299).

Distribution. Afghanistan, Azerbaijan, China, Iran, Kazakhstan, Mongolia.

6. *Phlebotomus* (*Paraphlebotomus*) *sergenti* Parrot, 1917

Selected published records. Perfil'ev (1966: 274–278, only important diagnostic characters of subsp. *sergenti* in comparison with subsp. *similis* Perfil'ev, 1963); Lewis (1982: 147, recognized two mentioned subspecies); Artemiev and Neronov (1984: 57, recognized *sergenti* and *similis* as two bona species); Wagner (1990: 14, as species); Wagner et al. (2002: 323); Melaun et al. (2014: 2299, as species).

Distribution. Afghanistan, Albania, Algeria, Armenia, Azerbaijan, Baleares, Bosnia and Herzegovina, Bulgaria, Canary I., China, Croatia, Cyprus, Egypt, France (Corsica), Georgia, Greece, India, Iran, Iraq, Israel, Italy, Jordan, Kazakhstan, Lebanon, Libya, Madeira, Macedonia, Mali, Malta, Morocco, Portugal, Romania, Saudi Arabia, Serbia, Slovenia, Somali Republic, Spain, Syria, Tunisia, Turkey, Ukraine, Yemen. Afrotropical and Oriental regions.

7. ***Phlebotomus* (*Paraphlebotomus*) *similis* Perfil'ev, 1963 (sensu Artemiev & Neronov, 1984)**

Phlebotomus (*Paraphlebotomus*) *sergenti similis* Perfil'ev, 1963

Selected published records. Perfil'ev (1966: 276); Lewis (1982: 148, as *sergenti similis*); Artemiev and Neronov (1984: 58–59, as a species).

Distribution. Perfil'ev (1966): southern coast of Crimea and southern Ukraine, Russia, northern Caucasus. Lewis (1982): same, Azerbaijan, Uzbekistan. Artemiev and Neronov (1984): Crimea, southern Ukraine, ?northern Caucasus, Albania, Romania, and former Yugoslavia. Melaun et al. (2014: 2299): Albania, Armenia, Azerbaijan, Bosnia and Herzegovina, Bulgaria, Croatia, Georgia, Greece, Macedonia, Romania, Serbia, Slovenia, Ukraine.

Subgenus *Larroussius* Nitzulescu, 1931

8. ***Phlebotomus* (*Larroussius*) *kandelakii* Shurenkova, 1929**

Selected published records. Perfil'ev (1966: 286, as *kandelakii*); Lewis (1982: 154, ssp. *kandelakii* and *burneyi* Lewis, 1967 recognized); Artemiev and Neronov (1984: 87, *burney* as a species); Wagner (1990: 15); Melaun et al. (2014: 2298).

Distribution. Afghanistan, Armenia, Azerbaijan, Dagestan, Georgia, Iran, Iraq, Lebanon, Turkey, Turkmenistan, Uzbekistan.

9. ***Phlebotomus* (*Larroussius*) *major syriacus* Adler & Theodor, 1931**

Selected published records. Perfil'ev (1966: 278–286, four subspecies recognized: *major*, *neglectus* Tonnoir, 1921, *syriacus* Perfil'ev, 1966 and *krimensis* Perfil'ev, 1966); Lewis (1982: 156–157, added *wui* Yang & Xiong, 1965); Artemiev and Neronov (1984: 92–95, considered *neglectus* as a separate valid species, but taxonomic position of *syriacus* is unclear due to the missing differential diagnosis in the original description); Wagner (1990: 16, recognized all four subspecies); Melaun et al. (2014: 2298, recognized *syriacus* as a species).

Distribution. Armenia, Azerbaijan, Crimea, Georgia, Greece (Crete), Israel, Italy (Sicily), Jordan, Lebanon, Palestine, Serbia, Syria, Turkey, Ukraine.

10. ***Phlebotomus* (*Larroussius*) *perfiliewi transcaucasicus* Perfiliew, 1937**

Selected published records. Perfil'ev (1966: 289–293, recognized three subspecies: *perfiliewi*, *transcucasicus*, and *galilaeus* Theodor, 1958); Lewis (1982: 160–161, 3 subspecies); Artemiev and Neronov (1984: 88, map 30, three subspecies); Wagner (1990: 16–17, three subspecies).

Distribution. Azerbaijan, Iran, Iraq, Russia, Uzbekistan.

11. *Phlebotomus* (*Larroussius*) *perniciosus* Newstead, 1911

Selected published records. Perfil'ev (1966: 299–300, only comments on systematic position); Lewis (1982: 161–162); Artemiev and Neronov (1984: 99–100); Wagner (1990: 17); Wagner et al. (2002: 323); Melaun et al. (2014: 2298).

Distribution. Albania, Algeria, Andorra, Azerbaijan, Baleares, Bosnia and Herzegovina, Bulgaria, Canary I., Corsica, Croatia, Cyprus, France, Germany, Greece, Italy, Libya, Macedonia, Malta, Morocco, Portugal, Romania, Sardinia, Serbia, Sicily, Slovenia, Spain, Switzerland, Tunisia, Turkey.

12. *Phlebotomus* (*Larroussius*) *tobbi* Adler & Theodor in Adler, Theodor & Lourie, 1930

Selected published records. Perfil'ev (1966: 296–300); Lewis (1982: 162–163); Artemiev and Neronov (1984: p. 101–102); Wagner (1990: 17); Melaun et al. (2014: 2298).

Distribution. Albania, Armenia, Azerbaijan, Bosnia and Herzegovina, Croatia, Cyprus, Georgia, Greece, Iran, Israel, Italy, Jordan, Lebanon, Palestine, Serbia, Sicily, Slovenia, Syria, Turkey.

Subgenus *Adlerius* Nitzulescu, 1931

13. *Phlebotomus* (*Adlerius*) *balcanicus* Theodor, 1958

Selected published records. Perfil'ev (1966: 316–317, as *P. chinensis balcanicus* Theodor, 1958); Lewis (1982: 165, as species); Artemiev and Neronov (1984: 114, as species); Wagner (1990, 2013: 17, as species); Melaun et al. (2014: 2298, as species).

Distribution. Albania, Azerbaijan, Bosnia and Herzegovina, Bulgaria, Caucasus, Crimea, Croatia, Georgia, Greece, Hungary, Iran (north-western), Macedonia, Romania, Serbia, Turkey, Ukraine.

14. *Phlebotomus* (*Adlerius*) *brevis* Theodor & Mesghali, 1964

Syn. *Phlebotomus chinensis ismailicus* Perfil'ev, 1966

Published records. Perfil'ev (1966: 314, as *P. chinensis ismailicus* Perfil'ev, 1966); Lewis (1982: 165); Artemiev and Neronov (1984: 115); Wagner (1990, 2013: 17); Melaun et al. (2014: 2298).

Comments on distribution. Artemiev and Neronov (1984): only Iran, southern Caucasus, Turkey. Wagner (1990, 2013) listed records from Moldova. Melaun et al. (2014) listed the species from Azerbaijan, Georgia, Greece and Malta.

15. *Phlebotomus* (*Adlerius*) *halepensis* Theodor, 1958

Selected published records. Perfil'ev (1966: 305–320, recognized 7 subspecies of *Phlebotomus chinensis* Newstead, 1916: *chinensis*, *simici* Nitzulescu, 1931, *longiductus* Parrot, 1928, *tauriae* Perfil'ev, 1966, *ismailicus* Perfil'ev, 1966, *balcanicus* and *halepensis*). Lewis (1982: 164–168, recognized five subspecies as valid species and supported synonymies proposed by Artemiev (1978, 1980): *tauriae* = *longiductus*, *ismailicus* = *brevis* Theodor & Mesghali, 1964); Artemiev and Neronov (1984: 107–125, discussed taxonomic problems); Wagner (1990: 18, as species); Melaun et al. (2014: 2298, as species).

Distribution. Armenia, Azerbaijan, Georgia, Iran, Israel, Kazakhstan, Kirghistan, Russia, Syria, Tajikistan, Turkey, Turkmenistan, Uzbekistan.

16. *Phlebotomus* (*Adlerius*) *simici* Nitzulescu, 1931

Selected published records. Perfil'ev (1966: 307, as subspecies *chinensis simici*, see *P. halepensis*); Lewis (1982: 168 as species); Artemiev and Neronov (1984: 123, as species); Wagner (1990: 18, as species).

Distribution. Armenia, Azerbaijan, Crete, Georgia, Greece, Jordan, Iran, Israel, Kazakhstan, Palestine, Romania, Russia, Syria, Turkey, former Yugoslavia.

Sergentomyia França & Parrot, 1920

Subgenus *Sergentomyia* s. str.

17. *Sergentomyia* (*Sergentomyia*) *dentata* Sinton, 1933

Selected published records. Perfil'ev (1966: 337); Lewis (1967: 25); Wagner (1990: 23).

Distribution. Azerbaijan, Greece, Iran, Iraq, Kazakhstan, Pakistan, Turkey, Turkmenistan, Uzbekistan.

Subgenus *Neophlebotomus* França & Parrot, 1920

18. *Sergentomyia* (*Neophlebotomus*) *pawlowskyi* (Perfiliev, 1933)

Selected published records. Perfil'ev (1966: 361–365, originally described the species as a *Phlebotomus*, Lewis (1967) later included it in *Sergentomyia* (*Rondanomyia*) Theodor, 1958; this subgenus was synonymized with *Neophlebotomus* (Lewis 1978: 269)); Wagner (1990: 22).

Distribution. Afghanistan, Armenia, Azerbaijan, Georgia, Iran, Iraq, Tajikistan, Turkey, Turkmenistan, Uzbekistan.

Subgenus *Parrotomyia* Theodor, 1958

19. *Sergentomyia* (*Parrotomyia*) *palestinensis* (Adler & Theodor, 1927)

Selected published records. Adler & Theodor (1927) originally described this species in *Phlebotomus*; Lewis (1967) later included it in the genus *Sergentomyia*, which was recognized as well by Perfil'ev (1966: 352–355); Perfil'ev (1968: 318, 326, in subgenus *Parrotomyia*); Lewis (1978: 264, in subgenus *Parrotomyia*); Wagner (1990: 25, subgenus *Parrotomyia*).

Distribution. Afghanistan, Armenia, Azerbaijan, Georgia, Iran, Iraq, Israel, Jordan, Palestine, Pakistan, Saudi Arabia. Oriental Region.

Psychodinae
Mormiini
Mormiina
Yomormia Ježek, 1984

20. *Yomormia petrovi* Ježek, 1985

Material examined. Armenia: Tavush Province, Bldan River, below Jukhtakvank monastery and the mineral water factory/plant, Arm 11, 28.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22537.

Comments. This species was known only from the original description from Sandanski, Bulgaria (Ježek 1985). Krek (1999) listed this species as *Limomormia* (*Limomormia*) *petrovi* (Ježek 1985: 122–123). New for Armenia.

Paramormiini
Paramormiina
Clogmia Enderlein, 1937

21. *Clogmia albipunctata* (Williston, 1893)

Material examined. Armenia: Yerevan, Tigran Mets Avenue, Arm 1, 30.viii.2015, 1 ♂, O leg., HC, NMPC slide 22575.

Comments. This is an expansive, often synanthropic circumtropical and circumsubtropical species (Ježek and Goutner 1995; Werner 1997; Wagner et al. 2002; Boumans 2009; Boumans et al. 2009; Ježek et al. 2012; Oboňa and Ježek 2012; Kvifte et al. 2013; Wagner 2013; Humala and Polevoi 2015; Afzan and Belqat 2016; Bejarano and Estrada 2016; Oboňa et al. 2016a; Afzan and Belqat 2016; Cazorla-Perfetti and Moreno 2017). New for Armenia.

Parajungiella Vaillant, 1972

21. *Parajungiella abchazica* Ježek, 1985

Material examined. Armenia: Tavush Province, Bldan River, above Dilijan City, Arm 10, 28.viii.2015, 1♂, O Ma H leg. SW, NMPC slide 22528; Bldan River, below Jukhtakvank monastery and the mineral water factory/plant, Arm 11, 28.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22538.

Comments. This species was known by its original description from Saken-narzan, Abkhazia (Ježek 1985: 52–55). New for Armenia.

22. *Parajungiella monikae* (Wagner & Joost, 1986), comb. n.

Comments. This species from Armenia was originally described by Wagner and Joost (1986: 112) as *Jungiella monikae*. Its generic placement was changed to *Parajungiella* Vaillant, 1972 (: 83, originally as subgenus of *Jungiella* Vaillant, 1972) by Ježek (1984: 166). This arrangement was subsequently followed by Krek (1999: 5), Kvifte et al. (2011), Salmela et al. (2014), and Omelková and Ježek (2017).

Paramormia Enderlein, 1935

Subgenus *Duckhousiella* Vaillant, 1972

23. *Paramormia* (*Duckhousiella*) *ustulata* (Walker, 1856)

Material examined. Armenia: Lori Province, tributary of Dzoraget River, above Pushkin village, Arm 27, 3.ix.2015, 1M, O Ma H leg. SW, NMPC slide 22541; Ararat Province, above the confluence of Azat and Gekhard rivers, Arm 17, 31.viii.2015,1 ♂, O Ma H leg. SW, NMPC slide 22582; Lori Province, tributary of Pambak River, at the H24 road switch-backs , Arm 22, 1.ix.2015, 1♀, O Ma H leg. SW, NMPC slide 22589; Kotayk Province, Hrazdan River, below Hrazdan Reservoir, Arm 6, 27.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22597.

Comments. This is a widespread species or complex of species occurring in the Holarctic region. Ježek and Yağci (2005) listed occurrences from the following countries: Afghanistan, Algeria, the Azores, Belgium, Bosnia-Herzegovina, Bulgaria, the Canary Islands, China, Corsica, Czech Republic, Denmark, France, Germany, Great Britain, Greece, Hungary, Iran, Ireland, Israel, Italy, Macedonia, Madeira, Mongolia, Morocco, the Netherlands, Poland, Romania, Sardinia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tunisia, Turkey and the USA. It has also been recorded from Finland (Salmela et al. 2014) and Mallorca (Kvifte et al. 2016). New for Armenia.

Subgenus *Paramormia* s. str.

24. *Paramormia* (*Paramormia*) *fratercula* (Eaton, 1893)

Comments. Eaton (1893: 128) described this species as *Pericoma fratercula* from Great Britain. Ježek (1984: 162, 163) considered it to be a *Paramormia* (*Paramormia*), within the tribe Paramormiini, and Wagner (1990: 49) placed it in *Parmormia fratercula* in tribe Telmatoscopini.

Distribution. Denmark, Germany, Great Britain, Hungary, the Netherlands, Sweden (Wagner 1990, 2013). As first record from Armenia by Wagner and Joost (1986: 111).

25. *Paramormia* (*Paramormia*) *polyascoidea* (Krek, 1971)

Material examined. Armenia: Shirak Province, tributary of Akhurian River, at Torosgyugh village, Arm 25, 3.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22600.

Comments. This is a common European and west Siberian species known from Austria, Bosnia and Herzegovina, Czech Republic, Estonia, Finland, Germany, Poland, Abkhazia, and Russia (Novosibirsk region) (Salmela and Piirainen 2005; Ježek and Omelkova 2012; Salmela et al. 2014). New for Armenia.

Peripsychoda Enderlein, 1935

26. *Peripsychoda auriculata* (Haliday in Curtis, 1839)

Material examined. Armenia: Tavush Province, Bldan River, below Jukhtakvank monastery and mineral water factory/plant, Arm 11, 28.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22536; Tavush Province, Bldan River, above Dilijan City, Arm 10, 28.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22527.

Comments. This is a large, black, conspicuous European and Transcaucasian species that is very common in lowlands to hilly regions. For detailed distributional data, see Wagner (1990, 2013) and Ježek (2004). New for Armenia.

Psychodini

Logima Eaton, 1904

27. *Logima albipennis* (Zetterstedt, 1850)

Material examined. Armenia: Ararat Province, Gekhard River, at Gerghard monastery (parking place), Arm 14, 30.viii.2015, 1 ♀, O Ma H leg. SW, NMPC slide 22574. **Azerbaijan:** Qabala district, S of Durca, light trap near stream, tributary of Qaraschay River, Aze 4, 30.v.2017, 1 ♀, H leg. LT, NMPC slide 24175.

Comments. This is a cosmopolitan species, very common from lowlands to mountains.

Distribution. In Europe, it is known from Austria, Azores, Belgium, Bosnia and Herzegovina, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Luxemburg, Madeira, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Sardinia, Serbia, Slovakia, Slovenia, and Sweden. In Asia from Afghanistan, China, India, Japan, North Korea, Syria and Turkey. In Afrika, from Algeria, the Canary Islands, Gambia, South Africa, Tunisia; also from Australia, New Zealand, South America, USA; Campbell Island, Juan Fernandez Island, Kerguelen Island, Macquarie Island (Ježek and Yağci 2005; Kvifte 2012; Afzan and Belqat 2016). New for Armenia and Azerbaijan.

28. *Logima satchelli* (Quate, 1955)

Material examined. Azerbaijan: Qabala district, S of Durca, light trap near stream, tributary of Qaraschay River, Aze 4, 30.v.2017, 1 ♀, H leg. LT, NMPC slide 24174.

Distribution. This is a common Holarctic species. In Europe, known from e.g. Austria, Czech Republic, Ireland, Italy, Norway, Russia, Slovakia, Slovenia, Sweden, the Netherlands, Ukraine and the former Yugoslavia; Canada, USA (Ježek and Goutner 1995; Svensson 2009; Kvifte et al. 2011; Ježek et al. 2017). New for Azerbaijan.

Psychoda Latreille, 1796

29. *Psychoda uniformata* Haseman, 1907

Material examined. Armenia: Kotayk Province, tributary of Marmarik River, above Meghradzor, behind railway, Arm 7, 27.vii.2015, 1 ♀, O Ma H leg. SW, NMPC slide 22601; Lori Province, tributary of Dzoraget River, above Pushkin village, Arm 27, 3.ix.2015, 1 ♀, O Ma H leg. SW, NMPC slide 22542.

Comments. This is a Holarctic species, recorded from Europe (Austria, Czech Republic, Italy, Slovakia, Slovenia, Greece), Turkey, Iran, Israel, Mongolia, Morocco and the USA (Ježek and Omelková 2012; Oboňa and Ježek 2014; Afzan and Belqat 2016). New for Armenia.

Psychodocha Ježek, 1984

30. *Psychodocha cinerea* (Banks, 1894)

Material examined. Armenia: Ararat Province, Gekhard River, at Gerghard monastery (parking place), Arm 14, 30.viii.2015, 1 ♀, O Ma H leg. SW, NMPC slide 22573;

Lori Province, tributary of Dzoraget River, above Pushkin village, Arm 27, 3.ix.2015, 1 ♀, O Ma H leg. SW, NMPC slide 22543.

Distribution. This is a very common cosmopolitan species, in Europe, it is known from Austria, Azores, Belgium, Bosnia and Herzegovina, Bulgaria, Canary Islands, Cyprus, Czech Republic, Denmark, Finland, France, Finland, Germany, Great Britain, Greece, Hungary, Ireland, Italy (including Sardinia), Madeira, the Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and Ukraine. In Asia, from Abkhazia, Afghanistan, Iran, and Israel. In Africa, from Algeria, Morocco, South Africa, and Tunisia. In the Americas, from Argentina; Brazil, Canada, Chile, Jaun Fernandéz Island, Puerto Rico, USA. Also known from Australia and New Zealand (Krek 1985; Ježek and Goutner 1995; Ježek and Yağci 2005; Kvifte et al. 2011; Kvifte 2012; Wagner 1990, 2013; Salmela et al. 2014; Afzan and Belqat 2016; Ježek et al. 2017). New for Armenia.

Tinearia Schellenberg, 1803

31. *Tinearia alternata* (Say, 1824)

Material examined. Armenia: Lori Province, tributary of Dzoraget River, above Pushkin village, Arm 27, 3.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22540; Lori Province, tributary of Aghstev River, above M8 road at Lermontov village, Arm 20, 31.viii.2015,1 ♀, O Ma H leg. SW, NMPC slide 22559; Ararat Province, above the confluence of Azat and Gekhard rivers, Arm 17, 31.viii.2015, 1 ♀, O Ma H leg. SW, NMPC slide 22581; Kotayk Province, Marmarik district, Marmarik near road H28, Arm 3, 26.viii.2015, 1 ♀, O Ma H leg. LT, NMPC slide 22583; Ararat Province, Azat River, at Lanjazat village, Arm 19, 31.viii.2015, 1 ♀, O Ma H leg. SW, NMPC slide 22595; Kotayk Province, Marmarik district, Marmarik Secondary School, Arm 2, 27.viii.2015, 1 ♂, O Ma H leg. BH, NMPC slide 22608. **Azerbaijan:** Khizi district, S of Sitalçay, wetland/pasture near Sumgayit bypass highway, Aze 1, 26.v.2017, 1 ♀, H leg. SW, NMPC slide 24173.

Distribution. This is a cosmopolitan species that is generally very common. In Europe, it is known from Austria, Balearic Islands, Belgium, Bulgaria, Crete, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Madeira, Norway, Poland, Romania, Sardinia, Slovenia, Spain, Sweden, Switzerland, the Netherlands, and Ukraine. In Asia, from Afghanistan and UAE. In Africa from Algeria, Cape Verde Islands, Canary Islands, D.R. Congo, Egypt, Gambia, Ghana, Morocoo, Nigeria, the Seychelles, Socotra Island, South Africa, Tanzania, and Tunisia. In Asia from Bangladesh, Borneo, Philippines, India, Jamaica, Japan, Malaysia, Mongolia, North Korea, Ryukyu Islands, Taiwan. From North and South America, including Panama, Puerto Rico, and Trinidad. Also from Australia, Hawaii, and from Micronesia, Macquarie Islands, New Zealand, and Samoa. (Ježek 1981; Ježek and van Harten 1996; Kvifte et al. 2011; Kvifte 2012; Ježek and Tkoč 2012; Wagner 2013; Afzan and Belqat 2016; Ježek et al. 2017). New for Armenia and Azerbaijan.

Pericomaini
Joostiella Vaillant, 1983

32. *Joostiella caucasica* Vaillant, 1983

Comments. The genus and species, originally described from near the Baksar River, Zlohl in the central Caucasus, were published by Vaillant (1983: 335). Wagner (1986: 87) later recorded this species from Turkey and Armenia, and additional records from Armenia were published by Wagner and Joost (1986: 111).

Pericoma Walker, 1856
Subgenus *Pachypericoma* Vaillant, 1978

33. *Pericoma* (*Pachypericoma*) *blandula* Eaton, 1893

Material examined. Armenia: Tavush Province, tributary of Gosh River, spring area at parking place and cafeteria, Arm 28, 4.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22546; Lori Province, tributary of the Pambak River, at the H24 road switch-backs, Arm 22, 1.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22588; Tavush Province, tributary of Aghstev River, above Teghut town, Arm 12, 29.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22592; Lori Province, small brook, in valley at road H23 to Pushkin Pass, Arm 26, 3.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22594; Lori Province, Zamanlu River, a tributary of Pambak River, at Vahagnadzor town, Arm 21, 1.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22607. **Azerbaijan:** Lankaran district, SW of Lankaran, stream with woody vegetation, tributary of Lankaran River, Aze 3, 3.vi.2017, 2 ♂, H leg. SW, NMPC slides 24171 and 24172.

Distribution. This species is widespread in Europe, known from Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, European Turkey, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Macedonia, Montenegro, Norway, Poland, Romania, European Russia, Sardinia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, the Netherlands and Ukraine. It is also recorded in Transcaucasia, Tunisia, and Morocco (Ježek 2004; Kvifte et al. 2011, 2013; Ježek et al. 2017). New for Armenia and Azerbaijan.

Subgenus *Pericoma* s. str.

34. *Pericoma bosniaca* Krek, 1966

Published records. Krek (1966: 249, 250; 1967a: 256, 257, 258; 1967b: 316; 1970: 98; 1972: 63; 1974: 60; 1979a: 1807, 1809; 1982: 154; 1985: 155, 179; 1999:

378, 379, as *Pericoma* (*Leptopericoma*) *bosniaca* Krek, 1967); Krek et al. (1976: 29); Mučibabič et al. (1984: 64); Vaillant (1978: 211, 219, pl. 66 fig 6, pl. 67 figs 7, 8); Wagner (1990: 38, as *bosnica* Krek, 1967); Wagner and Joost (1988: 30, the same).

Material examined. Armenia: Ararat Province, small tributary of Azat River, waterfall at road, Arm 16, 31.viii.2015, 2 ♂, O Ma H leg. SW, NMPC slides 22551 and 22552.

Distribution. Bosnia and Herzegovina, Bulgaria, Montenegro, Serbia, Macedonia. New for Armenia.

35. *Pericoma bunae* Krek, 1979

Published records. Krek (1979b: 127, 128, 129); Wagner (1990: 38, journal reference error); Krek (1999: 376, 377, as *Pericoma* (*Leptopericoma*) *bunae* Krek, 1979).

Material examined. Azerbaijan: Quba district, Xinaliq village, mountain stream, Aze 2, 27.v.2017, 1 ♂, H leg. SW, NMPC slide 24170.

Distribution: This species is known from Bosnia and Herzegovina, as well as Montenegro. New for Azerbaijan.

36. *Pericoma exquisita* Eaton, 1893

Material examined. Armenia: Shirak Province, tributary of Akhurian River, in valley below road from above Amasia town, Arm 24, 2.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22530; Tavush Province, tributary of Gosh River, spring area at the parking place and cafeteria, Arm 28, 4.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22547; Ararat Province, Gekhard River, below Garni Temple, Arm 15, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22578; Ararat Province, above the confluence of Azat and Gekhard rivers, Arm 17, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22587; Kotayk Province, Hrazdan River, above Solak town, Arm 5, 27.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22599.

Distribution. This species is widespread in Europe, North Africa (Algeria, Morocco, and Tunisia), and Transcaucasia (Armenia; Wagner 1981: 56). In Europe, it is known from Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Czech Republic, Crete, Croatia, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Macedonia, Montenegro, Poland, Serbia, Slovakia, Slovenia, Spain and Ukraine (Ježek 2004, 2009; Kvifte et al. 2013; Wagner 2013; Oboňa and Ježek 2014; Afzan and Belqat 2016; Ježek et al. 2017).

37. *Pericoma platystyla* Wagner, 1986

Material examined. Armenia: Ararat Province, small tributary of Azat River, waterfall at road, Arm 16, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22553.

Comments. Wagner (1986: 84) described this species from Turkey and Greece. New for Armenia.

Pneumia Enderlein, 1935

38. *Pneumia canescens* (Meigen, 1804)

Material examined. Armenia: Shirak Province, tributary of Akhurian River, in valley below road from above Amasia town, Arm 24, 2.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22534; Tavush Province, tributary of Gosh River, spring area at parking place and cafeteria, Arm 28, 4.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22534.

Comments. This is a common European and western Siberian species. In Europe it is known Austria, Belgium, Great Britain, Czech Republic, Denmark, European Turkey, France, Germany, Greece, Hungary, Slovakia, Sweden, the Netherlands and the former Yugoslavia. In Asia, it is known from Turkey, Kyrgyzstan, Afghanistan and China (Ježek 2006; Omelková and Ježek 2012; Ježek et al. 2013). New for Armenia.

39. *Pneumia joosti* (Wagner, 1981)

Material examined. Armenia: Kotayk Province, tributary of Marmarik River, above recreation centre, Arm 8, 27.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22606; Kotayk Province, Hrazdan River, above Solak town, Arm 5, 27.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22598; Lori Province, small brook, in valley at road H23 to Pushkin Pass, Arm 26, 3.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22593; Lori Province, small steppe brook, tributary of Dzoraget River, Arm 23, 2.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22591; Kotayk Province, Marmarik River, below Hankavan, Arm 4, 26.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22586; Lori Province, tributary of Aghstev River, above M8 road at Lermontov village, Arm 20, 31.viii.2015, 6 ♂, O Ma H leg. SW, NMPC slides 22561–22566; Shirak Province, tributary of Akhurian River, in valley below road from above Amasia town, Arm 24, 2.ix.2015, 3 ♂, O Ma H leg. SW, NMPC slides 22531–22533.

Comments. Wagner (1981) described this species from Armenia as *Satchelliella joosti*.

Distribution. This species is known only from Armenia and Transcaucasus (Wagner 1981).

40. *Pneumia pilularia* (Tonnoir, 1940)

Material examined. Armenia: Kotayk Province, tributary of the Marmarik River, above recreation centre, Arm 8, 27.viii.2015, 3 ♂, O Ma H leg. SW, NMPC slides 22549, 22605 and 22579; Ararat Province, small tributary of Azat River, waterfall at road, Arm 16, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22550; Lori Province, tributary of Aghstev River, above M8 road at Lermontov village, Arm 20, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22560; Ararat Province, Gekhard River, at Gerghard monastery (parking place), Arm 14, 30.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22572; Ararat Province, above the confluence of Azat and Gekhard rivers, Arm 17, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22580; Ararat Province, small tributary of Gekhard River, crossroad at factory, Arm 18, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22603; Gegharkunik Province, Dzknaget River, at Sevan Lake and M14 road, Arm 13, 29.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22604.

Comments. This species is distributed throughout almost all of Europe, including Spain, the British Isles and Scandinavia. It is also known from Algeria, Morocco, the Central Caucasus (Terskol, Russia) and Tajikistan, but it is relatively sporadic there (Wagner 1990; Vaillant and Joost 1983; Ježek 2004; Ježek et al. 2014). New for Armenia.

41. *Pneumia nubila* (Meigen, 1818)

Material examined. Armenia: Tavush Province, Bldan River, above Dilijan City, Arm 10, 28.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22526; Shirak Province, tributary of Akhurian River, in valley below road from above Amasia town, Arm 24, 2.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22529; Tavush Province, Bldan River, below Jukhtakvank monastery and mineral water factory/plant, Arm 11, 28.viii.2005, 1 ♂, O Ma H leg. SW, NMPC slide 22535; Lori Province, tributary of Dzoraget River, above Pushkin village, Arm 27, 3.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22539; Tavush Province, tributary of Gosh River, spring area at parking place and cafeteria, Arm 28, 4.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22544; Kotayk Province, tributary of Marmarik River, above the recreation centre, Arm 8, 27.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22548; Lori Province, tributary of Aghstev River, above M8 road at Lermontov village, Arm 8, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22567; Ararat Province, Gekhard River, at Gerghard monastery (parking place), Arm 14, 30.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22571; Lori Province, small steppe brook, tributary of Dzoraget River, Arm 23, 2.ix.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22590; Kotayk Province, Hrazdan River, below Hrazdan Reservoir, Arm 6, 27.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22596.

Distribution. This is a very common species, which is recorded from throughout Europe and the Canary Islands. In Europe, it is known from Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Luxembourg, Macedonia, Montenegro, Poland, Romania, Sardinia, Serbia, Slovakia, Slovenia, Spain, Switzerland, the Netherlands and Ukraine (Wagner 1981; Ježek and Goutner 1995; Krek 1999; Ježek 2002; Kvifte et al. 2013; Ježek et al. 2016). New for Armenia.

Saraiella Vaillant, 1973

42. *Saraiella ressli ressli* Wagner, 1981

Material examined. Armenia: Ararat Province, small tributary of Azat River, waterfall at road, Arm 16, 31.viii.2015, 5 ♂, O Ma H leg. SW, NMPC slides 22554–22558; Lori Province, tributary of Aghstev River, above M8 road at Lermontov village, Arm

20, 31.viii.2015, 2 ♂, O Ma H leg. SW, NMPC slides 22569 and 22570; Tavush Province, Lake Parz Lich and its tributary, lake Parz Lich, Arm 9, 28.8.2015, 1 ♂, O Ma H leg. SW, NMPC slides 22576 and 22577; Ararat Province, confluence of Azat and Gekhard rivers, Arm 17, 31.viii.2015, 2 ♂, O Ma H leg. SW, NMPC slides 22584 and 22585; Ararat Province, small tributary of Gekhard River, crossroad at factory, Arm 18, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22602. **Azerbaijan:** Quba district, Xinaliq village, mountain stream, Aze 2, 27.v.2017: 1 ♂, H leg. SW, NMPC slide 24169.

Comments. This species was described by Wagner (1981: 48) from northern Iran, from material collected at Veyshahr near Nowshahr on the Caspian Sea. Wagner and Joost (1983: 100) published central Caucasus record from Terskol, Russia. Ježek (1990: 27–30) published a record of a subspecies, *Saraiella ressli montana*, from Kerman province in south-eastern Iran. New for Armenia and Azerbaijan.

Thornburghiella Vaillant, 1973

43. *Thornburghiella veve* Oboňa & Ježek, 2017

Published record. Armenia: Oboňa et al. 2017a (Lori Province near Dzoraget village, tributary of Pambak River), 3 ♂, NMPC type material catalogue no. 34708-34710, inventory no. 22620-22622.

Ulomyia Walker, 1856

44. *Ulomyia cognata* (Eaton, 1893)

Material examined. Armenia: Lori Province, tributary of Aghstev River, above M8 road at Lermontov village, Arm 20, 31.viii.2015, 1 ♂, O Ma H leg. SW, NMPC slide 22568.

Distribution. This is a common European species known from Austria, Czech Republic, Finland, France, Germany, Great Britain, Italy, Lithuania, Poland, Slovakia, Slovenia and Ukraine (Ježek and Omelková 2012; Salmela et al. 2014; Ježek et al. 2017). It is known from the central Caucasus at Terskol, Russia (Vaillant and Joost 1983: 100). New for Armenia.

Discussion

According to Oboňa et al. (2016b, 2017b) and Hrivniak et al. (2018), as compared to many European countries, Transcaucasia (Georgia, Armenia, Azerbaijan) is insufficiently investigated for flies of the families Anisopodidae, Bibionidae, Dixidae, Drosophilidae, Limoniidae, Pediciidae, Ptychopteridae, and Scatopsidae. Only 17 species of Psychodidae are known for Armenia and 18 species are known from Azerbaijan (Perfil'ev 1966; Lewis 1982; Artemiev and Neronov 1984; Vaillant 1972; Wagner 1981, 1990, 2013; Wagner and Joost 1986; Wagner et al. 2002; Melaun et al. 2014; Oboňa et al. 2017a). In the present paper, we include 35 recent species (18 of them newly recorded) from Armenia. Included are 11 species of Phlebotominae and 24 species of Psychodinae. Similarly, our checklist of moth flies from Azerbaijan includes 24 species, of which 6 are newly recorded. The species recorded include 18 species of Phlebotominae and 6 of Psychodinae. Our results certainly represent only a small part of the Armenian and Azerbaijan psychodid fauna. Finding common and widespread species such as *Paramormia* (*Duckhousiella*) *ustulata*, *Psychodocha cinerea*, *Tinearia alternata*, *Pericoma* (*Pachypericoma*) *blandula*, *Pneumia canescens*, *Pneumia nubila*, or invasive species such as *Clogmia albipunctata*, for the first time from these countries shows that the psychodid fauna is still poorly known.

Knowledge of species distribution is important for studying biogeography and making effective conservation actions. This checklist will provide a baseline for further studies and for initiation of concerted conservation actions in Armenia and Azerbaijan. No doubt that future collecting in Georgia and Azerbaijan, done with the support of the International Visegrad Fund (project No. 21810533), will yield additional faunistic novelties of interest, as shown by similar studies in this region of other dipteran families (e.g. Oboňa et al. 2016b, 2017b; Negrobov et al. 2017; Starý et al. 2017; Hrivniak et al. 2018).

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RESEARCH ARTICLE

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Description of a new deep-water dogfish shark from Hawaii, with comments on the *Squalus mitsukurii* species complex in the West Pacific

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Abstract

Dogfish sharks of the genus *Squalus* are small, deep-water sharks with a slow rate of molecular evolution that has led to their designation as a series of species complexes, with low between-species diversity relative to other taxa. The largest of these complexes is named for the Shortspine spurdog (*Squalus mitsukurii* Jordan & Snyder), a medium-sized dogfish shark common to warm upper slope and seamount habitats, with a putative circumglobal distribution that has come under investigation recently due to geographic variation in morphology and genetic diversity. The Hawaiian population of *Squalus mitsukurii* was examined using both morphological and molecular analyses, putting this group in an evolutionary context with animals from the type population in Japan and closely-related congeners. External morphology differs significantly between the Hawaiian and Japanese *S. mitsukurii*, especially in dorsal fin size and relative interdorsal length, and molecular analysis of 1,311 base pairs of the mitochondrial genes ND2 and COI show significant, species-level divergence on par with other taxonomic studies of this genus. The dogfish shark in Hawaii represents a new species in the genus, and the name *Squalus hawaiiensis*, the Hawaiian spurdog, is designated after the type location.

Keywords

Chondrichthyes, DNA barcoding, Elasmobranchii, morphology, *Squalus hawaiiensis*, taxonomy

Introduction

Deep-water sharks like the dogfish sharks (Squaliformes, Squalidae) and the gulper sharks (Squaliformes, Centrophoridae) have proven confounding groups for systematists to resolve due to their highly conserved morphology, wide ranges, and patchy, infrequently-sampled distributions (Veríssimo et al. 2014; Cotton and Grubbs 2015; Veríssimo et al. 2017; Daly-Engel et al. submitted). Recent years have shown that DNA sequencing in conjunction with morphological analyses is an effective approach for elucidating the alpha taxonomy of deep-water sharks (Avise 2004; Ward et al. 2005; Last et al. 2007c; Ebert et al. 2010; Veríssimo et al. 2014; Pfleger et al. 2018). Findings from several studies have shown a mix of low genetic distances between well-established morphological species, and deep genetic splits between animals identified as conspecifics (Daly-Engel et al. 2010; Veríssimo et al. 2017; Daly-Engel et al. 2018; Pfleger et al. 2018; Daly-Engel et al. submitted).

Taxonomic delineation that incorporates DNA analysis has often relied upon consistencies among within- and between-species divergences in the barcoding gene (COI), as measured by percent nucleotide sequence variation (Avise 2004; Ward et al. 2005; Naylor et al. 2012). DNA barcoding is an effective, widely-used molecular method among taxonomists because the cytochrome oxidase I gene (COI) records a low rate of mutation compared with other loci (Avise 2004; Ward et al. 2005). While a reliable metric among distantly-related groups in which mutations accumulate consistently, COI may fail to elucidate shallow divergences among taxa with low genetic diversity. Complicating identification issues is the fact that deep-water sharks, whose cold environment results in low metabolic rates relative to other elasmobranchs, may undergo an overall slower rate of molecular evolution compared with shallow coastal species (Martin et al. 1992; Martin and Palumbi 1993). As a result, a number of investigators have found the more-rapidly evolving ND2 gene to be an effective genetic marker for estimating both inter- and intraspecific variation in dogfish sharks (Veríssimo et al. 2010; Naylor et al. 2012; Veríssimo et al. 2017; Pfleger et al. 2018).

Much work among shark systematists has focused on clarifying species delineations in dogfishes of the genus *Squalus*, an abundant, speciose, globally-distributed group of morphologically-similar, small-bodied demersal sharks that primarily inhabit circumglobal shelf and slope habitats from 100 - 1000 m depth (Compagno et al. 2005; Last et al. 2007c). Within this genus, genetic and morphological examinations have revealed a series of species complexes characterized by relatively shallow evolutionary divergences among putative species (Last et al. 2007c; Gaither et al. 2016; Veríssimo et al. 2017). Such complexes are not unusual in nature, having been observed across a variety of phyla from insects (Perring 2001) and nematodes (Chilton et al. 1995) to bony fishes (Barluenga and Meyer 2004), and have been shown to harbor "cryptic" diversity not always apparent from morphology alone (Daly-Engel et al. Submitted). Taxonomic reevaluation of *Squalus* in the Indo-Pacific and elsewhere has revealed many undescribed species that were historically lumped together under a single name (Ward et al. 2007; Naylor et al. 2012; Viana et al. 2016; Pfleger et al. 2018): although the relatively shallow spiny dogfish *Squalus acanthias* Linnaeus comprises just one wide-ranging species apart from the North

Pacific (Ebert et al. 2010; Veríssimo et al. 2010), we now know that both the Shortspine dogfish shark *Squalus mitsukurii* Jordan & Snyder and the shortnose dogfish shark *Squalus megalops* Macleay comprise global species complexes (Veríssimo et al. 2010; Naylor et al. 2012; Veríssimo et al. 2017; Pfleger et al. 2018; Daly-Engel et al. Submitted).

Recent taxonomic studies on *Squalus* have focused on *Squalus mitsukurii*, a putative circumglobal species found on continental and insular shelves and upper slopes and on seamounts between 100 and 950 m depth (Compagno et al. 2005). Re-examination of local *S. mitsukurii* stocks has revealed many new species, including four from the West Pacific alone: *S. formosus* White & Iglesias (2011), *S. chloroculus* Last, White, & Motomura (Last et al. 2007b), *S. montalbani* Whitley (Last et al. 2007b), and *S. griffini* Phillips (Duffy and Last 2007). Other revisions of *S. mitsukurii* have been done in the Atlantic using either genetic tools or morphological characters (Viana et al. 2016; Veríssimo et al. 2017), though not both (but see Pfleger et al. 2018).

Along the Hawaiian Archipelago in the Central Pacific, the Shortspine spurdog (*Squalus* cf. *mitsukurii*) is the only *Squalus* species known, aggregating in large numbers on or near the bottom at a depth of 100–950 m (Wilson and Seki 1994). Observable differences between specimens of *S. mitsukurii* in Hawaii and its conspecifics from the West Pacific first came to light during a genetic study (Daly-Engel et al. 2010), and subsequent research showed that growth (L_∞, k) and reproductive parameters (size-at-maturity) for *Squalus* cf. *mitsukurii* in Hawaii differed from published data (putatively as *S. mitsukurii*) from other regions (Cotton et al. 2011). Together with the relative geographic isolation of the Hawaiian Islands and the high levels of endemism observed there [25% of fish species in Hawaii are endemic, the most in the Indo-Pacific region (Roberts et al. 2002; Randall 2007; Briggs and Bowen 2011)] make this population a likely candidate for redescription.

We undertook a taxonomic evaluation of *Squalus mitsukurii* from the Hawaiian Islands using molecular and morphological data, couching these in the evolutionary context of closely-related, previously-recognized congeners from the West Pacific. DNA barcoding with COI can discriminate among species in the genus *Squalus* (Ward et al. 2007), but low resolution in this marker may fail to identify cryptic diversity. We therefore supplemented DNA sequences derived from the COI barcoding gene with data from ND2, a faster-evolving mitochondrial gene with the potential to distinguish evolutionary relationships with a high degree of resolution (Avise 2004; Naylor et al. 2012). In addition, morphological and meristic comparisons were made comparing *S.* cf. *mitsukurii* from Hawaii with measurements taken from the Japanese holotype, and reported by Last et al. (2007c) and in Viana et al. (2016).

Materials and methods

Tissue collections

Whole specimens and genetic samples of *Squalus* cf. *mitsukurii* were collected primarily during longline surveys conducted on the insular slope around the Hawaiian Island of Oahu. Survey methods are described in Daly-Engel et al. (2010) and Cotton et al.

(2011). Additional specimens and samples were collected from bottom fish surveys from Maui to Lisianski Atoll, spanning nearly 2,000 km of the Hawaiian Archipelago. Because the 2010 genetic study showed extremely low diversity, we deemed 5–10 specimens adequate for taxonomic evaluation, a number on par with other revisions in this genus (Ward et al. 2007; Pfleger et al. 2018). To that end, five whole mature females and three whole mature male *S.* cf. *mitsukurii* from Oahu were retained as voucher specimens for morphometric and genetic analyses. Small (< 1cm^3) samples of fin or muscle tissue were taken using scissors and stored in 2 mL vials containing 1.5 mL 20% dimethylsulfoxide (DMSO) saturated salt (NaCl) buffer (Seutin et al. 1991) or >70% ethanol (EtOH).

Genetic examination of 130 tissue samples from 25–30 *Squalus* dogfish species has shown that *S.* cf. *mitsukurii* from Hawaii clustered closely with *S. nasutus* Last, Marshall, & White from Australia, *S. japonicus* Ishikawa from Japan and elsewhere, and *S. mitsukurii* from Japan (Daly-Engel et al. Submitted), and well apart from other congeners in the region; hence the current analysis focuses on these species. Because it is impossible to extract undamaged DNA from the formalin-fixed *S. mitsukurii* holotype, we referenced DNA extracted from two specimens identified as *S. mitsukurii* by expert Japanese systematist Dr. Sho Tanaka (Tanaka et al. 1975; Yano and Tanaka 1984; Yano et al. 2017) collected from Suruga Bay in mainland Japan, which is approximately 100 kilometers from the type locality of Misaki. Tissue samples of *S. nasutus* (N = 2) and *S. japonicus* (N = 8) were obtained from Australia and Japan (Appendix 1).

Genetic analysis

DNA was extracted from fin clips using a DNeasy Blood & Tissue Kit from Qiagen (Germantown, MD). Primers were obtained from Integrated DNA Technologies, Inc. (Coralville, Iowa). PCR reactions consisting of 7 µL BioMix Red from Bioline (London, UK) at the recommended concentration, 1 µL (3 µg) template DNA, and 1 µL (1.0 µM) each primer (10 µL total PCR volume). PCR amplification on a C1000 Touch Thermal Cycler (Bio-Rad; Hercules, California) consisted of an initial denaturation at 95 °C for 4 minutes followed by 36 cycles of 1 min at 95 °C, followed by 30s at 58 °C, and 30s at 72 °C with a final extension at 72 °C for 20 minutes. DNA from two mitochondrial genes were sequenced for a total of 1,131 base pairs (bp; Table 1): Cytochrome Oxidase I (COI; 602 bp), and NADH dehydrogenase 2 (ND2; 529 bp). COI primers were standard barcoding primers by Folmer et al. (1994), and NADH 2 primers that were utilized were designed by Veríssimo et al. (2010; Table 1). PCR products were cleaned with ExoFAP (Thermo Fisher Scientific, Waltham, Massachusetts) and sequenced on an Applied Biosystems 3730XL DNA Analyzer at the University of Arizona Genetics Core.

DNA sequences were trimmed in Geneious v9.1.4 (Kearse et al. 2012) and aligned using Mafft (Katoh et al. 2002) implemented in Geneious. MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used to construct Bayesian inference phylogenetic trees: first, analyses of Markov Chain Monte Carlo (MCMC) chains were run for 10,000,000 generations while sampling one tree per 100

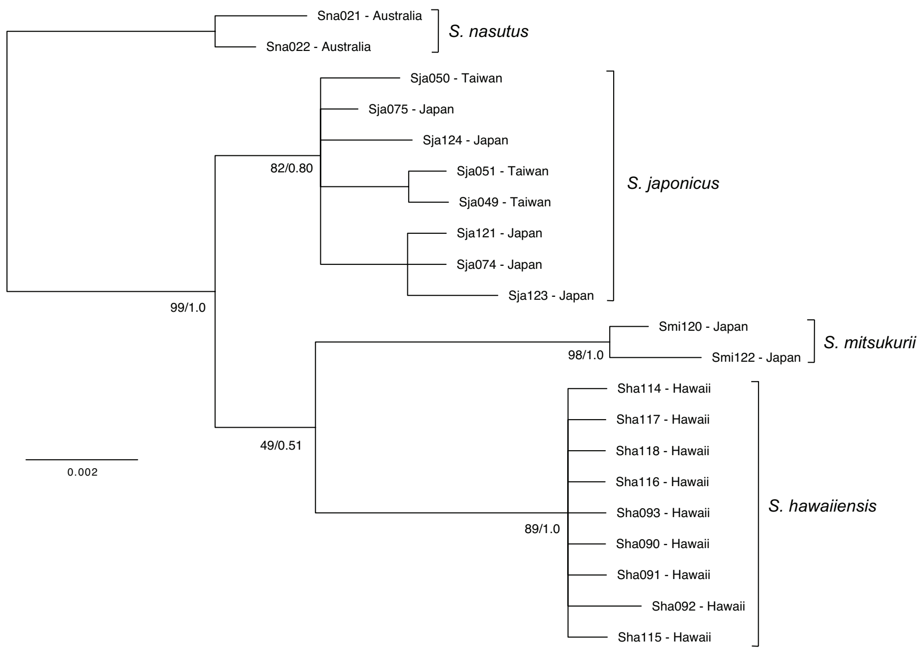

Figure 1. Phylogenetic tree of concatenated ND2 and COI sequences. Bayesian phylogenetic tree of concatenated mitochondrial ND2 and COI sequences for *Squalus* species used in this study, which was concordant with maximum likelihood methods. Numbers at nodes represent maximum likelihood bootstrap support/Bayesian posterior probability.

generations. Convergence between simultaneous runs was reached when the average standard deviation of split frequencies fell below 0.01 (Ronquist et al. 2005). Following a burn-in phase of 10,000 steps (10% of generations discarded), parameter values were averaged, and posterior clade probabilities were calculated and the likelihood scores for all the topologies averaged. PhyML v3.0 (Guindon et al. 2010) implemented in Geneious was used to construct a maximum likelihood (ML) tree with 10,000 bootstrap replicates, which was compared with the Bayesian topology (Figure 1). TCS networks (Clement et al. 2000) and gene diversity statistics were generated in PopART (Leigh et al. 2014), while mutational model was calculated for each gene independently using jModeltest (Table 1; Posada 2008). Genetic distance expressed as percent sequence divergence (Table 2) was calculated in MrBayes (Huelsenbeck and Ronquist 2001).

Morphometrics and meristics

Morphological measurements were used to discriminate between Japanese *S. mitsukurii*, including the holotype as measured by Last et al. (2007c) and Viana et al. (2016), and *S.* cf. *mitsukurii* collected from Hawaii. Measurements were performed on fresh

Table 1. Details and diversities of genetic loci amplified in *Squalus hawaiiensis* sp. n. Abbreviations: COI = Cytochrome oxidase I; ND2 = ND2 dehydrogenase 2; N = number of individual specimens included in the analysis; T_a = annealing temperature; π = nucleotide diversity; S = segregating sites; I = informative sites; H = number of haplotypes; h = haplotype diversity.

Gene	F primer	R primer	# bp	N	π	S	I	H	model	Citation
COI	LCO	HCO	602	20	0.0038	8	8	6	HKY	(Folmer et al. 1994)
ND2	ND2F	ND2R	529	20	0.0069	15	10	9	TRN	(Veríssimo et al. 2010)

Table 2. Genetic distances expressed as a percent divergence between *Squalus* species. Lower wedge is average between-species divergence in concatenated ND2 and COI genes; upper wedge is between-species divergence in each gene expressed as ND2/COI. Shaded boxes show within-species variation in concatenated sequences (top) and ND2/COI (bottom).

	S. mitsukurii	***S. japonicus***	***S. hawaiiensis* sp nov**	***S. nasutus***
S. mitsukurii (N = 2)	0.09 0.20/0.00	1.07/0.30	1.01/0.50	1.70/0.70
S. japonicus (N = 8)	0.71	0.12 0.13/0.18	0.84/0.50	1.07/0.70
S. hawaiiensis sp nov (N = 8)	0.73	1.90	0.00 0.00/0.00	1.01/0.80
S. nasutus (N = 2)	1.15	0.87	0.91	0.09 0.20/0.00

specimens in accordance with conventional techniques used for sharks (Compagno 1984), including taxon-specific adaptations (e.g. fin spine measurements) used in recent publications (Last et al. 2007c; Veríssimo et al. 2014). A suite of 82 morphological and meristic measurements were recorded for eight specimens. Measurements were taken by two readers for each individual, and the average measurement between the two readers is reported for three specimens to be designated as a holotype and two paratypes, along with the minimum and maximum values measured across the five remaining specimens. Vertebral meristic data were obtained for six specimens, including the three type specimens, using X-radiographs conducted at the Shepherd Spring Animal Hospital in Crawfordville, Florida. Dermal denticles from a male specimen (72.5 cm TL) were imaged by the Florida State University's Biological Science Imaging Resource (BSIR) using a scanning electron microscope with Everhart-Thornley Detector (SEM ETD; FEI Nova 400 NanoSEM; BAL-TEC CPD030 Critical Point Dryer) at 15 kV, with a spot size of 3 at magnifications of 195–800×.

Results

Genetic analyses

Mitochondrial DNA sampled from four conspecific shark taxa in the genus *Squalus* from the Central and West Pacific (*S. mitsukurii*, *S. nasutus*, *S. japonicus*, and

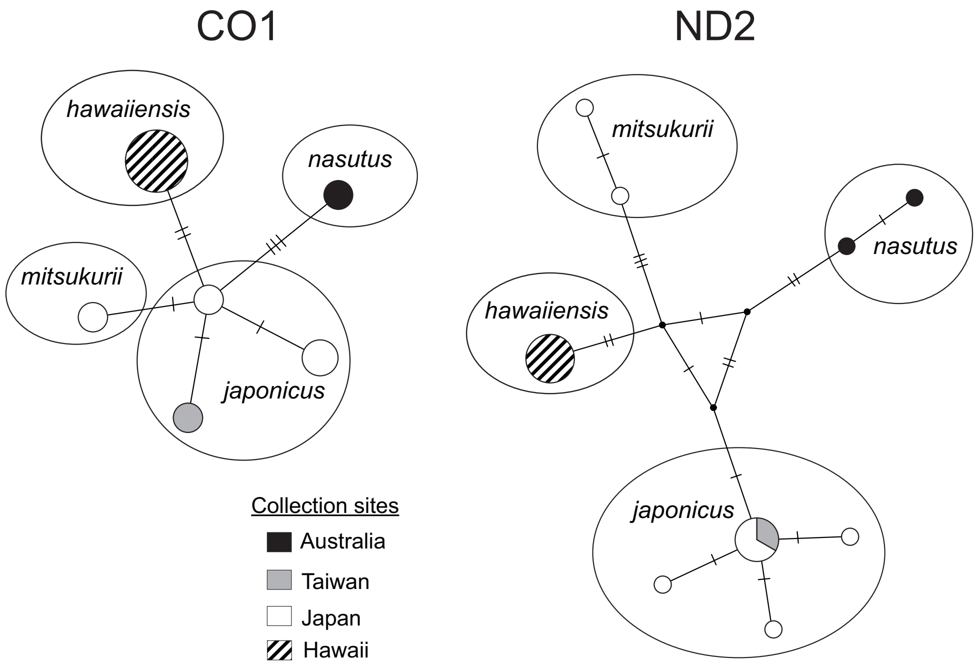

Figure 2. Haplotype networks for 20 individuals from four putative *Squalus* species. TCS plots show two the mitochondrial genes (COI and ND2) sequenced this study. The size of the circles or wedges represents the number of samples within each haplotype, and uninterrupted branches represent single mutational steps.

S. cf. *mitsukurii*) clustered into four genetically distinct genetic groups with a high degree of confidence using both Maximum Likelihood (89–98% bootstrap support) and Bayesian methodology (1.00 posterior probability), except for *S. japonicus* (80% bootstrap support, 0.80 posterior probability). COI and ND2 trees were concordant, though jModeltest showed slightly different best-fit models of molecular evolution for each (Table 1), and the concatenated tree is shown (Figure 1). As expected, ND2 showed roughly twofold-higher diversity (? = 0.0069) than COI (? = 0.0038), though the evolutionary patterns they describe are similar (Table 1). TCS networks illustrate distinct genetic separation between the four taxa, with some haplotypes being closely related, but none shared (Figure 2).

Among the four species examined here, interspecific divergence across 1,131 bp of concatenated mtDNA ranged from 0.71% between *S. mitsukurii* and *S. japonicus* to 1.90% between *S. japonicus* and *S.* cf *mitsukurii* (average = 1.05±0.18%). Average pairwise genetic distance between *S.* cf *mitsukurii* and the three named species was 1.18%, greater than the average distance linking named pairs (0.91%). Intraspecific divergence ranged from 0.00% among eight *S.* cf. *mitsukurii* to 1.12% in the same number of *S. japonicus*. Such lack of diversity is consistent with a 2010 population genetic study of *Squalus* from Hawaii that recovered only eight CO1 haplotypes in 112 individuals, and only five haplotypes in the 91 sharks sampled from Oahu (Daly-Engel et al. 2010).

Haplotype diversity was also low in *S. nasutus* and *S. mitsukurii*, likely because these were represented by just two samples each. Novel DNA sequences have been made publicly available via GenBank (Appendix 1).

***Squalus hawaiiensis* sp. n.**

http://zoobank.org/105A6FF0-9FFD-4425-BE9C-85019A911B25

Diagnosis. A large species of *Squalus* of the 'mitsukurii group' with the following combination of characters: body relatively slender, trunk height 8.7–12.4% TL (mean 10.1% TL, n=8; Figure 3); snout is angular and short to moderate in length, mouth width 1.35–1.60 (1.48) times horizontal prenarial length and pre-oral length is 1.92–2.06 (1.97) times the prenarial length (Figure 4); pre-first dorsal length 30.3–31.5 (30.2)% TL; pre-second dorsal length 63.6–67.0 (65.5)% TL; interdorsal space 26.7–30.0 (28.6)% TL; pelvic-caudal space 25.2–29.3 (27.1)% TL; relatively small, upright dorsal fins; first dorsal fin length 11.4–12.8 (12.2)% TL, height 6.5–7.8 (7.3)% TL, inner margin length 4.9–5.7 (5.4)%% TL; second dorsal fin length 10.6–11.7 (11.1)% TL, height 4.0–4.6 (4.4)% TL, inner margin length 4.3–4.9 (4.6)% TL; first dorsal fin spine length 46.6–64.6 (55.6)% of first dorsal fin height; second dorsal spine length 104.5–114.5 (109.0)% of second dorsal fin height; caudal bar triangular, extending from the caudal fork nearly to the anterior edge of the lower caudal, distinct upper caudal blotch and fringe in juveniles, upper caudal blotch diffuse in adults but extending to the posterior margin of the upper caudal fin, upper and lower caudal fins white tipped; flank denticles tricuspid (Figure 5A–C); teeth are similar in appearance in the upper and lower jaw, with numbers ranging from 26–28 in the upper jaw and 23 in the lower jaw; 41–45 monospondylous centra, 85–89 precaudal centra, 112–116 total centra; adult maximum size at least 101 cm TL.

Description. Morphometric data are provided in Table 3. *Squalus hawaiiensis* sp. n. is a relatively large dogfish shark with a fusiform body, a relatively short snout, and small dorsal fins. The nape is modestly humped over the pectoral fins, particularly in large females. Head length is 21.4–23.9% TL. The snout is relatively short but angular and relatively pointed in dorsal view, with a pre-narial length that is 49–52% of the pre-oral length and 1.06–1.31 times eye length. Pre-oral length is 2.04–2.42 times the internarial space. Pre-vent length is 50.4–53.6% of the TL. Mouth width is 0.69–0.83 times the pre-oral length. Eye is large (3.9–4.9% of TL) and strongly notched posteriorly. Upper and lower labial furrows pronounced. Upper labial furrow length 1.9–2.5% TL, 24.9–33.0% of mouth width, and 19.3–24.7% of pre-oral length. Inner nostril labial furrow space is 1.89–2.27 times labial furrow length. Pre-first dorsal fin length is 30.3–31.5% of TL, pre-second dorsal space is 63.6–67.0% of TL and the interdorsal space ranges from 26.7% to 30.0% of TL. The first dorsal fin is rounded at the apex. First dorsal fin length measures 1.62–1.81 times first dorsal fin height. First dorsal fin length is 1.02–1.16 times second dorsal fin length and the height of the first dorsal fin is 1.57–1.80 times the height of the second dorsal fin. Second dorsal fin length 2.36–2.79 times the second dorsal fin height. Dorsal fin spines are stout, with the spine on

Figure 3. Holotype**.** Lateral view of *Squalus hawaiiensis* sp. n. holotype (UF241161, female 750.5 mm TL).

Figure 4. Holotype**.** Ventral view of *Squalus hawaiiensis* sp. n. holotype (UF241161, female 750.5 mm TL).

the second dorsal fin typically longer (4.1–5.0%TL) than the spine on the first dorsal fin (3.6–4.6%TL). First dorsal spine length is 0.39–0.65 (mean: 0.53%) times the first dorsal fin height. Second dorsal spine length is 0.84–1.15 (mean: 1.04%) times the second dorsal fin height. The pectoral fins are well developed with an anterior margin that is 12.8–16.0% of the TL. The pectoral inner margin is 6.4–7.4% of total length and free rear tip is rounded (Figure 6A–C).

Squalus hawaiiensis is morphologically similar to other species in the "*mitsukurii*" group. It is distinguished morphologically by a very long inter-dorsal space which ranges from 26.7% to 30.0% of TL compared to 18.7–25.5% in *Squalus mitsukurii* (Last et al. 2007a) and 23.5–24.6% in *Squalus formosus* (White and Iglésias 2011), both from Taiwan and southern Japan, and to 23.5–25.6 in *S. edmundsi*, 20.6–23.8%

Table 3. Morphological data from *Squalus hawaiiensis* sp. n. and *S. mitsukurii* from Japan. Morphological data from type specimens of two *Squalus* species expressed as a percentage of total length (TL) in cm following the methods of Last et al. (2007b). Morphometrics from the holotype, two paratypes a and b, and the range of values from five additional specimens of *Squalus hawaiiensis* sp. n. are shown; type specimens are listed with FLMNH catalog number and genetic ID from Figure 1. Morphometrics for *S. mitsukurii* were taken directly from published studies, including two independent sets for the *S. mitsukurii* holotype: [1]Last et al. (2007b) and [2]Viana et al. (2016). Also shown are the minimum and maximum values from Last et al. (2007b) for four *S. mitsukurii* paratypes from Japan; "Min" and "Max" represent a range of values for these paratypes, not including holotype values. Abbreviations: ♂ = male, ♀ = female, bolded numbers indicate non-overlapping size ranges between *S. mitsukurii* and *S. hawaiiensis*.

		***S. hawaiiensis* sp. n.**					***S. mitsukurii* (Japan)**			
		Holotype (♀ UF241161, Sha116)	**Paratype[a] (♂, UF241162, Sha114)**	**Paratype[b] (♂, UF241163, Sha117)**	**Min**	**Max**	**Holotype[1]**	**Holotype[2]**	**Min**	**Max**
STL	Stretched total length	774.5	628	502.5			–	–	–	–
TL	Total length	750.5	608	487.5	535	836	719	710	266	855
PCL	Precaudal length	**81.3**	**82.6**	**80.3**	**80.3**	**83.1**	76.6	77.5	78.2	79.0
FL	Fork Length	90.2	91.3	89.8	88.4	92.5	–	–	–	–
PD2	Pre-second dorsal length	**65.8**	**65.6**	**64.6**	**63.6**	**67.0**	59.8	61.0	58.6	61.2
PD1	Pre-first dorsal length	31.5	30.4	30.9	30.3	31.3	30.9	32.4	28.5	32.3
SVL	Pre-vent length	53.1	52.4	51.1	50.4	53.6	51.5	50.0	48.9	52.2
PP2	Prepelvic length	52.2	50.1	48.6	48.9	52.4	48.5	47.9	47.4	50.1
PP1	Prepectoral length	22.5	24.3	23.1	22.0	23.3	23.3	24.6	19.9	23.9
HDL	Head Length	21.8	23.9	22.9	21.4	22.4	23.4	24.2	20.9	23.5
PG1	Prebranchial length	18.3	19.2	19.1	17.9	20.7	19.5	20.4	18.0	20.1
PSP	Prespiracular length	12.2	12.9	13.2	12.0	12.9	12.8	12.8	12.1	13.3
POB	Preorbital length	7.6	7.8	7.8	7.4	7.8	7.5	7.3	7.3	7.9
PRN	Prenarial length	4.9	5.2	5.4	4.8	5.1	5.5	5.6	5.0	5.4
PINL	Pre-inner nostril	5.0	5.2	5.3	4.9	5.1	–	–	–	–
POR	Preoral length	9.9	10.1	10.4	9.6	10.2	10.8	10.3	9.4	10.6
INLF	Inner nostril-labial furrow space	4.3	4.7	4.8	4.3	4.7	4.4	4.3	4.2	4.7
MOW	Mouth width	7.7	7.6	7.2	7.0	8.1	6.2	8.6	6.3	7.5
ULA	Labial furrow length	1.9	2.5	2.1	1.9	2.3	2.4	2.5	2.1	2.5
INW	Internarial space	4.4	4.9	4.4	4.0	4.8	4.8	4.7	4.0	4.9
INO	Interorbital space	8.0	7.8	8.0	6.7	7.8	8.1	9.3	7.9	8.4
EYL	Eye length	4.3	4.9	4.5	3.9	4.7	3.4	3.6	3.8	4.7
EYH	Eye height	3.0	3.1	2.9	1.7	3.5	1.3	0.9	1.8	2.5
SPL	Spiracle length	1.2	1.4	1.7	1.2	1.6	1.2	1.3	1.2	1.5

		***S. hawaiiensis* sp. n.**					***S. mitsukurii* (Japan)**			
		Holotype (♀, UF241161, Sha116)	**Paratype[a] (♂, UF241162, Sha114)**	**Paratype[b] (♂, UF241163, Sha117)**	**Min**	**Max**	**Holotype[1]**	**Holotype[2]**	**Min**	**Max**
GS1	First gill-slit height	1.7	1.6	1.4	1.5	1.9	1.9	1.7	1.6	1.7
GS5	Fifth gill-slit height	2.2	1.9	2.2	2.0	2.4	2.1	2.3	1.8	2.0
IDS	Interdorsal space	**27.8**	**28.9**	**26.7**	**28.1**	**30.0**	21.3	21.1	18.7	25.5
DCS	Dorsal-caudal space	10.2	12.1	11.4	10.9	11.6	9.8	10.6	9.9	11.2
PPS	Pectoral-pelvic space	26.2	22.9	22.8	23.6	27.7	22.5	21.8	21.3	24.5
PCA	Pelvic-caudal space	25.4	29.0	27.4	25.2	29.3	22.7	23.7	22.3	27.4
D1L	First dorsal length	12.5	11.4	11.9	11.6	12.8	14.5	13.6	12.5	15.7
D1A	First dorsal anterior margin	11.0	9.1	10.6	10.0	11.1	12.0	12.0	10.5	11.1
D1B	First dorsal base length	**7.2**	**6.2**	**6.9**	**6.4**	**7.4**	8.3	8.2	7.8	7.8
D1H	First dorsal height	7.7	6.5	7.8	6.9	7.7	8.5	9.8	4.5	8.3
D1I	First dorsal inner margin	5.5	5.2	5.4	4.9	5.7	6.3	6.2	4.9	6.4
D1P	First dorsal posterior margin	8.1	7.7	8.0	7.6	9.0	9.7	9.3	4.6	7.9
D1ES	First dorsal spine length	4.6	4.2	3.8	3.6	4.4	3.3	3.9	3.5	4.8
D1BS	First dorsal spine base width	0.9	0.8	0.9	0.7	1.0	0.8	1.0	0.6	0.8
D2L	Second dorsal length	9.4	9.7	9.7	9.2	9.9	–	–	–	–
D2L*	Second dorsal length (incl. cartilage)	**11.5**	**11.1**	**10.8**	**10.6**	**11.7**	12.7	12.3	12.0	13.9
D2A	Second dorsal anterior margin	6.9	6.5	7.3	6.7	7.4	–	–	–	–
D2A*	Second dorsal anterior margin (incl. cartilage)	**9.5**	**8.1**	**8.6**	**8.3**	**9.2**	10.2	10.2	10.4	10.7
D2B	Second dorsal base length	5.0	4.9	4.9	5.2	5.5	–	–	–	–
D2B*	Second dorsal base length (incl. cartilage)	**6.8**	**6.4**	**6.3**	**5.9**	**6.9**	7.2	7.2	8.0	9.2
D2H	Second dorsal height	4.3	4.1	4.6	4.0	4.6	4.5	6.8	3.0	4.6
D2I	Second dorsal inner margin	4.6	4.7	4.6	4.3	4.9	5.1	5.3	4.2	5.4
D2P	Second dorsal posterior margin	5.4	5.7	5.5	4.8	6.3	5.2	6.3	4.1	4.4
D2ES	Second dorsal spine length	4.1	4.7	5.0	4.1	4.6	3.8	4.2	3.8	5.0

		***S. hawaiiensis* sp. n.**					***S. mitsukurii* (Japan)**			
		Holotype (♀, UF241161, Sha116)	**Paratype[a] (♂, UF241162, Sha114)**	**Paratype[b] (♂, UF241163, Sha117)**	**Min**	**Max**	**Holotype[1]**	**Holotype[2]**	**Min**	**Max**
D2BS	Second dorsal spine base width	0.8	0.8	0.9	0.7	0.9	0.7	0.9	0.7	0.9
P1A	Pectoral anterior margin	16.0	12.8	13.6	12.9	15.6	15.0	15.2	11.7	16.1
P1I	Pectoral inner margin	6.6	6.8	7.1	6.4	7.4	8.2	9.5	7.0	7.5
P1B	Pectoral base length	5.6	5.4	5.3	5.0	5.8	6.8	5.3	5.0	6.1
P1P	Pectoral posterior margin	12.2	9.9	9.9	10.1	12.3	11.0	11.7	7.6	11.4
P2L	Pelvic length	9.9	11.4	10.9	9.3	10.7	10.8	11.5	9.6	10.3
P2H	Pelvic height	3.9	3.4	3.1	3.0	5.2	5.6	–	4.0	4.9
P2I	Pelvic inner margin	4.6	5.7	5.9	3.9	6.0	5.8	6.3	2.0	3.1
CDM	Dorsal caudal margin	20.7	20.1	21.1	19.4	21.4	22.6	24.4	21.2	21.3
CPV	Preventral caudal margin	11.2	9.9	10.3	10.2	12.0	12.3	12.1	10.2	12.2
CPU	Upper postventral caudal margin	16.6	15.0	15.4	14.3	16.6	16.4	–	13.2	16.2
CPL	Lower postventral caudal margin	5.2	4.0	3.2	3.7	5.4	4.8	–	3.4	5.6
CF.W	Caudal fork width	6.7	6.7	6.6	6.5	7.2	6.7	7.0	5.9	6.7
CF.L	Caudal fork length	8.5	8.1	8.5	8.2	9.3	9.2	–	9.3	10.3
HANW	Head width at nostrils	7.2	7.7	7.6	6.5	7.3	7.7	7.3	7.6	7.7
HAMW	Head width at mouth	10.8	11.1	10.5	9.9	10.6	11.5	12.2	10.1	10.8
HDW	Head width	13.6	11.7	10.8	11.7	15.8	14.8	22.5	11.5	13.8
TRW	Trunk width	15.0	10.6	10.7	11.7	14.2	–	18.3	8.2	10.7
ABW	Abdomen width	15.1	10.9	9.6	10.0	14.4	–	15.5	6.4	9.6
TAW	Tail width	7.1	7.1	5.9	5.9	7.9	6.3	–	4.7	6.7
CPW	Caudal peduncle width	3.0	3.0	2.7	2.4	3.4	2.5	–	2.4	3.1
HDH	Head height	8.2	8.3	8.2	8.1	10.7	8.5	12.7	7.5	11.7
TRH	Trunk height	9.2	8.7	8.8	8.8	12.4	–	10.3	7.9	9.1
ABH	Abdomen height	**11.1**	**9.3**	**9.2**	**8.6**	**14.2**	–	7.7	7.7	8.4
TAH	Tail height	6.3	5.9	5.3	5.7	8.8	7.2	–	5.3	6.2
CPH	Caudal peduncle height	2.3	2.2	2.2	2.3	2.5	2.6	–	2.3	2.5
CLO	Clasper outer length		4.5	3.8	5.1	5.1	–	–	1.7	2.6
CLI	Clasper inner length		7.7	6.3	8.4	8.4	–	–	5.2	6.0
CLB	Clasper base width		1.7	1.2	1.6	1.6	–	–	0.9	1.1

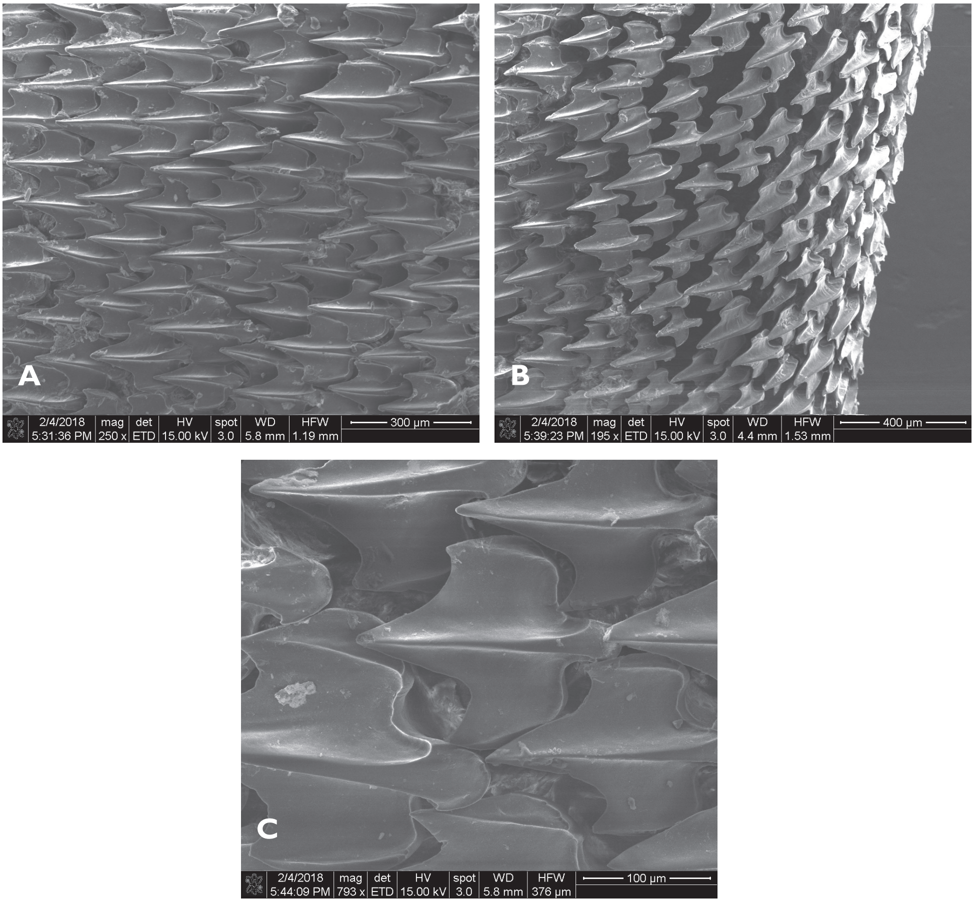

Figure 5. SEM images of dermal denticles. Three views of dermal denticles from adult male (TL = 72.5 cm) *Squalus hawaiiensis*.

in *S. grahami* (White et al. 2007), 21.7–25.9% in *S. montalbani* (Last et al. 2007b), all from Australia and 22.6–26.0% in *S. griffini* (Duffy and Last 2007) from New Zealand, but overlaps with *S. chloroculus* (23.7–27.5%) from Australia (Last et al. 2007b), *S. nasutus* (24.4–28.0%) from Australia, Indonesia, and the Philippines (Last et al. 2007a) and *S. japonicus* from Japan (28.0–29.5%TL) (Chen et al. 1979). *Squalus hawaiiensis* is further distinguished from *S. mitsukurii* by having smaller first and second dorsal fin lengths and anterior margins and a longer body or torso (longer pre-caudal and pre-second dorsal lengths but shorter dorsal caudal margin; Table 3). The longer torso is reflected in differences in the ranges of the following ratios between *S. mitsukurii* type specimens (reported in Last et al. 2007) and all *S. mitsukurii* measured here (N=8): pre-first dorsal length 1.45–1.73 vs. 1.01–1.16 times interdorsal space; prepectoral length 1.09–1.28 vs. 0.74–0.86 times interdorsal space; prepectoral length 1.02–1.07 vs. 0.78–0.89 times pelvic-caudal space. Based on data from Chen et al. (1979), *S. mitsukurii* has higher vertebral meristic counts (45–51 monospondylous centra, 87–93 precaudal centra, 118–127 total centra) than *S. hawaiiensis* (41–45

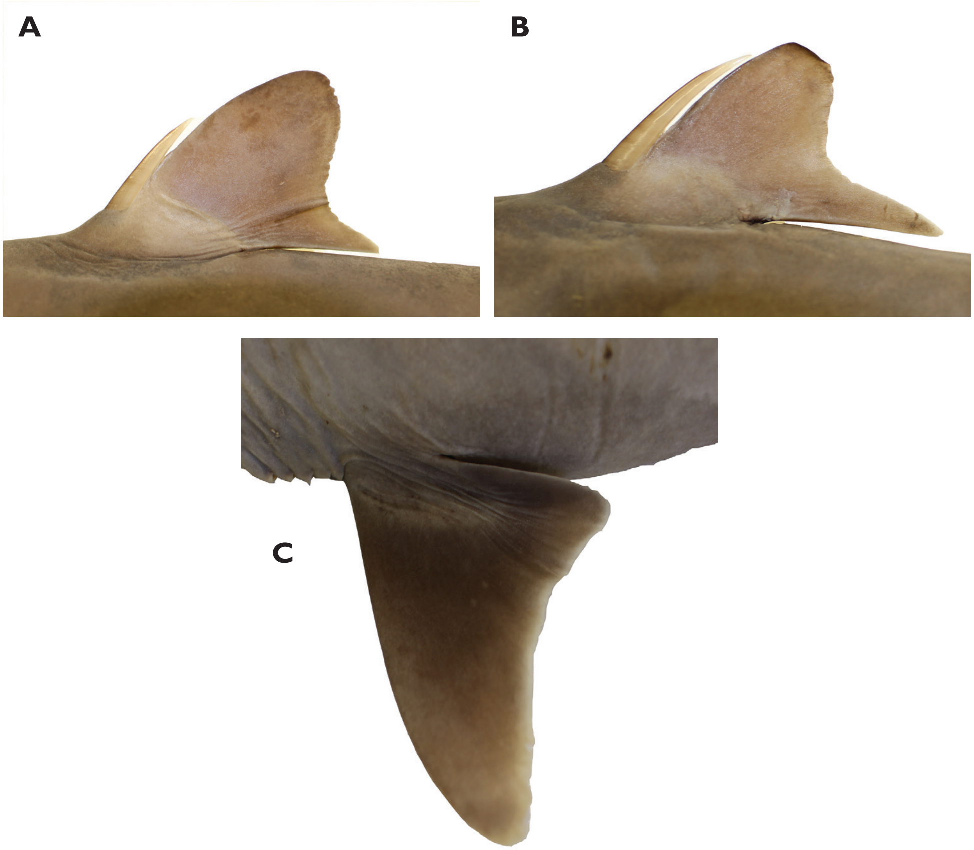

Figure 6. Holotype**. A** First dorsal fin **B** second dorsal fin **C** pectoral fin of *Squalus hawaiiensis* holotype (UF241161, female 750.5 mm TL).

monospondylous centra, 85–89 precaudal centra, 112–116 total centra). *Squalus chloroculus* has a caudal bar that extends much higher on the upper caudal fin and lacks the upper caudal blotch characteristic of *S. hawaiiensis* (Figure 7A–C). *Squalus chloroculus* also has much shorter first dorsal fin spines (2.3–3.3%TL) and second dorsal fin spines (2.5–3.9%TL) than *S. hawaiiensis*. *Squalus nasutus* has a much longer snout with pre-narial lengths of 5.9–7.5%TL and pre-oral lengths of 11.1–12.7%TL compared to 4.8–5.4%TL and 9.6–10.4%TL respectively for *S. hawaiiensis*. Based on the morphometrics from Chen et al. (1979), the closely related *S. japonicus* differs from *S. hawaiiensis* in having a smaller mouth (6.4–6.9%TL compared to 7.0–8.1%TL) and shorter first and second dorsal fin lengths. First dorsal fin length in *S. japonicus* is 10.1–11.0%TL compared to 11.4–12.8%TL in *S. hawaiiensis*. Second dorsal fin length is 7.9–8.4%TL *S. japonicus* compared to 10.6–11.7%TL in *S. hawaiiensis*.

Color. In life (based on many captured specimens): dorsal surface uniformly dark gray to brown, light gray to white ventrally. Dorsal fins uniformly gray to brown with think black tips that narrow with age, free rear tips slightly paler. Caudal fin mostly

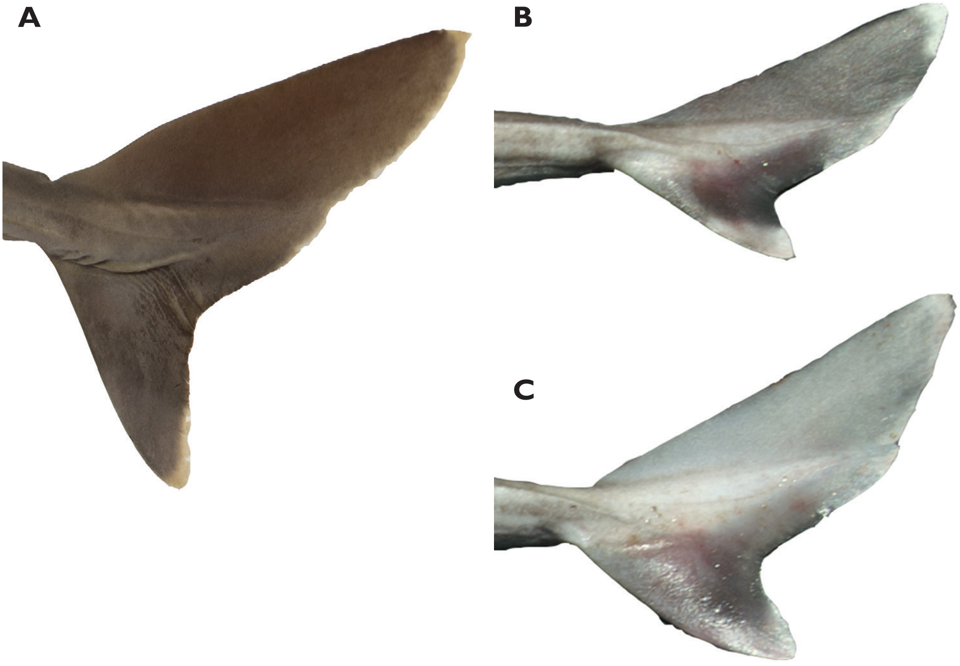

Figure 7. Caudal fin of *Squalus hawaiiensis* sp. n**.** **A** Holotype (UF241161, female 750.5 mm TL) **B** fresh adult male **C** fresh adult female.

dusky with a broken white trailing edge, dark caudal bar triangular, extending from the caudal fork nearly to the anterior edge of the lower caudal (Figure 8A–B). Upper caudal blotch diffuse in adults, extending to a short length of the posterior margin of the upper caudal fin, upper and lower caudal fins white tipped; pectoral and pelvic fins greyish dorsally, darker in the middle and with well-defined white posterior margin; Juveniles with much more pronounced fin markings; dorsal fins with black fringes, dark blotch in pectoral fins, caudal bar distinct on lower caudal from the fork to the anterior edge, well-defined and separated black upper caudal blotch and upper caudal fringe with upper caudal blotch not reaching the posterior margin of the upper caudal fin. In juvenile *S. mitsukurii* the upper caudal blotch is smaller and indistinct from the upper caudal fringe and the caudal bar is diagonal rather than triangular and does not reach the posterior edge of the lower caudal fin. In preservative: holotype similar, dark markings on fins faint but evident; caudal bar faint; broad, pale posterior margins on pectoral and pelvic fins well-defined. Eyes bright green in life (Figure 8C).

Size. Based on 197 Hawaii specimens surveyed, 156 females and 41 males (Daly-Engel et al. 2010; Cotton et al. 2011), the maximum observed length of females and males was 101 cm TL and 78 cm TL respectively. Cotton et al. (2011) reported that females reach maturity at ~64 cm TL and males reach maturity at ~47 cm TL.

Etymology. Derived from the type locality in the Hawaiian Archipelago

Vernacular. Hawaiian Spurdog

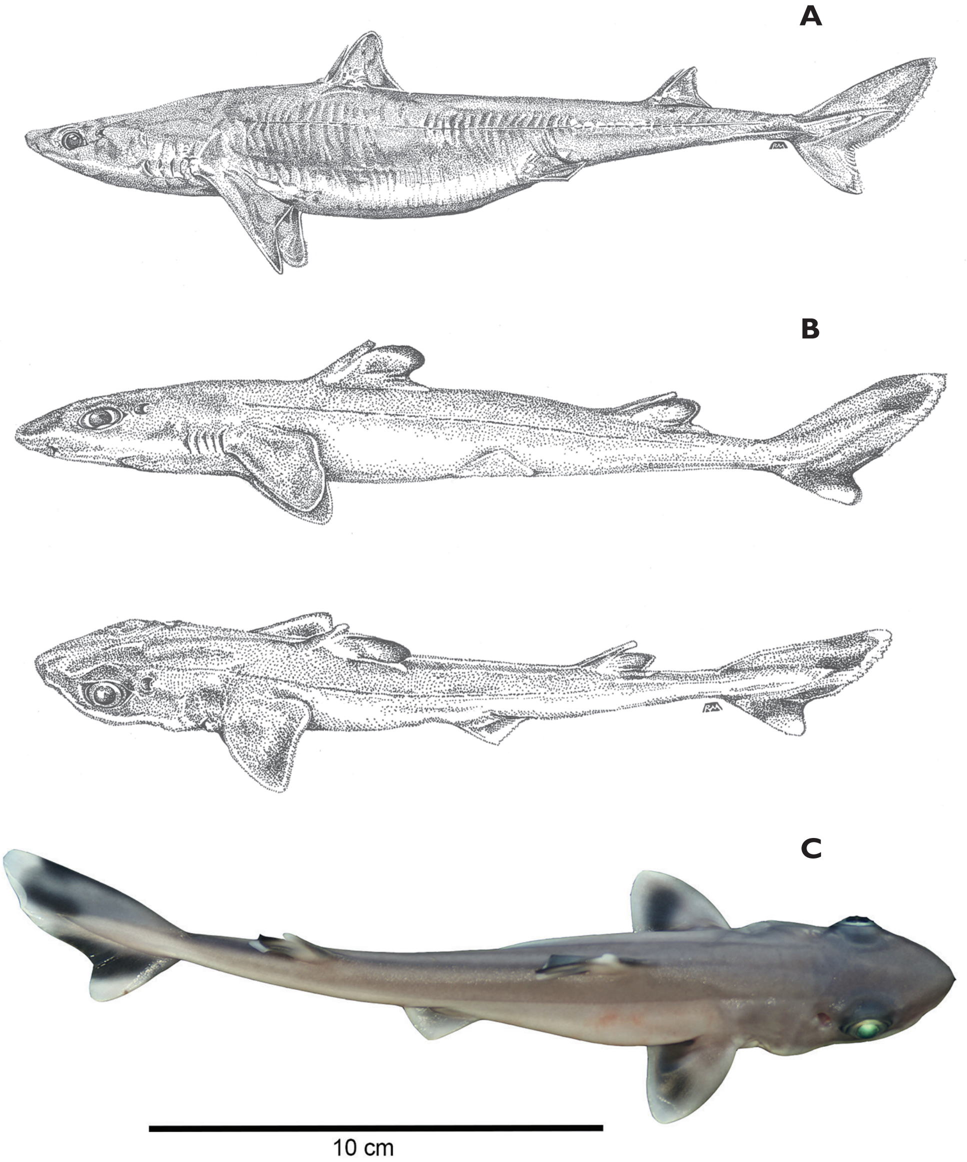

Figure 8. Images of the Hawaiian spurdog, *Squalus hawaiiensis*. **A** Lateral view of adult female *Squalus hawaiiensis*, drawing by R. McPhie **B** embryonic *Squalus hawaiiensis*, lateral and dorsal views, drawings by R. McPhie **C** embryonic *Squalus hawaiiensis*, dorsal view. Photo by RDG.

Discussion

We found marked genetic variation across 1,311 base pairs of mitochondrial DNA separating *Squalus hawaiiensis* from *Squalus mitsukurii* specimens collected from the Japanese type locality, as well as closely-related congeners from elsewhere in the Pacific (Figures 1, 2). Patterns of relatedness and inter- and intraspecific genetic distances were comparable to other phylogenetic studies on *Squalus*, including species descriptions (Ward et al. 2005; Ward et al. 2007; Naylor et al. 2012). Our data show that *S. mitsukurii* from Japan and *S. hawaiiensis* are demonstrably distinct sister species (Figures 1, 2), most closely related to *S. japonicus* from Japan and *S. nasutus* from Australia. Morphological examination also distinguished *S. hawaiiensis* from these three species by a combination of differences in the trunk length (interdorsal distance), snout length, fin and fin spine lengths, and caudal coloration. We conclude that *S. hawaiiensis* represents a novel, previously-unidentified species. A holotype and paratypes a and b have been deposited into the Florida Museum of Natural History (FLMNH catalog numbers UF241161, UF241162, and UF241163; Table 3).

The holotype of *Squalus mitsukurii* was first listed from Misaki, Japan by Jordan and Snyder (1901) before being officially described two years later by Jordan and Fowler (1903), though as in the 1901 paper, the accompanying drawing was of *Squalus acanthias*. *Squalus mitsukurii* from Hawaii was referenced by Jordan & Evermann shortly thereafter in the publication "Shore Fishes of the Hawaiian Islands" (1905), with a copy of the misattributed illustration from 1903. Little scientific investigation has been done on *Squalus* from the Central Pacific since then, with the exception of a 1994 account of the rapid depletion of the dogfish stock around Hancock Seamount in the Northwestern Hawaiian Islands as a result of bycatch in the armorhead fishery (Wilson and Seki 1994), and a more recent investigation of age, growth, and reproduction (Cotton et al. 2011). In 2010, the authors found that *S.* cf. *mitsukurii* from Hawaii has the lowest rate of both multiple paternity and genetic diversity estimated in a shark population to date, indicating that this species might have a particularly low rebound potential in the face of fishing pressure (Simpfendorfer and Kyne 2009; Daly-Engel et al. 2010).

Because taxonomic descriptions that incorporate molecular data may use different marker types, study taxa, and methods of estimating divergence, it can be difficult to directly compare genetic distances among studies, or define a genetic threshold for speciation. But a lack of shared haplotypes, plus variation between species that is generally an order of magnitude higher than variation within species, is a consistent pattern reported in many elasmobranch species descriptions (Spies et al. 2006; Ward et al. 2007; Veríssimo et al. 2014; Daly-Engel et al. 2018; Pfleger et al. 2018). Among the four closely-related species we studied, average concatenated sequence divergence between species (1.045±0.183%) was nearly fourteen times the average within-species divergence (0.075±0.026), and therefore consistent with species-level differences reported for other elasmobranchs, including *Squalus* (Ward et al. 2007; Ebert et al. 2010; Viana et al. 2016).

In addition to being taxonomically unresolved, members of genus *Squalus* are often subject to high fishing pressure as bycatch in commercial trawl fisheries, sometimes resulting in severe population depletion (Wilson and Seki 1994; Graham et al. 2001; Kyne and Simpfendorfer 2007; Dulvy et al. 2014; Pfleger et al. 2018). Furthermore, their long reproductive intervals (12–24 months) and slow growth results in a low rate of replacement (Musick 1999; Musick and Ellis 2005; Cotton et al. 2011; Cotton and Grubbs 2015), compounding the depleting effect of fishing pressure. In general, *S. mitsukurii* is classified by the International Union for Conservation of Nature (IUCN) as Data Deficient globally (Cavanagh et al. 2007), but life history parameters among *Squalus* species likely varies due to undiagnosed taxonomic variation. The combination of these variables may result in the extirpation or extinction of deep-water stocks and species before they are described by science, so taxonomic evaluation is of vital importance to ensure the survival of species that may not yet be managed as distinct evolutionary units. Further, though the barcoding gene, COI, has great utility for species identification, it may not provide sufficient resolution for diagnosing differences between organisms with low rates of molecular evolution, such as deep-water sharks. Because so many potential species remain unexamined, the name *S. mitsukurii* now represents a series of geographically distinct Evolutionary Significant Units (ESUs), each meriting its own taxonomic examination (Daly-Engel et al. Submitted).

Conclusions

Morphological and genetic differences indicate that the dogfish shark in Hawaii represents a novel species, designated here as *Squalus hawaiiensis*, the Hawaiian spurdog shark, named for the type location. Further, *Squalus mitsukurii* in Japan is subject to taxonomic confusion even among experts, and may comprise multiple distinct species, one of which likely includes the holotype. There, thorough morphological and genetic examination is warranted to elucidate the subtle differences between co-occurring populations that are morphologically indistinguishable but genetically unique. Given the number of previously-cryptic species identified in the *S. mitsukurii* complex alone, analysis of other populations will likely yield further identification of cryptic diversity within the genus *Squalus*.

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Appendix I

Demographic data for specimens used in this study. ID numbers refer to those used in Figure 1; GenBank Accession numbers are listed by gene (ND2/CO1); CSIRO = Commonwealth Scientific and Industrial Research Organisation Marine & Atmospheric Research (Hobart, Tasmania).

ID #	*Squalus* species	GenBank Accession #s	Source	Collector/ identifier	Origin	Latitude	Longitude	Collection date	Depth (m)	Sex	TL (mm)
Sna021	*nasutus*	MG654959.1/ MK005141	CSIRO	P. Last & W. White	West Australia, W of Shark Bay	25.0633S	112.1483E	23 Apr 2006	340		
Sna022		MG654960.1/ MK005140	CSIRO	W. White	West Australia, W of Leander Point	29.3100S	113.9467E	06 Feb 1991	505		
Sja049	*japonicus*	MG654922.1/ MK005129	CSIRO	W. White	Taiwan, Tashi fish market, near I-Lan (NE coast)			24 May 2005			
Sja050		MG654923.1/ MK012557	CSIRO	W. White	Taiwan, Tashi fish market, near I-Lan (NE coast)			23 May 2005			
Sja051		MG654924.1/ MK005128	CSIRO	P. Last	Taiwan, Tashi fish market, near I-Lan (NE coast)			23 May 2005			
Sja074		MG654925.1/ MK005130	A. Veríssimo	N. Straube	Suruga Bay, Japan						
Sja075		MG654926.1/ MK005127	A. Veríssimo	N. Straube	Suruga Bay, Japan						
Sja121		MG654927.1/ MG792167.1	A. Yamaguchi	Sho Tanaka	Suruga Bay, Japan			May 5 2011		F	945
Sja123		MG654928.1/ MG792169.1	A. Yamaguchi	Sho Tanaka	Suruga Bay, Japan			21 Mar 2007		F	885
Sja124		MG654929.1/ MG792170.1	A. Yamaguchi	Sho Tanaka	Suruga Bay, Japan			24 Apr 2009		M	774
Smi120	*mitsukurii*	MG654933.1/ MG792166.1	A. Yamaguchi	Sho Tanaka	Suruga Bay, Japan			21 Mar 2007		M	778
Smi122		MG654934.1/ MG792168.1	A. Yamaguchi	Sho Tanaka	Suruga Bay, Japan			10 May 2011		F	1096
Sha090	*hawaiiensis*	MG654906.1/ MK005139	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4916N	157.7525W	17 Aug 2015	360	F	58.4
Sha091		MG654907.1/ MK005138	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4916N	157.7525W	17 Aug 2015	360	F	64.8
Sha093		MG654908.1/ MK005136	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4916N	157.7525W	17 Aug 2015	360	F	63.5
Sha114		MG654909.1/ MK005131	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4983N	157.7310W	29 Jan 2016	305	M	60.8
Sha115		MG654910.1/ MK005135	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4983N	157.7310W	29 Jan 2016	305	M	57.5
Sha116		MG654911.1/ MK005134	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4983N	157.7310W	29 Jan 2016	305	F	75.1
Sha117		MG654912.1/ MK005133	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4983N	157.7310W	29 Jan 2016	305	M	48.8
Sha118		MG654913.1/ MK005132	J.M. Anderson	J.M. Anderson	Kaneohe Bay, Oahu, Hawaii	21.4983N	157.7310W	29 Jan 2016	305	M	55.5