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The present state of the leech fauna (Annelida, Hirudinea) in the Upper Irtysh cascade of water reservoirs

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Abstract

Hirudinea is a small and ecologically important group of aquatic organisms. However, up to date, the leech fauna of Kazakhstan is poorly studied. The presence of large under-collected areas, such as the Upper Irtysh basin, makes biodiversity studies concerning these invertebrates from Kazakhstan relevant. In this paper, the latest information on species diversity of the freshwater hirudofauna of the Upper Irtysh cascade of water reservoirs, the Kazakhstan part of Irtysh River, is presented. It includes 10 free-living and parasitic species, of which 7 and 9 inhabit the Shulbinsk and the Bukhtarma reservoirs, respectively. These species belong to 2 orders, 3 families and 6 genera. The faunal list highlights four potentially new morphological species (*Alboglossiphonia* sp., *Erpobdella* sp., *Piscicola* sp. 1 and *Piscicola* sp. 2). Besides them, another three species *Erpobdella vilnensis*, *Helobdella stagnalis* and *Theromyzon tessulatum* recorded for the first time in the area. The exact systematic position is stated for all leech taxa. Each species from the list accompanied with information on taxonomic synonymy, data on its geographic distribution, and brief summary of morphological and ecological characteristics.

Keywords

Hirudinea, species diversity, Upper Irtysh water reservoirs

Introduction

The Irtysh River with a catchment area of more than 300 000 km^2 is the main water artery of the Eastern Kazakhstan, which meets the regional requirements of water resources. The hydrological level of the river is controlled by the Upper Irtysh water cascade consisting of Bukhtarma, Shulbinsk and Ust-Kamenogorsk storage reservoirs. Using these reservoirs, a multi-year, seasonal and weekly regulation of the river flow is consistently implemented (Beysembaeva and Dubrovskaya 2014, Vinokurov et al. 2010). The Bukhtarma reservoir is the largest of them; it serves as the main regulator of the cascade as a whole. The close proximity of the mining resource industry and nonferrous metallurgy resulted in ecologically strenuous conditions on the Bukhtarma reservoir (Kulikov et al. 2011). A specificity of biota inhabiting the Bukhtarma reservoir was formed by both the natural dispersal of species from the flooded water bodies and by the use of artificial colonization (Zadoenko et al. 1985, Evseeva 2011). The Ust-Kamenogorsk reservoir is located in the mountain valley of canyon type; it regulates weekly and daily river flow. This reservoir is characterized by significant water exchange, cold water and absence of the littoral zone. A high flowage of the reservoir, with an extremely unstable water exchange and low water temperature are limiting factors for many species of aquatic organisms. The aquatic fauna of this reservoir was formed as a result of biological invasions from upstream reservoirs (Evseeva 2012). The Shulbinsk reservoir in turn completes the cascade of artificial reservoirs, constructed in the Upper Irtysh. The reservoir conducts the seasonal adjustment of lateral afflux (Ulba River and Uba River) in the area between Bukhtarma and Shulbinsk hydropower stations. Operating mode of the Shulbinsk reservoir has a negative impact on its ichthyofauna and causes a significant reduction in biodiversity of benthic organisms (Kulikov et al. 2011). Thus, the exploitation of hydroelectric power stations, increased water consumption and the development of floodplain areas have led to changes in the hydrological regime of the Irtysh River and decrease in the natural potential of the river ecosystem (Beysembaeva and Dubrovskaya 2014). Instability of the hydrological level in the Irtysh cascade reservoirs, industrial impacts, and acclimatization activities conducted earlier necessitate the studying of natural biodiversity and its preserving.

Freshwater Hirudinea is one of the most important ecological groups of hydrobionts. Leeches are of scientific interest as an important link in the food chain of aquatic ecosystems, as well as bio-indicators of water pollution (Bezmaternykh 2007, Romanova and Klimina 2010, Kaygorodova et al. 2014). Moreover, parasitic leeches are involved in the abundance regulation of host species. As it was established, Hirudinea sp. may be directly related to transmission of bacterial and viral infections (Ahne 1985, Mulcahy et al. 1990, Cruz-Lacierda et al. 2000, Faisal et al. 2009, Faisal et al. 2011), as well as hematozoa including trematodes, cestodes, and nematodes (Demshin 1975) and parasitic flagellates (Khan 1976, Khamnueva and Pronin 2004, Burreson 2007), which are considered to be pathogenic organisms for aquatic animals. Moreover, ulceration, hemorrhage, and inflammation associated with leech attachment sites weaken the host and may pose an opportunity hosts to bacterial infections.

Previously, special-purpose research of leech fauna has never been conducted within the Upper Irtysh cascade. There are only scant data on the occurrence of a few leech species in the Bukhtarma water reservoir. As part of the fish parasite fauna, two species of leeches were identified – *Piscicola geometra* (Linnaeus, 1758) and *Caspiobdella fadejewi* (Epstein, 1961) (Izumova 1977). Later on, the presence of *P. geometra* has been confirmed, and the species list has been supplemented by *Hemiclepsis marginata* (Müller, 1774) and *Erpobdella octoculata* (Linnaeus, 1758) (Deviatkov 2012). The presence of *C. fadejewi* is doubtful, since this species belongs to the European faunistic complex and has a limited distribution within the basins of the rivers that flow into the Azov and Black Seas (Epstein 1987) and the Volga River (Lapkina and Komov 1983). Data on its occurrence outside this region are absent. Most likely, the leech species affiliation could be incorrectly identified. Thus, the species composition of the Irtysh leech fauna needs to be clarified, especially since the previously mentioned papers exclude the possibility of a verification due to the absence of morphological descriptions.

The aim of the study was to determine the current state of the species diversity and its spatial distribution and to fulfil a comparative analysis of the parasitic annelid fauna of the Upper Irtysh water cascade.

Materials and methods

Expedition works were conducted in August-September 2014. The biological material was collected in the dam areas of Bukhtarma and Shulbinsk water storage reservoirs (Fig. 1). Catching leeches was performed manually or by using hydrobiological nets in the coastal zone of each reservoir in a depth range of 0.5–1.5 m. The specimens were fixated in 80% ethanol with preliminary animal anaesthesia by low percent alcohol solution.

Morphological analysis of ethanol-fixed samples was performed using a stereomicroscope MSP-2 var. 2 (LOMO). The leech species affiliation was ascertained under existing systematic keys (Lukin 1976, Nesemann and Neubert 1999) in accordance with the present-day classification of the group. Reference specimens are stored in the annelid collection of the Limnological Institute.

Results

This is the updated checklist of the Irtysh leech species inhabiting the Upper Irtysh cascade of water reservoirs. At present, 10 species were documented. The species diversity includes leeches from two orders (Rhynchobdellida and Arhynchobdellida), three families (Glossiphoniidae, Piscicolidae, Erpobdellidae) and six genera (*Alboglossiphonia*, *Helobdella*, *Hemiclepsis*, *Theromyzon*, *Piscicola*, and *Erpobdella*). Species composition includes both free-living and parasitic freshwater leeches. Parasitic forms are represented by seven species, and include representatives of 5 genera – *Theromyzon tessulatum*, *Hemiclepsis marginata*, *Alboglossiphonia* sp., *Helobdella stagnalis*, *Piscicola geometra*, *Piscicola* sp. 1,

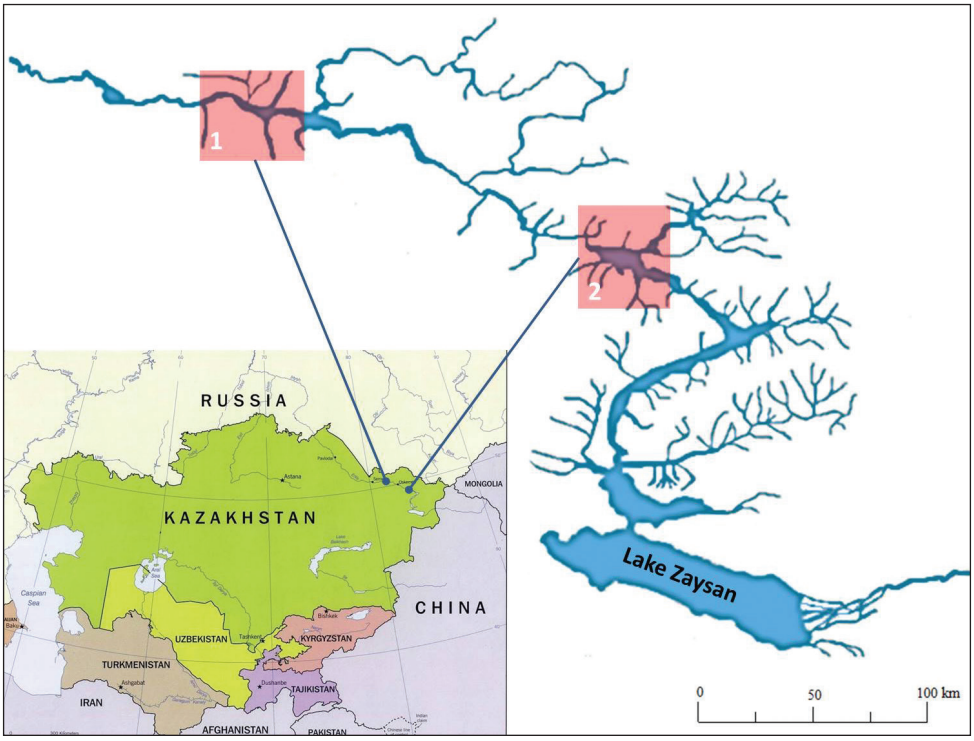

Figure 1. Geographical location of the study region with indication of sampling areas: **1** Shulbinsk water reservoir **2** Bukhtarma water reservoir.

Piscicola sp. 2. Among free-living macrophagous leeches, there are only three erpobdellid species, namely *Erpobdella vilnensis*, *Erpobdella octoculata*, and *Erpobdella* sp.

The taxonomic list includes both Palaearctic species (*H. marginata*, *P. geometra*, *T. tessulatum*, *E. octoculata*, *E. vilnensis*), and widespread Holarctic species (*H. stagnalis*). Four species in the checklist, including *Alboglossiphonia* sp., *Erpodella* sp. and two representatives of *Piscicola* (Table 1), were referred in this paper, with caution, to unidentified species since their morphology differed from all currently described species. With high probability, these four unidentified species are potentially new to science. All four of these, for the first time were recorded within the Irtysh River basin.

Hirudofauna of the Bukhtarma and Shulbinsk water reservoirs is represented by 9 and 7 leech species, respectively (Table 1). Higher species diversity found in the Bukhtarma storage reservoir, is probably related to more favourable environmental conditions for freshwater leeches. This reservoir has a greater area and more tributaries (Fig. 1). Moreover, 50 years ago exactly in the Bukhtarma reservoir, experiments on acclimation of fish, molluscs, mysids and amphipods have been conducted. Sixteen alien species (6 species of fish and 10 species of invertebrates) have successfully taken root (Zadoenko et al. 1985, Evseeva 2011), which significantly expanded the number of potential hosts for parasitic leeches.

Table 1. Leech fauna of the Upper Irtysh water cascade

#	Taxon	Bukhtarma reservoir	Shulbinsk reservoir
1.	*Alboglossiphonia* sp.	+	+
2.	*Helobdella stagnalis*	+	+
3.	*Hemiclepsis marginata*	+*	+
4.	*Theromyzon tessulatum*	+	
5.	*Piscicola geometra*	+	+
6.	*Piscicola* sp. 1		+
7.	*Piscicola* sp. 2	+	
8.	*Erpobdella octoculata*	+	+
9.	*Erpobdella vilnensis*	+	+
10.	*Erpobdella* sp.	+	
Total:		**9**	7

* according to Deviatkov (2012).

Systematics

Phylum: ANNELIDA Lamarck, 1809
Class: CLITELLATA Michaelsen, 1919
Subclass: HIRUDINEA Lamarck, 1818 (synonym Hirudinida)
Order: RHYNCHOBDELLIDA Blanchard, 1894
Family: GLOSSIPHONIIDAE Vaillant, 1890
Subfamily: GLOSSIPHONIINAE Autrum, 1939

***Alboglossiphonia* Lukin, 1976**

Geographic distribution. Holarctic.

Type species. *Alboglossiphonia heteroclita* (Linnaeus, 1761)

***Alboglossiphonia* sp.**
Fig. 2

New species records. The Shulbinsk and the Bukhtarma water reservoirs.

Morphological characteristics. Small leaf-shaped leech. Mature individuals are about 3–6 mm in length and 2–4 mm in width. The number and arrangement of eyes is typical for representatives of the genus. Body surface is smooth, papillae are mild. Live animals are light yellow, the colour practically disappears when fixing. A characteristic feature of this species is a specific arrangement of the sparse pigment cells, main concentration of which falls on the anterior and posterior end of the body surface. *Alboglossiphonia* sp. is bears offspring on the ventral surface; dimensions of young are up to 1 mm in length with a width of 0.2 mm.

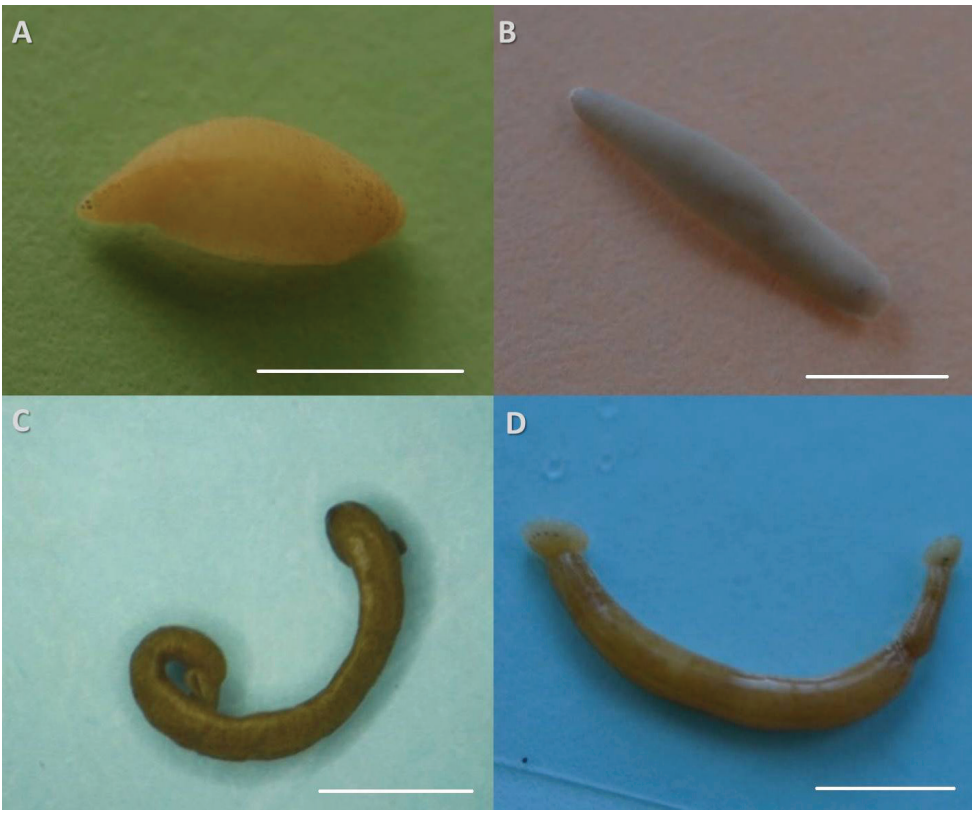

Figure 2. External morphology of new species from the Upper Irtysh reservoirs: **A** *Alboglossiphonia* sp. **B** *Erpobdella* sp. **C** *Piscicola* sp. 1 **D** *Piscicola* sp. 2. Scale bar is 5 mm.

Ecological characteristics. This species is a benthic ectoparasite of snails. It parasitizes mainly gastropods. Single specimens were found often on aquatic vegetation, less often on solid substrates.

Helobdella Blanchard, 1876

Geographic distribution. Cosmopolite.

Type species. *Helobdella stagnalis* (L., 1758)

Helobdella stagnalis (Linnaeus, 1758)

Synonymy. *Hirudo stagnalis*: Linnaeus 1758; *Hirudo pulligera*: Daudin 1800; *Glossiphonia perate*: Johnson 1816; *Erpobdella bioculata*: Lamark 1818; *Clepsine bioculata*:

Savigny 1822; *Glossiphonia punctata* Johnson 1825; *Clepsine stagnalis*: Fillipi 1837; *Glossiphonia bioculata*: Maquin-Tandon 1846; *Glossiphonia circulans*: Maquin-Tandon 1846; *Clepsine modesta*: Verrill 1972; *Glossiphonia modesta*: Vaillant 1890; *Glossiphonia stagnalis*: Blanchard 1894; *Glossiphonia* (*Helobdella*) *stagnalis*: Moore 1922; *Bakedebdella gibbosa*: Sciacchitiano 1939.

Geographic distribution. Cosmopolite. Shulbinsk and Bukhtarma water reservoirs.

Morphological characteristics. One pair of eyes. Leeches are oval in shape; body has jagged edges. The body surface is smooth, no papillae. Intravital coloration can be from light green to yellowish or even with tints of gray. Preserved specimens become dirty-white or completely lose colouring. A distinctive feature of this species is the presence of chitin plate on the dorsal side. Small leeches, reaching a body length of 2–11 mm and width of 1–4 mm.

Ecological characteristics. This species is considered one of the most common freshwater leeches in the world. It inhabits almost all types of freshwaters, the most abundant in slow running and stagnant waters with well-developed aquatic vegetation. This species is a benthic ectoparasite. It sucks the hemolymph of freshwater invertebrates such as oligochaetes, larvae of insects, and molluscs.

Hemiclepsis Vejdowsky, 1884

Geographic distribution. Palaearctic region.

Type species. *Hemiclepsis marginata* (Müller, 1774)

Hemiclepsis marginata (Müller, 1774)

Synonymy. *Hirudo marginata*: Müller 1774; *Hirudo variegata*: Braun 1805; *Hirudo cephalota* Carena 1820; *Hirudo oscillatoria*: Saint-Amas 1825; *Piscicola tesselata*: Maquin-Tandon 1826; *Piscicola linearis*: Kollar 1842; *Glossobdella cephalota*: Blainville 1827; *Haemoharis marginata*: Filippi 1837; *Glossiphonia marginata*: Maquin-Tandon 1846; *Hirido flava*: Dalyell 1853; *Glossiphonia flava*: Johnston 1865; *Glossiphonia marginata*: Blanchard 1892.

Geographic distribution. Palaearctic region. Shulbinsk water reservoir.

Morphological characteristics. Two pairs of eyes, the first pair is a smaller and located close to the front edge of the body. Clavate head is well separated from the body. Colour in life is from light green to light brown. Alcohol-fixed specimens are either greyish green or light pink colour, respectively. The dorsal side is granular and pigmented, with the exception of the posterior part and suckers with a characteristic pattern. Small leeches with length of 2–12 mm and 2–6 mm of width.

Ecological characteristics. This species is found in differing types of running and stagnant waters. This leech is an ectoparasite sucking blood of different species of fish, amphibians and birds.

Theromyzon Philippi, 1867

Geographic distribution. Palaearctic region.

Type species. *Theromyzon tessulataum* (Müller, 1774)

Theromyzon tessulatum (Müller, 1774)

Geographic distribution. Palaearctic region. Bukhtarma water reservoir.

Synonymy. *Hirudo tessulata*: Müller 1774; *Nephelis tessulatum*: Savigny 1822; *Erpodella vulgaris* var. *tessulatum*: Blainville 1828; *Clepsine tessulata*: Müller 1844; *Glossiphonia tessulatum*: Moquin-Tandon 1846; *Glossiphonia vitrina*: Johnston 1865; *Theromyzon tessulatum*: Philippi 1867; *Hemiclepsis tessulatum*: Vejdovsky 1884; *Protoclepsis tessulatum*: Livanow 1902.

Morphological characteristics. Four pairs of eyes. The shape and colour of the body varies and depends on satiety of leeches. Colour in life is from light green to brown-red. Posterior sucker is well developed, with radial bright spots on it. The dorsal surface is almost smooth, with six rows of paramedial underdeveloped papillae on it. The crop has 7 pairs of caeca. Leeches are up to 15 mm in length and 10 mm in width.

Ecological characteristics. It was found in warmed backwaters with well-developed aquatic vegetation. *Theromyzon tessulatum* is a temporary ectoparasite feeding on vertebrates, mostly waterfowl.

PISCICOLIDAE Johnston, 1865 (synonym Icthyobdellidae Leuckart, 1863)

Piscicola De Blainville, 1818

Geographic distribution. Holarctic region

Type species. *Piscicola geometra* (Linnaeus, 1758)

Piscicola geometra (Linnaeus, 1758)

Geographic distribution. Holarctic region. Both the Shulbinsk and the Bukhtarma water reservoirs.

Synonymy. *Hirudo geometra*: Linnaeus 1758; *Hirudo galearia*: Braun 1805; *Ichthyobdella geometra*: Blainville 1828; *Ichthyobdella percae*: Templeton 1836; *Piscicola piscium*: Apáthy 1888; *Piscicola lippa*: Olsson 1893; *Piscicola perspicax*: Olssen 1893.

Morphological characteristics. Two pairs of eyes are typical for the family. Elongated small sized cylindrical leeches with well-developed (conspicuous) suckers. Cranial sucker is smaller than caudal one. There are 10 ocelli on caudal sucker. Body colour varied from greenish to brown-olive. A characteristic feature of the species is

cross-shaped pattern on the dorsal surface. Leeches are 5–12 mm of length and 1.5–2 mm of width.

Ecological characteristics. This species inhabits both the Bukhtarma and Shulbinsk water reservoirs. Ectoparasite of various fish species.

***Piscicola* sp. 1**
Fig. 2

New species record. the Bukhtarma reservoir and the Shulbinsk reservoir.

Morphological characteristics. Number and arrangement of the eyes is typical for Piscicolidae. Suckers are small. Posterior sucker with diameter of 1.5 mm is slightly larger than anterior sucker. There is an achromatous medial stripe on the dorsal surface. Ethanol fixed specimen has a light green coloration, with dark pigment cells, which are evenly distributed over the entire body surface. Body length of 17 mm and a width of 2 mm

Ecological characteristics. A single specimen was found in a floating piece of rotten wood in the coastal zone of the Shulbinsk reservoir. Host is unknown.

***Piscicola* sp. 2**
Fig. 2

New species record. the Bukhtarma water reservoir.

Morphological characteristics. Two pairs of eyes, their arrangement as in *P. geometra*. The body is in the form of an elongated cylinder. It has a light brown coloring. Distinguishing features are rather large copulatory area and diamond-shaped depigmented pattern on the dorsal side. In fixed specimens, body length of 12–16 mm and a width of 1.5–2.5 mm.

Ecological characteristics. Three specimens were collected in swampy backwater of the Bukhtarma reservoir near the New Bukhtarma village in the water plant thickets, where many invertebrates and young fish live. Exact host is unknown.

Order: ARHYNCHOBDELLIDA Blanchard, 1894
Suborder: Erpobdelliformes Sawyer, 1986
Family: ERPOBDELLIDAE Blanchard, 1894

***Erpobdella* de Blainville, 1818**

Geographic distribution. Holarctic region

Type species. *Erpobdella octoculata* (Linnaeus, 1758)

Erpobdella octoculata (Linnaeus, 1758)

Synonymy. *Hirudo octoculata*: Linnaeus 1758; *Hirudo vulgaris*: Müller 1774; *Nephelis octoculata*: Moquin-Tandon 1826; *Nephelis sexoculata*: Scheider 1883; *Nephelis scripturata*: Scheider 1885; *Nephelis atomaria*: Blanchard 1893; *Nephelis crassipunctata*: Scheider 1893; *Nephelis seripturata*: Blanchard 1893; *Nephelis sexoculata*: Blanchard 1893; *Herpobdella octoculata*: Johannson 1910; *Herpobdella octomaculata*: Pawlowski 1935.

Geographic distribution. Palaearctic region. Shulbinsk and Bukhtarma water reservoirs.

Morphological characteristics. Four pair of eyes. The basic colour of live specimens is light brown, with dark reticulate pigmentation in dorsal side. Body surface is covered with multiple papillae of various size. Genital pores are separated by 2.5 annuli. Leeches are up to 32 mm in length and 4 mm in width.

Ecological characteristics. Eurythermal species is very tolerant of a lack of oxygen and can live in highly polluted waters (Lukin, 1976). Within the studied area, *E. octoculata* is widespread, but not abundant in the Bukharma water reservoir. This macrophagous leech feeds on small invertebrates, remains of dead animals, and with the critical shortage of natural food can be cannibals.

Erpobdella vilnensis (Liskiewicz, 1925)

Synonymy. *Herpobdella atomaria* var. *monostriata*: Gedrouć 1916; *Herpobdella octoculata* subsp. *vilnensis*: Liskiewicz 1925; *Herpobdella octoculata* f. *monostriata*: Gedroyć and Pawlowski 1936; *Herpobdella vilnensis*: Liskiewicz 1934; *Erpobdella octoculata* f. *monostriata*: Gedroyć and Pawlowski 1937; *Erpobdella monostriata*: Gedroyć and Pawlowski 1948.

Geographic distribution. Palaearctic region. *Erpobdella vilnensis* is rather a common leech species that occurs in Central, Eastern and South-Eastern Europe. In Central Asia, it was first discovered in the Shulbinsk and the Bukhtarma water reservoirs during this study.

Morphological characteristics. Four pairs of eyes. In living leeches, the basic body is dark brown to almost black. Dorsally, there is one pair of dark paramedian longitudinal stripes. After fixation colour is changed to light brown. Genital pores are separated by 2.5 annuli. Body length is up to 22 mm at width of 3–4 mm.

Ecological characteristics. Predator of small invertebrates. The specimens were collected in coastal part of the Bukhtarma and Shulbinsk reservoirs. In the latter, it is a very common leech.

Erpobdella sp.

Fig. 2

New species record. The Bukhtarma water reservoir.

Morphological characteristics. Four pair of eyes with typical for the family location. Small-sized erpobdellids, with body length of 5–15 mm and width of 2–2.5 mm. The leeches have no pigmentation. Genital pores are separated by 2.5 annuli.

Ecological characteristics. This species was found in Northern part of the Bukhtarma reservoir only, where it very abundant.

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RESEARCH ARTICLE

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A new species of the genus *Castoponera* (Araneae, Corinnidae) from Sarawak, Borneo, with comparison to a related species

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Abstract

A new species of the genus *Castoponera* Deeleman-Reinhold, 2001, *Castoponera christae* **sp. n.**, is described here. The species is closely related to *C. lecythus* Deeleman-Reinhold, 2001, but can be distinguished by the structures of the male palp and the female genitalia.

Keywords

Castianeirinae, taxonomy, myrmecomorphy, Southeast Asia

Introduction

The genus *Castoponera* Deeleman-Reinhold, 2001 is endemic to Southeast Asia, and is currently comprised of three species (Deeleman-Reinhold 2001; World Spider Catalog 2016). From Borneo, two species, *C. scotopoda* Deeleman-Reinhold, 1993 and *C. lecythus* Deeleman-Reinhold, 2001, have been recorded. *Castoponera* species closely resemble Ponerinae ants and they commonly occur on the forest floor. The morphological resemblance to ants is known as myrmecomorphy and is a common phenomenon in the Corinnidae (Cushing 1997, 2012; Haddad 2013; Raven 2015; Reiskind 1969).

Our group has conducted several investigations in Borneo to reveal the association between ant-mimicking spiders and ants. Although the Corinnidae fauna in Southeast Asia has been comprehensively reviewed by Deeleman-Reinhold (1993, 2001), our investigations have resulted in the discovery of an undescribed corinnid species. We here describe it, in comparison with the closely related species, *C. lecythus*.

Materials and methods

Specimens examined here were collected from the forest floors in Danum Valley Field Centre, Tawau Hills Park and Poring Hot Spring, Sabah, and Lambir Hills National Park, Sarawak, Borneo (Fig. 1). Collected spiders were preserved in 75% ethanol. The morphology was examined using a Nikon SMZ1270 microscope, and specimens were sorted and identified on the basis of descriptions in Deeleman-Reinhold (2001). Multi-focused montage images were produced using Helicon Focus ver. 4.2.9 from several series of source images. The habitus images were obtained using a Canon EOS 60D camera with a Canon MP-E 65mm macro lens and the images of the male palp and female genitalia were obtained by the same camera attached to a Nikon AZ100 microscope.

Methodology and terminology for the description follow Deeleman-Reinhold (2001). The leg spination of each segment is described as a row of spines from proximal to distal parts on each side (dorsal, ventral, prolateral and retrolateral sides). However, recognition of spine position on distal part of metatarsi III and IV was very difficult due to the narrow segments. For the distal spines on these segments the total number of spines is given, without positional information. For the width of the eye region, the width of posterior eye row was measured. All measurements are given in millimeters. Abbreviations used in the present paper are as follows: ALE, anterior lateral eyes; AME, anterior median eyes; d, dorsal; pl, prolateral; PLE, posterior lateral eyes; PME, posterior median eyes; rl, retrolateral; v, ventral.

The holotype designated here is deposited in the Forest Research Centre, Sarawak, Malaysia (FRCS), and the paratypes in FRCS and the Museum of Nature and Human Activities, Hyogo, Japan (MNHAH).

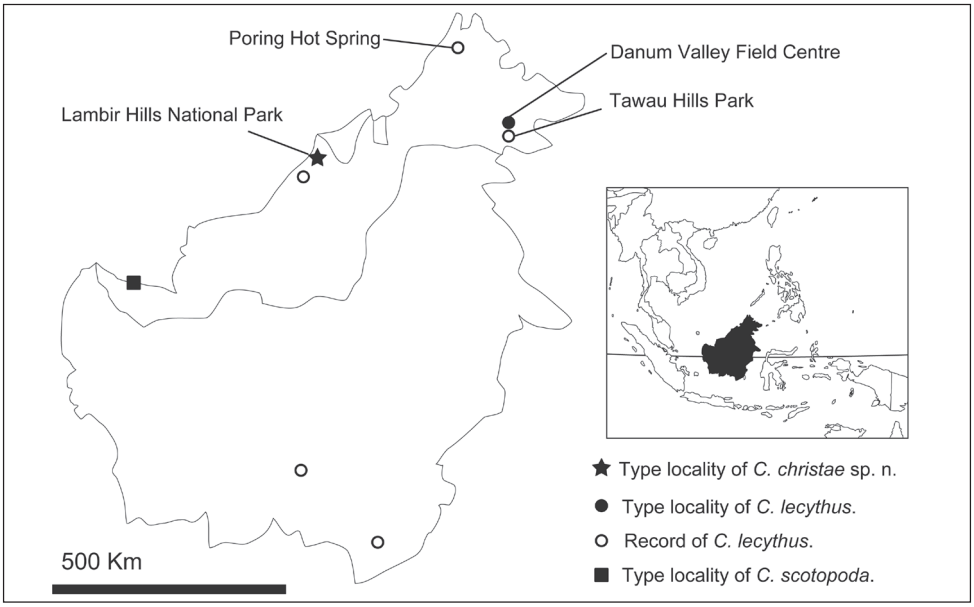


Figure 1. Study sites and distribution of *Castoponera* species on Borneo (modified from Deeleman-Reinhold 2001).

Taxonomy

Castoponera **Deeleman-Reinhold, 2001**

Castoponera christae **Yamasaki, sp. n.**
http://zoobank.org/F527D81F-E1D1-4FC9-A0C2-69C5333D96BA
Figs 2–18

Type material. Holotype male (FRCS; LCo20090226 Itioka), Sungai Liku (Liku River), 4°14'N, 114°03'E, Lambir Hills National Park, Sarawak, Borneo, 29-II-2009, T. Itioka leg. **Paratypes:** 1 female (FRCS; LCo20070822-AMS2), same locality as the holotype, 22-VIII-2007, Y. Hashimoto & T. Endo leg.; 1 male (MNHAH; LCo20140331-HYO1), same locality as the holotype, 31-III-2014, F. Hyodo leg.

Diagnosis. In males, *C. christae* sp. n. is distinguishable from *C. ciliata* (Deeleman-Reinhold, 1993) and *C. scotopoda* by the long embolus (Figs 6, 15, cf. figs 445, 449 in Deeleman-Reinhold 2001), and from *C. lecythus* by the tapering distal region of the bulb: lateral margins are more or less parallel in *C. lecythus* (Figs 6, 15 vs. Figs 23, 32). In females, *C. christae* sp. n. is distinguishable from *C. ciliata* and *C. scotopoda* by the long and curved insemination ducts (Figs 14, 18, cf. figs 448, 451 in Deeleman-Reinhold 2001), and from *C. lecythus* by the position of the copulatory opening on the copulatory atrium, the position of insemination duct where it joins the bursa, and the rounded shape of the bursa (Figs 13–14, 17–18 vs. Figs 30–31, 34–35).

Figures 2–8. *Castoponera christae* sp. n., male. **2** habitus, dorsal view **3** habitus, lateral view **4** habitus, ventral view **5** chelicera and fang, ventral view **6** palp, ventral view **7** palp, retrolateral view **8** palp, dorsal view. Scales: 1.0 mm (**2–4**), 0.5 mm (**5–8**).

Measurements (holotype male/paratype female). Total length 7.4/8.5. Carapace length 3.07/4.20; width 1.87/2.43; height 0.97/1.20. Clypeus height 0.28/0.37. Eye size: AME 0.18/0.21; ALE 0.12/0.15; PME 0.14/0.18; PLE 0.14/0.18. Width of eye

region 0.68/0.98. Distance between PMEs 0.13/0.15. Abdomen length 4.70/8.80; width 1.67/2.33.

Male (Figs 2–5). Carapace oval, with granulated surface (Fig. 2). Chelicera with three promarginal and two retromarginal teeth on fang furrow (Fig. 5). Retrocoxal hymen obviously smaller than ALE, approximately 0.06 mm in diameter. Pedicel wrapped in tube-like sclerite extending from abdomen (Figs 3–4). Abdomen slender pear-shaped, constricted at middle part; entire surface strongly sclerotized (Fig. 2).

Male palp (Figs 6–8, 15–16). Cymbium slender (Fig. 8). Bulb slender teardrop-shaped, including tapering anterior part and globular posterior part (Figs 6, 15); distal part curved toward retrolateral side (Figs 6, 15). Sperm duct beginning at retrolateral surface of bulb, strongly curving once at retrolateral and twice at ventral surfaces (Figs 6–7, 15–16), then extending to embolus (Figs 6, 15). Embolus very slender, strongly curved on horizontal plan against longitudinal axis (Figs 6, 15).

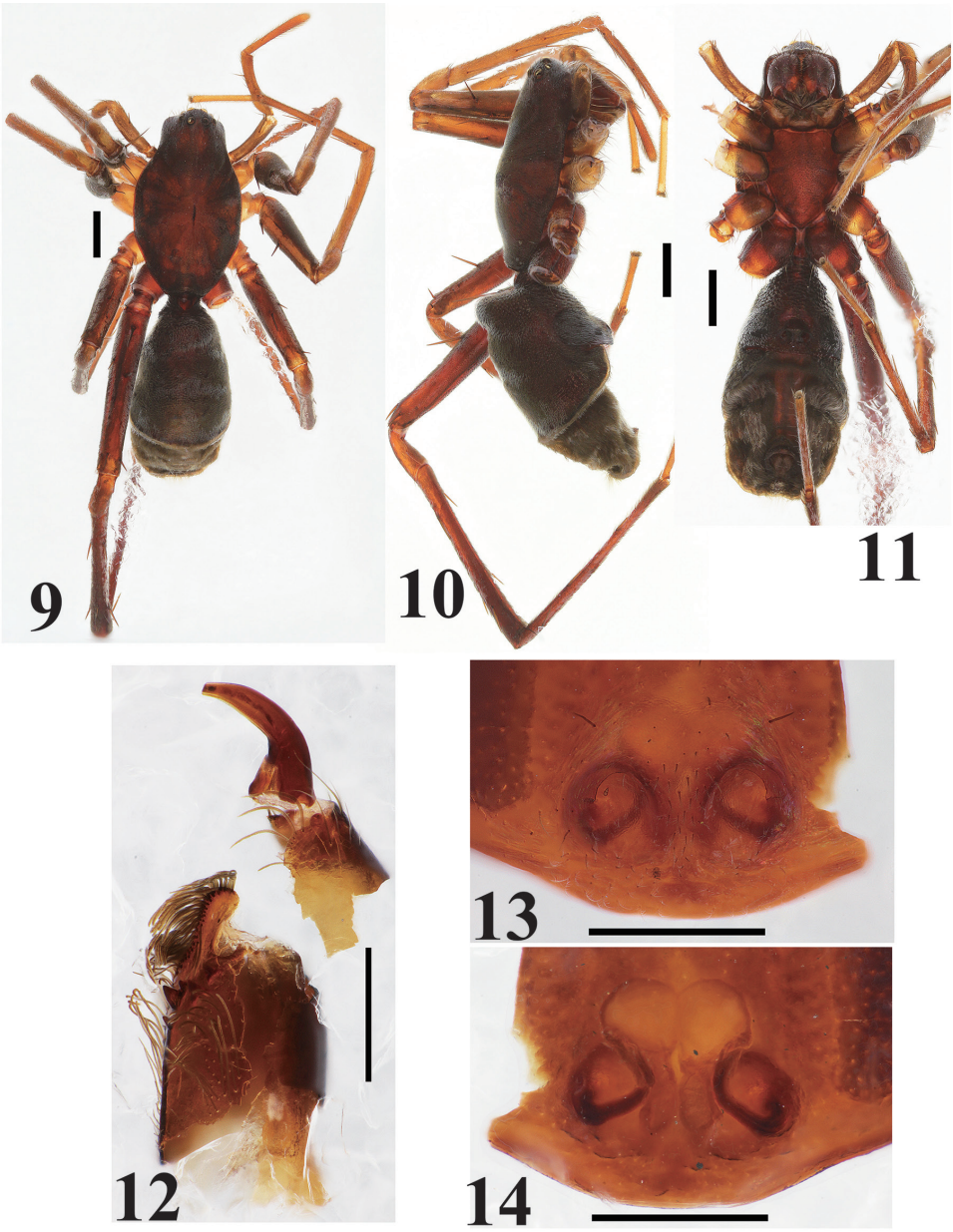
Leg spination. Femur I 1-1-1d, 1pl; tibia I 2-2-2v; metatarsus I 2-2v; femur II 1-1-1d, 1pl; tibia II 2-2-2v; metatarsus II 2-2v; femur III 1-1-1d, 1-1pl, 1-1rl; tibia III 1d, 1-2v, 1-1pl, 1-1rl; metatarsus III 2-2v, 1-1pl, 1-1rl with 5 distal spines; femur IV 1-1-1d, 1-1pl, 1-1rl; tibia IV 1d, 1-2-2v, 1-1pl, 1-1rl; metatarsus IV 2-2v, 1-1pl, 1-1rl with 5 distal spines.

Coloration and setation (Figs 2–4). Carapace dark brown, covered with short fine setae; anterior surface near eye region covered with white plumose setae. Chelicera brown; anterior surface sparsely covered with long gray setae and short transparent setae; promargin of fang furrow densely fringed with long thick setae whose surfaces are rough (Fig. 5). Labium, maxilla, sternum brown. Legs covered with black setae, black plumose setae and transparent plumose setae; plumose setae sparse in tarsi; coxae I, II and III brownish cream; coxa III more darker than I and II; coxa IV brown; trochanters almost same coloration as in coxae; femora light brown, tinged with black in femora I, II and III; patellae yellowish cream to brownish cream; tibiae I and II grayish yellow, III and IV light brown; metatarsi almost same coloration as in tibiae; tarsus I cream, tarsi II and III brownish cream, tarsus IV light brown. Pedicel dark brown. Abdomen blackish brown; entire surface covered with white fine plumose setae, posterior surface additionally covered with long setae; thick white plumose setae forming following markings: a pair of patches and transverse band on anterior dorsum, transverse band encircling abdominal constriction, two or three patches and transverse band on posterior dorsum; posterior end bearing tuft of white long plumose setae.

Female (Figs 9–12). Almost same as in male, except for abdomen. Abdomen without distinct constriction; anterior half covered with strongly sclerotized surface (Figs 10–11).

Female genitalia (Figs 13–14, 17–18). Copulatory atrium round; copulatory opening located at outer margin of atrium (Figs 13, 17). Insemination duct curving, connecting to outer margin of bursa (Figs 14, 18). Bursa round, accompanying slender spermatheca on posterior margin (Figs 14, 18).

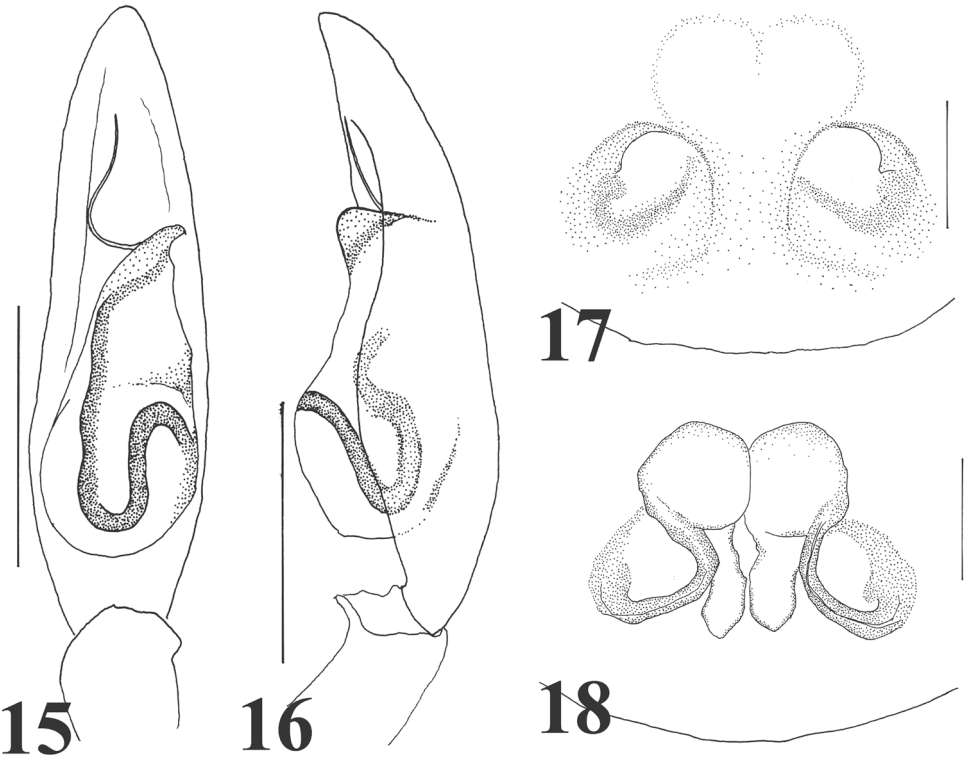
Leg spination. Femur I 1-1-1d, 1pl; tibia I 2-2-2v; metatarsus I 2-2v; femur II 1-1-1d, 1pl; tibia II 2-2-2v; metatarsus II 2-2v; femur III 1-1-1d, 1-1pl, 1-1rl; tibia III 1d, 1-2v, 1-1pl, 1-1rl; metatarsus III 2-2v, 1-1pl, 1-1rl with 5 distal spines; femur IV



Figures 9–14. *Castoponera christae* sp. n., female. **9** habitus, dorsal view **10** habitus, lateral view **11** habitus, ventral view **12** chelicera and fang, ventral view **13** epigyne, ventral view **14** internal structures of genitalia, dorsal view. Scales: 1.0 mm (**9–11**), 0.5 mm (**12–14**).

1-1-1d, 1-1pl, 1-1rl; tibia IV 1d, 1-1-2v, 1-1pl, 1-1rl; metatarsus IV 2-2v, 1-1pl, 1-1rl with 5 distal spines.

Coloration and setation. Almost same as in male.



Figures 15–18. *Castoponera christae* sp. n. **15** male palp, ventral view **16** male palp, retrolateral view **17** epigyne, ventral view **18** internal structures of genitalia. Scales: 0.5 mm (**15–16**), 0.25 mm (**17–18**).

Etymology. The specific epithet is a patronym in honor of Dr. Christa L. Deeleman-Reinhold, who has made great contributions in studies of corinnid spiders from Southeast Asia.

Distribution. Lambir Hills National Park, Sarawak.

Remarks. For the female paratype, some morphological characters of the abdomen were not observed because the specimen had once been dried and the soft part of the abdomen was shrunken. However, the sclerotized parts of the abdomen and the genitalia have been well preserved and the identification is possible on the basis of these characters.

Castoponera christae sp. n. is closely related to *C. lecythus*. The male of *C. christae* sp. n. can be distinguished from the male of *C. lecythus* by the medially constricted abdomen (Figs 2–4 vs. Figs 19–21), shape of apical part of the bulb and route of the sperm duct running on the surface of the bulb (Figs 6–7, 15–16 vs. Figs 23–24, 32–33). Additionally, among our specimens of each species, the posterior bulb of *C. christae* sp. n. is more swollen than that of *C. lecythus*. In the females it is relatively difficult to distinguish the species using superficial characters. However, the internal genitalic structures are clearly distinct (Figs 14, 18 vs. Figs 31, 35).

Castoponera lecythus **Deeleman-Reinhold, 2001**
Figs 19–35

Castoponera lecythus Deeleman-Reinhold, 2001: 314, figs 452–463.

Material examined. 1 male (MNHAH; AMS-Hy6), Danum Valley Field Centre, 4°58'N, 117°48'E, Sabah, Borneo, 18-XII-2006, Y. Hashimoto leg.; 1 female (MNHAH; AMS3), same locality, 9-I-2008, Y. Hashimoto & T. Endo leg.; 1 female, Tawau Hills Park, 4°23'N, 117°53'E, Sabah, Borneo, 17-XI-2009, T. Yamasaki leg.; 1 male, Poring Hot Spring, Kinabalu Park, 6°02'E, 116°42'E, Sabah, Borneo, 12-XI-2010, T. Yamasaki leg.

Measurements (Male: AMS-Hy6/Female: AMS3). Total length 7.4/8.7. Carapace length 3.13/3.45; width 1.87/2.13; height 0.98/1.05. Clypeus height 0.26/0.28. Eye size: AME 0.18/0.20; ALE 0.12/0.13; PME 0.15/0.18; PLE 0.15/0.18. Width of eye region 0.70/0.80. Distance between PMEs 0.13/0.16. Abdomen length 3.10/5.00; width 1.58/2.50.

Male (Figs 19–22). Carapace oval, with granulated surface (Fig. 19). Chelicera with three promarginal and two retromarginal teeth on fang furrow (Fig. 22). Retrocoxal hymen obviously smaller than ALE, approximately 0.05 mm in diameter. Pedicel wrapped in tube-like sclerite extending from abdomen (Figs 19–21). Abdomen slender oval, slightly constricted at anterior part; entire surface strongly sclerotized (Fig. 20).

Male palp (Figs 23–25, 32–33). Cymbium slender (Fig. 25). Bulb teardrop-shaped but retrolateral corner of anterior bulb squarish; posterior part spherical, slightly asymmetrical (Figs 23, 32). Sperm duct beginning at retrolateral surface of bulb, curving twice at retrolateral and once at ventral surfaces, then running directly into embolus through center of bulbal surface (Figs 23–24, 32–33).

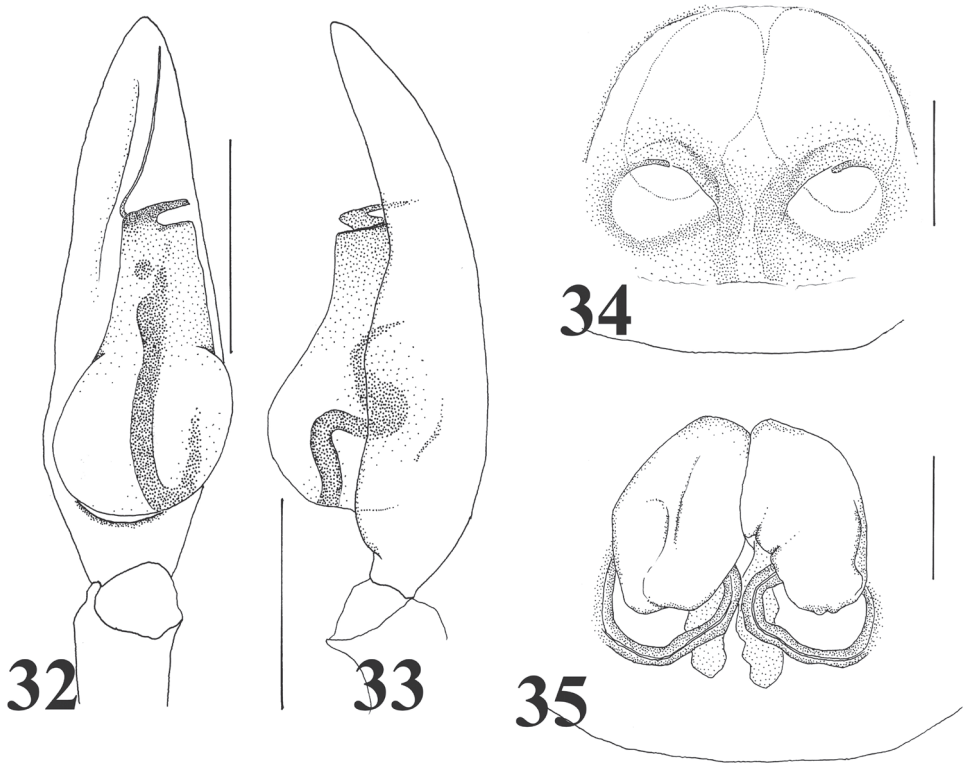
Leg spination. Femur I 1-1-1d, 1pl; tibia I 2-2-2v; metatarsus I 2-2v; femur II 1-1-1d, 1pl; tibia II 1-2-2v; metatarsus II 2-2v; femur III 1-1-1d, 1-1pl, 1-1rl; tibia III 1d, 1-2v, 1-1pl, 1-1rl; metatarsus III 2-2v, 1-1pl, 1-1rl, with 5 distal spines; femur IV 1-1-1d, 1-1pl, 1rl; tibia IV 1d, 1-1-2v, 1-1pl, 1-1rl; metatarsus IV 2-1v, 1-1pl, 1-1rl, with 5 distal spines.

Coloration and setation (Figs 19–22). Carapace dark brown, covered with short fine setae; anterior surface near eye region covered with white plumose setae. Chelicera brown; anterior surface sparsely covered with long gray setae and transparent plumose setae; promargin of fang furrow densely fringed with long thick setae whose surfaces are rough (Fig. 22). Labium, maxilla, sternum brown. Legs covered with black setae, black plumose setae and transparent plumose setae; plumose setae sparse in tarsi; coxae I, II and III brownish cream; coxa III darker than I and II; coxa IV brown; trochanters almost same coloration as in coxae; femora brown; patellae I and II yellowish brown, III and IV light brown; tibiae I and II yellowish brown, III light brown, IV brown; metatarsi almost same coloration as in tibiae; tarsi yellowish brown, IV more darker than others. Pedicel dark brown. Abdomen blackish brown; entire surface covered

Figures 19–25. *Castoponera lecythus*, male. **19** habitus, dorsal view **20** habitus, lateral view **21** habitus, ventral view **22** chelicera and fang, ventral view 23 palp, ventral view **24** palp, retrolateral view **25** palp, dorsal view. Scales: 1.0 mm (**19–21**), 0.5 mm (**22–25**).

with plumose setae, some white and some light brown, and posterior surface covered with long setae; thick white plumose setae forming following markings: transverse band on anterior dorsum, transverse band encircling middle part, large patch on posterior dorsum; posterior end bearing tuft of long white plumose setae.

Figures 26–31. *Castoponera lecythus*, female. **26** habitus, dorsal view **27** habitus, lateral view **28** habitus, ventral view **29** chelicera and fang, ventral view **30** epigyne, ventral view **31** internal structures of genitalia, dorsal view. Scales: 1.0 mm (**26–28**), 0.5 mm (**29–31**).



Figures 32–35. *Castoponera lecythus*. **32** male palp, ventral view **33** male palp, retrolateral view **34** epigyne, ventral view **35** internal structures of genitalia, dorsal view. Scales: 0.5 mm (**32–33**), 0.25 mm (**34–35**).

Female (Figs 26–29). Almost same as in male, except for abdomen. Abdomen without distinct constriction; anterior half covered with strongly sclerotized surface (Figs 26–28).

Female genitalia (Figs 30–31, 34–35). Copulatory atrium round; copulatory opening located at anterior margin (Figs 30, 34). Insemination duct, curving, connecting to inner margin of posterior bursa (Figs 31, 35). Bursa oval, accompanying slender spermatheca on posterior margin (Figs 31, 35).

Leg spination. Femur I 1-1-1d, 1-1pl; tibia I 1-2-2v; metatarsus I 2-2v; femur II 1-1d, 1pl; tibia II 2-2-2v; metatarsus II 2-2v; femur III 1-1d, 1pl, 1rl; tibia III 1d, 1-2v, 1-1pl, 1-1rl; metatarsus III 2-2v; 1-1pl, 1-1rl, with 5 distal spines; femur IV 1-1-1d, 1-1pl, 1-1rl; tibia IV 1d, 1-1-2v, 1-1pl, 1-1rl; metatarsus IV 2-2v, 1-1pl, 1-1rl, with 5 distal spines.

Coloration and setation (Figs 26–29). Almost same as in male.

Distribution. Danum Valley Feild Centre, Sabah (Deeleman-Reinhold 2001); Tawau Hills Park, Sabah; Poring Hot Spring, Kinabalu Park, Sabah; Niah cave National Park, Sarawak (Deeleman-Reinhold 2001); Kaharian, 2°02S, 113°40'E, SE

Figures 36–37. *Diacamma* spp. **36** *Diacamma* sp., Poring Hot Spring, Borneo **37** *Diacamma* sp. Java.

Kalimantan (Deeleman-Reinhold 2001); Aranio district, SE Kalimantan (Deeleman-Reinhold 2001).

Remarks. We examined 1 male and 1 female collected from the type locality, and these specimens agreed with the original description of *C. lecythus*. For the comparison with *C. christae* sp. n., see Diagnosis and Remarks in *C. christae* sp. n.

Notes on *C. christae* sp. n. and *C. lecythus*

The members of Castianeirinae are considered to be myrmecomorphies (Cushing 1997; Deeleman-Reinhold 2001). In the fields, *C. christae* sp. n. or *C. lecythus* occur sympatrically with Ponerinae ants such as *Diacamma* Mayr, *Odontoponera* Mayr and *Leptogenys* Roger. These ants might be the suitable models of Batesian mimicry for *C. christae* sp. n. and *C. lecythus* because they are common and abundant in the forest floor, and have a sting. *Castoponera christae* sp. n. and *C. lecythus* are especially similar to *Diacamma* spp. in the coloration and setation of the abdomen (Figs 36–37). The transversal bands of white setae on the abdomen emphasize the similarity to *Diacamma* ants.

Acknowledgements

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RESEARCH ARTICLE

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Sinolatindia petila gen. n. and sp. n. from China (Blattodea, Corydiidae, Latindiinae)

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Abstract

Sinolatindia petila **gen. n.** and **sp. n.** (Blattodea: Corydiidae: Latindiinae) is reported from Yunnan Province, China. Description, illustrations and a distribution map of the new taxon are provided. Comparisons with the type genus *Latindia* Stål, 1860 and the genus *Homopteroidea* Shelford, 1906 are given.

Keywords

Blattodea, China, cockroaches, Corydiidae, Latindiinae, new genus, new species, *Sinolatindia*, *Sinolatindia petila*

Introduction

Latindiinae, a group of small sized Corydiidae cockroaches, is a poorly studied subfamily that superficially differs from the typical Corydiidae. Despite superficial differences, the diagnostic character of Corydiidae, "anal area of hind wing usually flat in resting position and simply folded over the anterior field" (Roth 2003), still applies to Latindiinae. Previously, Latindiinae species were generally treated under the subfamily Corydiinae/Corydinae (Kirby 1904; Shelford 1912; Hebard 1917, 1921; Karny

1921; Rehn and Hebard 1927; Hanitsch 1931; Rehn 1932, 1937; Bruijining 1959) or the family Corydidae (Brunner 1865). Then Handlirsch (1925) erected the subfamily Latindiinae to include these small, delicate cockroaches with *Latindia* Stål, 1860 designated as the type genus. This subfamily status was accepted by some researchers (Princis 1950; Rehn 1951), but it was later raised to family level (Brues and Melander 1932; Princis 1960, 1963). Princis (1963) listed twelve genera under the family Latindiidae. Roth (2003) kept the subfamily Latindiinae, but didn't list the genera under it. Pellens and Grandcolas (2008) followed the subfamily status and listed four Brazilian genera in Latindiinae. Beccaloni (2014) only lists the two genera *Latindia* and *Bubloblatta* in the subfamily Latindiinae, and many of the genera listed in Princis (1963) are treated as undetermined genera. Several phylogenetic works (Djernæs et al. 2015; Legendre et al. 2015) have shown Latindiinae as being closely related to Nocticolidae; but due to the limited taxon sampling of Nocticolidae, Latindiinae and other Corydiidae (Djernæs et al. 2015), the current taxonomy is maintained.

Recently, the cockroach collection of the Institute of Zoology, Chinese Academy of Science, Beijing (IZCAS) was examined, and two peculiar cockroach specimens were found. They are very small and delicate. After careful study of these two specimens, it was established that they should be classified as a new species belonging to a new genus under the subfamily Latindiinae. This new genus resembles the type genus *Latindia* Stål and may be confused with the southeast Asian genus *Homopteroidea* Shelford. A comparison is made of this new genus with *Latindia* and *Homopteroidea*.

Materials and methods

Specimens studied and examined during this research are deposited in the following institutions:

IZCAS Institute of Zoology, Chinese Academy of Sciences, Beijing, China
NRM Swedish Museum of Natural History (Naturhistoriska riksmuseet), Stockholm, Sweden
OUM Oxford University Museum of Natural History, Oxford, UK

Morphological terminology used in this paper mainly follows Hanitsch (1929), Roth (1995a, 1995b) and Klass (1997), and venation terms mainly follow Kukalová-Peck and Lawrence (2004) with the modification by Li and Wang (2015). Original and important taxonomic references are cited after taxon names. Some figures in this article are without scales because the original figures lack scales or are illustrated with magnification.

The venation terms and their abbreviations in parentheses in this article are listed as below: subcosta (**Sc**), radius (**R**), radius anterior (**RA**), presutural vein, media (**M**), cubitus anterior (**CuA**), cubitus posterior (**CuP**), CuP in claval furrow (**CuP in cfr**), anal fold (**afd**), anal anterior (**AA**), and anal posterior (**AP**). The presutural vein is an important character in *Homopteroidea*, which may be a separated part of CuA, the area between

it and the sutural margin totally hyaline, which is known as presutural zone, the terms "presutural vein" and "presutural zone" follow Hanitsch (1929) and Roth (1995a, 1995b).

The current Latindiinae only contains two genera (Beccaloni 2014), and many of the former Latindiinae genera are treated as subfamily undetermined (Roth 2003). It is inadequate to establish this new genus by only comparing with the current two genera, we have carefully reviewed the ten genera of Latindiidae listed in Princis (1963) (excepting *Biolleya* and *Stenoblatta*, which have been moved into Blaberidae by Roth (2003)) and we found *Sinolatindia* gen. n. is very similar to *Latindia*. We also compared the new genus with *Homopteroidea*, since they are all distributed in Oriental Region and they may be confused by some common characters.

The genital segments of the examined specimens were macerated in 10% NaOH and observed in glycerin jelly using a Motic® K400 stereomicroscope and a Leica® M205A stereomicroscope. All drawings were made with the aid of Adobe Photoshop® CS5, a Leica® M205A stereomicroscope and a Motic® K400 stereomicroscope. Photographs of the specimens were taken using a Canon® 50D plus a Canon® EF 100mm f/2.8L IS USM Macro lens combined with Helicon Focus® software; photos of other characters were taken using a Leica® M205A stereomicroscope. All photographs mentioned above were modified in Adobe Photoshop® CS5. The map was downloaded from www.d-maps.com and modified using Adobe Photoshop® CS5.

Taxonomy

Subfamily Latindiinae Handlirsch, 1925

Latindiinae Handlirsch, 1925: 491, designated subfamily with one male *Latindia* sp., mentioning *Latindia* and *Paralatindia* as examples; Rehn 1951: 29; Roth and Slifer 1973: 23; Roth 2003: 34, cited as "Latindiinae Beier"; Pellens and Grandcolas 2008: 18; Djernæs et al. 2015: 297.

Latindiidae Brues & Melander, 1932: 81, key to order Blattariae; Princis 1960: 437; Princis 1963: 98, catalogue.

Corydiinae Hebard, 1917: 205; Karny 1921: 191; Rehn and Hebard 1927: 280; Bruijining 1959: 18.

Type genus. *Latindia* Stål, 1860

Remarks. Based on former studies (Handlirsch 1925; Brues and Meander 1932; Rehn 1951), the Latindiinae is characterized as follows: body small, delicate, legs sparsely with spines, cerci long, subgenital plate of female valved or seam divided, venation simple or less branched, tegmina with an irregular network of large cells made by the cross veins, wings with venation reduced but not as extreme as in Holocompsinae.

This subfamily is badly in need of revision. First, recent molecular phylogenetic studies (Djernæs et al. 2015; Legendre et al. 2015) suggest that the subfamily may be more closely related to Nocticolidae than other Corydiidae. Second, although Princis

(1963) listed twelve genera in Latindiidae (now Latindiinae), the validity of these genera has not been verified. What's more, Roth (2003) moved the twelve genera listed in Princis (1963) out of Latindiinae, and kept ten of them in Polyphagidae (now Corydiidae) as subfamily undetermined. This management is also unreasonable, which made Latindiinae without any genera. Third, the definition of Latindiinae is too simple, a careful study on the type genus especially the male genitalia must be done to redefine Latindiinae.

Genus *Sinolatindia* gen. n.
http://zoobank.org/14E2B5FE-322F-42BF-8BA5-E2C253129AB2
纤蠊属

Type species. *Sinolatindia petila* sp. n. 素色纤蠊

Diagnosis. Male. Small size, form elongate elliptical. Body flat, gracile, pubescent. Head transversely triangular, eyes wide apart, interocular space greater than the distance between antennal sockets, ocelli missing. Pronotum suboval, pubescent. Front femur short and robust, type C_1 spination (Fig. 4C), tarsal claws symmetrical, serrated. Tegmina and wings fully developed, right tegmen with wide, hyaline zone, CuA of wings with 2-3 branches. Supra-anal plate symmetrical, transverse, cerci long. Subgenital plate simple, styli similar. Genitalia complex, with a very elongate L3.

This genus is very close to the type genus *Latindia* Stål, 1860. We have examined the type specimen of *Latindia maurella* Stål, 1860 (Fig. 2G–I. Deposited in NRM, the type species of *Latindia*) and one *Latindia dohrniana* Saussure & Zehntner, 1894 (Fig. 2C–D. Deposited in NRM, determined by Rehn in 1930). Along with the descriptions (Rehn and Hebard 1927; Rehn 1937, 1951), it was found that *Sinolatindia* can be distinguished from *Latindia* by the following characters: 1) pronotum subtransparent, disc without a Y-shaped sulcation (Fig. 2A), whilst in *Latindia*, pronotum rough, median with a distinct Y-shaped sulcation (Fig. 2C); 2) right tegmen with a hyaline area (Fig. 4G), while not with a hyaline area in *Latindia* (Rehn & Hebard, 1927); 3) in tegmina, CuA with transverse branches that generally parallel with CuA (Fig. 4F–G), while branches of CuA are oblique, paralleled to each other in *Latindia* (Fig. 2L). In addition, *Latindia* is restricted to north and south America, while *Sinolatindia* gen. n. is found in East Asia.

This genus may be confused with *Homopteroidea*, both of which have hyaline part of right tegmen and serrated tarsal claws, and all distributed in Oriental Region. *Homopteroidea* used to be determined as a member of Latindiidae (Princis 1963), but Roth (1995a, 2003) treated it as subfamily undetermined. We have examined some *Homopteroidea* collections that were studied by Roth (all deposited in OUM) and in combination with the description (Roth 1995a), we found *Sinolatindia* can be distinguished from *Homopteroidea* by the following characters: 1) head wide, vertex nearly truncated (Figs 2B, 4A), without ocelli (Fig. 4A), while head long, vertex round, with

Figure 1. Recorded distribution of *Sinolatindia petila* gen. n. and sp. n.

reduced ocelli (Fig. 2F) in *Homopteroidea*; 2) body subtransparent, pubescent, while body horny, smooth and shining, sometimes with a few setae in *Homopteroidea*; 3) venation of tegmina and wings not distinct, right tegmen without presutural vein and presutural zone, but with a large transparent part, the boundary between the colored part and transparent part unclear (Fig. 4G), while venation clear with dark coloration, presutural vein present, right tegmen with a hyaline presutural zone (Fig. 2J–K, except in *H. aberrans*, see Roth 1995a and 1995b) in *Homopteroidea*; 4) left phallomere with L3 very elongate, apex curved (Fig. 5B–C), while L3 short, apex usually like a sickle (Fig. 5E–F) in *Homopteroidea*.

Female. Unknown.

Distribution. China (Yunnan).

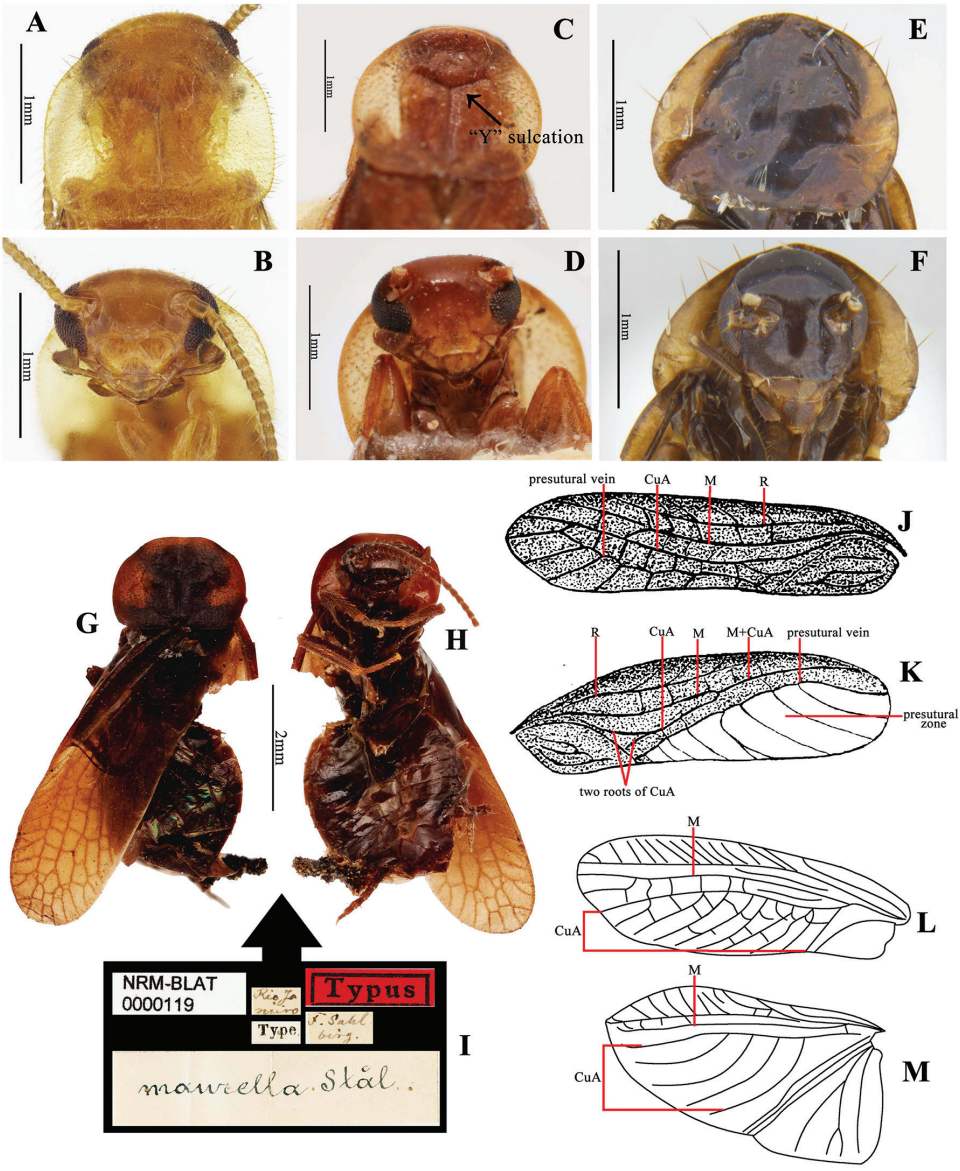

Figure 2. A–F Pronotum and head features **A** pronotum of *Sinolatindia petila*, holotype **B** head of *Sinolatindia petila*, holotype **C** pronotum of *Latindia dohrniana* **D** head of *Latindia dohrniana* **E** pronotum of *Homopteroidea minor*, lectotype **F** head of *Homopteroidea minor*, lectotype **G–I** *Latindia maurella*, holotype, female (originally reported as male, but latter corrected as female (Rehn, 1937)) **G** in dorsal view **H** in ventral view **I** label **J–K** original figures of *Homopteroidea shelfordi*, from Hanitsch, 1929 **J** left tegmen **K** right tegmen **L–M** *Latindia dohrniana*, after Rehn, 1951 **L** tegmen **M** wing **C–D** and **G–I** photographed by Gunvi Lindberg, Swedish Museum of Natural History, Stockholm (NRM) **E–F** photographed by Katherine Child and provided by Amoret Spooner, Oxford University Museum of Natural History, Oxford (OUM).

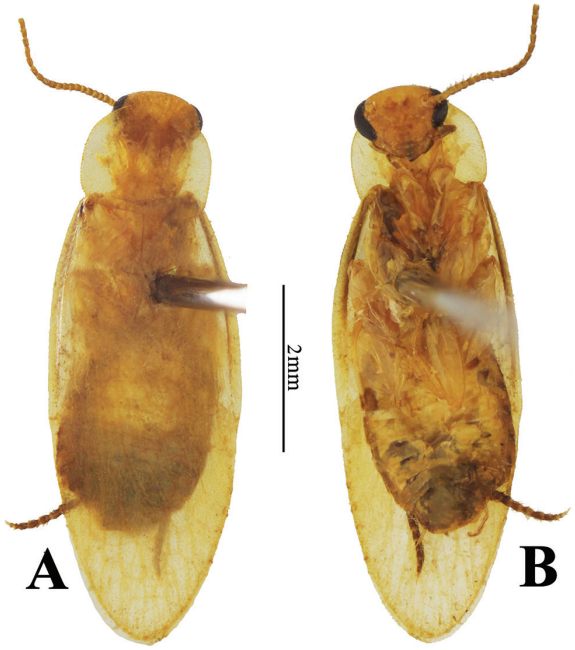

Figure 3. A–B *Sinolatindia petila* gen. n. and sp. n., male **A** paratype, in dorsal view **B** same, in ventral view.

Etymology. This generic epithet comes from the Latin word "*Sino*" and "*Latindia*". "*Sino*" refers to China, "*Latindia*" in reference to the genus being similar to the Latin American genus *Latindia*.

Remarks. This genus contains all the diagnostic characters of Latindiinae. It also quite resembles *Latindia.* Both of the genera hold in common the following characters: 1) body small, form elongate elliptical, very flat, pubescent; 2) head transversely triangle, eyes wide apart, ocelli absent (Fig. 2B, D); 3) pronotum suboval, with hind margin truncated (Fig. 2A, C); 4) femur stout; arolia absent; 5) tegmina with irregular network of large cells made by cross veins, Sc without branches, wings with venation slightly reduced, only the first AP (known as axillary in Rehn 1951) branched. Based on Rehn (1951), this genus should belong to tribe Latindiini whose Sc of tegmen is free from R, and M is stalked basally with CuA, thus this tribe current with two genera, viz. *Latindia* and *Sinolatindia*.

***Sinolatindia petila* sp. n.**
http://zoobank.org/46E73A6D-3BA1-41CF-9C06-974BD02FE49F
素色纤蠊
Figs 1, 2A-B, 3, 4, 5A–D

Type material. Holotype: Yunnan: ♂ (IZCAS), 40 km from southeast Jinggu County (景谷县), Puer City, 1000m, 13.V.1957, D. V. Panfilov leg.; **Paratype: Yunnan:**

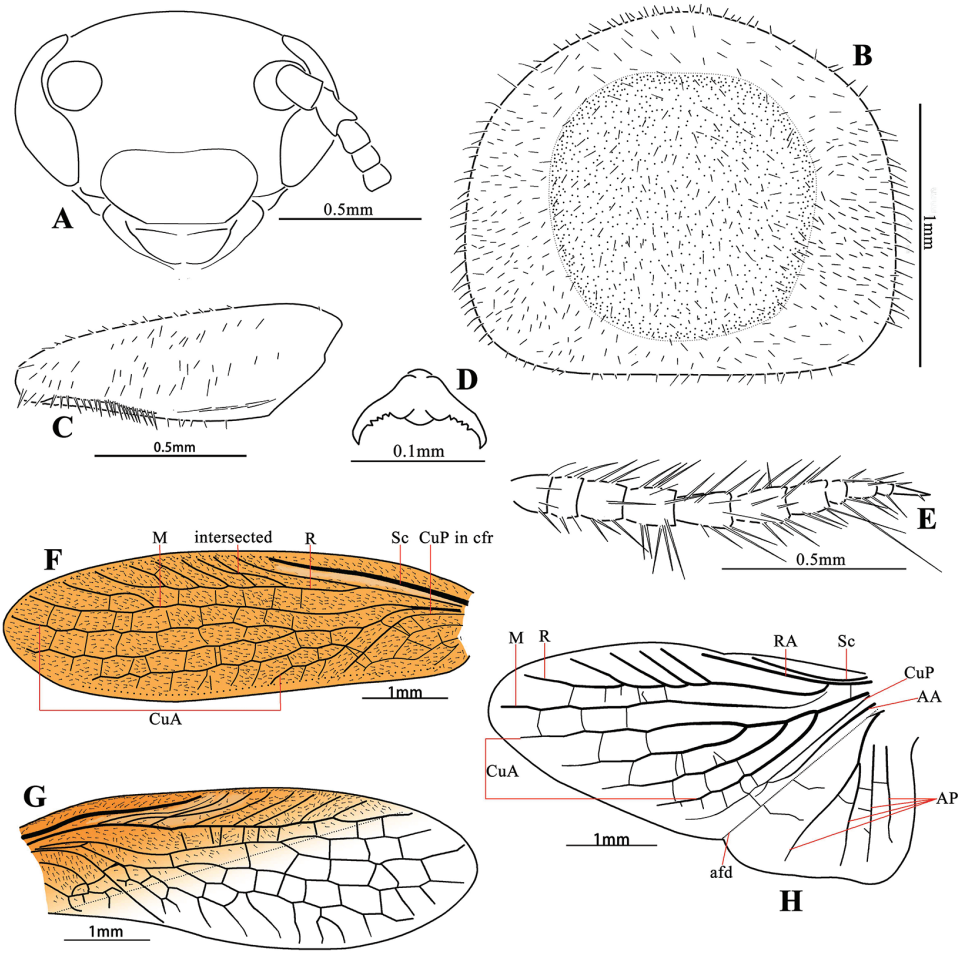

Figure 4. Features of *Sinolatindia petila*, male **A** head **B** pronotum **C** front femur **D** tarsal claw **E** cercus **F** left tegmen **G** right tegmen **H** wing.

1♂ (IZCAS), Mengla County (勐腊县), Xishuangbanna Prefecture, 620-650m, 2.VI.1959, Suo-Fu Li leg.

Diagnosis. As for the genus (*vide supra*)

Description. Male. Body length 5.9–6.0 mm; overall length including tegmen 6.8–7.0 mm; pronotum length × width 1.2–1.3 × 1.5–1.6 mm.

Coloration: Body generally light brownish yellow, transparent (Fig. 3A–B). Head yellowish brown, eyes black, antenna brown. Pronotal disk brownish yellow, with hyaline anterior, posterior and lateral areas (Fig. 2A). Left tegmen brownish yellow, right tegmen brownish yellow with wide hyaline area (Fig. 4F–G). Wings hyaline, distal portion light brownish yellow. Venation of tegmina and wings light-colored. Legs brownish yellow. Cerci brown.

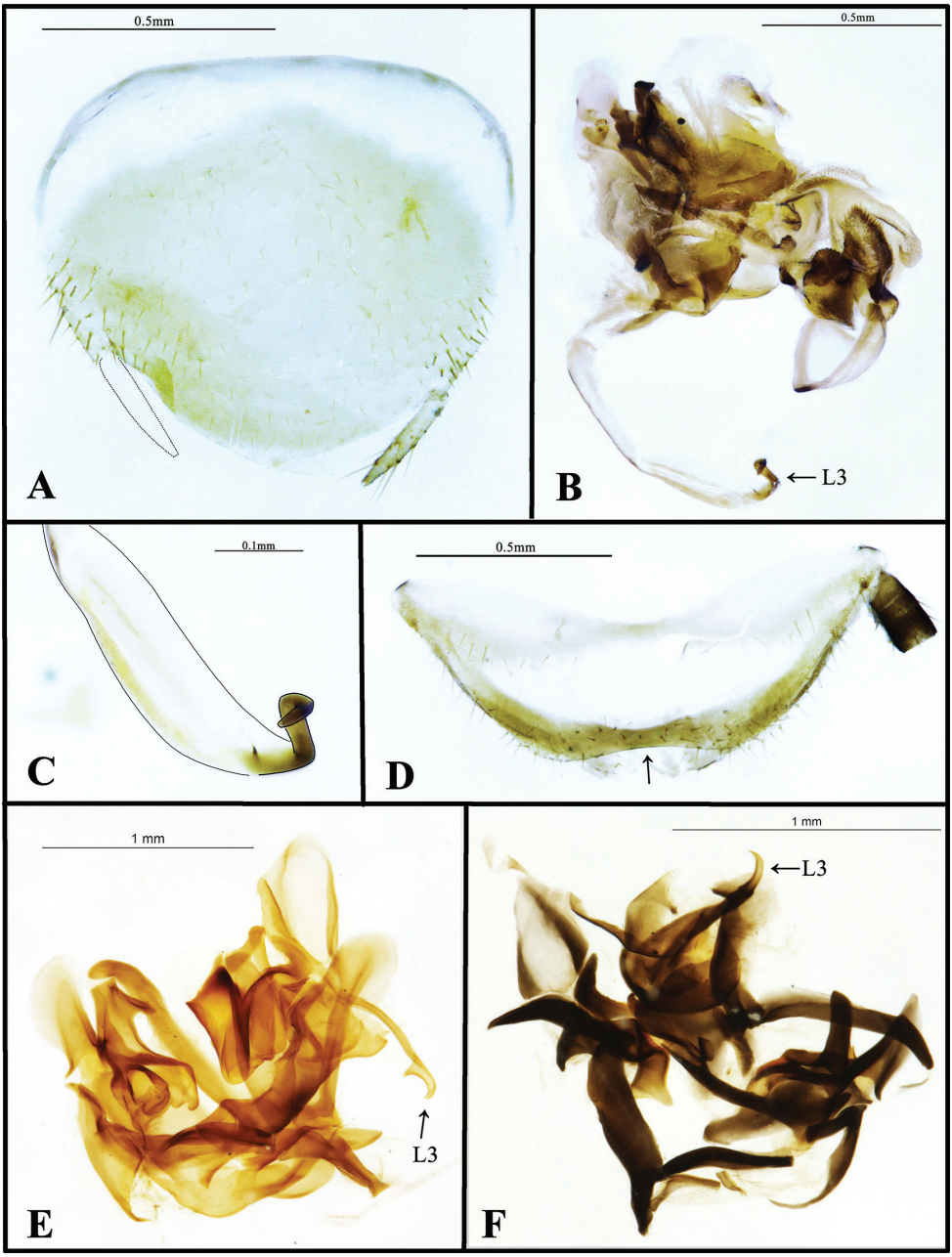

Figure 5. A–D Male features of *Sinolatindia petila* **A** Subgenital plate, in ventral view **B** genitalia, in dorsal view **C** L3, in dorsal view **D** supra-anal plate, in dorsal view **E–F** male genitalia of *Homopteroidea* spp. **E** *H. nigra*, in dorsal view (this is the original position of the drawing pictured in Roth (1995a), and Roth said it was "dorsal") **F** *H. brachyptera*, holotype, in dorsal view (annotation the same as 5E) **E–F** photographed by Katherine Child and provided by Amoret Spooner, Oxford University Museum of Natural History (OUM).

Body very flat, narrow, well pubescent. **Head:** exposed dorsally, triangular, longer than its width, vertex nearly straight, face flat, eyes lateral, wide apart, surface with the individual facets convex, interocular space greater than the distance between antennal sockets, ocelli absent (Figs 2B, 4A). **Pronotum:** suboval, pubescent, anterior margin slightly protruded, lateral of anterior margins oblique, lateral margins nearly parallel, hind margin truncated, with lateral corners bluntly rounded (Figs 2A, 4B). **Tegmina and wings:** fully developed extending beyond the end of abdomen, venations not distinct. Tegmina pubescent except the hyaline region of right tegmen, both tegmina with free, long, simple and strong Sc, R with 7-8 oblique branches, the second and third branches intersected (Fig. 4F–G), RA simple, M bifurcated distally, and stalked with CuA basally, CuA with 2-3 branches, major veins reticulate with some cross veins, forming many polygonal cells (Fig. 4F–G). Wings with Sc shorter than RA, M simple, or bifurcate distally, CuA with 2-3 branches, reticulate with very a few cross veins, CuP slender, AA connects CuP medially or distally, the first AP bifurcate (Fig. 4H). **Legs:** Pubescent, front femur stout, apex of the hind margin with one small spine on each side, and followed with contiguous spinules (type C_1), pulvilli and arolia absent, tarsal claws small, symmetrical, slightly serrated (Fig. 4C–D). **Abdomen:** Supra-anal plate in dorsal view transverse, symmetrical, apex widely depressed (see the arrow in Fig. 5D), anterior and lateral margins pubescent, median hyaline broadly (Fig. 5D), cerci slender, well pubescent, apex acute (Fig. 4E). Subgenital plate generally symmetrical, pubescent, lateral parts with distinctly longer and thicker setae, apex slightly protruding, styli similar, with several long setae (Fig. 5A). **Genitalia:** Very complex, as Figure 5B. Left phallomere with a very elongated L3, the apex of which is curved rectangularly three times as in Figure 5C.

Female. Unknown.

Distribution. China: South Yunnan (Fig. 1).

Etymology. The species epithet is from the Latin word "petilus" meaning thin and little in reference to its narrow and small body.

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RESEARCH ARTICLE

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Cryptotermes colombianus a new drywood termite and distribution record of *Cryptotermes* in Colombia

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Abstract

A new species of drywood termite (Kalotermitidae), *Cryptotermes colombianus*, is described and new records for *Cryptotermes cylindroceps* and *Cryptotermes mangoldi* are presented from the Caribbean coast of Colombia. *C. colombianus* is described from two soldiers and genetic sequences. This unusual species differs noticeably from other regional *Cryptotermes* species for its weak and inconspicuous definition of the frontal and genal horns and its acute angle of the frons with respect to the vertex. *C. colombianus* clustered with species from the Ethiopian and Oriental region and it is closely related to *Cryptotemes havilandi*. *C. cylindroceps* is widely distributed along the Colombian Caribbean coast, commonly associated with dead wood in mangrove habitats. It also is commonly found in wooden furniture, constituting an important household pest. *C. mangoldi* is reported from the Caribbean mainland for the first time.

With these new records, there are now five *Cryptotermes* species for Colombia, including the pest species *Cryptotermes brevis* and *Cryptotermes dudleyi*. This new description raises the numbers of Neotropical *Cryptotermes* to a total of 34 species, of which 2 are fossils, 4 introduced, and 28 endemic.

Keywords

Cryptotermes colombianus new species, *C. cylindroceps*, *C. mangoldi*, tropical dry forest, Colombian Caribbean coast

Introduction

Cryptotermes is one of the best studied and economically most significant genus of drywood termites (Krishna 1961, Chhotani 1970, Gay and Watson 1982, Lenz et al. 1985, Bacchus 1987, Constantino 1998, Scheffrahn and Křeček 1999, Korb 2009, Krishna et al. 2013). Sixty-nine species have been described with 33 distributed in the Neotropics (2 fossil, 4 introduced and 27 native species, including *C. venezolanus - nomen dubium*).

Cryptotermes has been poorly studied in Colombia, only three species have been recorded: *Cryptotermes brevis* (Walker 1853), *Cryptotermes dudleyi* Banks 1918, and *Cryptotermes cylindroceps* Scheffrahn and Křeček 1999, (Gile et al. 2011). The first two species are important pests. *C. dudleyi* has been introduced to the Neotropics and often appears in disturbed outdoor habitats (Scheffrahn and Křeček 1999, Constantino 2002) and *C. brevis* whose origin was established for the Atacama Desert region of coastal northern Chile and southern Peru is widespread in the Neotropics, (Scheffrahn et al. 2009). Until this study *Cryptotermes mangoldi* Scheffrahn and Křeček 1999, was only known from the Dominican Republic.

Morphological identification of termite species can be difficult as diagnostic morphological markers can be rare and are often restricted to soldiers or alates. For such taxa, sequencing of gene fragments (DNAbarcoding) is now an important molecular tool widely used to elucidate phylogenetic relationships between taxa and to identify species (Inward et al. 2007a, 2007b, Legendre et al. 2008). Mitochondrial markers have been extensively used in termites, e.g. Miura et al. 2000, Lo et al. 2004, Ohkuma et al. 2004, Bergamaschi et al. 2007, Li et al. 2009, Singla et al. 2013, Hausberger et al. 2011, Scheffrahn et al. 2015. In termites, sequencing fragments of the cytochrome oxidase subunit II (COII) proved to be an especially suitable marker (e.g., Legendre et al. 2008, Hausberger et al. 2011): Cytochrome oxidase subunit I (COI), the standard 'tree of life gene', is less suitable as it does not amplify well in termites and have too low resolution to distinguish species.

Most studies on Colombian termites have been directed towards species of economic importance as pests in agriculture and forestry (Weidner 1980, Galvis 1985, Scheffrahn et al. 1999, Scheffrahn 2010, 2011, Gutierrez et al. 2004, Medina and Pinzon-Florian 2011, Abadía et al. 2013). The total number of termite species in Colombia remains unknown, but Vargas-Niño et al. (2005) list 26 genera of Termitidae from Colombia. Given that Colombia has 37 types of ecosystems (Instituto de Hidrología, Meteorología y Estudios Ambientales et al. 2007) and more than 26,000 plants (Bernal et al. 2015), 2,569 from tropical dry forests (Instituto de Investigación Alexander von Humboldt 2014), the number of termite species for Colombia is expected to be high.

The purpose of this paper is to describe a new *Cryptotermes* species, *Cryptotermes colombianus*, and to provide new information on the status, biology and distribution of genus *Cryptotermes* in Colombia.

Materials and methods

Specimens were gathered as part of a research project on termite assemblages in the Colombian Caribbean between 2014 and 2015. Termites were collected using a standardized sampling protocol (Jones and Eggleton 2000, Hausberger and Korb 2015). Termites were also collected in structural wood from buildings and furniture. All *Cryptotermes* were preserved in 100% ethanol for DNA analysis, and 80% ethanol for museum curation. Additional *Cryptotermes* localities from Colombia are included in this paper, from an unpublished survey in 2009 by R. Scheffrahn.

Identification

Taxonomic keys from Scheffrahn and Křeček (1999) were used to determine *Cryptotermes* species. The specimens of the new species could not be identified with this key. Hence, it was sequenced together with specimens from all samples, except *C. mangoldi*, for genetic species identification. In addition, eleven other *Cryptotermes* species and *Blatta orientalis* were used for comparison (Table 1). Fragments of the mitochondrial gene *cytochrome oxidase subunit II* (*COII*; total length ~740 bp), 12S rRNA (~385 bp) and 16S rRNA (total length ~480bp) were used and sequenced as described in Hausberger et al. (2011). DNA sequences were aligned with MEGA 6.0. (Tamura et al. 2013) and a Bayesian inference phylogeny was created with MrBayes 3.2.1. (Ronquist and Huelsenbeck 2003) (10^7 generations, 50% discarded as burn-in). The resultant tree was visualized using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Additionally, we also used MEGA 6.0. to calculate *p*-distance between species.

Imaging and measurements

Specimens were suspended in Sagrotan® Hand Sanitizer and images were taken with a Nikon SMZ25 stereomicroscope coupled to a Nikon Model DS-Fi2 digital camera. The software Helicon Focus® was used to stack pictures. Morphological definitions and measurements were done following Roonwal (1969), Gay and Watson (1982) and Scheffrahn and Křeček (1999).

Deposit

Voucher specimens are held at Freiburg University. The holotype, dealated morphotype and pseudergates from type colony of *Cryptotermes colombianus* will be deposited in the Natural History Museum of the Alexander von Humboldt Institute of Bogotá (MIAvH) and Paratype soldier in the collection of the American Museum of Natural

Table 1. GenBank accession numbers of the mitochondrial genes.

Species	GenBank ID		
	COII	12S rRNA	16S rRNA
Blatta orientalis	DQ874267.1	-	-
Cryptotermes cavifrons	FN377806.1	-	-
Cryptotermes colombianus	KU510330	KX267100	KX267099
Cryptotermes cylindroceps	KU510331	-	-
Cryptotermes declivis	HQ012042.1	-	-
Cryptotermes domesticus	AF189085.1	-	-
Cryptotermes dudleyi	FN377808.1	-	-
Cryptotermes havilandi	FN377809.1	-	-
Cryptotermes longicollis	FN377810.1	-	-
Cryptotermes primus	AF189090.1	-	-
Cryptotermes queenslandis	AF189092.1	-	-
Cryptotermes secundus	AF189093.1	-	-
Cryptotermes simulatus	AF189094.1	-	-
Cryptotermes tropicalis	AF189095.1	-	-

History, New York. Specimens of *Cryptotermes cylindroceps* will be part of the collection of the Department of Chemistry and Biology at the University del Norte, Barranquilla, Colombia. Other Colombian material is housed in the University of Florida Termite Collection in Davie, Florida.

Systematics

Family Kalotermitidae Froggatt, 1897
Genus *Cryptotermes* Banks, 1906

***Cryptotermes colombianus* sp. n.**
http://zoobank.org/9D27B3AE-E8A0-4512-8A1E-D9E54A88A46C
Fig. 1

Description. Dealated (Fig. 1A–B). General color brown. Frons pale brown, vertex brown. Pronotum and abdominal tergites brown. Antennae pale brown. Labrum pale brown. Femora brown, tibiae pale brown. Abdominal sternites pale brown and very pale brown laterally. Head suboval; cranial sutures fine, but distinct. Eyes moderately large, non-protruding, and oval. Ocelli moderately large, oval, and touching eyes. Antenna with 6 and 8 articles but incomplete, with formulae 2>3<4=5=6. Pronotum wider than long, usually with distinctive midline mark. Arolia present. Measurements are reported in Table 2.

Soldier. (Fig. 1C–F). Head in dorsal view with frontal flange and front horns very dark; 3/4 of anterior vertex almost black chestnut, grading to chestnut brown;

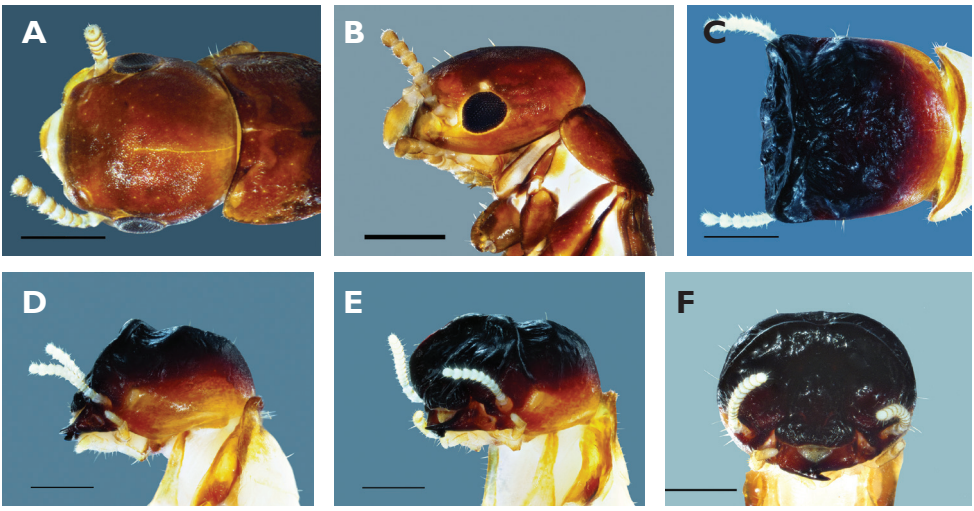

Figure 1. *Cryptotermes colombianus* sp. n. Dealated imago: Head in dorsal (**A**) and lateral view (**B**). Soldier: Head in dorsal (**C**), lateral (**D**), oblique (**E**), and frontal view (**F**). Scale bar: 0.5 mm.

Table 2. Measurements (in mm) of *Cryptotermes colombianus* sp. n. dealated imago.

No.	Measurements in mm (n=1) from 1 colony	
1	Head length with labrum	1.27
2	Head length to postclypeus	1.08
3	Head width, maximum at eyes	0.86
4	Eye diameter, maximun	0.30
5	Eye to head base, minimum	0.16
6	Ocellus diameter	0.08
7	Pronotum, maximum width	0.90
8	Pronotum, maximum length	0.73
9	Total length without wings	4.60
10	Total length with wings	–
11	Fore wing length to suture	–
12	Fore wing, maximun width	–

posterior it turns ferruginous orange to pale yellow (Figure 1C). Head in lateral view with anterodorsal region almost black, which grades steeply to chestnut brown then to pale yellow under eye spot and occipital foramen (Figure 1D). Mandibles chestnut brown. Anterior margin of pronotum chestnut brown posterior margin pale yellow (Fig. 1E–F).

Head in dorsal view abruptly truncated in front; frontal flange forming a rim surrounding a few undulations on frons. Head widest behind flange, gradually narrowing toward the occiput (Figure 1C). Frontal flange coalesces with frontal horn and postclypeus to form pentagonal rim occupying the entire frontal view. In lateral view, margin of frons and occiput from acute ca. 60 degree angle (Fig. 1D–E). Vertex widely striated

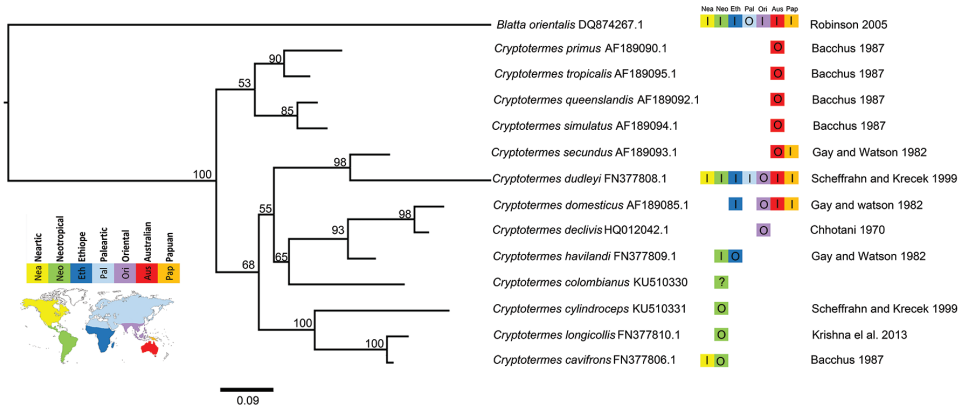

Figure 2. Tree topology and branch lengths inferred with MRBAYES from COII sequence data (Bootstrap values above branches). Origin (O), unknown (?) and established introductions from other regions or land masses (I): Neartic=Nea, Neotropic=Neo, Ethiopian=Eth, Paleartic=Pal, Oriental=Ori, Australian=Aus, Papuan=Pap.

with several robust undulations; frontal horns very broad and shallow; genal horns reduced to tiny protrusions anterior to antennal sockets. Mandibles short humped and slightly bended forward, right mandible tip under tip of left mandible, tips are under labrum in frontal view. Labrum short, hyaline and tongue-shaped. Anteclypeus white; postclypeus trapezoidal with undulating rugosity. Eye spots large, narrowly elliptical. Antenna moniliform between 10 and 12 articles, formula variable 2> 3 = 4 = 5 <6. Legs with three apical spurs on each tibia, formula 3:3:3. Pronotum slightly incised in front, slightly narrower than head capsule. Measurements are reported in Table 4.

Genetic characterization. Thirteen COII mtDNA sequences were aligned for *Cryptotermes* species using *Blatta orientalis* as an outgroup. Information from NCBI is largely limited to COII (see Suppl. material 1), hence we could not include comparative analysis for nuclear and mitochondrial 12S and 16S rRNA genes. Note, COII is very informative to identify termite species (Hausberger et al. 2011).

The COII tree topology for *Cryptotermes* revealed two major clusters, one group composed of eastern Australian species (53% bootstrap value) and the other comprising clusters of Northwest Australian-Papuan (98% bootstrap value), Ethiopian-Oriental (65% bootstrap value) and Neotropical species (100% bootstrap value) (Figure 2). *C. colombianus* is located on a separate basal branch within the Ethiopian–Oriental cluster. Based on additional sequence comparisons, its closest relative among the studied species is *C. havilandi* (p-distance = 0.148) (Table 3).

Phylogeny and phylogeography of the *Cryptotermes* is debated (Chhotani 1970, Gay and Watson 1982, Bacchus 1987, Thompson et al. 2000, Scheffrahn and Křeček 2009). Bourguignon et al. (2014) proposed that Kalotermitidae evolved at the cusp of Gondwana dissolution with *Cryptotermes* originating after the separation of land masses. The current distribution of *Cryptotermes* species can be explained with transo-

Table 3. Estimates of Evolutionary Divergence between Sequences (*p*-distance between species).

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Cryptotermes cavifrons*													
2	*Cryptotermes longicollis*	0.030												
3	*Cryptotermes cylindroceps*	0.157	0.165											
4	*Cryptotermes primus*	0.174	0.186	0.184										
5	*Cryptotermes tropicalis*	0.158	0.172	0.167	0.096									
6	*Cryptotermes queenslandis*	0.167	0.177	0.162	0.130	0.117								
7	*Cryptotermes simulatus*	0.165	0.188	0.160	0.137	0.132	0.064							
8	*Cryptotermes secundus*	0.174	0.183	0.179	0.179	0.163	0.153	0.165						
9	*Cryptotermes dudleyi*	0.200	0.202	0.188	0.209	0.190	0.188	0.205	0.137					
10	*Cryptotermes havilandi*	0.150	0.160	0.167	0.160	0.137	0.151	0.167	0.170	0.183				
11	*Cryptotermes domesticus*	0.165	0.172	0.190	0.160	0.146	0.174	0.177	0.188	0.216	0.113			
12	*Cryptotermes declivis*	0.169	0.176	0.183	0.167	0.150	0.181	0.177	0.176	0.203	0.108	0.059		
13	*Cryptotermes colombianus*	0.183	0.186	0.167	0.172	0.160	0.169	0.162	0.186	0.202	0.148	0.150	0.160	
14	*Blatta orientalis*	0.287	0.296	0.257	0.247	0.256	0.256	0.254	0.270	0.285	0.264	0.237	0.249	0.278

Table 4. Measurements (in mm) of *Cryptotermes colombianus* sp. n. soldier.

No.	Measurements in mm, n=2 from 1 colony	(Holotype)	(Paratype)	Mean
1	Head length to tip of mandibles	1.54	1.38	1.46
2	Head length to frontal horns	1.33	1.23	1.28
3	Frontal flange width	1.32	1.22	1.27
4	Frontal horns, outside span	1.32	1.22	1.27
5	Head width, maximum	1.32	1.22	1.27
6	Head height, excluding postmentum	1.01	0.88	0.94
7	Pronotum, maximum width	1.16	1.14	1.15
8	Pronotum, maximum length	0.82	0.77	0.79
9	Left mandible length, tip to ventral condyle	–	–	–
10	Total length	4.18	3.95	4.07

ceanic dispersal via drift wood (Scheffrahn et al. 2009, Bourguignon et al. 2016) and more recently through human introductions during colonization and trade (Li et al. 2009, Scheffrahn et al. 2009, Evans 2011). The geographic pattern on the phylogeny with regional specific clades may also indicative for some continent specific radiations. The origin of *C. colombianus* is unclear, it may have arrived in Colombia via infested drift wood. Data presented here are not conclusive. More genetic analyses, including different populations, are needed to reveal the origin of *C. colombianus* and track the evolutionary history and dispersal of *Cryptotermes* species.

Material examined. Type-locality: Colombia, Magdalena: Santa Marta, Tayrona National Park, Gayraca Bay, 11°18.84'N; 74°6.34'W, tropical dry forest, 23 June 2015.

Holotype-colony: Colombia. Magdalena Santa Marta Tayrona National Park, Gayraca Bay, 23.VI.2015 (collected by R. Casalla) in a piece of dry wood on soil, at elevation of 12 m a.s.l (11°18.84'N; 74°6.34'W), sample COLPT1LII-56: 2 soldiers, 1 dealated, 23 pseudergates; 3 for DNA isolation. Holotype: Soldier from the previous sample (COLPT1LII-56), it will be deposited at the Arthropod Collection of the Natural History Museum of the Alexander von Humboldt Institute of Bogotá, Colombia (MIAvH). Paratypes from sample COLPT1LII-56: 1 soldier, 1 reproductive dealate. Paratypes will be deposited as follows: 1 soldier will be deposited at the American Museum of Natural History New York, United States, 1 dealated at MIAvH. Pseudergates will be part of the collection of the Department of Chemistry and Biology at the University del Norte, Barranquilla, Colombia. All measurements for dealated reproductive, holotype and paratype soldiers are reported in Tables 2, 4.

Diagnosis. The diminutive frontal and genal horns and the truncated frons and converging genal margins of the head capsule (in dorsal view) distinguish the *C. colombianus* soldier from all other Neotropical congeners.

Etymology. Named for its country of origin, Colombia.

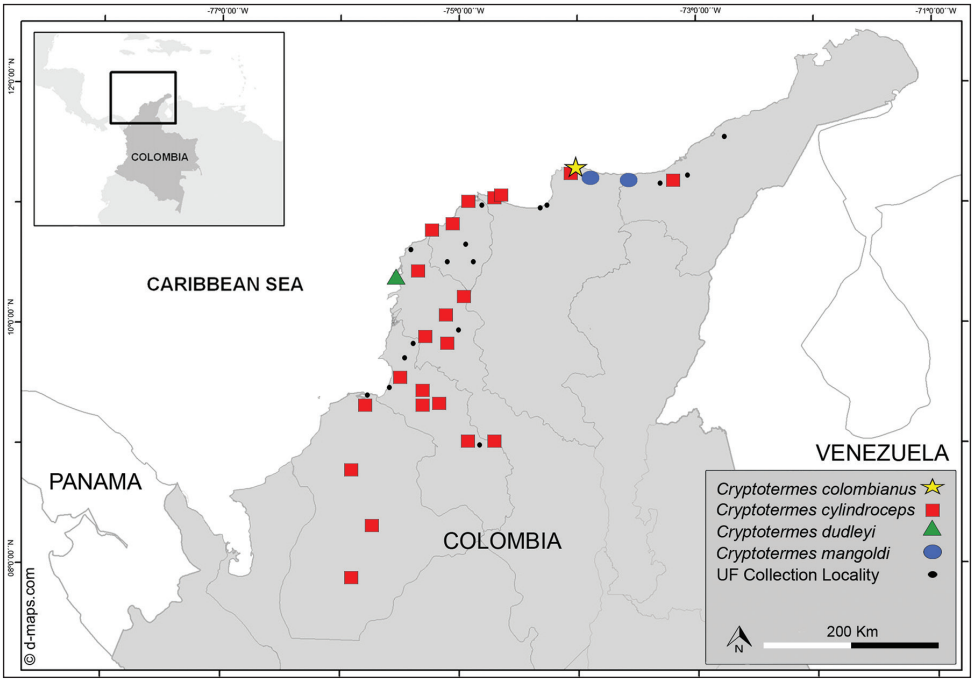

Figure 3. Distribution of the genus *Cryptotermes* in Colombia. *C. brevis* not shown, but widespread.

Discussion

We extend the distribution of *Crytotermes* to Colombia and we herein report *C. mangoldi* for the first time, along the Caribbean coast (Figure 3). We found *C. cylindroceps* in infested drywood trunks of *Glírícidia sepium, Prosopis juliflora*, *Manilkara zapota*, *Hura crepitans* and *Avicennia germinalis*, which are often used for wooden artefacts, furniture and as structural material (Figure 4). Along the coast, *C. cylindroceps* was common in dead branches and trunks of the black mangrove, *Avicennia germinalis*. In line transects that covered a total of 500m x 2m, *C. cylindroceps* accounted for 24 % of all termite encounters (N = 241) (Casalla and Korb, unpublished data). *C. cylindroceps* also occurred up to 100 km inland (Figure 3). Hence, *C. cylindroceps* can be considered an economically important pest to this part of the Caribbean. *C. mangoldi* was only known from the Dominican Republic (Scheffrahn and Křeček 1999). In 2009, R. Scheffrahn found three samples from two localities near Santa Marta, Colombia (Figure 4).

Genetically, *C. cylindroceps* clustered with the other Neotropical endemics, *C. cavifrons* and *C. longicollis* (100% bootstrap value) (Figure 2). Our data provided strong branch support at the regional level, but more resolution from different species are needed to attain a well-corroborated phylogeny of the *Cryptotermes*.

With these new records, there are now five *Cryptotermes* species recorded for Colombia: *C. brevis*, *C. colombianus*, *C. cylindroceps*, *C. dudleyi* and *C. mangoldi*. Further

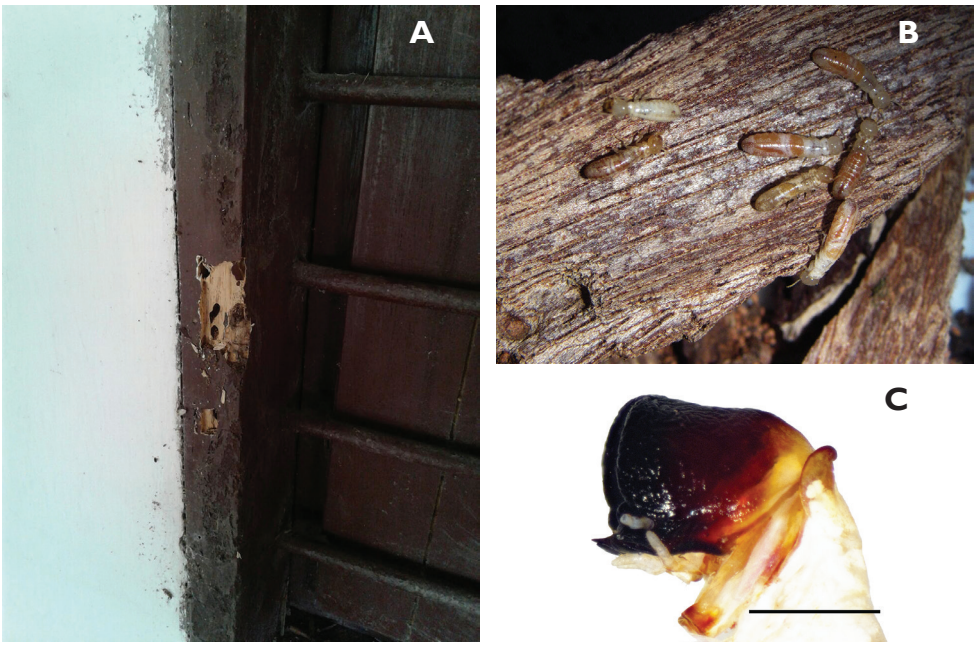

Figure 4. Window frame damaged by *C. cylindroceps* (**A**), workers (white-reddish) (pseudergates *sensu lato*) and neotenic reproductives (brownish) (**B**), soldier of *C. cylindroceps* (**C**). Scale bar: 1 mm

studies on the diversity of termites will determine if there are more *Cryptotermes* in northern and western Colombia, especially at the pacific coast which has important mangroves areas.

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Supplementary material 1

List of *Cryptotermes* of the world and sequences reported in the NCBI for mtDNA genes

Authors: Robin Casalla, Rudolf Scheffrahn, Judith Korb

Data type: Data table Excel

Explanation note: Hits for mtDNA sequences to COII, 12S, 16S and CytB in *Cryptotermes*. NCBI Filter: ("*Cryptotermes*"[Organism] OR cryptotermes[All Fields]) AND (animals[filter] AND biomol_genomic[PROP] AND mitochondrion[filter]). Updated 05.05.16.

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DNA Barcoding of genus *Hexacentrus* in China reveals cryptic diversity within *Hexacentrus japonicus* (Orthoptera, Tettigoniidae)

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Abstract

DNA barcoding has been proved successful to provide resolution beyond the boundaries of morphological information. Hence, a study was undertaken to establish DNA barcodes for all morphologically determined *Hexacentrus* species in China collections. In total, 83 specimens of five *Hexacentrus* species were barcoded using standard mitochondrial cytochrome c oxidase subunit I (COI) gene. Except for *H. japonicus*, barcode gaps were present in the remaining *Hexacentrus* species. Taxon ID tree generated seven BOLD's barcode index numbers (BINs), four of which were in agreement with the morphological species. For *H. japonicus*, the maximum intraspecific divergence (4.43%) produced a minimal overlap (0.64%), and 19 specimens were divided into three different BINs. There may be cryptic species within the current *H. japonicus*. This study adds to a growing body of DNA barcodes that have become available for katydids, and shows that a DNA barcoding approach enables the identification of known *Hexacentrus* species with a very high resolution.

Keywords

BOLD, China, DNA Barcoding, *Hexacentrus*, species delineation

Introduction

DNA barcoding employs short, standardized gene regions (5' segment of mitochondrial cytochrome oxidase subunit I for animals) as an internal tag to enable metazoan species identification (Hebert et al. 2003). Schmidt et al. (2015) found that DNA barcoding largely supported 250 years of classical taxonomy for central European bees. Unlike distinct species, closely related species offer a great challenge for phylogeny reconstruction and species identification with DNA barcoding due to overlapping genetic variation (Dai et al. 2012). For example, Versteirt et al. (2015) found that DNA barcoding offered a reliable framework for mosquito species identification in Belgium except for some closely related species. Zhou et al. (2012) found that molecular identification with DNA barcoding supported most traditional morphological species of genus *Ruspolia* in China.

In this study, our objective is to assess the utility of DNA barcoding for closely related katydid species, belonging to the genus *Hexacentrus* (Serville, 1831) in China. *Hexacentrus* is mainly distributed in Australian, Afrotropical and Oriental realms. *Hexacentrus* is a particularly speciose genus, containing 24 known species (Eades et al. 2016). *Hexacentrus* was the single genus within Hexacentrinae, which has been reported in China according to "Orthptera Species File" (Eades et al. 2016). Up to now, a total of six *Hexacentrus* species have been reported, including *H. japonicus* (Karny, 1907), *H. unicolor* (Serville, 1831), *H. yunnaneus* (Bey-Bienko, 1962), *H. fuscipes* (Matsumura & Shiraki, 1908), *H. mundus* (Walker, 1869) and *H. expansus* (Wang and Shi 2005). Due to the rather difficult morphological discrimination between *Hexacentrus* species, an interspecific molecular delineation was needed. To make *Hexacentrus* more accessible to the scientific community, the open access project "BHC" had been initiated in the Barcoding of Life Data systems (BOLD) (Ratnasingham and Hebert 2007). There are a few DNA barcoding study concentrated on *Hexacentrus*, and the number of barcode sequences was limited in BOLD. The goals of this study are as following: (i) it will allow scientists with molecular capability but insufficient knowledge of *Hexacentrus* taxonomy and systematics to recognize species and document the biodiversity of *Hexacentrus*. (ii) For Tettigoniidae taxonomists, it contributes to integrative taxonomic approaches, such as the elucidation of related species and clarification of problematic species groups, association of the sexes within one species, and the identification of new species (Gibbs 2009, 2011, Packer et al. 2009, Schmidt et al. 2015). To this end, we checked for the presence of species barcode gaps and cryptic diversity within species. BOLD's barcode index number (BIN) analysis tool (Ratnasingham and Hebert 2013) was used to analyze *Hexacentrus*.

Material and methods

Collection of specimens

All specimens were collected by hand or sweeping method during their active season (July–November). *Hexacentrus* species were all gathered in China from 12 localities, with

the latitude from 18.70°N to 41.80°N and the longitude from 97.83°E to 123.38°E. One or more specimens were chosen from each locality in order to include as many morphologically distinguishable individuals per site as possible. Specimens were collected and stored in 100% ethanol at -20 °C and were deposited in the Hebei University Museum. Species-level identification was based on the original morphological descriptions, locality data and additional information. Details on all specimens (sampling location, GPS coordinates, voucher number, BOLD number, etc.) are available within the "DNA Barcoding of *Hexacentrus* in China, BHC" project in the Barcode of Life Data Systems (BOLD. www.barcodinglife.org).

DNA extraction, amplification and sequencing

Total DNA was extracted from the muscle of one hind leg of each specimens using TIANamp Genomic DNA Kit in accordance with the manufacturer's instructions. The standardized gene regions of animals DNA barcoding was amplified using the primers COBU (5'-TYT CAA CAA AYC AYA ARG ATA TTG G-3') and COBL (5'-TAA ACT TCW GGR TGW CCA AAR AAT CA-3') (Pan et al. 2006). The 50 μL polymerase chain reaction (PCR) mixture contained 3 μL of template DNA, 5 μL of 10 × buffer, 4 μL of dNTP mix, 5 μL of each primer (10 μM each), 0.5 μL of *Taq* polymerase (5 U/μL), and 27.5 μL of water. The thermal profile was: 94 °C for 3 min, 34 cycles at 94 °C for 30 s, 49 °C for 30 s, and 72 °C for 90 s, and final extension at 72 °C for 8 min. PCR products were visualized in 1% agarose gels electrophoresis. PCR products were sequenced directly using ABI BigDye Terminator chemistry on ABI3730 automated sequencer (Applied Biosystems) in Genewiz Inc. (Beijing, China), and in both directions to minimize PCR artifacts, ambiguities and base calling error.

Data analysis

Consensus sequence of both directions was assembled using SeqMan in Lasergene and verification of ambiguities and unexpected stop codons were performed in EditSeq (Burland 2000). Sequence alignments were conducted using Clustal X 1.81 (Thompson et al. 1997) with default parameters. The both ends of the sequences matching the primer sequences were excised to remove artificial nucleotide similarity introduced by PCR amplification, resulting in the final data sets for barcoding analysis.

The analyses were restricted to the subset of sequences, which met barcode standards (sequence length > 500bp, < 1% ambiguous bases, bidirectional sequencing, country specification). Intra- and inter-specific genetic distances were based on the Kimura-2-parameter (K2P) model (Kimura 1980) using the 'distance summary' tool in BOLD. The barcode gap was defined by intraspecific vs. interspecific [nearest neighbor (NN)] genetic distance of species. A globally unique identifier (i.e. BIN) then was assigned to each sequence cluster, creating an interim taxonomic system because the

members of a particular BIN often correspond to a biological species. Character based DNA barcoding used the nucleotide variation in each position across DNA regions as diagnostic characters.

Results

COI sequences were recovered from 86 of the 91 specimens that were analyzed with barcode compliant records from 83 specimens representing five species. Three records have no barcode compliant records because of low quality of trace file. A number of 80 barcodes belong to four previously identified species whereas three analyzed specimens were only identified to genus level because they are female; they probably are *H. yunnaneus* due to collection in Yunnan and separated from other specimens. The 658bp length sequences without indel (insertion/deletion) had full-length records. COI sequences were translated to amino acid sequences to check for stop codons and shifts in reading frame that might indicate the presence of nuclear mitochondrial copies (numts), but none were detected. Diagnostic character analysis was consistent with that of traditional external appearance discrimination. *H. expansus* only having one specimen was not analyzed, thus lacking diagnostic character.

Distance summary and Barcode Gap analysis

Mean intraspecies divergence was 1.32% (ranged between 0.57% and 2.43%), and maximum intraspecies divergence 4.43% was observed in *H. japonicus* (Table 1). When correcting for the uneven sample sizes of species, the within-species divergence decreased from 1.32% to 1.23%. Between *Hexacentrus* species, the average K2P genetic distance was 12.54%, whereas minimum genetic distance only 3.79% (Table 1).

Singleton species (*H. expansus*) were excluded from barcode gap analysis. Except for *H. japonicus*, barcode gap was present in the remaining *Hexacentrus* species (Fig. 1). Although the maximum intraspecific divergence of *H. unicolor* was more than 2% (ranged between 0 and 3.79%), but still less than minimum interspecific between *H. unicolor* and its NN (Nearest Neighbor) *Hexacentrus* spp. However, the maximum intraspecific divergence of *H. japonicus* (4.43%) produced a minimal overlap (0.64%).

Table 1. Mean and maximum intraspecific and nearest neighbor (NN) distance for all specimens.

Species	Mean intraspecific distance	Max intraspecific distance	Nearest neighbor	Distance to NN
Hexacentrus expansus	N/A	N/A	*Hexacentrus unicolor*	13.19
Hexacentrus japonicus	2.43	4.43	*Hexacentrus mundus*	3.79
Hexacentrus mundus	0.57	0.93	*Hexacentrus japonicus*	3.79
Hexacentrus sp.	0.72	1.08	*Hexacentrus unicolor*	9.72
Hexacentrus unicolor	1.19	3.79	*Hexacentrus* sp.	9.72

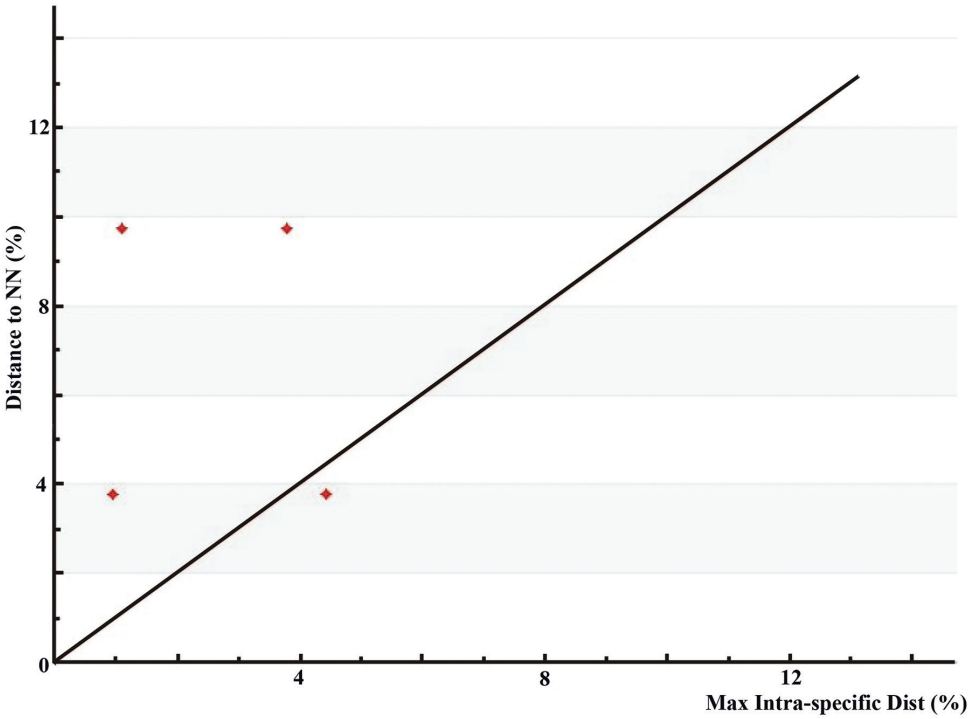

Figure 1. Barcode gap plot showed the distance to the nearest neighbor (NN) vs. the maximum intraspecific distance Kimura-2-parameter (K2P) for 83 specimens. Dots above the 1:1 line indicated the presence of a barcode gap.

Taxon ID tree analysis

The taxon ID tree was divided into seven clades represented by different BINs (Fig. 2). *Hexacentrus unicolor*, *H. mundus* and *H. expansus* were composed well-supported monophyletic groups, which were fully congruent with the morphological species. *Hexacentrus japonicus* were divided into three different BINs. Although no morphological differences were observed among these three BINs, there might be cryptic species within the current *H. japonicus*.

Diagnostic characters analysis

Forty-four diagnostic characters were found in the study (Table 2). Four *Hexacentrus* species had diagnostic characters and the success rate was 80%. The number of diagnostic character sites of *H.* spp. was no less than 20 (Table 2), which may be caused by scarce specimens and by the relative distance of the phylogenetic relationship to others.

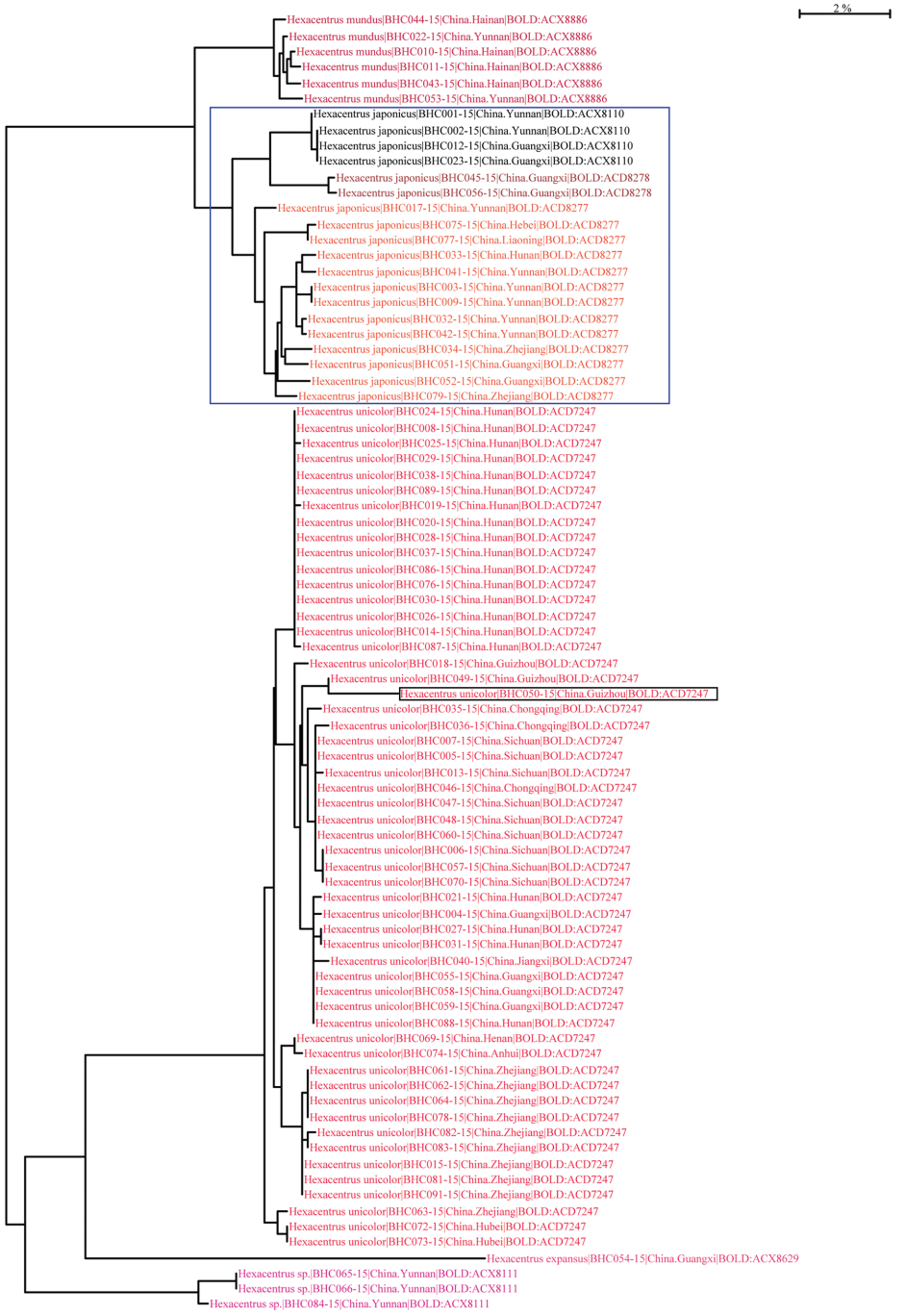

Figure 2. Taxon ID tree revealed seven well-differentiated haplogroups. Process ID, location, and BINs were shown in the tree. The clusters with a blue box indicated there may be two new putative 'cryptic species' within *H. japonicus*. The clade with a black box indicated the specimen had more mutation within *H. unicolor*.

Table 2. Character-based DNA barcodes for four *Hexacentrus* species of COI gene. A_7* means A is at the 7th position.

Species	Diagnostic characters	Characters no.	Specimen no.
Hexacentrus sp.	A_7* G_184 C_247 A_301 A_304 T_346 G_391 C_400 G_424 C_463 C_472 C_500 T_517 G_550 G_586 C_607 A_619 C_622 C_625 G_628	20	3
Hexacentrus unicolor	T_25 T_136 T_223 A_227 T_322 T_379 T_424 G_487 A_502 C_517 T_529 C_530 G_532 C_550 C_586 G_619 T_631	17	54
Hexacentrus mundus	T_34 T_118 C_187 T_397 A_643	5	6
Hexacentrus japonicus	T_460 C_514	2	19

Discussion

The present study evaluated the efficacy of using DNA barcodes for the identification of *Hexacentrus* in China and provided a group of sequences associated with the identified species. Using these DNA barcoding, not only can one delineate the boundaries between species, but also assign taxonomic status to unknown specimens from known species.

Hexacentrus unicolor was controversial, and *H. plantaris* (Burmeister, 1838) and *Tedla sellata* (Walker, 1869) were considered as its synonyms. *H. unicolor* is distributed in south of the Yangtze River. In this study, however, one specimen was collected from Henan. In fact, molecular data support *H. unicolor* as a single group, with all specimens sharing one BIN (BOLD: ACD 7247). The specimen with black box (Fig. 2) came from Guizhou, and had more mutations compared to the other *H. unicolor* specimens. We cannot be completely certain that this phenomenon was due to geographic isolation rather than sequencing or calibrating errors. *Hexacentrus japonicus* was closely related to *H. unicolor*, and they are widely distributed in the southwest, central and south areas of China. The tegmina of male *H. japonicas* was short and broad (about 2.75–3.00 times), whereas *H. unicolor* was long and narrow (about 2.95–3.30 times) as long as broad (Wang and Shi 2005). Nevertheless, the analyses revealed that *H. japonicus* ranged to Hebei and Liaoning. Interestingly, *H. japonicus* contained three BINs (BOLD: ACX 8110, BOLD: ACD 8277, BOLD: ACD 8278) with high intraspecific distance (> 2%). This group included 20 specimens, in which no morphological differences were found. Therefore it is necessary to clarify the status of this species complex as it may include more species than currently recognized. *Hexacentrus mundus* was only recorded in Guangxi and Yunnan. In this study, four specimens from Hainan were also identified as *H. mundus* because only 2–3 larger teeth in the middle part of stridulatory file, however there are 6–7 large teeth in *H. japonicas* and *H. unicolor* (Wang and Shi 2005). Thus *H. mundus*'s distribution was enlarged. All specimens of *H. mundus* were assigned as 1 BIN, which clearly confirm a consistency between molecular and morphological analyses. *Hexacentrus expansus*, due to the obviously inflated male tegmina, was easy to identify only by the morphological method. BIN assignments also revealed that *H. expansus* was a separated clade with only a single male available for analysis. The specimens from Yunnan almost certainly represent the 'true' *H. yunnaneus* because the type loca-

tion of this species (Hekou, Yunnan) was in close proximity. Hence, further specimens are needed to be analyzed, especially male material.

DNA barcoding, as one effective tool in insect taxonomy, had been already applied widely. It can rapidly acquire molecular data, simplifying species classification and identification. Yet, DNA barcodes has been argued to be unreliable for consistent species identification by many authors due to a number of drawbacks (DeSalle et al. 2005; Will et al. 2005; Rubinoff et al. 2006; Ebach 2011). Recent speciation, incomplete lineage sorting, interspecific hybridization and infection by endosymbiotic bacteria such as *Wolbachia* (Funk and Omland 2003) may all interfere with the performance of DNA barcoding in insects (Virgilio et al. 2010). In this context, most of the species can be amplified successfully; however, five specimens cannot be translated and three specimens only had one represented trace file, which cannot meet the DNA barcoding standards. The deep inspection of trace files indicated that most of these failures arose from co-amplification of the bacterial endosymbiont *Wolbachia*, which disturbed normal interpretation of trace file. It had been estimated that *Wolbachia* is present in two-thirds of all insect species (Hilgenboecker et al. 2008). There was no reason to doubt the absence of *Wolbachia* in *Hexacentrus*. Zhou et al. (2014) found that the nuclear sequence of mitochondrial (numts) reported in *Mecopoda niponensis* may form a separate clade. The same case was reported in *Podisma pedestris* (Bensasson et al. 2000). But in this study, the clades in *H. japonicus* were not caused by numts for all sequences analyzed without translation early termination, base indel, frameshift mutations. On the other hand, geographic isolation was also rejected, for only the specimens from Yunnan included three BINs in this group. The most probable reason was the existence of cryptic species compared to other *Hexacentrus* species.

Species boundaries were hard to delimit only based on morphologyas, and analyses including additional sources of information such as molecular data, biogeography, behavior and ecology has been called integrative taxonomy which has been shown to be very useful (Dayrat 2005). We are convinced that DNA barcoding can promote the *Hexacentrus* species identification. Our study showed that all known *Hexacentrus* species could be delimitating rapidly through DNA barcoding in China, except for *H. japonicus*. *Hexacentrus japonicus* was problematic when using BOLD analysis. Finally, regardless of the promising results, the incorporation of nuclear genes is valuable for species delimitation and might strengthen the results, as they are independent of the maternal inherited mitochondrial genes.

The ideal situation would be that each species was represented by sequence from its type material, particularly the holotype. Type specimens were also dried specimens and DNA degraded at different level, so not only amplification was difficult, but also the damage of specimens can't be neglected. Recently, Prosser et al. (2016) successfully obtained sequences from century-old type specimens using next-generation sequencing (NGS). We believed that DNA barcoding is useful in revealing cryptic biodiversity, potentially facilitating traditional taxonomy in future.

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Revision on Palaearctic species of *Periclistus* Förster with description of a new species and its host plant gall (Hymenoptera, Cynipidae)

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Abstract

Palaearctic species of *Periclistus* Förster has been systematically described, but a new inquiline gall-wasp, *Periclistus qinghainensis* **sp. n.**, is described from China. This species was obtained from an unknown stem gall induced on *Rosa* sp. Diagnosis, distribution and biology of the new species are described in this paper. After examining the types of *P. idoneus* Belizin, 1973 and *P. capillatus* Belizin, 1968, it is concluded that *P. idoneus* belongs to genus *Aulacidea*, and *P. capillatus* is a valid species of *Periclistus*. A key to the Palaearctic *Periclistus* species is also given.

Keywords

Cynipidae, Gallwasp, inquiline, *Periclistus*, taxonomy, revision, China

Introduction

Synergini is an important tribe of the family Cynipidae (Hymenoptera) with a worldwide distribution. They are biologically characterized for being inquilines: although they have lost the ability to induce galls, they are still able to directly modify the gall tissue that surrounds them, inducing the characteristic nutritive tissue usually found in the larval chambers of the gall-inducers (Melika 2006). All inquilines are wholly phytophagous, some of them being lethal if they compete with the inducer for the food in the same larval chamber. This lifestyle represents a unilateral relationship only beneficial for the inquiline (Askew 1984).

The Synergini includes 186 species of inquilines grouped in nine genera (Pénzes et al. 2012*). Six genera are inquilines of cynipid galls on Fagaceae (*Agastoroxenia* Nieves-Aldrey & Medianero, 2010, *Ceroptres* Hartig, 1840, *Saphonecrus* Dalla Torre & Kieffer, 1910, *Synergus* Hartig, 1840, *Synophrus* Hartig, 1843 and *Ufo* Melika & Pujade-Villar, 2005); species of *Synophromorpha* (Ashmead, 1903) are also found in *Diastrophus* galls on *Rubus* (Rosaceae); *Rhoophilus* inquilines are found in lepidopteran galls induced by a *Scyrotis* moth (cecidosid) on species of *Rhus* (Anacardiaceae); and inquilines of *Periclistus* Förster are associated with cynipid galls on roses (Diplolepidini).

Periclistus is a small genus with 14 species distributed across the Holarctic region, three of them having an uncertain status: *P. idoneus* Belizin, 1973, *P. mongolicus* Belizin, 1973 and *P. capillatus* Belizin, 1968 (Taketani and Yasumatsu 1973).

Despite being morphologically similar to *Synophromorpha* Ashmead, *Periclistus* can be distinguished by the following characters (Ritchie and Shorthouse 1987): uniformly and delicately coriaceous mesoscutum (graniculate or smooth in *Synophromorpha*); notauli never complete, forming two short sulci not posteriorly broadened (complete and distinctly broadened notauli in *Synophromorpha*), ventral margin of subalar triangle with a row of setigerous punctures (without a row of setigerous punctures in *Synophromorpha*), closed radial cells (opened radial cells in *Synophromorpha* and Japanese species of *Periclistus*), and the male's third flagellomere usually strongly notched and distally broadened (third flagellomere weakly curved, broadly notched and weakly expanded distally in *Synophromorpha*). Both genera form a monophyletic group, as has been demonstrated by several authors (Ritchie and Shorthouse 1987; Ronquist and Liljeblad 2001; Nylander 2004, among others). Here genus *Periclistus* is firstly reported from China, with a new species *Periclistus qinghainensis* sp. n., found in a gall on an unidentifies species of *Rosa* induced by an unknown species in *Diplolepis*.

Materials and methods

The types of *P. idoneus* and *P. capillatus* described by Belizin from Hurfeish (Israel) and Primorskij Kraj (Russian Far East) respectively, have been examined in this study.

* In the monent to publish this manuscript the Synergini is reestructred according to Ronquist et al. (2015)

They are deposited in ZIN (Zoological Institute of the Russian Academy Sciences, St. Petersburg, Russia).

The galls of the new species described here were collected on May 2010 in the north western province of Qinghai of China. During this month the weather is still cold, the branches of trees are still covered by snow and the useful characters to determine the *Rosa* species are not present in the plant, so it was impossible to identify it; in addition, in China there are approximately 100 described species of *Rosa*, making it hard to establish a potential candidate. Hence, the galls were sent to Y. Wang without determination of *Rosa* species.

The current terminology describing the cynipid gall-wasp morphology follows Liljeblad and Ronquist (1998) and Melika (2006). Abbreviations for the forewing venation are taken from Ronquist and Nordlander (1989) and those for the cuticular surface from Harris (1979). Measurements and abbreviations used here include F1–F12 for first and subsequent flagellomeres. Other abbreviations are: post-ocellar distance (POL), the distance between the inner margins of the posterior ocelli; ocellar–ocular distance (OOL), the distance from the outer edge of the posterior ocellus to the inner margin of the compound eye; and lateral-ocullar distance (LOL), the distance between lateral and frontal ocelli. The width of the forewing radial cell was measured from the margin of the wing to the Rs vein.

Measurements were made under a Leica MZ 12.5 stereomicroscope (Wetzlar, Germany), and photos were taken with a digital camera (Q-Imaging, Micropublisher 3.3 RTV) attached to the Leica MZ APO stereomicroscope (Wetzlar, Germany) using software of Synoptics Auto- Montage version 5.0.

Specimens of the new species are deposited in the Hymenoptera Collection in Zhejiang A & F University (ZAFU) and the University of Barcelona (UB), respectively.

Results

Periclistus capillatus Belizin, 1968

Periclistis capillatus Belizin, 1968: 718–719.

Type material. 1 ♀ deposited in ZIN, with the following labels: "Kedrovaya pad' [Nature Reserve] Primorie [= Primorskiy kray] O. Kovalev 17 V 60" (black label, handwritten in Russian), "From galls on leaves of Rosa" (red label, handwritten in Russian), "*Periclistus capillatus* ♀ m. V. Belizin det" (black label, handwritten), "Primorskiy kray, 'Kedrovaya pad' 'Nature Reserve. From galls on Rosa (leaves). 17. V. 60 g. O.V. Kovalev" (black label, handwritten in Russian), "Lectotype ♀ of *Periclistus capillatus* Belizin, 1968, det JP-V 2015" (red label, printed).

Diagnosis. This species is characterized by the following characters: black head and mesosoma, chestnut brown to black metasoma, testaceous antennae and legs; 12-segmented antenna, F1 and F2 subequal in length (4:5); an alutaceous mesoscutum with

piliferous points and sparse pubescence; notauli and posterior medial sulcus present, short, both extending to ¼ of total scutum length; parapsidal lines and anterior parallel lines present; smooth mesopleuron; closed radial cell (although both R1 and its projection in margin of forewing nearly inconspicuous), short, 3 times as long as broad; areola visible; metasomal tergites fused (T2+T3) and smooth, with an anterolateral patch of white setae; the subsequent segments are micropunctuated and glabrous.

Comments. This species presents characters belonging to Asian species (scutal and mesopleural sculpture) and characters belonging to European species (radial cell length and shape). A key provided at the end differentiates this species from its congeners.

Aulacidea idoneus (Belizin, 1973), comb. n.

Periclistis idoneus Belizin, 1973: 26.

Type material. 1 ♀ deposited in ZIN, with the following labels: Herfeish, 22.IV, Israel, V. Trjapitzin ' 966" (black label, handwritten in Russian), " Holotype *Periclistus idoneus* ♀ m., V. Belizin det" (red label, handwritten), *Aulacidea idoneus* Belizin, 1973, det. JP-V 2015" (white label, printed).

Comments. After examining the holotype, we conclude that this species belongs to genus *Aulacidea*. After determining the specimen following the Palaearctic *Aulacidea* species key made by Melika (2006) we conclude that this species is a valid species related to *A. laureae* Nieves-Aldrey, 1992 and *A. follioti* Barbotin, 1972. The three species present the head broader than high, 13-segmented antenna, F1 shorter than F2, incomplete notauli and ciliated forewing margin. *Aulacidea idoneus* differs from *A. follioti* in presenting median mesoscutal line, like *A. laureae*; *A. idoneus* can be distinguished from *A. laureae* by the following characters: short and narrow scutelar foveae, OOL 3.0 times longer than the diameter of lateral ocellus, space between totuli and clypeus without radiating carina, having shorter notauli and medial mesoscutal line shorter (both extending 1/3 of scutum length) and radial cell (slightly more than 2.0 times longer than broad) and having a second metasomal tergite with only some dorsal points while being laterally smooth.

Periclistus mongolicus Belizin, 1973, species dubia

Periclistis mongolicus Belizin, 1973: 26.

Remarks. This species described from Mongolia was considered by Abe et al. (2007) as having an uncertain status until the types were revised. Because of the loss of the type material (S. Belokobylskij pers. comm.) this species is definitively considered as '*species dubia*' according to the description, which does not permit assessment of its validity nor its placement in the genus *Periclistus*.

***Periclistus qinghainensis* sp. n.**
http://zoobank.org/C0EA8F5E-6EAB-4B2F-B77B-1F97332B6066
Figs 1–2

Diagnosis. *Periclistus qinghainensis* sp. n. differs from all of the known *Periclistus* species in the absence of notauli. *Periclistus qinghainensis* sp. n. is morphologically similar to two Japanese species (*P. natalis* Taketani & Yasumatsu and *P. quinlani* Taketani & Yasumatsu) and the Far East Russian species (*P. capillatus*) in having a smooth and shiny mesoscutum (very weakly alutaceous in *P. capillatus*) with dispersed piliferous points and smooth mesopleuron, but it differs from all these species in having a partially closed radial cell (radial cell opened in *P. natalis* and *P. quinlani* while closed and shorter in *P. capillatus*), shorter F1 than F2 (F1 and F2 subequal in *P. natalis* and *P. quinlani*) and the absence of notauli (present in the other three species). *Periclistus qinghainensis* sp. n. differs from the European species in having the radial cell partially closed (closed in *P. caninae* (Hartig) and *P. brandtii* Ratzeburg), a smooth and shiny mesoscutellum (uniformly and delicately coriaceous scutellum with a dense and short pilosity without piliferous points in the European species) and the length and width of the radial cell (more than 4.0 times as long as wide in *P. qinghainensis* while around 3.0 times in *P. brandtii* and *P. caninae*).

Description. Length. Female. Body length 2.1 mm, and fore wing 2.8 mm.

Colour. Body black, except yellow tegulae and antennae, scapus and apical flagellomere darker; coxae dark brown, rest of the legs yellowish; forewing hyaline, with brown veins.

Head (Fig. 1a, b). Head coriaceous, with sparse setae, 2.0 times wider than long in dorsal view, 1.4 times wider than high in front view and slightly wider than mesosoma. Gena delicately coriaceous and not broadened behind eyes. Clypeus very small, impressed quadrangular and delicately coriaceous, ventrally slightly rounded; slightly higher than wide, with distinct small anterior tentorial pits, epistomal sulcus and clypeo-pleurostomal lines indistinct. Lower face with striae radiating from clypeus, not reaching eyes and antennal socket, median elevated area delicately coriaceous and striated. Malar space 0.3 times longer than eye height. Diameter of antennal torulus 2.0 times longer than inter-toruli distance and 1.1 times longer than eye-torulus distance. POL: OOL: LOL=1.7: 0.6: 1.3. Frons, vertex, and gena behind eyes and postgena with sparse setae. Frons largely smooth, with some very small and distinct punctures but without lateral frontal carina. Vertex and occiput uniformly punctured.

Antenna. Female (Fig. 2a). 12-segmented, slightly shorter than body; pedicel subglobose, only slightly longer than wide; F1 2.5 times as long as pedicel; F2 around 1.2 times as long as F1 and only slightly longer than F3; the antennal formula is: 9: 4: 10: 13: 11: 11: 10: 9: 9: 8: 7: 14. **Male** (Fig. 2b, c). antenna 14- segmented, F1 medially incised and apically swollen, 2.3 times as long as pedicel, 0.9 times as long as F2; F2 as long as F3; F4 slightly longer than F3; F6–F8 equal in length; F9-10 equal in length; the antennal formula is: 3.0: 2.0: 4.2: 4.9: 5.0: 5.5: 5.0: 4.5: 4.5: 4.5: 4.0: 4.0: 3.5: 4.0.

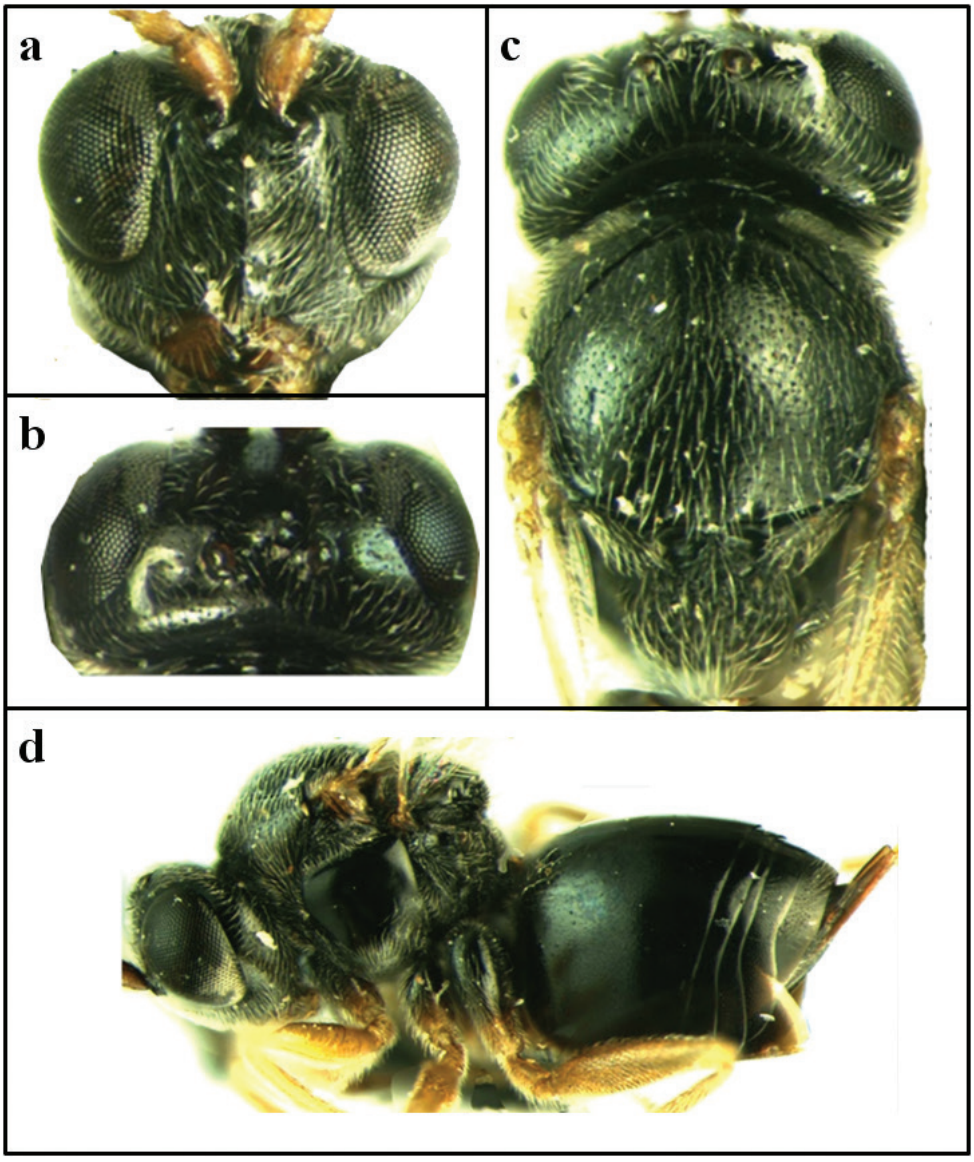

Figure 1. *Periclistus qinghainensis* sp. n.: **a** head of female in front view **b** head of female in dorsal view **c** head and mesoscutum of female in dorsal view **d** general habitus of female in lateral view.

Mesosoma (Fig. 1c, d). Mesosoma slightly compressed dorso-ventrally and longer than high in lateral view, and with white setae. Pronotum dorsal an lateral surface uniformly and delicately coriaceous, lacking wrinkles and lateral pronotal carina but having rounded anterior corners in dorsal view. Mesoscutum smooth and shiny with some dispersed piliferous points, slightly broader than long. Notauli and median mesoscutal line absent; anterior parallel lines distinct, extending to 1/4 of entire mesoscutum length. Parapsidal lines present, shallow, extending to 1/4 of mesoscutum length.

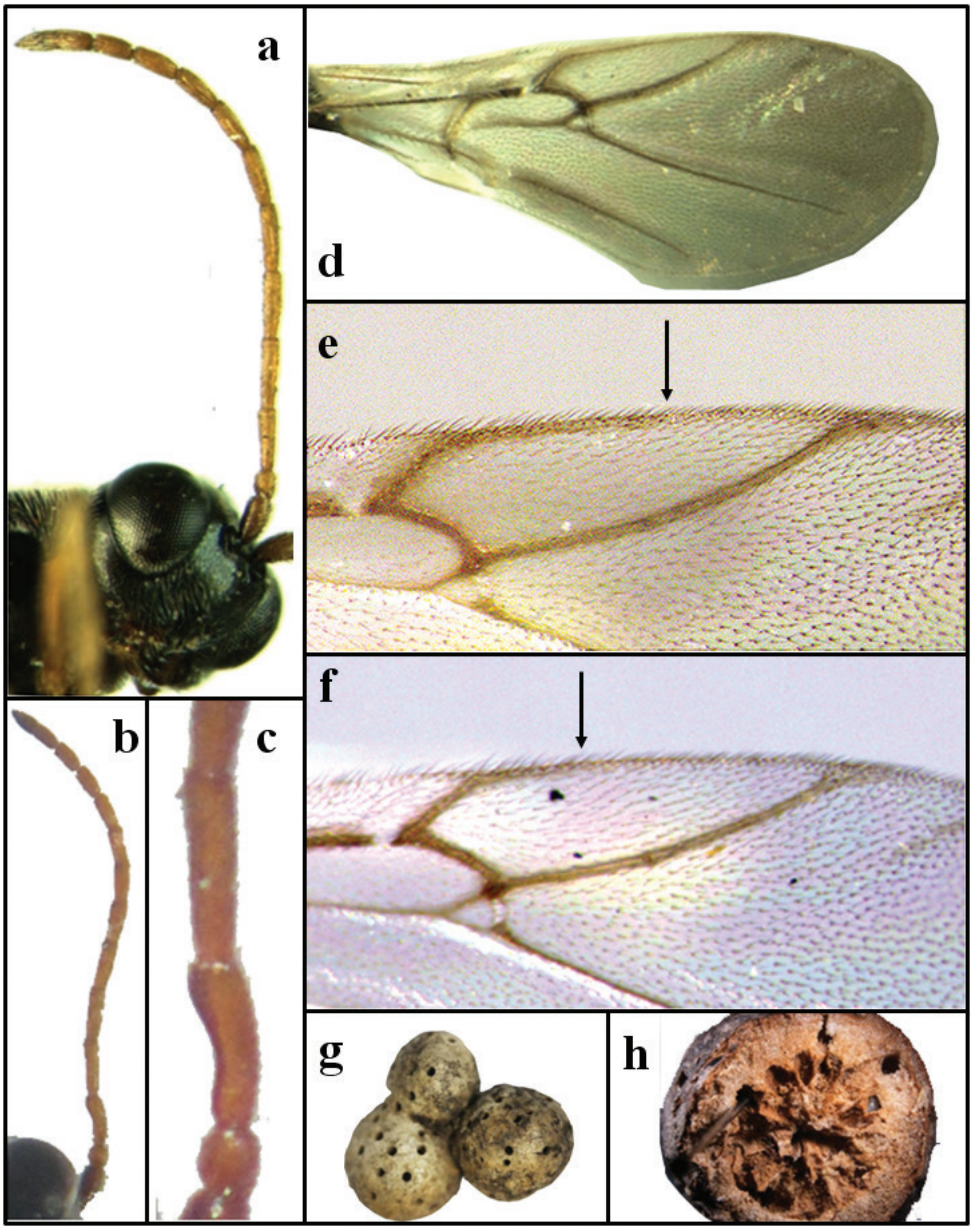

Figure 2. *Periclistus qinghainensis*, sp. n.: **a** antenna and head of female in lateral view **b–c** male antenna and detail of first flagellomeres **d** forewing **e–f** detail of radial cell indicating the R1 prolongation in margin of forewing **g** galls **h** longitudinal section of gall.

Mesoscutum rugose, more sculptured toward central scutellar disk and between scutellar foveae, metanotum slightly overhanging. Scutellar foveae transversely ovate, only slightly wider than high, well-delimited around, with smooth and shiny deep bottom but without setae, separated by distinct medial carina. Mesopleuron smooth and shiny,

without striae, with dense setae ventrally, especially postero-ventrally; mesopleural triangle alutaceous, with sparse setae. Metapleural sulcus reaching the mesopleuron at 4/5 of its height; lateral propodeal carinae straight and parallel, with some setae; central propodeal area coriaceous, with setae; lateral propodeal area uniformly and delicately coriaceous, with relatively dense white setae.

Fore wing (Fig. 2d–f). Forewing longer than body, wing margin with long cilia; radial cell 4.3 times as long as the wide, partially closed (R1 vein projected about 1/3-1/2 on radial cell margin), Rs and R1 veins slightly curved, areolet distinct; vein Rs+M distinct, nearly reaching basalis.

Metasoma. Female (Fig. 1d). metasoma nearly as long as head plus mesosoma, distinctly longer than height in lateral view; metasomal tergites 2+3, with patches of dense setae at laterals in its base, fifth and sixth metasomal tergites broadly punctuate dorso-posteriorly; prominent part of ventral spine of hypopygium very short. **Male.** second and third metasomal tergites not fused, separated by a suture.

Type material examined. Holotype. ♀, China: Qinghai, Huzhu, Bei Mountain (102°32'E, 36°51'N), 2010-V-6, Guo Rui, reared in galls on *Rosa* sp. Paratypes. 6♀♀1♂, same labels as the holotype (1♀ paratype UB).

Distribution. China (Qinghai).

Biology. Reared from stem galls on *Rosa* sp. (Fig. 1g and h). The young gall is juicy, soft, covered with small raised tubercles, and multilocular with greenish-purple spots, 1.0-2.0 cm in diameter. Adults emerge in September.

Etymology. The new species is named after the province where it was collected.

Discussion

Periclistus includes 12 species in the Holarctic region, seven species known from America to the north of Mexico (*P. arefactus* McCracken & Egbert; *P. californicus* Ashmead; *P. obliquus* Provancher; *P. piceus* Fullaway; *P. pirata* (Osten Sacken); *P. semipiceus* (Harris); and *P. smilacis* (Ashmead) (Burk 1979; Ritchie and Shorthouse 1987); two (Fig. 3) from the western Palaearctic (*P. brandtii* (Ratzeburg) and *P. caninae* (Hartig)); and three (Fig. 3) from the eastern Palaearctic: *P. capillatus* Belizin from Russian Far East, *P. natalis* Taketani & Yasumatsu and *P. quinlani* Taketani & Yasumatsu from Japan (Belizin 1973; Taketani and Yasumatsu 1973).

Periclistus species are associated with *Diplolepis* and *Liebelia* galls, except *P. smilacis*, a Nearctic species known from Florida reared in galls of *Diastrophus smilacis* (Ashmead 1896; Penzes et al. 2012), although M. Buffington and M. Gates (pers. comm.) disagree and consider *P. smilacis* should be associated with some *Diplolepis* species.

Abe et al. (2007) placed *Periclistus capillatus* and *P. mongolicus* in an 'uncertain status' and the original description of *P. idoneus* does not allow one to discriminate this species from *P. caninae* and *P. brandtii*, except for the shorter radial cell present in *P. idoneus*. After examining the type material of *Periclustus capillatus* we considered it is a valid species. Unfortunately, the type material of *P. mongolicus* is lost, so we were not

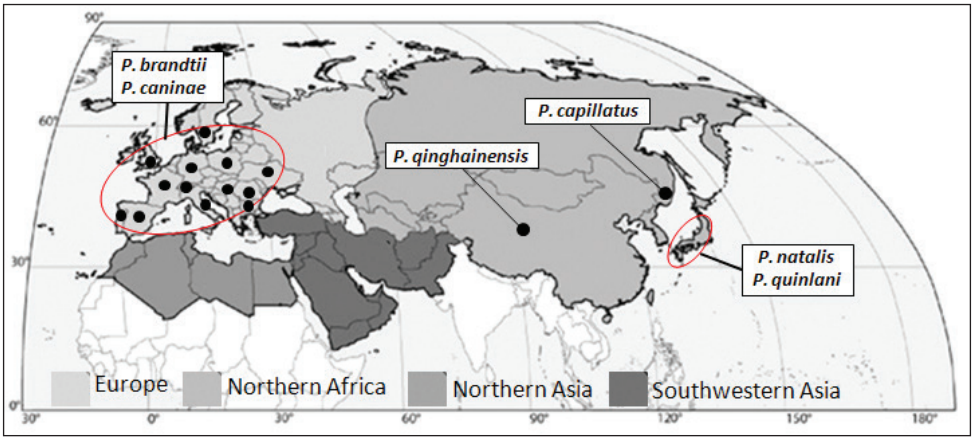

Figure 3. Distribution map of *Periclistus* species in the Palaearctic regions.

able to study it and we considered this species '*incertae sedis*'. Finally, when examining the holotype of *P. idoneus* we concluded that it was a valid species belonging to the genus *Aulacidea*.

Periclistus natalis and *P. quinlani* are morphologically very similar (both having complete shallow notauli, smooth and shiny mesopleuron, and opened radial cell of the forewing), and share the same gall host (*Diplolepis japonica* (Walker)) and host plant (*Rosa polyantha* Sieb. & Zucc.); however, the authors of these species (Taketani and Jasumatzu 1973) described biological differences between them. Abe (1998) studied the type material of these two species and concluded that there was only one morphological character different between the two species, viz. the pits of the notauli are weakly present anteriorly in *P. natalis*, and absent in *P. quinlani*. Nevertheless, this difference is very superficial based on our knowledge of morphology of Cynipidae; with additional data, it is very probable that both species will be synonymized.

The species described here, *Periclistus qinghainensis*, is similar to two Japanese species (*P. natalis* and *P. quinlani*) and a Far Eastern Russian species (*P. capillatus*). They share a punctured mesoscutum and smooth and shiny mesopleuron. These characters are exclusive of these four species from the rest of the Eastern Palaearctic *Periclistus*. *Periclistus qinghainensis* presents a partially closed radial cell, an intermediate characteristic between the open radial cell of the Japanese species and the remaining of Palaearctic species (*P. caninae* and *P. brandtii* both present a closed radial cell). As mentioned above, *P. capillatus* is intermediate between the Japanese and Chinese species and the remaining of Palaearctic species

Key to Palaearctic species of *Periclistus*

1 Mesopleuron entirely smooth, shiny, without striae; mesoscutum smooth or alutaceous, shiny, with sparse setae and piliferous points..... **2**

– Mesopleuron with more or less delicate striae; mesoscutum dull and uniformly coriaceous, with dense setae **5**

2 Forewing with the radial cell partially closed (Fig. 2e–f); notauli absent (Fig. 1c); metasoma black in females ..... ***P. qinghainensis* sp. n.**

– Forewing with radial cell opened or closed; notauli shallow but distinct; metasoma reddish-brown in females **3**

3 Radial cell short, around 3.0 times as long as the width; forewing hyaline .... ***P. capillatus* Belizin, 1968**

– Radial cell longer, around 4.0 times as long as the width; forewing with small clouded macula posterior to anterior margin near apex of radial cell **4**

4 Notaular pits present anteriorly but weakly impressed; and metasoma reddish-brown ..... ***P. natalis* Taketani & Jasumatzu, 1973**

– Notaular pits absent; and metasoma blackish brown..... ..... ***P. quinlani* Taketani & Jasumatzu, 1973**

5 Notauli complete; mesopleuron entirely striated, without smooth and shiny patch; fused second and third metasomal tergites of females and third metasomal tergite in males without punctuation or only with some punctures in dorso posterior part..... ***P. brandtii* Ratzeburg, 1831**

– Notauli incomplete, absent or very indistinct in the anterior half; mesopleuron mainly striate but with a smooth and shining patch posteriorly; the fused second and third metasomal tergites of females and third metasomal tergite in males with a narrow band of punctuation in posterior part..... ..... ***P. caninae* (Hartig, 1840)**

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Description of a new species of *Aphanogmus* Thomson (Hymenoptera, Ceraphronidae) that parasitizes acarivorous gall midges of *Feltiella* (Diptera, Cecidomyiidae) in Japan

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Abstract

In 2008–2009, we reared small ceraphronids (about 0.5 mm in body length) from cocoons that had been made possibly by two acarivorous species, *Feltiella acarisuga* (Vallot) and *F. acarivora* (Zehntner) (Diptera: Cecidomyiidae) in Okinawa, Japan. Detailed morphological observation revealed that the ceraphronid was a new species of *Aphanogmus* Thomson (Hymenoptera: Ceraphronidae). We describe it as *Aphanogmus flavigastris* Matsuo, **sp. n.** Identification of the *Aphanogmus* species is essential to evaluate its possibly negative effects on the predatory activity of *Feltiella* species that have been used as control agents against tetranychid mites.

Keywords

Aphanogmus flavigastris, *Feltiella acarisuga*, *Feltiella acarivora*, taxonomy

Introduction

In 2008–2009, small (about 0.5 mm in body length) species of ceraphronids (Hymenoptera) were reared from cocoons that had been made possibly by two acarivorous species, *Feltiella acarisuga* (Vallot) and *F. acarivora* (Zehntner) (Diptera: Cecidomyiidae) in Okinawa, Japan (Abe et al. 2011, Ganaha-Kikumura et al. 2012). Preliminary identification revealed that the ceraphronid was a member of *Aphanogmus* Thomson (Hymenoptera: Ceraphronidae), which contains at least 100 species worldwide (Johnson and Musetti 2004, Evans et al. 2005, Buhl et al. 2010). About 20% of them have been known as parasitoids of various insects including Cecidomyiidae (Diptera), Bethylidae, Ichneumonidae (Hymenoptera) and Cybocephalidae (Coleoptera) (Oatman 1985, Gilkeson et al. 1993, Polaszek and Dessart 1996, Evans et al. 2005). Host information for the remaining 80% has not been provided. At present, two species, *Aphanogmus floridanus* Ashmead and *A. fulmeki* Szelényi (=*A. parvulus* Roberti) have been known to parasitize *Feltiella* species in the Holoarctic region. The former is an endoparasitoid of *Feltiella acarivora* (Oatman 1985, Johnson and Musetti 2004) and the latter attacks *F. acarisuga*, *F. acarivora*, *Aphidoletes aphidimyza* (Rondani), and *Mycodiplosis* sp. (Diptera: Cecidomyiidae) (Dessart 1992).

A few taxonomic studies have focused on Japanese species of *Aphanogmus*. Ashmead (1904) first recorded *Aphanogmus* from Japan, describing *A. hakonensis* Ashmead based on individuals collected from Hakone, Kanagawa. Polaszak and Dessart (1996) detected several cryptic species of *Aphanogmus hakonensis* and proposed the species complex of *A. hakonensis.* Ishii (1937) reported an unidentified species of *Aphanogmus* as a parasitoid of *Cybocephalus* species (Coleoptera: Cybocephalidae) that feed on *Unaspis yanonensis* (Kuwana) (Hemiptera: Diaspididae) on citrus in Japan. Evans et al. (2005) considered that *Aphanogmus* sp. reported in Ishii (1937) was identical to *A. inamicus* Evans and Dessart. In total, two nominal species, *Aphanogmus hakonensis* and *A. inamicus* have been known in Japan.

Larvae of all known *Feltiella* species feed on tetranychid mites (Acari: Tetranychidae) (Gagné 1995, Gagné and Jaschhof 2014). In particular, *Feltiella acarisuga* is regarded as an important natural enemy against tetranychid mites that frequently develop pesticide resistance and cause serious damage to various agricultural products (Barnes 1933, Wardlow and Tobin 1990). Therefore, the purpose of this study is to identify the *Aphanogmus* found in Okinawa, as this is essential to evaluate its effect on mortality of *Feltiella* species.

Material and methods

We collected more than one larva or cocoon of *Feltiella* from each collecting site in Okinawa, Japan in 2008–2009. They were kept in petri-dishes to rear *Aphanogmus* and *Feltiella* species. Adults that emerged were preserved in 75% ethanol for morphological observation. If possible, host species of parasitoid wasp should be identified by

examining remnants of host insect but the male genitalia of host cecidomyiid, which is important for species identification, would not be included in the remnants. Otherwise, host species should be identified before the attack of parasitoid wasps. However, this is not always applicable under natural conditions. Therefore, we regarded host cecidomyiid to be identical to either *F. acarisuga* or *F. acarivora* when *A. flavigastris* emerged from cocoons that coexisted on the same plant with either *F. acarisuga* or *F. acarivora*, respectively because we have seldom seen *F. acarisuga* and *F. acarivora* on the same plant.

For microscopic study, the ethanol-stored specimens were dried by the method described in Matsuo and Yukawa (2009). Fore wings were mounted on slides in Canada balsam using ethanol and xylene. Several specimens were gold-coated for microphotography with a JEOL JSM-5600LV scanning electronic microscope. High resolution image was taken with the methods described in Matsuo et al. (2012). Adult morphological terminology follows Mikó and Deans (2009), except for wing venation, which follows Dessart (1963). The holotype and paratypes are deposited in the collection of the Biosystematics Laboratory, Faculty of Social and Cultural Studies, Kyushu University, Japan.

Results and discussion

***Aphanogmus flavigastris* Matsuo, sp. n.**
http://zoobank.org/4725144C-E843-4706-8DE2-D58805F78F41

Ceraphronidae sp.: Abe et al. 2011: 277.
Ceraphronidae sp.: Ganaha-Kikumura et al. 2012: 323.

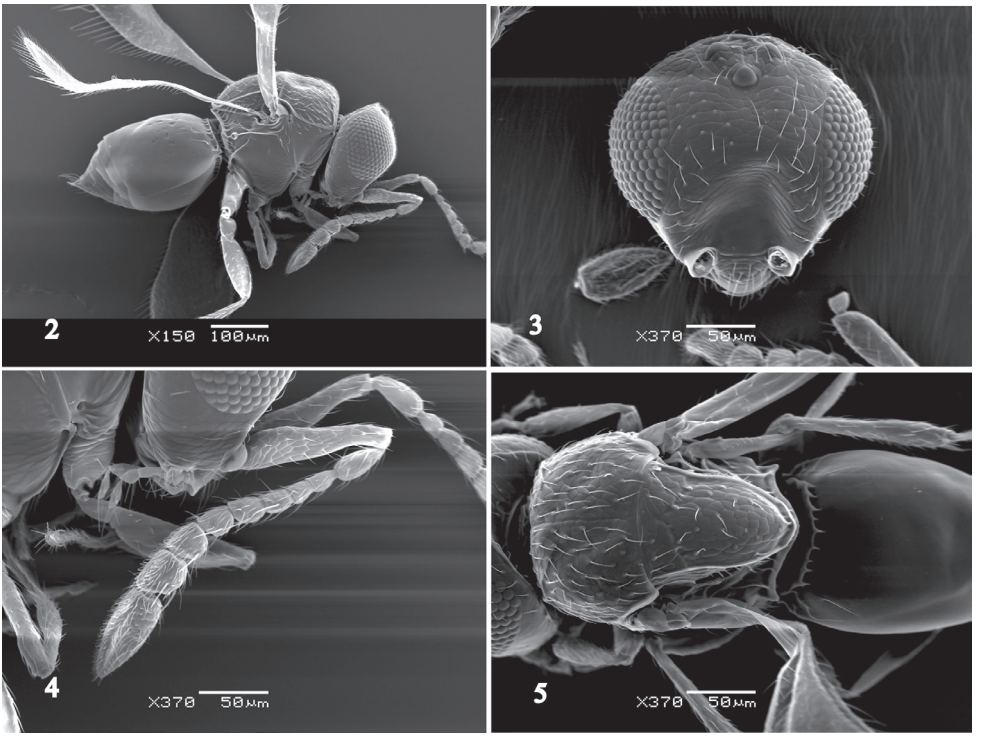
Etymology. The specific name, *flavigastris*, is Latin meaning yellowish gaster, derived from the color of the female metasoma.

Type material. See Table 1.

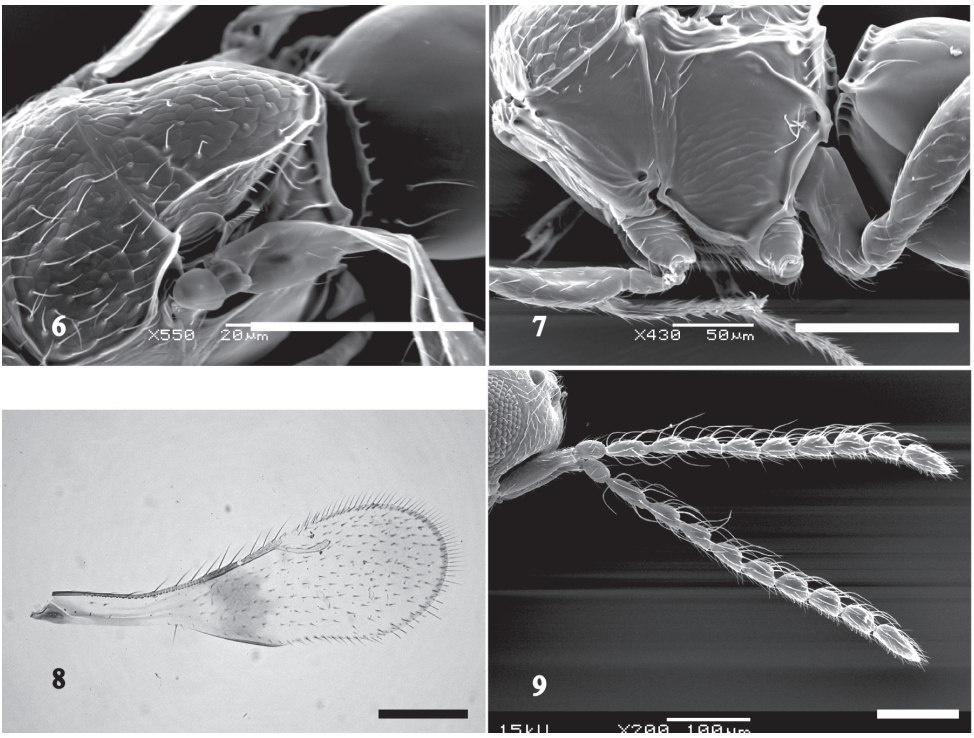
Description. FEMALE. Body length 0.5–0.6 mm (Figs 1, 2). Head dark brown. Scape yellow; pedicel and all flagellomeres yellowish brown. Mesosoma dark brown. Fore wing with an infuscate area. Fore and mid coxae dark brown, sometimes yellowish in apical half; fore and mid femora yellow, sometimes brownish; hind leg and all tibiae yellow. Metasoma yellow, darker dorsally.

Head in dorsal view 1.5–1.7 times as wide as long, 1.2–1.4 times as wide as mesosoma; POL: OOL: LOL = 1.8: 1.5: 1.0. Head in frontal view (Fig. 3) 1.0–1.1 times as wide as high; malar space 0.3–0.5 times as long as eye height; lateral margin of torulus distinctly raised; intertorular carina distinct; frontal depression transversely reticulate; ocellar foveae absent; preocellar pit absent; facial pit absent; preoccipital furrow present and extends from anterior ocellus to occipital foramen; preoccipital carina absent; preoccipital lunula absent; occipital carina present; occipital depression absent; occiput smooth. Antenna (Fig. 4) 10 segmented; scape about 0.6 times as long as height of

Figure 1. A female of *Aphanogmus flavigastris*. Scale bar: 100 μm.



Figures 2–5. *Aphanogmus flavigastris*. **2** female body, lateral view **3** female head, frontal view; **4** female antenna, lateral view **5** female mesosoma and metasoma, dorsal view. Scale bars: **2**: 100 μm; **3–5**: 50 μm.



Figures 6–9. *Aphanogmus flavigastris*. **6** female scutellum, antero-dorsal view **7** female mesosoma, lateral view **8** female fore wing, upper surface **9** male antenna, lateral view. Scale bars: **6**: 20 µm; **7**: 50 µm; **8, 9**: 100 µm.

head, as long as distance between inner orbits; pedicel 2.0–2.5 times as long as flagellomere 1; the following segments gradually widened; flagellomere 7 about 2.0 times as wide as flagellomere 1; club 1 segmented.

Mesosoma 1.2–1.4 times as long as wide; 1.3–1.5 times as high as wide; ventral pronotal pit distinct; mesoscutum reticulate, sparsely setose (Fig. 5); setal base slightly pustulate; median mesoscutal sulcus complete; notaulus absent; parapsidal line absent; interaxillar sulcus present; scutoscutellar sulcus angled medially, foveolate, continuous with interaxillar sulcus; dorsal axillar area and mesoscutellum sculptured as mesoscutum, with distinct lateral carina which connects posterior mesoscutellar sulcus (Fig. 6); mesoscutellum 1.4–1.6 times as long as wide; anterior mesopleural sulcus distinct (Fig. 7); anterior mesopleural area finely reticulate with several setae; dorsal mesometapleural carina straight; anterior mesopleural sulcus perpendicularly intersecting dorsal mesometapleural carina; metapleural carina distinct, extends near dorsal mesometapleural carina.

Fore wing about 3.0 times as long as wide, with a darkly pigmented band (Fig. 8); radial vein 1.4–1.5 times as long as marginal vein. Metacoxa bare dorsally; longitudinal metacoxal carina present at base.

Table 1. A list of type specimens of *Aphanogmus flavigastris*. All specimens are kept in the collection of the Biosystematics Laboratory, Faculty of Social and Cultural Studies, Kyushu University, Japan.

Possible host	Associated plant*	Collecting site (collector**)	Host collecting	No. specimens	Notes
F. acarisuga	*Pueraria montana*	Senbaru, Nishihara, Okinawa, Japan (SO)	22 vii 2008	1 female	Holotype
F. acarisuga	*P. montana*	Senbaru, Nishihara, Okinawa, Japan (SO)	22 vii 2008	1 male	Paratype
F. acarisuga	*Mallotus japonicus*	Uka, Kunigami, Okinawa, Japan (SO, TGK)	1 viii 2008	1 female	Paratype
F. acarisuga	*Ma. japonicus*	Uehara, Ogimi, Okinawa, Japan (SO, TGK)	6 viii 2008	1 female	Paratype
F. acarisuga	*Broussonetia papyrifera*	Gesashi, Higashi, Okinawa, Japan (SO)	21 ii 2009	1 female	Paratype
F. acarivora	*Bauhinia variegata*	Senbaru, Nishihara, Okinawa, Japan (SO)	18 vii 2008	2 females	Paratypes
F. acarivora	*Melanolepis multiglandulosa*	Hentona, Kunigami, Okinawa, Japan (SO)	31 vii 2008	2 males	Paratypes
F. acarivora	*Mucuna macrocarpa*	Oku, Kunigami, Okinawa, Japan (SO, TGK)	1 viii 2008	2 females	Paratypes
F. acarivora	*P. montana*	Iramina, Yomitan, Okinawa, Japan (SO)	2 x 2008	1 female	Paratype
F. acarivora	*Morus australis*	Kijoka, Ogimi, Okinawa, Japan (SO)	16 x 2008	1 female	Paratype

* The plant, from which *Feltiella* species were collected.

** Name of collectors. SO: Suguru Ohno, TGK: Tomoko Ganaha-Kikumura.

Syntergum with distinct transverse carina anteriorly, smooth, with 2–3 setae anterolaterally, occupying more than half of total length of metasoma; longitudinal striae of syntergum absent.

MALE. Differs from female as follows: Antenna (Fig. 9) 11 segmented; flagellar setae long, about 2.0 times width of flagellomeres.

Distribution. Japan.

Host insects. *Feltiella acarisuga* and *F. acarivora*. Usually one, occasionally two or three adults emerged from a single host cocoon.

Diagnosis

Evans et al. (2005) proposed the following three species groups based on characteristics of the mesosoma and metasoma:

clavicornis group: mesoscutal median furrow and metasomal basal carina absent. *tenuicornis* group: mesoscutal median furrow absent, metasomal basal carina present. *fumipennis* group: mesoscutal median furrow and metasomal basal carina present.

According to the morphological features of these species groups, the new species belongs to the *fumipennis* group, while *Aphanogmus fulmeki* and *A. floridanus* that have been known as parasitoids of *Feltiella* species belong to the *clavicornis* group and *tenuicornis* group, respectively. Therefore, the new species can be distinguished from *Aphanogmus fulmeki* and *A. floridanus*.

Among members of the *fumipennis* group, the new species shares the following characteristics with species in the *Aphanogmus hakonensis* complex *sensu* Polaszak and Dessart (1996): median mesoscutal sulcus present; dorsal axillar area and mesoscutellum with distinct lateral carina; syntergum with distinct transverse carina anteriorly. However, *Aphanogmus flavigastris* does not belong to the *A. hakonensis* complex based on the following characters: fore wing with a darkly pigmented band (hyaline in *A. hakonensis* complex); antenna of female with flagellomere 2–7 not transverse (transverse in *A. hakonensis* complex).

The new species is most similar to *Aphanogmus inamicus* as it shares the following characters: median mesoscutal sulcus present; dorsal axillar area and mesoscutellum with distinct lateral carina; syntergum with distinct transverse carina anteriorly; fore wing with a darkly pigmented band; antenna of female with flagellomere 2–7 not transverse. However, *Aphanogmus flavigastris* can be distinguished from *A. inamicus* by the following characters: club of antenna 1 segmented (3 segmented in *A. inamicus*); lateral carina on dorsal axillar area and mesoscutellum more raised than that of *A. inamicus*; longitudinal striae of syntergum absent (present in *A. inamicus*); mesosoma dark brown (reddish yellow in *A. inamicus*); infuscate area on fore wing smaller (from marginal vein to posterior margin of fore wing in *A. inamicus*).

According to a key to the Palaearctic species of *Aphanogmus* (Szelényi 1940), the new species runs to *A. fasciolatus* Förster based on the following characters: antenna clavate; club 1 segmented and longer than the preceding two segments combined; ra-

dial vein longer than marginal vein. However, the new species could be distinguished from *Aphanogmus fasciolatus* by having longer pedicel that is distinctly longer than flagellomere 1 while *A. fasciolatus* has the pedicel that is shorter than flagellomere 1.

We need to monitor the seasonal abundance of *Aphanogmus flavigastris* for the successful application of *Feltiella* species, because its congener *A. floridanus* that attacks *F. acarivora* has been regarded to act as a negative force in controlling *Tetranychus urticae* Koch (Acari: Tetranychidae) on strawberry in California (Oatman 1985). Shimoda et al. (2016) recently developed a remarkable system for trapping *Feltiella* species and other predators of spider mites using pots of *Brassica rapa* Linnaeus var. *perviridis* L.H.Bailey (Brassicaceae), 'komatsuna' in Japanese, which bore *Tetranychus urticae*. They could rear an unidentified species of *Aphanogmus* from *Feltiella acarisuga* with the trapping system. This method may be useful to collect plenty of individuals of *Feltiella* and its parasitoids from 'komatsuna' in the fields. Further field surveys are needed to verify the efficacy of this method as a monitoring tool for *Aphanogmus flavigastris*.

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RESEARCH ARTICLE

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Revision of the *Gonioctena nivosa* species-group (Coleoptera, Chrysomelidae, Chrysomelinae) in the Holarctic region, with descriptions of two new species

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Abstract

The *Gonioctena nivosa* species-group of the genus *Gonioctena* Chevrolat, 1836 is defined and reviewed. It contains six species including two new to science: *G. gracilicornis* (Kraatz, 1879), *G. nivosa* (Suffrian, 1851), *G. norvegica* (Strand, 1936), *G. springlovae* (Bechyně, 1948), *G. amurensis* Cho & Borowiec, **sp. n.** and *G. jani* Cho & Borowiec, **sp. n.** Six new synonyms are proposed: *G. nivosa* (= *G. arctica alberta* Brown, 1952, **syn. n.**, *Phytodecta linnaeana bergrothi* Jacobson, 1901, **syn. n.**, *P. linnaeanus* var. *mutatus* Achard, 1924, **syn. n.**, *P. linnaeanus* var. *simplex* Achard, 1924, **syn. n.** and *P. nivosa* var. *cedehensis* Ronchetti, 1922, **syn. n.**) and *G. norvegica* (= *G. janovskii* Medvedev, 1976, **syn. n.**). *Phytodecta flavicornis* var. *limbatipennis* Achard, 1924 and *P. nivosa* var. *bicolor* Heyden, 1883 are removed from synonymy with *G. nivosa* (Suffrian, 1851) and are synonymized with *G. flavicornis* (Suffrian, 1851). Distribution maps, a key to species, color variation, geographic variation of male genitalia and host plants are provided. Ovoviviparity is newly recorded in *G. gracilicornis* and *G. nivosa*. Lectotypes are designated for *G. affinis*, *G. arctica*, *G. linnaeana bergrothi* and *G. nivosa*.

Keywords

Leaf beetles, taxonomic revision, geographic variation, ovoviviparity

Introduction

The genus *Gonioctena* Chevrolat, 1836 with about 100 described species in nine subgenera is one of the largest genera within the subfamily Chrysomelinae (Cho and Borowiec 2016). The nominotypical subgenus is the largest and contains 47 species that are widely distributed in the Holarctic and Oriental regions (Cho 2016). Many species of the nominotypical subgenus have received much attention due to their extremely high variability in coloration. Although the color pattern of several species has been revealed (Bechyně 1948, Silfverberg 1994b, V. L. Medvedev 2003, etc.), a similar color pattern between closely related sympatric species has produced a number of synonyms and misidentifications. The structure of male genitalia is generally used as the only source of reliable diagnostic characters. However, the shape of aedeagus is often geographically variable in several species with wide distributions or it is very similar between closely related species. The taxonomic status of these forms is still unclear. Kippenberg (2010) mentioned that 13 species of the subgenus *Gonioctena* s. str. in the catalogue of Palaearctic Coleoptera are characterized by the external morphology because they often have the similar shape of aedeagus. For example, the taxonomic status of the following taxa has been interpreted controversially: *G. arctica* Mannerheim, 1853 from Alaska, *G. decaspilota* (Achard, 1924) from the Scandinavian Peninsula, *G. dinah* (Bechyně, 1948) from Siberia, *G. nivosa* (Suffrian, 1851) from the Alps and *G. salicis* Motschulsky, 1860 from Transbaikalia.

In the present study, we define and review the *Gonioctena nivosa* species-group of the subgenus *Gonioctena* s. str. Six species including two new species are recognized by the following characters: apical antennomere more than twice longer than wide; first tarsomere of fore legs in male swollen; apical process of aedeagus narrow, with apex rather truncate in dorsal view, apical process pointed and slightly bent downward at apex in lateral view. We have attempted to solve its taxonomic problems based on the external morphology, geographic variation of male genitalia, coloration and distribution. Biological information on host plant and ovoviviparity is also provided.

Material and methods

Specimens were examined with a Nikon SMZ800 microscope. Male genitalia were dissected from adult specimens softened in the closed Petri dish with wet tissue paper for 12–24 hours, cleared in 10% sodium hydroxide solution, and rinsed in distilled water. Photographs were taken by a Nikon D5200 digital camera attached to a Nikon SMZ1500 microscope, and were edited by Helicon Focus 5.3.12 and Adobe Photoshop CS5. A double slash (//) in the collecting data separates the data on different labels. Type localities are cited in the original spelling. Specimens examined in the study are deposited in the following collections:

ABC Andrzej O. Bieńkowski Collection, Moscow, Russia
AWC Andrzej Warchałowski Collection, Wrocław, Poland

BMNH	The National History Museum, London, UK
CNCI	Canadian National Collection of Insect, Ottawa, Canada
DBET	Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, Wrocław, Poland
ELEU	Entomological Laboratory, Ehime University, Matsuyama, Japan
ELKU	Entomological Laboratory, Kyushu University, Fukuoka, Japan
FKC	František Kantner Collection, České Budějovice, Czech Republic
HCC	Hee-Wook Cho Collection, Andong, South Korea
JBC	Jan Bezděk Collection, Brno, Czech Republic
LMC	Lev N. Medvedev Collection, Moscow, Russia
MLUH	Institut für Zoologie, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany
MNHN	Museum National d'Historie Naturelle, Paris, France
MSNM	Museo Civico di Storia Naturale, Milano, Italy
MZHF	Finnish Museum of Natural History, University of Helsinki, Finland
NHMB	Naturhistorisches Museum Basel, Basel, Switzerland
NHRS	Naturhistoriska Riksmuseet, Stockholm, Sweden
NMPC	Národní Muzeum, Prague, Czech Republic
SDEI	Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany
SEHU	Systematic Entomology, Hokkaido University Museum, Sapporo, Japan
TARI	Taiwan Agricultural Research Institute, Applied Zoology Division, Wufeng, Taiwan
TLMF	Horst Kippenberg Collection, Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
UZIU	Zoological Museum, Uppsala University, Uppsala, Sweden
ZIN	Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia
ZMHB	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany
ZMUC	Zoological Museum, University of Copenhagen, Copenhagen, Denmark

Taxonomy

Gonioctena nivosa species-group

Diagnosis. Body length 4.05–7.00 mm. Males usually with much longer antennae than females; antennae in male reaching elytral humeri, as long as or longer than half length of body (Figs 1, 3, 5, 7, 9, 11); in female reaching elytral humeri or not; apical antennomere in both sexes more than twice longer than wide (Figs 13, 17, 26, 29, 55, 63). First tarsomere of fore legs in male swollen, almost as wide as or wider than third; in female not modified. Aedeagus parallel-sided or moderately narrowed apically, with apical process narrow, apex rather truncate in dorsal view; moderately or strongly curved, with apical process pointed and slightly bent downward at apex in lateral view (Figs 14, 18, 27, 30, 56, 64).

Key to species of *Gonioctena nivosa* species-group

1 Antennae as long as or longer than half length of body in male (Figs 1, 3, 5, 7, 11), almost or fully reaching elytral humeri in female **2**
– Antennae much shorter than half length of body in male (Fig. 9), not reaching elytral humeri in female. From Norway to Mongolia..... ***norvegica*** **(Strand)**
2 Antennae as long as half length of body in male (Figs 1, 5), almost reaching elytral humeri in female **3**
– Antennae much longer than half length of body in male (Figs 3, 7, 11), fully reaching elytral humeri in female **4**
3 Pronotum with obscure black spots or marking (Fig. 15); aedeagus rather thin with apical process long, very slightly tapered apically (Fig. 14). Mongolia, Russia (Far East) ..... ***amurensis*** **Cho & Borowiec, sp. n.**
– Pronotum with distinct black spots or marking (Fig. 28); aedeagus rather thick with apical process short, very slightly widened apically (Fig. 27). Russia (East Siberia, Far East)..... ***jani*** **Cho & Borowiec, sp. n.**
4 Smaller, body length 4.05–6.00 mm; first tarsomere of all legs in male much strongly swollen (Fig. 7); aedeagus strongly curved in lateral view (Fig. 30). Transholarctic..... ***nivosa*** **(Suffrian)**
– Larger, body length 5.70–7.00 mm; first tarsomere of all legs in male less strongly swollen (Figs 3, 11); aedeagus moderately curved in lateral view (Figs 18, 64) **5**
5 Pronotum feebly rounded laterally (Fig. 12); aedeagus thin (Fig. 64). Sakhalin, Hokkaido ..... ***springlovae*** **(Bechyně)**
– Pronotum strongly rounded laterally (Fig. 4); aedeagus rather thick (Fig. 18). Russia (East Siberia, Far East, Sakhalin), Mongolia, China (Heilongjiang), Korea ..... ***gracilicornis*** **(Kraatz)**

***Gonioctena* (*Gonioctena*) *amurensis* Cho & Borowiec, sp. n.**
http://zoobank.org/A9B743E2-DDE4-4F4B-A8AF-26C9A3B74D0F
Figs 1–2, 13–16

Type material. Holotype: ♂ (ZIN), Russia, Amur Oblast, Svobodnensky District, between the Malaya Pera and Bolshoy Ergel Rivers, 2.VII.1958, Zinoviev leg. // HOLOTYPUS *Gonioctena* (*Gonioc.*) *amurensis* sp. n. Cho & Borowiec 2015. Paratypes: 5♂♂ (ZIN), same data as holotype; 1♂, 1♀ (ZIN), Russia, Amur Oblast, Svobodnensky District, Klimoutsy Village, 40 km W Svobodny City, 14.VII.1957, Zinoviev leg.; 1♂ (ZIN), Russia, Amur Oblast, Tyndinsky District, between Djeltulak and Sosnovaya, 30.VII.1928, Obolenskiy leg.; 1♂ (NHMB), Russia, Primorsky Krai, Ussuriysk Reserve, VI.1956, L.N. Medvedev; 1♂ (TLMF), Russia, Primorsky Krai, Khasansky District, Kedrovaya Pad Nature Reserve, 1956, L. Medvedev; 1♀ (NHMB), Mongolia, Central Aimak, 21.VI.1974, V. Janovsky leg. Each paratype

Figures 1–6. Dorsal habitus and pronotum. **1–2** *Gonioctena amurensis* sp. n., holotype **3–4** *G. gracilicornis* **5–6** *G. jani* sp. n., holotype. Scale bars = 1.0 mm.

specimen has a type label: PARATYPUS *Gonioctena* (*Gonioc.*) *amurensis* sp. n. Cho and Borowiec 2015.

Diagnosis. *Gonioctena amurensis* sp. n. is closely related to *G. jani* sp. n. in having small body size and similar length of antennae, however it can be distinguished by pronotum with small and moderately dense punctures on median region and large and dense punctures on lateral region (sparse punctures on median region and moderately dense punctures on lateral region in *G. jani* sp. n.) and rather thin aedeagus with relatively long apical process (rather thick with relatively short apical process in *G. jani* sp. n.).

Description. Measurements in mm (n = 5): length of body: 5.00–5.50 (mean 5.16); width of body: 2.90–3.25 (mean 3.09); height of body: 1.85–2.30 (mean 2.09); width of head: 1.40–1.45 (mean 1.41); interocular distance: 0.95–1.05 (mean 0.99); width of apex of pronotum: 1.50–1.65 (mean 1.55); width of base of pronotum: 2.45–2.60 (mean 2.51); maximum width of pronotum: 2.52–2.62 (mean 2.56); length of pronotum along midline: 1.30–1.40 (mean 1.32); length of elytra along suture: 3.65–4.10 (mean 3.85).

Body oblong oval and moderately convex (Fig. 1). Head black. Mandibles black, with reddish brown band near apex. Maxillary palps reddish brown or dark brown, with apical palpomere blackish brown. Antennomeres 1–7 yellowish brown, 1 and 7 darkened, 8–11 dark brown to blackish brown. Pronotum reddish brown, with 2–3 obscure

spots or an obscure marking (Fig. 15). Scutellum black. Elytra reddish brown, with 3–5 pairs of black spots. Venter black, with hypomera and apical margin of last abdominal ventrite reddish brown. Legs black, with tibiae except base and tarsi reddish brown.

Head. Vertex weakly convex, covered with coarse and dense punctures. Frontal suture V-shaped, coronal suture absent or weak. Frons flat, strongly depressed anteriorly, covered with moderately dense punctures. Clypeus narrow and trapezoidal. Anterior margin of labrum distinctly concave. Mandibles with 2 sharp apical teeth and a deep excavation for apical maxillary palpomere at outer side. Maxillary palps 4-segmented, with apical palpomere distinctly widened, truncate apically in male; slightly widened in female. Antennae in male as long as half length of body; antennomere 1 robust; antennomere 2 shorter than 3; antennomere 3 longer than 4; antennomeres 7–11 each distinctly longer than wide; antennomere 11 longest, about 2.48 times as long as wide (Fig. 13). Antennae in female almost reaching elytral humeri; antennomere 11 about 2.38 times as long as wide.

Pronotum. Lateral sides widest near base, roundly moderately narrowed anteriorly, anterior angles strongly produced (Fig. 2). Anterior and lateral margins bordered, lateral margins invisible in dorsal view. Trichobothria present on posterior angles. Disc covered with moderately dense punctures; lateral sides covered with much coarser and denser punctures, becoming larger toward base, partially confluent near basal margin; interspaces covered with fine and sparse punctures. Scutellum slightly wider than long, narrowed posteriorly.

Elytra. Lateral sides moderately widened posteriorly, widest beyond middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with 11 regular rows of large punctures, including a short scutellar row; punctures rather irregular between 6th and 8th striae in apical half; interspaces shagreened, covered with fine and sparse punctures. Epipleura wholly visible in lateral view. Hind wings well developed.

Venter. Hypomera weakly rugose, with a few punctures near anterolateral corners of prosternum. Prosternum covered with coarse and dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with moderately dense punctures. Metasternum covered with small and moderately dense punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with dense punctures bearing short setae.

Legs. Moderately robust. Tibiae widened apically; fore tibia with a blunt tooth-like projection; mid and hind tibiae each with a tooth-like projection. Fore legs with tarsomere 1 strongly enlarged, distinctly wider than 3 in male; slightly narrower than 3 in female. Tarsal claws appendiculate.

Genitalia. Aedeagus moderately narrowed apically, with apical process rather long, very slightly tapered apically, apex truncate in dorsal view; moderately curved, with apical process pointed and slightly bent downward at apex in lateral view (Fig. 14). Spermatheca absent.

Etymology. Named after the type locality, Amur region.

Distribution. Mongolia, Russia (Far East) (Fig. 16).

***Gonioctena* (*Gonioctena*) *gracilicornis* (Kraatz, 1879)**
Figs 3–4, 16–25, 66

Phytodecta gracilicornis Kraatz, 1879b: 135 (type locality: Amur); Weise 1893: 1129; Jacobson 1901: 128; Bechyně 1948: 115.
Gonioctena gracilicornis: Marseul 1888: 37; L. N. Medvedev 1968: 76; Jolivet 1973: 266; L. N. Medvedev and Voronova 1976: 228; L. N. Medvedev and Korotyaev 1980: 86; L. N. Medvedev and Zaytsev 1980: 105 (larva); L. N. Medvedev and Roginskaya 1988: 100 (host plant); Dubeshko and L. N. Medvedev 1989: 132 (biology); Li 1992: 184; Mikhailov and Hayashi 2000: 82.
Phytodecta (*Phytodecta*) *gracilicornis*: Weise 1916: 177; Winkler 1930: 1295; Chen 1935: 127, 1936: 86; Chûjô 1941: 74.
Gonioctena (*Gonioctena*) *gracilicornis*: Gressitt and Kimoto 1963: 358, 361; L. N. Medvedev and Zaytsev 1978: 119 (larva); L. N. Medvedev 1982: 92, 179, 252 (incl. larva); Takizawa 1985: 9; L. N. Medvedev 1992: 575; L. N. Medvedev and Dubeshko 1992: 118; V. L. Medvedev 1999: 14; Lee and An 2001: 102; V. L. Medvedev 2004: 41; Lopatin et al. 2004: 122; L. N. Medvedev 2006a: 139; Cho and Lee 2008: 105, 107; Zaytsev and L. N. Medvedev 2009: 145 (larva); Cho and Lee 2010: 58; Kippenberg 2010: 433; Warchałowski 2010: 559.
Phytodecta (*Phytodecta*) *gracilicornis* var. *kiberi* Chûjô, 1941: 74 (type locality: Korea, Keiki-Do, Hosen-Gun, Mt. Syoyo-Zan); Gressitt and Kimoto 1963: 362 (as synonym of *G. gracilicornis*).
Phytodecta (*Phytodecta*) *gracilicornis* var. *munaguro* Chûjô, 1941: 75 (type locality: Korea, Kankyo-Hokudo, Mt. Kwambo-Zan); Gressitt and Kimoto 1963: 362 (as synonym of *G. gracilicornis*).
Phytodecta (*Phytodecta*) *gracilicornis* var. *signaticollis* Chûjô, 1941: 75 (type locality: E Siberia); Gressitt and Kimoto 1963: 361 (as synonym of *G. gracilicornis*).
Gonioctena sunkangensis Kimoto and Kawase, 1966: 44 (type locality: Manchuria, Laoheishan); L. N. Medvedev 1982: 252 (as synonym of *G. gracilicornis*).
Gonioctena sungkangensis [sic!]: Takizawa 1985: 7.
Gonioctena (*Gonioctena*) *sungkangensis* [sic!]: Takizawa 1985: 9; Lee and An 2001: 103.
Gonioctena (*Gonioctena*) *sunkangensis*: V. L. Medvedev 2004: 41; Kippenberg 2010: 434; Yang et al. 2014: 368, 2015: 50.
Gonioctena (*Gonioctena*) *coreana*: L. N. Medvedev 1992: 573 (part) (misidentification).
Gonioctena springlovae: Li 1992: 189 (misidentification).

Type material. *Gonioctena gracilicornis*: Syntypes 1♂ (SDEI), Amur // Coll. Kraatz // Dtsch Ent. Inst. Eberswalde // Lectotypus *Gonioctena gracilicornis* Kz.; 1♂ (SDEI), Amur // Paralectotypus // Coll. Kraatz // Dtsch Ent. Inst. Eberswalde; 2♂♂, 5♀♀ (SDEI), Amur // Paralectotypus // Coll. Kraatz // *P. gracilicornis* Kr. // Dtsch Ent. Inst. Eberswalde; 1♂ (SDEI), Amur, Christoph 77 // Paralectotypus // *P. gracilicornis* Kr. // Dtsch Ent. Inst. Eberswalde; 1♂ (BMNH), Cotype // Amur // Brit. Mus, 1937-250 // *Phytodecta gracilicornis* Kr. // Coll. Kraatz // Typus.

Phytodecta gracilicornis var. *kiberi*: Holotype in TARI.

Phytodecta gracilicornis var. *munaguro*: Holotype and paratype in TARI.

Phytodecta gracilicornis var. *signaticollis*: Type depository unknown.

Gonioctena sunkangensis: Holotype ♂ (ELKU), Manchuria, Laoheishan, 17.X.1918 // *Gonioctena sunkangensis* Kimoto & Kawase // HOLOTYPE.

Other material. Russia: 1♂ (NHMB), Vladivostok, Russia, 1933, N. Filippov; 1♂ (NHMB), Russia, Primorsky Krai, Ussuriysk Reserve, VI.1956, L.N. Medvedev; 1♂ (NHMB), Magadanska oblast, 13 km N of Klepka, 27.VI.1975 // *Salix*; 1♂ (NHMB), pr. Kamenushka 30 km E Ussurijsk, 20–25.VI.1990 // USSR Ussuri, Maritime Terr., S. Kasantsev; 1♀ (NHMB), Transbaikal; 1♂ (NHMB), Tschita, Transbaikalien. Hermann Frieb.; 1♂ (NHMB), Sutschan, Ussuri; 1♂ (NHMB), Sibiria orient., Sotka-Sora, B. v.Bodemeyer; 2♂♂, 1♀ (JBC), Russia, Krasnojarskij K. Sajanogorsk, Maina, 3–9.VII.1994, leg. Kletecka; 1♂ (ABC), Russia, Primorskiy Kray, Zarechnoye 10 km SE, Ussuriysk, 43.37N 132.18E, 11.VI.1993, 200 m, leg. L. Zerche; 3♂♂, 1♀ (ABC), Russia, Tuva, S. Slopes of E. Tanu-Ola Mts., envir. Samagaltai vill., 1400–1800 m, 21.V.–11.VI.2002, Vashchenko leg.; 3♂♂ (HCC), Russia, NE Siberia, Yakutia reg., Khandyga, VII.1993, Maglis leg.; 7♂♂, 2♀♀ (HCC), Russia, S Siberia, Tuva, S slope of E Tannu Ola Mts, Samag altai v., 1600m, 10.VI.2004, S. Vaschenko leg.; 2♂♂, 1♀ (ELEU), Far East Russia, nr. Anisimovka, Primor Territ., 3.VII.1999, Y. Notsu leg.; 1♀ (ELEU), Russia, Bistraya River, Kamchatka (53.55N, 157.42E), 16.VIII.2000, T. Yamamoto leg.; 16♂♂, 5♀♀ (FKC), Russia, Primorskij kr., Arsenev env., VI.1991, leg. M. Štrba; 10♂♂, 5♀♀ (FKC), Russia, Krasnojarskij kr., Sajangorsk, Maina, 3.VII.1994, leg. Z. Kletecka; 1♂ (LMC), Saghalien, Toyohara, 16.VII.1922, Teiso Esaki; 1♂ (SDEI), Russia: Primorsky Kray, Sikhote-Alin, Biof. Stat. 35 km SE, Chuguyevka // 44.05N 134.02E, 31.V.1993, 650 m, leg. L. Zerche et al.; 1♂ (SDEI), Russia, Primorsky Kray, Krounovka, Medveditsa river, 40 km SW Ussuriysk, 250 m // 43°3'N, 131°15'E, 2–6.VIII.1993, leg. E.K. Groll; 1♂ (SDEI), Amur, Christoph 77 // Coll. Kraatz; 1♂, 1♀ (ZIN), Russia, Magadan Oblast, Tenkinsky District, Kolyma River, Duskanya Village (or river outlet), 8.VIII.1979, Migovich leg.; 1♂ (ZIN), Russia, Magadan Oblast, Tenkinsky District, Kolyma River, Duskanya Village (or river outlet), 3.VIII.1979, Russ leg.; 1♂ (ZIN), Russia, Primorsky Krai, Ussuriysky Urban Okrug, Kaimanovka Village (Suputinsky Dok Village), 14.VI.1960, Kabakov leg.; 1♂, 1♀ (MNHN), Museum Paris Siberie env. D'Irkoutsk, Nilova Poustine, D. Busson 1913; 1♀ (NMPC), Transbaikalien, Leder Reitter // Collectio A. Fleischer // Ovoviviparous, Det. H.W. Cho; 1♂ (TLMF), Kamchatka, Elisovo (53°20'N, 158°25'E), 13.VIII.1995, leg. S. Bohl; 2♂♂ (TLMF), VII.–VIII., Russia, Primorsky Krai, Kedrovaja pad, leg. + det. L. Medvedev; 2♂♂, 2♀♀ (TLMF), Transbaicalia, Selenga-Tal; 1♂ (TLMF), Blagovetchensk, leg. Zaizev; 3♂♂, 3♀♀ (TLMF), Russia, Tuva, Shuurmag, Khorumnug-Tayga, 800m, 29.VI.–1.VII.1998, leg. Vashchenko; 7♂♂, 10♀♀ (TLMF), E-Sibiria, Chabarowsk, Ochotsk surr., Ulia-river, 13.VII.–7.VIII.1985, leg. Ryvkin & Veselova; 1♂ (TLMF), Russia, Amur reg., Selemdgin distr., Tamsche, 4.IX.2004, leg. Ryvkin; 1♀ (TLMF), Russia, Amur reg., Selemdgin distr., Norsk vill., 2.VIII.2004, leg. Ryvkin & Veselova. **Mon-**

golia: 2♀♀ (FKC), Mongolia, 50 km E of Ulanbatar, Tuul riv., 22.VI.2003, J. Halada lgt.; 1♂, 1♀ (NMPC), Nordl. Mongolei. Changai, Leder. // Coll. Achard Mus. Pragense; 1♂ (NMPC), Mongolia, Reitter // Coll. Achard Mus. Pragense. **China:** 1♂ (SDEI), China, Charbin, v. Bennigsen // Fleischer det.; 1♂ (SDEI), Erzendjanzsy, Manshukuo, leg. W. Alin, 21.VI.1940; 1♂ (SDEI), Erzendjanzsy, Manshukuo, leg. W. Alin, 15.VI.1941; 2♂♂ (MNHN), Museum Paris Mandjourie Ourga a Tsitsikhar, J. Chaffnjon 174-96; 1♂ (NMPC), Charbin v. Bennigsen // Collectio A. Fleischer // *Ph. gracilicornis* ab. *innocens* Mader 1945 Det. J. Bechyně. **North Korea:** 1♂ (NHMB), PuRyong, N. Korea; 1♂, 1♀ (SEHU), Rangrim, Nth Korea, 1.VII.1980. **South Korea:** 3♂♂ (HCC), Korea, Gyeongbuk Prov., Bonghwa-gun, Socheon-myeon, Buncheon-ri, 13.V.2006, H.W. Cho; 7♂♂, 1♀ (HCC), Korea, Gangwon Prov., Pyeongchang-gun, Mt. Gyebangsan, 30.V.2006, H.W. Cho; 3♂♂, 1♀ (HCC), Korea, Gangwon Prov., Pyeongchang-gun, Mt. Odaesan, 30.V.2006, H.W. Cho; 1♂ (HCC), Korea, Gangwon Prov., Pyeongchang-gun, Mt. Odaesan, 6.VI.2009, H.W. Cho; 1♂ (HCC), Korea, Gangwon Prov., Hongcheon-gun, Nae-myeon, Myeonggae-ri, 13.VII.2002, D.Y. Lee; 1♂ (HCC), Korea, Gangwon Prov., Hongcheon-gun, Nae-myeon, Unduryeong, 11.VI.1997, S.B. Ahn.

Diagnosis. This species is very similar to *G. springlovae* in having large body size, long antennae and similar shape of aedeagus. However, *Gonioctena gracilicornis* can be distinguished by pronotum with strongly rounded lateral sides (feebly rounded in *G. springlovae*), pronotum reddish brown, with or with a large black marking, sometimes entirely black (always entirely black in *G. springlovae*) and aedeagus rather thick (thin in *G. springlovae*).

Redescription. Measurements in mm (n = 5): length of body: 6.20–7.00 (mean 6.66); width of body: 3.70–4.20 (mean 3.99); height of body: 2.60–3.20 (mean 2.87); width of head: 1.77–1.95 (mean 1.84); interocular distance: 1.17–1.30 (mean 1.24); width of apex of pronotum: 2.00–2.30 (mean 2.13); width of base of pronotum: 3.02–3.40 (mean 3.22); maximum width of pronotum: 3.10–3.47 (mean 3.28); length of pronotum along midline: 1.57–1.70 (mean 1.63); length of elytra along suture: 4.60–5.35 (mean 5.03).

Body oblong oval and moderately convex (Fig. 3). Coloration extremely variable. Head black, with reddish brown band near apex of mandibles. Antennomeres 1–5 yellowish brown, sometimes darkened, 6–7 dark brown to blackish brown, 8–11 black. Pronotum reddish brown, with or without a large black marking, sometimes entirely black (Fig. 25). Scutellum black. Elytra reddish brown, with or without 5 pairs of black spots, sometimes enlarged and connected with each other. Venter black, with hypomera and apical margin of last abdominal ventrite reddish brown. Legs black, with tibiae reddish brown except base and inner margin and tarsi dark brown to blackish brown, sometimes tibiae and tarsi largely black. Rarely body almost completely black except antennae.

Head. Vertex weakly convex, covered with sparse punctures, becoming denser toward sides. Frontal suture V-shaped, coronal suture weak. Frons flat, strongly depressed anteriorly, covered with moderately dense punctures. Clypeus very narrow and

trapezoidal. Anterior margin of labrum distinctly concave. Mandibles with 2 sharp apical teeth and a deep excavation for apical maxillary palpomere at outer side. Maxillary palps 4-segmented, with apical palpomere distinctly widened, truncate apically in male; slightly widened in female. Antennae in male longer than half length of body; antennomere 1 robust; antennomere 2 shorter than 3; antennomere 3 longer than 4; antennomeres 7–11 elongate; antennomere 11 longest, about 3.44 times as long as wide (Fig. 17). Antennae in female reaching elytral humeri; antennomere 11 about 2.72 times as long as wide.

Pronotum. Lateral sides widest near base, roundly moderately narrowed anteriorly, anterior angles strongly produced (Fig. 4). Anterior and lateral margins bordered, lateral margins well visible in dorsal view. Trichobothria present on posterior angles. Disc covered with sparse punctures; lateral sides covered with much coarser and denser punctures, becoming larger toward base, partially confluent near basal margin; interspaces covered with fine and sparse punctures. Scutellum variable in length, as long as wide, longer than wide or wider than long.

Elytra. Lateral sides slightly widened posteriorly, widest beyond middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with 11 regular rows of large punctures, including a short scutellar row; interspaces shagreened in some specimens, covered with fine and sparse punctures. Epipleura wholly visible in lateral view. Hind wings well developed.

Venter. Hypomera weakly rugose, with dense punctures on anterior side. Prosternum covered with coarse and dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with sparse punctures. Metasternum covered with small and sparse punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with dense punctures bearing short setae.

Legs. Moderately robust. Tibiae widened apically, with a tooth-like projection. Fore legs with tarsomere 1 enlarged, slightly wider or narrower than 3 in male; distinctly narrower than 3 in female. Tarsal claws appendiculate.

Genitalia. Aedeagus rather thick, moderately narrowed apically, with apical process rather thick in dorsal view; moderately curved, with apical process pointed and slightly bent downward at apex in lateral view (Figs 18–24). Spermatheca absent.

Distribution. Russia (East Siberia, Far East, Sakhalin), Mongolia, China (Heilongjiang), North Korea, South Korea (Fig. 16).

Host plant. Salicaceae: *Salix caprea*, *S. rorida*, *S. sachalinensis* (L. N. Medvedev 1968); *Salix* spp. (L. N. Medvedev and Zaytsev 1978, L. N. Medvedev 1982, 1992, L. N. Medvedev and Roginskaya 1988, L. N. Medvedev and Dubeshko 1992, Zaytsev and L. N. Medvedev 2009).

Remarks. *Gonioctena gracilicornis* is widely distributed in the Northeastern Palearctic region (Fig. 16) and is slightly variable in the shape of aedeagus (Figs 18–24). *Gonioctena gracilicornis* var. *kiberi*, *munaguro*, *signaticollis* were described by Chûjô (1941) and synonymized with *Gonioctena gracilicornis* by Gressitt and Kimoto (1963). However, the type specimens of these variations have not been examined and their taxonomic status needs to be re-examined. Medvedev (1982) synonymized *G. sunk-*

angensis with *G. gracilicornis*, however he did not examine the type of *G. sunkangensis*. We examined types of both species and confirm that both are conspecific. Lectotype label of *Gonioctena gracilicornis* by L. N. Medvedev has not been published, and thus invalid. Li's record (1992) is probably based on misidentified *G. gracilicornis* because *G. springlovae* has not been recorded from China. Female laid larvae which were enclosed within chorion on leaves of *Salix* sp. in South Korea, therefore this species is ovoviviparous (Fig. 66).

***Gonioctena* (*Gonioctena*) *jani* Cho & Borowiec, sp. n.**
http://zoobank.org/63AE87F3-E9F9-4481-812C-EC824EA4DFFF
Figs 5–6, 16, 26–28

Type material. Holotype: ♂ (ZIN), Russia, Sakha Republic, Amginsky District, Krestyah Village, 18.VII.1928, ex Museum of Yakutia // HOLOTYPUS *Gonioctena* (*Gonioc.*) *jani* sp. n. Cho & Borowiec 2015. Paratypes: 3♂♂, 1♀ (ZIN), same data as holotype; 2♂♂ (NHMB), Oberer Amur // ex Orig. Samlg. J. Breit Wien; 1♂ (ABC), Eastern Yakutia Republic, Suntar-Khayata range, 1290 m, on *Salix*, 8.VII.2002, O. Khruleva leg.; 1♂ (ABC), Amur Reg., Zeya Distr., Zeyskiy Reservoir, Tukurlinga ridge, 21–24.VI.2006, E.V. Guskova leg.; 1♂ (TLMF), Russia, Primorsky Krai, Khasansky District, Kedrovaya Pad Nature Reserve, VII–VIII.1956, Medvedev; 1♂ (TLMF), Russia, Amur oblast, Blagoveshchensk; 3♂♂, 9♀♀ (TLMF), Russia, Yakutia Republic, Khandyga, VII.1993, L. Naglis. Each paratype specimen has a type label: PARATYPUS *Gonioctena* (*Gonioc.*) *jani* sp. n. Cho & Borowiec 2015.

Diagnosis. *Gonioctena jani* sp. n. is closely related to *G. amurensis* sp. n. in having small body size and similar length of antennae, however it can be distinguished by pronotum with sparse punctures on median region and moderately dense punctures on lateral region (small and moderately dense punctures on median region and large and dense punctures on lateral region in *G. amurensis* sp. n.) and aedeagus rather thick with relatively short apical process (rather thin with relatively long apical process in *G. amurensis* sp. n.).

Description. Measurements in mm (n = 5): length of body: 5.00–5.70 (mean 5.30); width of body: 3.00–3.40 (mean 3.20); height of body: 2.10–2.40 (mean 2.18); width of head: 1.42–1.60 (mean 1.52); interocular distance: 1.02–1.12 (mean 1.07); width of apex of pronotum: 1.57–1.75 (mean 1.66); width of base of pronotum: 2.47–2.77 (mean 2.63); maximum width of pronotum: 1.80–2.75 (mean 2.45); length of pronotum along midline: 1.30–1.45 (mean 1.37); length of elytra along suture: 3.70–4.40 (mean 3.96).

Body oblong oval and moderately convex (Fig. 5). Head black. Mandibles black, with reddish brown band near apex. Maxillary palps reddish brown or dark brown, with apical palpomere black. Antennomeres 1–7 yellowish brown, 1 and 7 slightly darkened, 8–11 reddish brown to dark brown. Pronotum reddish brown, with 3 spots or a large marking (Fig. 28). Scutellum black. Elytra reddish brown, with or without 5

pairs of black spots. Venter black, with hypomera, apical and lateral parts of abdominal ventrites 3–5 reddish brown. Legs black, with tibiae reddish brown except base and tarsi dark brown to reddish brown.

Head. Vertex weakly convex, covered with coarse and dense punctures. Frontal suture V-shaped, coronal suture absent or weak. Frons flat, strongly depressed anteriorly, covered with dense punctures. Clypeus narrow and trapezoidal. Anterior margin of labrum distinctly concave. Mandibles with 2 sharp apical teeth and a deep excavation for apical maxillary palpomere at outer side. Maxillary palps 4-segmented, with apical palpomere distinctly widened, truncate apically in male; slightly widened in female. Antennae in male almost as long as half length of body; antennomere 1 robust; antennomere 2 shorter than 3; antennomere 3 longer than 4; antennomeres 7–11 each distinctly longer than wide; antennomere 11 longest, about 2.22 times as long as wide (Fig. 26). Antennae in female almost reaching elytral humeri; antennomere 11 about 2.33 times as long as wide.

Pronotum. Lateral sides widest near base, roundly moderately narrowed anteriorly, anterior angles strongly produced (Fig. 6). Anterior and lateral margins bordered, lateral margins invisible in dorsal view. Trichobothria present on posterior angles. Disc covered with sparse punctures; lateral sides covered with much coarser and denser punctures, becoming larger toward base, partially confluent near basal margin; interspaces covered with fine and sparse punctures. Scutellum slightly wider than long, narrowed posteriorly.

Elytra. Lateral sides moderately widened posteriorly, widest beyond middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with 11 regular rows of large punctures, including a short scutellar row; punctures rather irregular between 6th and 8th striae in apical half; interspaces shagreened in female, covered with fine and sparse punctures. Epipleura wholly visible in lateral view. Hind wings well developed.

Venter. Hypomera weakly rugose, with a few punctures near anterolateral corners of prosternum. Prosternum covered with coarse and dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with sparse punctures. Metasternum covered with small and sparse punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with dense punctures bearing short setae.

Legs. Moderately robust. Tibiae widened apically, with a tooth-like projection. Fore legs with tarsomere 1 strongly enlarged, distinctly wider than 3 in male; slightly narrower than 3 in female. Tarsal claws appendiculate.

Genitalia. Aedeagus rather thick, parallel-sided in middle, with apical process rather short, very slightly widened apically, apex rather truncate in dorsal view; moderately curved, with apical process pointed and slightly bent downward at apex in lateral view (Fig. 27). Spermatheca absent.

Etymology. Dedicated to Jan Bezděk (Brno, Czech Republic), the well-known specialist in Chrysomelidae.

Distribution. Russia (East Siberia, Far East) (Fig. 16).

Host plant. One specimen was collected on *Salix* sp. (Salicaceae) in Sakha Republic.

***Gonioctena* (*Gonioctena*) *nivosa* (Suffrian, 1851)**
Figs 7–8, 29–54, 67

Chrysomela affinis Gyllenhal, 1808: 257 nec Fabricius, 1787: 67 (type locality: Lapponia); Suffrian 1851: 218 (part).

Gonioctena affinis: Chevrolat 1836: 403; Mannerheim 1852: 369; Letzner 1864: 142; Weise 1884: 500; Marseul 1888: 41; Székessy 1934: 33; L. N. Medvedev 1968: 77; L. N. Medvedev and Voronova 1976: 228; L. N. Medvedev and Korotyaev 1980: 81, 86; L. N. Medvedev and Zaytsev 1980: 104, 106 (larva); L. N. Medvedev and Roginskaya 1988: 100 (host plant); Dubeshko and L. N. Medvedev 1989: 130 (biology); Steinhausen 1996: 74 (larva).

Phytodecta affinis: Gradl 1882: 330; Kraatz 1879a: 53; Kittel 1884: 32; Weise 1893: 1130, 1906: 561; Holdhaus and Lindroth 1939: 208; Bechyně 1948: 92, 118, 124; L. N. Medvedev 1963: 114.

Phytodecta (*Phytodecta*) *affinis*: Weise 1916: 176; Winkler 1930: 1296; Chen 1935: 127; Mohr 1966: 185.

Gonioctena (*Gonioctena*) *affinis*: L. N. Medvedev and Zaytsev 1978: 119 (larva); L. N. Medvedev 1982: 91, 179, 252 (incl. larva), 1992: 575; L. N. Medvedev and Dubeshko 1992: 118; V. L. Medvedev 1999: 14; L. N. Medvedev 2006a: 139; Zaytsev and L. N. Medvedev 2009: 145 (larva).

Chrysomela nivosa Suffrian, 1851: 222 (type locality: Austria, Kärnten).

Gonioctena nivosa: Letzner 1864: 143 (biology); Marseul 1888: 45; Holdhaus and Lindroth 1939: 208 (as synonym of *G. affinis*); Wilcox 1972: 22; Steinhausen 1996: 75 (larva); Clark et al. 2004: 110.

Phytodecta nivosa: Kraatz 1879a: 54; Weise 1884: 500, 1891: 160, 1893: 1129; Székessy 1934: 33 (as synonym of *G. affinis*); Kippenberg 1994: 84.

Phytodecta nivosus: Weise 1906: 561; Achard 1924: 32; Bechyně 1948: 92, 118, 125.

Phytodecta (*Phytodecta*) *nivosa*: Reitter 1913: 129; Cantonnet 1968: 40, 43.

Phytodecta (*Phytodecta*) *nivosus*: Weise 1916: 178; Winkler 1930: 1295; Mohr 1966: 185.

Gonioctena (*Gonioctena*) *nivosa*: Daccordi et al. 1991: 96; Steinhausen 1994: 277 (larva); Warchałowski 1994: 104, 118, 2003: 311; Winkelman and Debreuil 2008: 142; Kippenberg 2010: 433; Warchałowski 2010: 559.

Chrysomela stenomera Dufour, 1851: 353 (type locality: Eaux-Bonnes); Weise 1906: 561 (as aberration of *G. nivosa*).

Phytodecta nivosus var. *stenomera*: Achard 1924: 32.

Gonioctena arctica Mannerheim, 1853: 257 (type locality: Kenai); Crotch 1873: 52; Holdhaus and Lindroth 1939: 208 (as synonym of *G. affinis*); Brown 1952: 340; Silfverberg 1992: 69, 1994a: 508, 1994b: 32; Mikhailov and Hayashi 2000: 82; Mikhailov 2001: 61; Silfverberg 2004: 82.

Chrysomela arctica: Suffrian 1858: 382.

Phytodecta arctica: Stål 1865: 329; Kraatz 1879a: 55, 56 (as synonym of *G. nivosa*); Schaeffer 1924: 140; Brown 1942: 100.

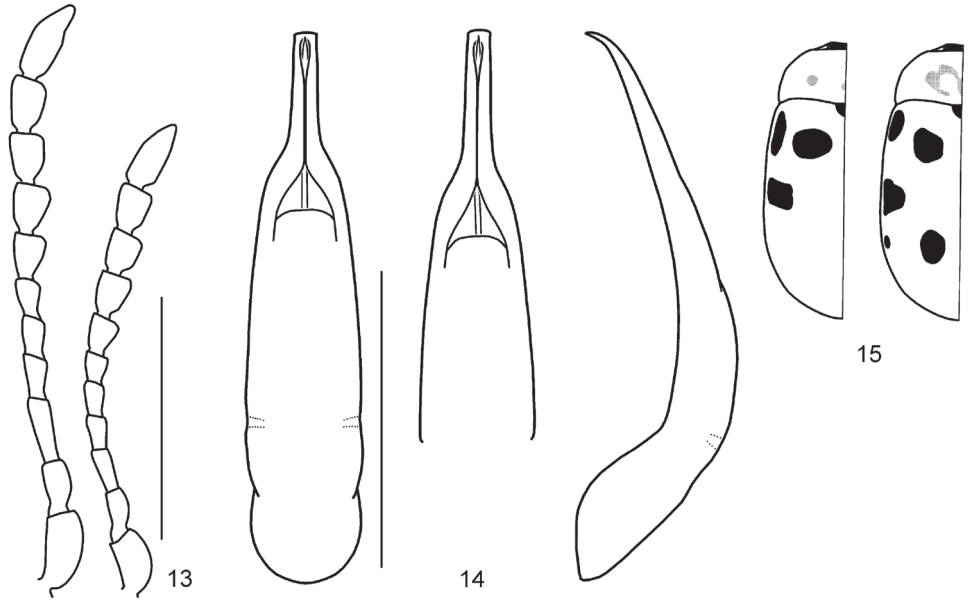
Gonioctena nivosa arctica: Wilcox 1972: 22.

Gonioctena (*Gonioctena*) *nivosa arctica*: Riley et al. 2003: 44.
Gonioctena (*Gonioctena*) *arctica*: Bieńkowski 2004: 67; Kippenberg 2010: 432; Warchałowski 2010: 559; Yang et al. 2014: 360, 2015: 49.
Gonioctena salicis Motschulsky, 1860: 223 (type locality: Daourie); Marseul 1888: 42; L. N. Medvedev 1982: 252 (as synonym of *G. affinis*), 2006b: 416 (as synonym of *G. affinis*).
Phytodecta salicis: Kraatz 1879b: 136; Jacobson 1901: 128.
Phytodecta (*Phytodecta*) *salicis*: Weise 1916: 179; Winkler 1930: 1296; Chen 1935: 128.
Phytodecta nivosa var. *rufula* Kraatz, 1879a: 55 (type locality: Tyrol); Weise 1884: 501; Marseul 1888: 45 (as synonym of *G. nivosa*).
Phytodecta nivosa var. *rufulus*: Achard 1924: 32.
Phytodecta affinis var. *clythroides* Gradl, 1882: 331 (type locality: Tirol).
Phytodecta nivosus ab. *clytroides* [sic!]: Weise 1916: 178 (as aberration of *G. nivosa*).
Phytodecta nivosus var. *clytroides* [sic!]: Achard 1924: 33.
Phytodecta affinis var. *marginata* Gradl, 1882: 331 (type locality: Tirol); Weise 1916: 178 (as aberration of *G. nivosa*).
Phytodecta nivosus var. *marginatus*: Achard 1924: 33.
Phytodecta affinis var. *nana* Gradl, 1882: 330 (type locality: Tirol); Weise 1916: 178 (as aberration of *G. nivosa*).
Phytodecta nivosus var. *nanus*: Achard 1924: 32.
Phytodecta affinis var. *nigricollis* Gradl, 1882: 331 (type locality: Tirol); Weise 1916: 178 (as aberration of *G. nivosa*).
Phytodecta affinis var. *octopunctata* Gradl, 1882: 330 (type locality: Tirol); Weise 1916: 178 (as aberration of *G. nivosa*).
Phytodecta nivosus var. *octopunctatus*: Achard 1924: 32.
Phytodecta affinis var. *tyrolensis* Gradl, 1882: 331 (type locality: Tirol); Weise 1916: 178 (as aberration of *G. nivosa*).
Phytodecta nivosus var. *tyrolensis*: Achard 1924: 33.
Phytodecta nivosa var. *aethiops* Heyden, 1883: 53 (type locality: Stilfserjoch); Weise 1906: 561 (as aberration of *G. nivosa*).
Phytodecta nivosus var. *aethiops*: Achard 1924: 33.
Phytodecta nivosa var. *apicalis* Heyden, 1883: 53 (type locality: Stilfserjoch); Weise 1906: 561 (as aberration of *G. nivosa*).
Phytodecta nivosa var. *eppelsheimi* Weise, 1884: 501 (type locality: Stilfser); Marseul 1888: 45 (as synonym of *G. nivosa*).
Phytodecta nivosus var. *eppelsheimi*: Achard 1924: 33.
Phytodecta nivosa var. *funesta* Weise, 1884: 501 (type locality: not indicated); Marseul 1888: 45 (as synonym of *G. nivosa*).
Phytodecta nivosa var. *personata* Weise, 1884: 501 (type locality: Tirol); Marseul 1888: 45 (as synonym of *G. nivosa*).
Phytodecta scutellaris Sahlberg, 1887: 55 nec Baly, 1862: 27 (type locality: Alaska, Porte Clarence); Brown 1942: 100 (as synonym of *G. arctica*); Kippenberg 2010: 437 (as nomen dubium).

Phytodecta (*Phytodecta*) *scutellaris*: Weise 1916: 181; Winkler 1930: 1296.
Phytodecta nivosa var. *ruficollis* Weise, 1891: 160 (type locality: Brenner); Weise 1906: 561 (as aberration of *G. nivosa*).
Phytodecta nivosus var. *ruficollis*: Achard 1924: 32.
Phytodecta linnaeana bergrothi Jacobson, 1901: 128 (type locality: Fl. Jenissej). **syn. n.**
Phytodecta (*Phytodecta*) *linnaeanus* var. *bergrothi*: Winkler 1930: 1295.
Phytodecta nivosa var. *cedehensis* Ronchetti, 1922: 89 (type locality: Monte Cevedale). **syn. n.**
Phytodecta affinis var. *decaspilotus* Achard, 1924: 32 (type locality: Norvège, Dowre); Winkler 1930: 1296 (as aberration of *G. affinis*).
Gonioctena decaspilota: Silfverberg 1977: 94.
Phytodecta decaspilota: Kippenberg 1994: 84.
Gonioctena (*Gonioctena*) *decaspilota*: Warchałowski 1994: 103, 104 (incl. larva), 2003: 311; Lopatin et al. 2004: 121; L. N. Medvedev 2014: 36.
Phytodecta affinis var. *hamatus* Achard, 1924: 32 (type locality: Lapponia); Winkler 1930: 1296 (as aberration of *G. affinis*).
Phytodecta nivosus var. *immarginatus* Achard, 1924: 33 (type locality: Helvetia); Winkler 1930: 1295 (as aberration of *G. nivosa*).
Phytodecta (*Phytodecta*) *nivosus* var. *immarginatus*: Chen 1936: 86.
Phytodecta (*Phytodecta*) *nivosus immarginatus*: Chen 1935: 128.
Phytodecta linnaeanus var. *simplex* Achard, 1924: 31 nec Suffrian, 1858: 383 (type locality: Kureika). **syn. n.**
Phytodecta linnaeanus var. *mutatus* Achard, 1924: 31 (replacement name for *P. linnaeanus* var. *simplex*). **syn. n.**
Phytodecta nivosa var. *undulatus* Pic, 1924: 27 (type locality: Alpes, Col du Pallet); Winkler 1930: 1295 (as aberration of *G. nivosa*).
Phytodecta dinah Bechyně, 1948: 118, 123 (type locality: Siberia).
Gonioctena (*Gonioctena*) *dinah*: Gressitt and Kimoto 1963: 358, 361; L. N. Medvedev 1992: 575 (as synonym of *G. affinis*); V. L. Medvedev 2004: 41; Kippenberg 2010: 433.
Phytodecta occidentalis: Bechyně 1948: 118, 124 (misidentification).
Gonioctena arctica alberta Brown, 1952: 340 (type locality: Alberta, Nordegg). **syn. n.**
Gonioctena nivosa alberta: Wilcox 1972: 22.
Gonioctena (*Gonioctena*) *nivosa alberta*: Riley et al. 2003: 44.

Type material. *Chrysomela affinis*: Lectotype ♂ (UZIU), hereby designated, 168 // LECTOTYPUS *Chrysomela affinis* Gyllenhal, 1808 des. H.W. Cho 2014 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014. Paralectotypes: 1♂ (UZIU), 34; 1♀ (UZIU), Lappon., Schh. [= Lapponia, Schönherr]; 1♀ (UZIU), Bog. [= Carl Johan Bogeman]; 1♀ (UZIU), Lappon., Schh.; 1♀ (UZIU), *C. affinis* var., e. Lappon., Mannerheim; 2♀♀ (UZIU), gg. // Lappon., Schh.; 3♂♂, 3♀♀ (UZIU), no data; each specimen has a label, PARALECTOTYPUS *Chrysomela affinis* Gyllenhal, 1808 des. H.W. Cho 2014 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014. 1♀ (UZIU), 36 // *C. affinis* var., e. Lappon., Mannerheim; 1♂ (UZIU), 482 // Dej. [=

Figures 7–12. Dorsal habitus and pronotum. **7–8** *Gonioctena nivosa* **9–10** *G. norvegica*, syntype **11–12** *G. springlovae*. Scale bars = 1.0 mm.



Figures 13–15. *Gonioctena amurensis* sp. n. **13** Antenna (♂, ♀) **14** Aedeagus (dorsal, apical and lateral views) **15** Color variation. Scale bars = 1.0 mm.

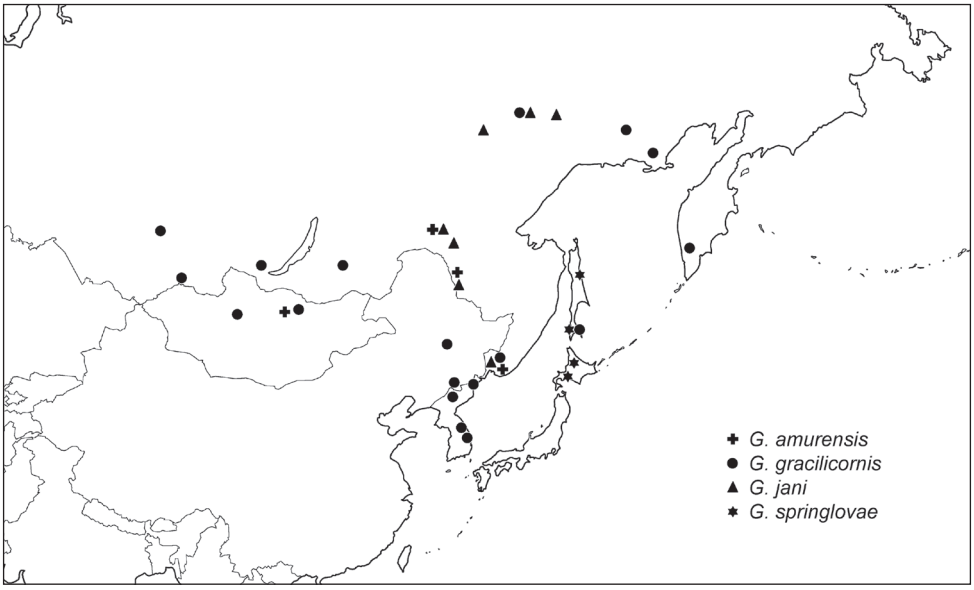


Figure 16. Distribution of *Gonioctena amurensis*, *G. gracilicornis*, *G. jani* and *G. springlovae* based on specimens in Asia.

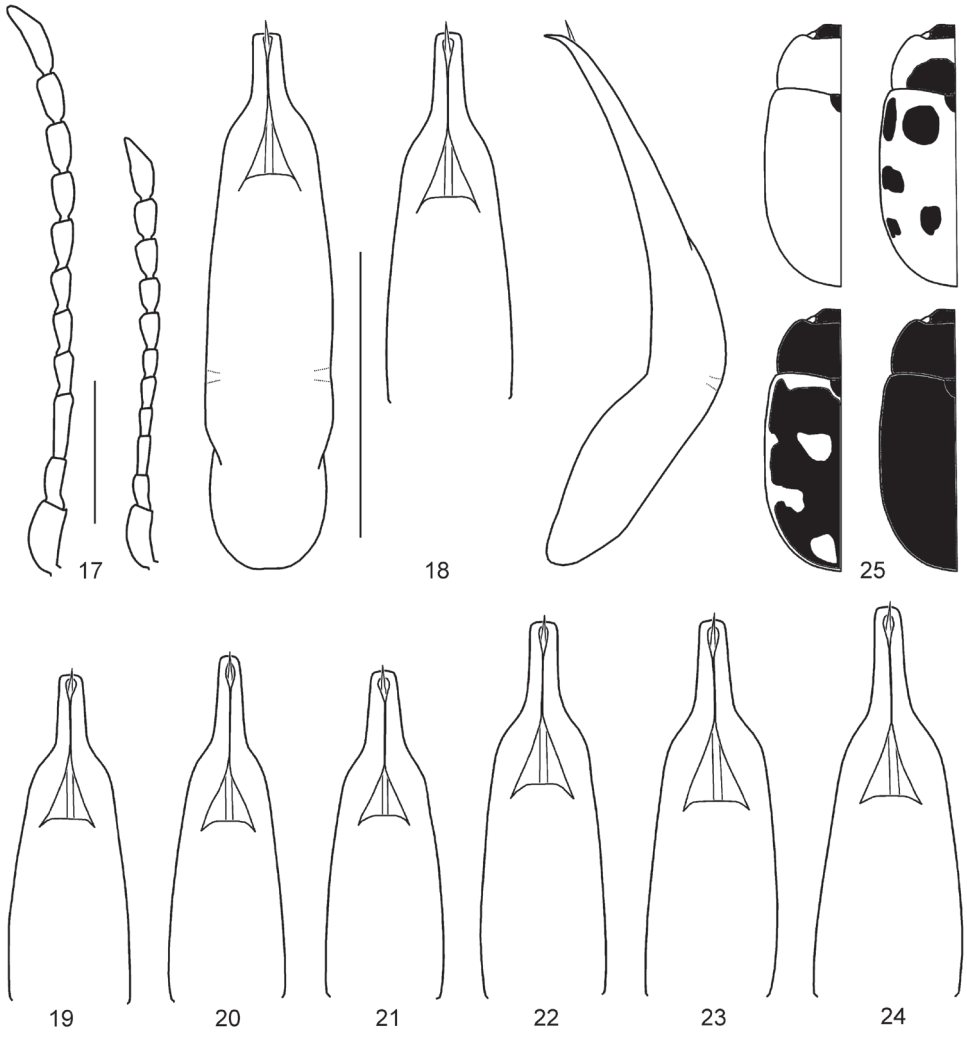
Pierre F.M.A. Dejean]; 1♂, 2♀♀ (UZIU), Dej.; 1♀ (UZIU), no data; each specimen has a label, PARALECTOTYPUS *Chrysomela affinis* Gyllenhal, 1808 des. H.W. Cho 2014 // *Gonioctena linnaeana* (Schrank, 1781) det. H.W. Cho 2014.

Chrysomela nivosa: Lectotype ♂ (MLUH), hereby designated, 23950 (Kärnten) // MLU Halle, WB Zoologie, S.-Nr. 7/1/8 // LECTOTYPUS *Chrysomela nivosa* Suffrian, 1851 des. H.W. Cho 2014. Paralectotypes: 1♂ (MLUH), 9930 (Switzerland) // MLU Halle, WB Zoologie, S.-Nr. 7/1/8; 1♂ (MLUH), 9931 (Switzerland) // MLU Halle, WB Zoologie, S.-Nr. 7/1/8; 1♂ (MLUH), 9932 (Switzerland) // MLU Halle, WB Zoologie, S.-Nr. 7/1/8; 1♂ (MLUH), 14692 (Kärnten) // MLU Halle, WB Zoologie, S.-Nr. 7/1/8. Each paralectotype specimen has a type label: PARALECTOTYPUS *Chrysomela nivosa* Suffrian, 1851 des. H.W. Cho 2014.

Chrysomela stenomera: Type depository unknown.

Gonioctena arctica: Lectotype ♂ (MZHF), hereby designated, Kenai // Holmberg // *Gonioctena arctica* Mannerh. Kenai d.j. // LECTOTYPUS *Gonioctena arctica* Mannerheim, 1853 des. H.W. Cho 2014 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014. Paralectotypes: 5♂♂, 9♀♀ (MZHF), Kenai // Holmberg // PARALECTOTYPUS *Gonioctena arctica* Mannerheim, 1853 des. H.W. Cho 2014 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014.

Gonioctena salicis: Lectotype (designated by L. N. Medvedev, 2006b): ♂ (LMC), type // *Gonioctena salicis* Motsch. Sib. Armenia // Lectotypus *Gonioctena salicis* Motsch. L. Medvedev design. Paralectotypes: 2♂♂, 3♀♀ (LMC), Paralectotypus *Gonioctena salicis* Motsch. L. Medvedev design.; 1♂ (BMNH), Type Motsch. // *Gonioctena salicis* Motsch. Siberia orient. Type Motsch. Schaufuss Janson // Baly Coll. // Syntype // PA-



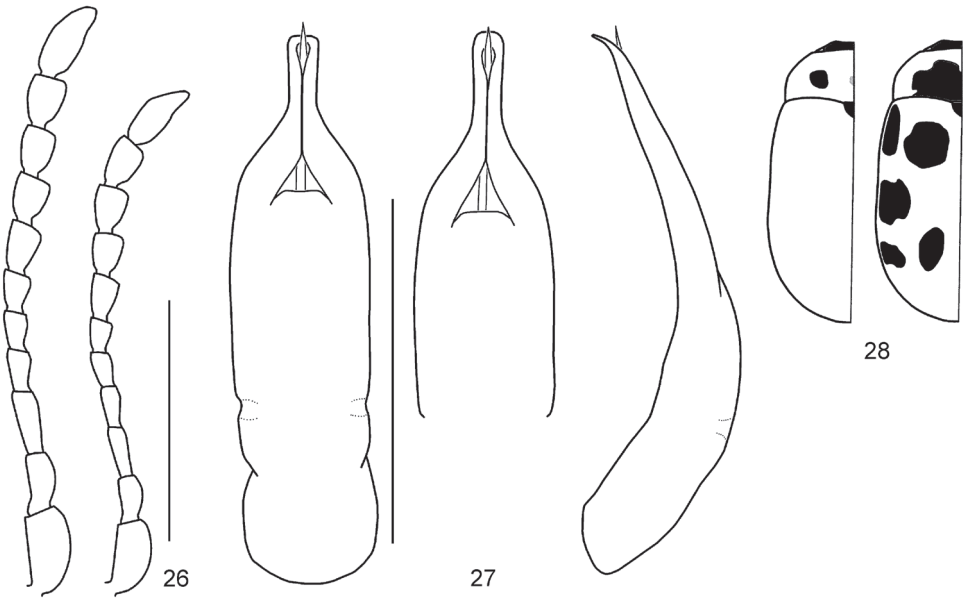
Figures 17–25. *Gonioctena gracilicornis*. **17** Antenna (♂, ♀) **18** Aedeagus (Amur) **19** Aedeagus (Tuva, Russia) **20** Aedeagus (Ulan Bator, Mongolia) **21** Aedeagus (Transbaikalia) **22** Aedeagus (Charbin, China) **23** Aedeagus (Pyeongchang, South Korea) **24** Aedeagus (Anisimovka, Russia) **25** Color variation. Scale bars = 1.0 mm.

RALECTOTYPUS *Gonioctena salicis* Motschulsky, 1860 des. L.N. Medvedev 2006 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho.

Phytodecta nivosa var. *rufula*: Type depository unknown (possibly in SDEI).

Phytodecta affinis var. *clythroides*: Syntype 1♂ (NMPC), 17 / 894. // Tirol, Coll. Gradl // TYPUS // *Ph. nivosus* TYPE ab. *clytroides* Gradl n. a. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 103.

Phytodecta affinis var. *marginata*: Syntypes 1♂ (NMPC), 17 / 896. // Tirol, Coll. Gradl // TYPUS // *Ph. nivosus* TYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně.



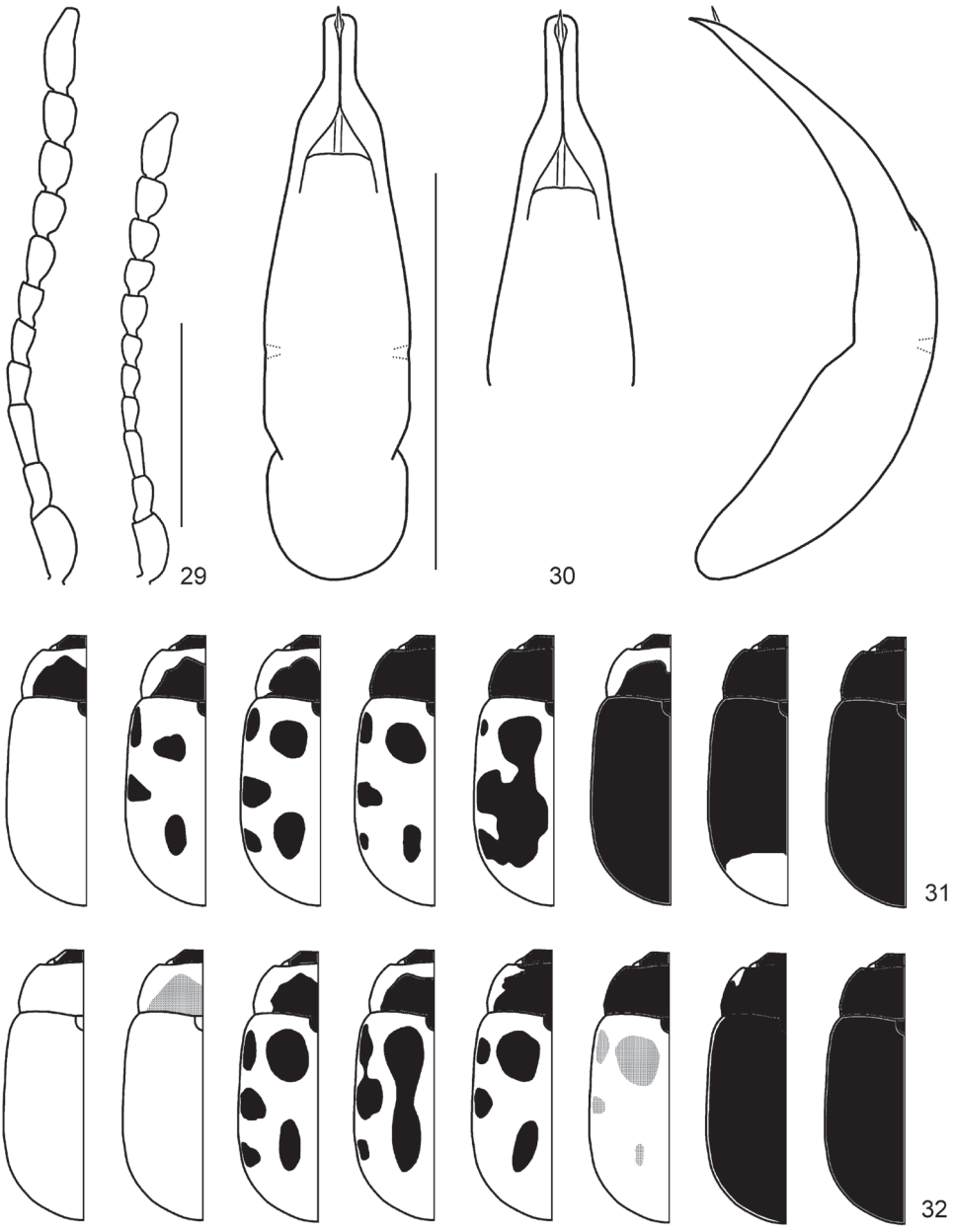
Figures 26–28. *Gonioctena jani* sp. n. **26** Antenna (♂, ♀) **27** Aedeagus **28** Color variation. Scale bars = 1.0 mm.

// Mus. Nat. Pragae Inv. 19 114; 1♀ (NMPC), 17 / 891. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* COTYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 115; 1♀ (NMPC), 17 / 892. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* COTYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 116; 1♂ (NMPC), 17 / 895. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* COTYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 117; 1♀ (NMPC), 17 / 897. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* COTYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 118; 1♀ (NMPC), 17 / 893. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* COTYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 119; 1♀ (NMPC), 17 / 899. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* COTYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 120; 1♀ (NMPC), 17 / 898. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* COTYPE ab. *marginatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 121.

Phytodecta affinis var. *nana*: Syntype 1♀ (NMPC), 17 / 884. // Tirol, Coll. Gradl // TYPUS // *Ph. nivosus* TYPE ab. *nanus* Gradl n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 104.

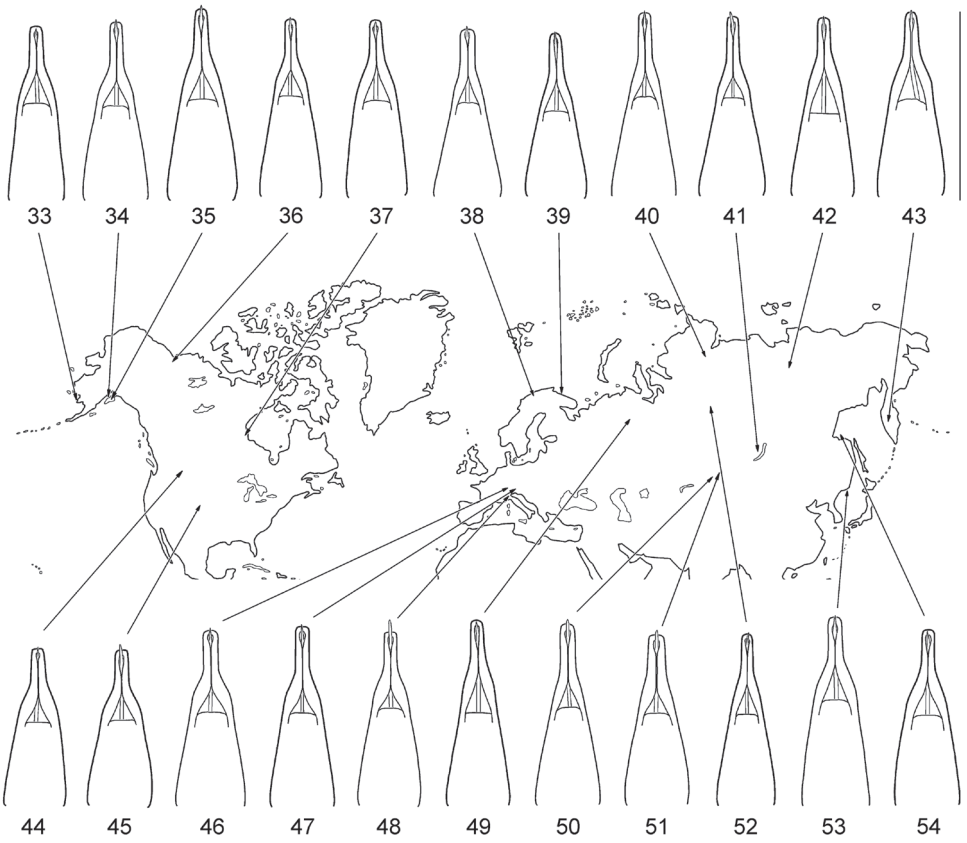
Phytodecta affinis var. *nigricollis*: Syntype 1♀ (NMPC), 2 / 846 // Tirol, Coll. Gradl // TYPUS // *Ph. nivosus* Suffr. a. *nigricollis* Gradl TYPE 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 128.

Phytodecta affinis var. *octopunctata*: Syntypes 1♀ (NMPC), 17 / 262. // Tirol, Coll. Gradl // TYPUS // *Ph. nivosus* TYPE *8-punctatus* Gradl n. ab. 1945 Det. J. Bechyně.



Figures 29–32. *Gonioctena nivosa*. **29** Antenna (♂, ♀) **30** Aedeagus (Hohe Tauern, Austria) **31** Color variation (Palaearctic region) **32** Color variation (Nearctic region). Scale bars = 1.0 mm.

// Mus. Nat. Pragae Inv. 19 107; 1♂ (NMPC), 23 / 953. // Tirol, Coll. Gradl // COTYPE // *Ph. nivosus* Suffr. COTYPE ab. *8-punctatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 108; 1♂ (NMPC), 17 / 263. // Tirol, Coll. Gradl //



Figures 33–54. Geographic variation in male genitalia of *Gonioctena nivosa*. **33** Porte Clarence, Alaska **34** Kenai, Alaska **35** Summit Lake, Alaska **36** Aklavik, Canada **37** Churchill, Canada **38** Tromso, Norway **39** Kildin Island, Russia **40** Maimecha River, Russia **41** Baikal area, Russia **42** Verkhoyansky, Russia **43** Kamchatka, Russia **44** Glacier Park, USA **45** Niwot Ridge, USA **46** Hohe Tauern, Austria **47** Karnten, Austria **48** Lombardia Val Brembana, Italy **49** Troitsko-Pechorsky, Russia **50** E Kazakhstan **51** Altai, Russia **52** Podkamennaya Tunguska, Russia **53** Vladivostok, Russia **54** Shantar Islands, Russia. Scale bar = 1.0 mm.

COTYPE // *Ph. nivosus* COTYPE ab. *8-punctatus* Gradl 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 109.

Phytodecta affinis var. *tyrolensis*: Syntype 1♀ (NMPC), 17 / 890. // Tirol, Coll. Gradl // TYPUS // *Ph. nivosus* TYPE a. *tyrolensis* Gradl n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 127.

Phytodecta nivosa var. *aethiops*: Type depository unknown (possibly in SDEI).

Phytodecta nivosa var. *apicalis*: Type depository unknown (possibly in SDEI).

Phytodecta nivosa var. *eppelsheimi*: Syntypes 1♂ (ZMHB), Stilfser Joch...[illegible] // *stenomera* Dufour, *eppelsheimi* m. // ex. coll. J. Weise; 1♂ (ZMHB), Brenner // Strasser // ex. coll. J. Weise; 1♂ (ZMHB), Stilfser Joch. Mts. Cristallo, v. Bodemeyer // ex. coll. J. Weise; 1♀ (ZMHB), *nivosa*, *eppelsh*. // ex. coll. J. Weise; 1♀ (ZMHB), ex. coll. J. Weise.

Phytodecta nivosa var. *funesta*: Type probably lost.

Phytodecta nivosa var. *personata*: Syntypes 1♂ (ZMHB), Tirol // v. *personata* // ex. coll. J. Weise; 1♀ (ZMHB), Savoyen, manuel // ex. coll. J. Weise; 2♂♂, 1♀ (ZMHB), ex. coll. J. Weise; 1♂ (SDEI), Tirol, Reitter // 323 // v. *personata* Weise.

Gonioctena scutellaris: Holotype ♂ (NHRS), Porte Clarence (Alaska) // Exped. Vega. // Spec. typ. // 206 // Typus // *Gonioctena scutellaris* J. Sahlb // NHRS-JLKB 000023152 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho.

Phytodecta nivosa var. *ruficollis*: Syntype 1♀ (ZMHB), Brenner // Strasser // var. *ruficollis* // ex. coll. J. Weise.

Phytodecta linnaeana bergrothi: Lectotype ♂ (ZIN), hereby designated, Fl. Jenisej // J. Sahlb. // J. Sahlberg 900. // *linnaeana bergrothi* // LECTOTYPUS *Phytodecta linnaeana bergrothi* Jacobson, 1901 des. H.W. Cho 2014 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014.

Phytodecta nivosa var. *cedehensis*: Type probably in MSNM.

Phytodecta affinis var. *decaspilotus*: Syntype 1♀ (NMPC), Norv. Dowre ex coll. Donckier // *P. affinis* Sch! J. Achard det in Mars // Coll. Achard Mus. Pragense // TYPUS // *Ph. affinis* TYPE ab. *decaspilotus* Achard 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 085 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014.

Phytodecta affinis var. *hamatus*: Syntype 1♂ (NMPC), Lapponia // J. Sahlb. // *Phytodecta* s. str. *affinis* J. Achard det. // Coll. Achard Mus. Pragense // TYPUS // *Ph. affinis* TYPE ab. *hamatus* Achard 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 090 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014.

Phytodecta nivosus var. *immarginatus*: Syntypes 1♂ (NMPC), Helvetia // Coll. Achard Mus. Pragense // TYPUS // *Ph. nivosus* TYPE ab. *immarginatus* Achard 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 123; 1♂ (NMPC), Helvetia, Reitter. // Coll. Achard Mus. Pragense // COTYPE // *Ph. nivosus* COTYPE ab. *immarginatus* Achard 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 124; 1♂ (NMPC), Helvetia // Coll. Achard Mus. Pragense // COTYPE // *Ph. nivosus* COTYPE ab. *immarginatus* Achard 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 125.

Phytodecta linnaeanus var. *simplex*: Type probably lost.

Phytodecta nivosa var. *undulata*: Syntype 1♂ (MNHN), Col du Pallet // type // v. *undulata* Pic // TYPE // Museum Paris Coll. M. Pic // SYNTYPE *Phytodecta viminalis* var. *undulata* Pic, 1924.

Phytodecta dinah: Holotype ♂ (NMPC), Sibérie, coll. Donckier // *Ph. dinah* TYPUS n. sp. ♂ 1945 Det. J. Bechyně. // TYPUS // Coll. Achard Mus. Pragense // Mus. Nat. Pragae Inv. 19 079 // *Gonioctena* (*Gonioctena*) *dinah* (Bechyně) Det. S. GE 2004 // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014.

Gonioctena arctica alberta: Holotype (CNCI), not examined. Paratypes 1♂ (CNCI), Glacier Park Mont., 23 July 1924 // PARATYPE *Gonioctena arctica alberta* Brown, No. 6006; 1♀ (CNCI), Nordegg, Alta., 10.VI.1921, J. McDunnough // PARATYPE *Gonioctena arctica alberta* Brown, No. 6006.

Phytodecta flavicornis var. *limbatipennis*: Syntype 1♀ (NMPC), Schlüsseljoch [= Allemagne] // Germania Reitter // TYPUS // *Ph. flavicornis* TYPE ab. *limbatipennis*

Ach. 1945 Det. J. Bechyně. // Coll. Achard Mus. Pragense // Mus. Nat. Pragae Inv. 18 906 // *Gonioctena flavicornis* (Suffrian, 1851) det. H.W. Cho 2013.

Phytodecta nivosa var. *bicolor*: Syntypes 2♀♀ (SDEI), 507. // Engadin, Strl // *Phytodecta nivosa* var. *bicolor* Heyden, 1883 // *Gonioctena flavicornis* (Suffrian, 1851) det. H.W. Cho 2014.

Other material. Norway: 1♂, 2♀♀ (NHMB), Umg. Tromso, Norwegen; 1♂, 1♀ (NHMB), J. Schneider, Tromso // Ovoviviparous, det. H.W. Cho 2014; 1♀ (NHMB), Ivalo, Finland; 1♀ (BMNH), N Norway: Arnoy, VI.–VII.1958, P.J.M. Greenslade, B.M. 1969-168.; 1♀ (AWC), Norvegia, ad Tromso, 1898; 1♂ (NMPC), Norge, 7.13; 1♂ (NMPC), Norv. Dowre // Coll. Achard Mus. Pragense // Mus. Nat. Pragae Inv. 19 086. **Sweden:** 1♂ (NMPC), Lpl. Abisko, 21.VI.–2.VII.1948, T.Palm leg.; 1♂ (BMNH), Lapland. S. of Riksgransen, Vindskydd [= Karsatjakko], 800–900 m. VII–VIII.1957 // N. SWEDEN: B.G. Gardiner. B.M.1957-657; 1♂ (SDEI), Lappland, Kvikkjokk, 24.VI.–7.VII.1901, Thurau S. **Finland:** 1♂ (ZMHB), Halssch. Eppelsh. // ex. coll. J. Weise; 1♂ (MZHF), Fl. Nuorti, Envald; 1♀ (MZHF), Petsamo, Hellén; 1♂ (SDEI), Lac. Inari // Thuneberg // Finland; 1♀ (NMPC), Ivalo // Thuneberg // Finland // Mus. Nat. Pragae Inv. 19 088; 1♂ (NMPC), Syd-Varanger // Fennia // Krogerus // Coll. Achard Mus. Pragense // TYPUS // *Ph. affinis* TYPE ab. *fennicus* n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 089; 1♀ (TLMF), Utsjoki, leg. Hellén; 1♂ (TLMF), Kilpisjärvi, leg. V. Löfgren; 1♂ (TLMF), Kevo, 23.VI.1989. **France:** 4♂♂ (MNHN), Parc National de la Vanoise, 16.VII.1897; 1♀ (NMPC), Berarde – 1925, Hautes – Alpes, Ga. ing. Jedlička; 1♂ (TLMF), Massif du Mt. Cenis, la Petit Turra, 2500m, 22.VII.2003, leg. Knapp; 1♂ (TLMF), Vanoise, Pralognan, Ref. du Grd. Bec, 2400m, 19.VII.2002, leg. Knapp; 1♂, 1♀ (TLMF), Haute Maurienne, Bonval, Sentier Balcon, 2600m, 27.VII.2003, leg. Knapp; 1♀ (TLMF), Col de la Bonette, 2500m, 26.VII.1977, leg. Kippenberg; 1♂ (TLMF), F/73 Savoie, Haute, Maurienne, Bonval, Sentier Balcon 2600 m, 27.VII.2003, leg. Knapp. **Switzerland:** 1♂, 4♀♀ (NHMB), Vals, Switzerland, VII-1925; 1♂, 1♀ (NHMB), Vals, Switzerland, 11.VII.1925; 3♂♂, 2♀♀ (NHMB), Vals, Tomül, Switzerland, 11.VII.1908; 3♂♂, 1♀ (NHMB), Switzerland, Val Tasna, Engadin, 12.VII.1949, W. Schlier; 3♂♂, 1♀ (NHMB), GR Sur, Alp Flix CH, Val Savriez, Plan Bel, 2400 m, 771.8/154.5, 20.VI.2002, leg. W. Marggi; 1♂ (ABC), Mt. Rosa. W. Bohmlander // Helvetia mer.; 5♂♂ (BMNH), Arolla, Switz. G.C.C. // G.C. Champion Coll. B.M. 1927-409; 1♂ (AWC), Helvetia (Wallis), mons Eggis-Horn, leg. B. Malkin; 1♂ (NMPC), Val. Piora (Switzerland), E. 6. 08; 1♀ (TLMF), Churfirsten, Brisi, 2260m, 27.VI.2004, leg. Kapp; 1♀ (TLMF), Unterwalden: Susten-Pass, 2200–2400m, 23. VII.1991, leg. Hiermeier; 7♂♂, 4♀♀ (TLMF), Umbrail-Pass, 2500–2700m, 4. IX.1974, leg. Kippenberg; 2♂♂, 1♀ (TLMF), Umbrail-Pass, 2300–2500m, 16. VIII.1975, leg. Kippenberg; 1♀ (TLMF), Stilfser Joch, 2700m, 6.IX.1986, leg. Kippenberg; 1♂ (TLMF), Albula-Pass, 2000–2200m, 6.VI.1993, leg. Kippenberg; 1♂ (TLMF), Greina-Ebene, 2300m, 5.V.1988, leg. W. Marggi; 1♂ (TLMF), Greina-Gebiet, 2500m, VII.1988, leg. W. Marggi; 1♂ (TLMF), Greina-Süd, Alp Motterascio 2200m, VII.1988, leg. W. Marggi; 1♂ (TLMF), Oberalp-Pass, 2100–2200m,

23.VII.1991, leg. Hiermeier; 1♀ (TLMF), Furka-Pass, 2450m, 30.VII.1982, leg. Kippenberg; 1♂, 1♀ (TLMF), Vispertinem, Gebidem, Nanztal, 2400m, 23.VI.2002, leg. W. Marggi; 1♀ (TLMF), Zinal, Come de Borebois, 2800m, 25.VI.2002, leg. Gollkowski; 1♂, 2♀♀ (TLMF), Grimsel-Pass, 2180m, 26.IX.1990, leg. I. Wolf. **Austria:** 1♂ (NHMB), Austria, Tilisuna See, Montafon, 18–2200 m, leg. Dr. Mandl, VII.1954; 1♂, 1♀ (ABC), Bachlenke Troyer Tal hochalpin // Osttirol Holdhaus; 1♀ (SDEI), Austria: S Ferleiten (Hohe Tauern), 47°07'27''N, 12°49'17''E, 18.VII.1999, 2300 m, leg. C. Lange & J. Ziegler; 2♂♂ (SDEI), 1♂ (TLMF), Austria: Salzb., Hohe Tauern, Fusch, 2300 m, 6.VII.1993, leg. Zerche; 1♀ (NMPC), Tirol // Collectio A. Fleischer // TYPUS // *Ph. nivosus* TYPE ab. *latefasciatus* n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 136; 1♀ (NMPC), Rhaetia // Collectio A. Fleischer // TYPUS // *Ph. nivosus* TYPE ab. *limitata* n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 126; 1♀ (NMPC), 17 / 889. // Tirol, coll. Gradl // *Ph. nivosus* Suffr. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 091; 1♀ (NMPC), 17 / 885. // Tirol, coll. Gradl // *Ph. nivosus* TYPE ab. *excisus* n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19; 7♂♂, 5♀♀ (ZMUC), Österreich, Triol, Ötztaler, Alpen: Vent 7 km S, Umg. Martin-Busch-Hutte (2700 m, feuchtes Schotterfeld, saß auf Stein), 01. VII.2008, leg. D. Luckow; 1♀ (TLMF), Ulmer Hütte, 30.VI.1928, leg Ratter; 1♂ (TLMF), Kühtai, Feldringer Alm, 25.VI.2006, leg. M. Egger; 1♂ (TLMF), Ötztal, Chemnitzer Hütte, 2600m, 2.IX.1951; 1♂, 1♀ (TLMF), Ötztal, Kreuzspitze, 3000m, 5.VIII.1948; 1♂ (TLMF), Ötztal, Obergurgl, Gaisbergtal, 2200–2400m, 17.IX.1997, leg. Kippenberg; 1♀ (TLMF), Nößlachjoch, 2100m, 28.IX.1975, leg. K. Burmann; 1♂ (TLMF), Hohe Tauern, Innergschlöß, Prager Hütte, 2300–2400m, 5.IX.1983, leg. Kippenberg; 2♀♀ (TLMF), Hohe Tauern, Kals, Ködnitztal, Fanatscharte, 2600–2700m, 3.IX.1985, leg. Kippenberg; 1♀ (TLMF), Hohe Tauern, Matrei, Tauernhaus, 11.VII.1991, leg. M. Egger; 1♂ (TLMF), Schladminger Tauern, Steirische Kalkspitze, 2200m, 15.VIII.1986, leg. Kippenberg; 1♂ (TLMF), Hohe Tauern, Heiligenblut, Pasterzenhaus, 2200m, 10.VII.1993, leg. Zerche. **Italy:** 2♂♂, 2♀♀ (JBC), Italy, Val d'Ayas (Aosta), 12.VII.1978, S Zoia; 1♂ (ABC), Vinschgau. Ti.G.Kuchta // Italien S.-Tirol; 1♂ (TLMF), I-Cuneo, Alpi Cozie, Colle dell'Agnello, leg. Kahlen // S-Seite 2700 m, 20.VI.2000, Schneeboeen, *Salix* herbacea-Rasen; 1♂ (SDEI), Lombardia, Val Brembana, Lago Colombo, L. Ceresa // coll. K. H. Mohr, DEI Eberswalde; 1♂ (SDEI), Fiery d'Ayas, Val d'Aosta, VII.1910, A. Dodera // O. Leonhard; 1♂ (SDEI), Italy, Sudtirol, 5.VII.1928, Linke leg.; 1♀ (DBET), Stelvio; 1♀ (NMPC), Alpy Penninske. Italia; 1♂ (NMPC), Stelvio Ti, 15.7.05 // TYPUS // *Ph. nivosus* TYPE ab. *marginipennis* n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 134; 5♂♂ (ZMUC), Italien, Lombardia: Stilfser Joch (P. so di Stelvio) 1,5 km NW (Ri. Umbrailpass) (2600 m, Mattenbereich, viel Schnee, unter einem Stein) leg. D. Luckow, 12.VI.2008.102; 2♀♀ (TLMF), Umgebung Brenner, Schlüsseljoch, 25.VI.1964, leg. Kippenberg; 1♀ (TLMF), Cogne, Valnontey, Rif. V. Sella, Lago del Loson, 2660m, 8.VII.2002, leg. Kopetz; Valle d'Aosta; 1♀ (TLMF), Piccolo S. Bernardo, 2100m, 29.VI.1976, leg. Krätschmer; 1♀ (TLMF), Picc. S. Bernardo, 2100m, 13.VI.1981, leg. Kippenberg; 4♂♂ (TLMF), Alpie Cozie, Colle dell' Agnello, 2700m, leg. Kahlen,

Salix herbacea-Rasen; 1♀ (TLMF), Colle del Mulo, 2400m, VII.1968. **Slovenia:** 1♂ (AWC), Slovenia, Alpi Julian. Cervinia, 7.VII.1970. **Russia:** 1♀ (NHMB), Tschita, Transbaikal, Mandi // Ovoviviparous, det. H.W. Cho 2014; 1♂ (NHMB), Vladivostok, Russia, 1933, N. Filippov; 2♂♂ (NHMB), Russia, Taymyrsky Dolgano-Nenetsky District, Maimecha River, 7.VII.1971; 2♂♂ (JBC), Russia, Altai rep., Kalguty & Ak-Alacha junction Ukok plateau, 2150–2500 m, 49°23'N, 87°38–40'E, 8–12. VII.2009, L. Čížek leg.; 5♂♂ (ABC), Russia, Tuva, S. Slopes of E. Tanu-Ola Mts., envir. Soglyi vill., 1800–2700 m, 14.V.–24.VI.2002, Vashchenko leg.; 1♂ (ABC), Subpolar Ural Mts., Northern Maldy ridge, 1–18.VII.2000, A.A. Medvedev; 1♂ (ABC), Transbaikal Krai, Kodarsky ridge, 40 km NW from Chara Vill., 16–25. VII.1996, A.E. Brinev leg.; 1♂ (ABC), Murmansk reg., Tuloma river and Not-lake, 4–6.VIII.1906, Soldatov leg.; 3♂♂, 1♀ (HCC), Russia, S Siberia, SW Tuva reg., W Tannu Ola Mt. mg., Sogly v., 2000 m, 5.VII.2003, S. Vastchenko leg.; 1♀ (HCC), Yakutia, Chandyga distr., 7/93; 1♂ (FKC), Russia, S Siberia, SW Tuva reg., S Tannu-Ola Mts. 1800 m, 14.V.2002; 1♂ (FKC), USSR-Tungur, Gorno altaysk, 5.VII.1990, V. Lenserk lgt.; 1♂ (LMC), Kamchatka, 22.VI.1958, L. Ivliev coll.; 1♂ (SDEI), Altai // COTYPUS // Coll. Kraatz // *Phytodecta affinis* v. *pernigra* m.; 1♂ (AWC), Russia (Sib. Occ.), Kuznetzky Alatau, Malyj Zub, 31.VII.1996, leg. J. Mikhailov; 1♂ (AWC), Russia, W. Siberia, Kuznetsky Alatau mts., Malyj Zub mt. On *Salix*, 31.VII–1. VIII.1996, Yu. Mikhailov leg.; 13♂♂, 14♀♀ (TLMF), Yakutien, Chandyga, VII.1993, leg. Naglis; 1♀ (TLMF), Amur region, Selemdgin distr., Tamsche, 7.VI.2005, leg. Ryvkin & Veselova; 2♂♂, 1♀ (TLMF), Chabarowsk region, Ochotsk, Ulia river, 29.VII.–6.VIII.1985, leg. Ryvkin & Veselova; 1♂ (TLMF), SW Tuva, W Tanu-Ola Mts., near Solglyi vill., 2000–2800m, 13.V.–1.VI.2003, leg. Vashchenko; 1♂ (TLMF), Tuva, Chandagayt, 10.VII.1971; 2♂♂ (TLMF), Tuva, 80km S Taly, 23–26.VI.1972, leg. Korotyaev; 1♂ (ZIN), Fl. Jenisej // J. Sahlb. // 1274 // J. Sahlberg 900. // *linnaeana bergrothi* v. *correspondens* // *Gonioctena nivosa* (Suffrian, 1851) det. H.W. Cho 2014; 1♂ (ZIN), Russia, Khabarovsk Krai, Shantar Islands, 22.VII.1911, V. Soldatov leg.; 2♂♂ (ZIN), Russia, border of Irkutsk Oblast and Republic of Buryatia, approx. 51–52°N, 101–102°E, between the rivers Kitoy, Bogdashka and Belaya, 20.V.1973, Gartung leg.; 1♂ (ZIN), Russia, Murmansk Oblast, Kildin Island, 29.VII.1900, Il'in leg.; 1♂ (ZIN), Russia, Republic of Tuva, 80 km N Teeli, 25.VI.1972, B. Korotyaev leg.; 2♂♂ (ZIN), Ochotsk // F. Sahlb // J. Sahlberg 900; 1♂ (ZIN), Russia: Altai Kuray Mt. R., NE Aktash, upp. Tarlyamry R., 50°19'10''N, 87°45'14''E, 2100–2300 m, 30.VI.2005, B. Kataev leg.; 1♂ (ZIN), Russia, Krasnoyarsk Krai, Nizhnyaya Tunguska River, upper than Vivi River mouth, 28.VII.1873, Chekanovsky leg.; 1♂ (ZIN), Russia, Amur Oblast, Zeyskiy District, upper of Erakingra River, on Tukuringra Mountains, 19.VI.1957, Zinoviev leg.; 1♂ (ZIN), Russia, Amur Oblast, Zeyskiy District or Magdagachinsky District, Ulunga River, 25.V.1910, Mishin leg.; 2♂♂ (ZIN), Russia, Murmansk Oblast, Kolsky District, Notozero Lake and Tuloma River, 4–6. VIII.1906, Soldatov leg.; 1♂ (ZIN), Russia, Murmansk Oblast, Kolsky District, Nota [= Noti] River, 28.VII.1906, Soldatov leg.; 1♂ (ZIN), Russia, Krasnoyarsk Krai, Evenkiysky District, Podkamennaya Tunguska River, Big (7-verst) rapids, approx. 61N

94E, 20.VI.1928, Valdaev leg.; 2♂♂ (ZIN), Russia, Krasnoyarsk Krai, Evenkiysky District, Podkamennaya Tunguska River, Muchnoi rapids, 61.81N, 94.43E, 25. VI.1928, Valdaev leg.; 2♂♂ (ZIN), Russia, Komi Republic, Troitsko-Pechorsky District, Aranets River, 25–26.VI.1905, Zhuravskiy leg.; 1♂ (ZIN), Russia, Komi Republic, Troitsko-Pechorsky District, Bolshaya Synya River, Mount. "Voi", Sablya, "Izb.", 16.VI.1908, Zhuravsky leg.; 1♂ (ZIN), Russia, Sakha Republic, Verkhoyansky District, tundra along river Dogdo, Yana River basin, 16–18.VI.1901, Hertz leg.; 1♂ (ZIN), Russia, Sakha Republic, 17.VII.1925, L. Bianki leg.; 3♂♂, 1♀ (ZIN), Russia, Tyumen Oblast, Yamalo-Nenets Autonomous Okrug, Priuralsky District, Sob' River basin, Obdorskiy Krai, 15.VII.1925, Fridolin leg.; 1♂ (ZIN), Russia, Tyumen Oblast, Yamalo-Nenets Autonomous Okrug, Shuryshkarsky District, between Varchaty Lake and Maly Ural Mountains, Obdorskiy Krai, 4.IX.1925, Fridolin leg.; 1♂ (ZIN), Russia, Sakhalin Oblast, Sakhalin Island, Holmsky pass (approx. 47°N, 142°E), 1. VII.1982, Smirnov leg. **Kazakhstan:** 1♂ (JBC), Kazakhstan, East, 2300–2500 m, 49°30'N, 86°22'E, 13–14.VI.2006; 1♂ (TLMF), Ussuria, ad chasan, 42°25'N, 130°45'E, 1–8.VII.2000, Melnik; 1♂ (TLMF), RU: Sibirien, E-Sayan 54 km west, Mondy, 8.VII.2012, 1900 m, St. FloBmann leg.; 1♂, 1♀ (TLMF), Kazakhstan, Altaj, Sarym-Sakty, 2500 m, 11.VI.1999, leg. Vashchenko; 2♂♂, 2♀♀ (TLMF), 100km SSE Ust-Kamenogorsk, Panteleymonovka vill., 16–21.VI.1993, leg. A. Napolov. **Mongolia:** 1♂ (ZIN), Mongolia, NE, Khentii Mountains, Rivers Manza and Sharotay, 19.VII.1897, Klementz leg. **Canada:** 1♂ (ABC), Aklavik, N.W.T., 24.VI.1956, E.F. Cashman leg.; 2♂♂ (CNCI), ALB., Banff Nat. Pk. Sunwapta Pass, 9.VII.1955, W.J. Brown; 6♂♂, 5♀♀ (CNCI), Reindeer Depot, Mackenzie Delta, 28.VI.1948, W.J. Brown; 20♂♂, 5♀♀ (CNCI), Aklavik, N.W.T., 16.VI.1956, E.F. Cashman; 2♂♂, 1♀ (CNCI), Saw Mill Bay, N.W.T., 16.VI.1948, D.F. Hardwick // on willow; 11♂♂, 7♀♀ (CNCI), Churchill. Man., 3.VII.1937, W.J. Brown // On *Salix*; 2♀♀ (CNCI), Toad River, B.C. Mi440 Alaska Hwy, 19.VI.1959, 4500' R.E. Leech; 6♂♂, 1♀ (CNCI), Kluane, Y.T., 28.VII.1948, Mason & Hughes; 1♂, 3♀♀ (CNCI), North Richardson Mts. Yukon, VII.1982, On *Salix* m. *polaris*, D.M. Wood; 4♂♂, 2♀♀ (CNCI), Y.T., British Mts. June Cr. 600 m, 69°14'N, 140°08'W, J.M. Campbell; 1♂, 1♀ (CNCI), Banff Natl. Pk., Alta, 9.VII.1955, W.J. Brown // On *Salix*. **United States:** 9♂♂, 11♀♀ (CNCI), Moose Pass, Kenai Pen., 30.VI.1951, W.J. Brown; 3♂♂, 6♀♀ (CNCI), Paxon Lodge, Gulkana, Alaska, 4.VIII.1951, W.R.M. Mason; 2♂♂, 3♀♀ (CNCI), Summit Lake, B.C. Mi392 Alaska Hwy, 26–27.VI.1959, 4500' R.E. Leech; 1♂ (CNCI), Niwot Ridge, COLO. nr. Ward, 11,500', 4.VI.1961, W.R.M. Mason; 1♂ (TLMF), USA - Alaska, Fairbanks, 3.VIII.2009, K. Renner // Murphy Dome, 870 m, Shrubs; 1♂ (ZMUC), Colo. Morr. // Col. H. H. Meeske // Zool. Museum DK Copenhagen. **Uncertain localities:** 1♀ (NMPC), Alpes // Coll. Achard Mus. Pragense // TYPUS // *Ph. nivosus* TYPE a. *vicinus* n. ab. 1945 Det. J. Bechyně. // Mus. Nat Pragae lnv. 18 113; 1♂ (NMPC), Alpes // *nivosus* v. *trinotatus* m. // Coll. Achard Mus. Pragense // TYPUS // *Ph. nivosus* TYPE ab. *trinotatus* [Achard i. l.] n. ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 105; 1♂ (NMPC), Alpes // Coll. Achard Mus. Pragense // TYPUS // *Ph. nivosus* TYPE ab. *hexangularis* n.

ab. 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 19 106; 1♂ (NMPC), N America // Coll. Achard Mus. Pragense // Mus. Nat. Pragae Inv. 19 081.

Diagnosis. *Gonioctena nivosa* differs in having small body size, long antennae, first tarsomere of all legs in male much strongly swollen, aedeagus moderately narrowed apically in dorsal view and strongly curved in lateral view.

Redescription. Measurements in mm (n = 10): length of body: 4.05–6.00 (mean 5.19); width of body: 2.25–3.60 (mean 3.03); height of body: 1.50–2.50 (mean 2.10); width of head: 1.30–1.65 (mean 1.51); interocular distance: 0.90–1.17 (mean 1.06); width of apex of pronotum: 1.55–1.87 (mean 1.72); width of base of pronotum: 1.92–2.85 (mean 2.51); maximum width of pronotum: 1.97–2.85 (mean 2.49); length of pronotum along midline: 1.02–1.47 (mean 1.29); length of elytra along suture: 2.90–4.40 (mean 3.77).

Body oblong oval and moderately convex (Fig. 7). Coloration extremely variable. Head black. Mandibles black, with reddish brown band near apex. Maxillary palps reddish brown or dark brown, with apical palpomere black. Antennomeres 1–5 yellowish brown, partially darkened, 6–7 darkened, 8–11 dark brown or blackish brown. Pronotum entirely black or reddish brown with a large black marking, rarely entirely reddish brown (Figs 31–32). Scutellum black, rarely entirely reddish brown. Elytra entirely black or reddish brown, with or without 4–5 pairs of black spots. Venter black, with hypomera reddish brown or black and apical margin of last abdominal ventrite reddish brown. Legs black, with tibiae reddish brown except base and inner margin and tarsi blackish brown or reddish brown, sometimes legs entirely black to dark brown.

Head. Vertex weakly convex, covered with dense punctures. Frontal suture V-shaped, coronal suture weak or absent. Frons flat, strongly depressed at anterior margin, covered with dense punctures. Clypeus narrow and trapezoidal. Anterior margin of labrum distinctly concave. Mandibles with 2 sharp apical teeth and a deep excavation for apical maxillary palpomere at outer side. Maxillary palps 4-segmented, with apical palpomere distinctly widened, truncate apically in male; slightly widened in female. Antennae in male longer than half length of body; antennomere 1 robust; antennomere 2 shorter than 3; antennomere 3 longer than 4; antennomeres 7–11 each distinctly longer than wide; antennomere 11 longest, about 2.68 times as long as wide (Fig. 29). Antennae in female reaching elytral humeri; antennomere 11 about 2.31 times as long as wide.

Pronotum. Lateral sides widest near base, roundly moderately narrowed anteriorly, anterior angles strongly produced (Fig. 8). Anterior and lateral margins bordered, lateral margins not or hardly visible in dorsal view. Trichobothria present on posterior angles. Disc covered with rather dense punctures; lateral sides covered with much coarser and denser punctures, becoming larger toward base, partially confluent near basal margin; interspaces covered with fine and sparse punctures. Scutellum slightly wider than long, narrowed posteriorly.

Elytra. Lateral sides moderately widened posteriorly, widest beyond middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with

11 regular rows of large punctures, including a short scutellar row; sometimes punctures rather irregular between 6th and 8th striae in apical half; interspaces shagreened, covered with fine and sparse punctures. Epipleura wholly visible in lateral view. Hind wings well developed.

Venter. Hypomera weakly rugose, with a few punctures near anterolateral corners of prosternum. Prosternum covered with coarse and dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with sparse punctures. Metasternum covered with small and sparse punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with moderately dense punctures bearing short setae.

Legs. Moderately robust. Tibiae widened apically, with a tooth-like projection. Fore legs with tarsomere 1 strongly enlarged, distinctly wider than 3 in male; very slightly narrower than 3 in female. Tarsal claws appendiculate.

Genitalia. Aedeagus moderately narrowed apically, with apical process rather short in dorsal view; strongly curved, with apical process pointed and slightly bent downward at apex in lateral view (Fig. 30). Spermatheca absent.

Distribution. Transholarctic species: Austria, Finland, France, Germany, Italy, Kazakhstan, Liechtenstein, Norway, Slovenia, Spain, Sweden, Switzerland, Mongolia, Russia (North European Territory, West & East Siberia, Far East, Sakhalin), Canada (Alberta, British Columbia, Manitoba, Northwest Territories, Yukon), United States (Alaska, Montana).

Host plant. Salicaceae: *Salix* spp. (Mannerheim 1853, Motschulsky 1860, Stål 1865, Brown 1942, Wilcox 1972, L. N. Medvedev and Zaytsev 1978, L. N. Medvedev and Roginskaya 1988, L. N. Medvedev 1992, L. N. Medvedev and Dubeshko 1992, Steinhausen 1994, Bieńkowski 2004, Zaytsev and L. N. Medvedev 2009); *Salix retusa* (Reitter 1913, Cantonnet 1968, Kippenberg 1994); *Salix retusa*, *S. herbacea* (Daccordi et al. 1991); *Salix* spp., *S. bebbiana* (Clark et al. 2004). Rosaceae: *Spiraea* spp. (L. N. Medvedev and Roginskaya 1988, L. N. Medvedev 1992).

Remarks. The taxonomic status of *G. nivosa*, and its relationships to *G. affinis* and *G. arctica* has been disputed for a long time. Kraatz (1879a) treated *G. arctica* as a synonym of *G. nivosa*. Székessy (1934) synonymized *G. arctica* and *G. nivosa* with *G. affinis*, while Bechyně (1948) established them as distinct species. Mohr (1966) regarded *G. affinis* and *G. nivosa* as phylogenetically young group that are almost identical. Wilcox (1972) treated *G. arctica* as a subspecies of *G. nivosa*. However, *Chrysomela affinis* Gyllenhal, 1808 is a junior homonym of *Chrysomela affinis* Fabricius, 1787, therefore Silfverberg (1977) proposed the name *G. decaspilota* as the oldest available name. He again treated *G. decaspilota* and *G. nivosa* as distinct species. All these taxa have been confused until now by many authors (see list above). After examining all type specimens of the discussed taxa and many other specimens from the Holarctic region we conclude that all these taxa are conspecific. The shape of body and aedeagi from Europe, Siberia, Far East and North America are identical, although aedeagi and color patterns slightly vary even within the same population (Figs 31–54). Three type localities of *G. nivosa* are

given in the original description: Kärnten, Berner Alpen and Switzerland. Due to the designation of lectotype, the restricted type locality becomes "Kärnten [= Carinthia in Austria]" (ICZN 1999: Recommendation 74E). Six paralectotypes of *Chrysomela affinis* Gyllenhal, 1808 belong to *Gonioctena linnaeana* (Schrank, 1781).

We examined the type of *Phytodecta linnaeana bergrothi* and found it is conspecific with *G. nivosa*. *Phytodecta linnaeana bergrothi* has been misidentified since its original description and is here synonymized with *G. nivosa*. The name *Phytodecta linnaeanus bergrothi* var. *simplex* published by Jacobson (1901b) is infrasubspecific. It is available from Achard (1924) who first used it for a variety of species, *P. linnaeanus* var. *simplex* (ICZN 1999: Article 45.5.1). However, this name and its incorrectly proposed replacement name *P. linnaeanus* var. *mutatus* are removed from synonymy with *G. linnaeana* and are synonymized with *G. nivosa* based on the original description. *Phytodecta nivosa* var. *cedehensis* is for the Alpine specimen having black elytra with a large yellow marking at tip and is synonymized with *G. nivosa*.

We also examined the types of *Phytodecta nivosa* var. *bicolor* Heyden, 1883 and *P. flavicornis* var. *limbatipennis* Achard, 1924 and found that they are conspecific with *G. flavicornis* (Suffrian, 1851). Therefore, they are removed from synonymy with *G. nivosa* and are synonymized with *G. flavicornis*.

Several larvae were dissected from the female specimens collected in Norway and Transbaikalia, therefore this species is ovoviviparous (Fig. 67). The previous record of the occurrence of ovoviviparity by Notman (1921) is based on misidentified *G. notmani* (Schaeffer, 1924).

***Gonioctena* (*Gonioctena*) *norvegica* (Strand, 1936)**
Figs 9–10, 55–62

Phytodecta norvegicus Strand, 1936: 104 (type locality: Målselv, Rundhaugen, Nordmo); Palmén 1946: 230.
Gonioctena norvegicus: L. N. Medvedev and Korotyaev 1980: 81 (as synonym of *G. affinis*).
Gonioctena norvegica: Silfverberg 1992: 69, 1994b: 32, 2004: 82.
Gonioctena (*Gonioctena*) *norvegica*: Bieńkowski 2004: 67; Kippenberg 2010: 434.
Phytodecta charitonowi Palmén, 1946: 231 (type locality: Siberia); Kippenberg 2010: 434 (as synonym of *G. norvegica*).
Gonioctena janovskii L. N. Medvedev, 1976: 234 (type locality: Mongolia, Central Aimak, Tereldzhin gol forestry); L. N. Medvedev and Voronova 1976: 229; Zaytsev and L. N. Medvedev 1977: 368 (larva); L. N. Medvedev and Zaytsev 1980: 106 (larva); L. N. Medvedev and Roginskaya 1988: 101 (host plant); Dubeshko and L. N. Medvedev 1989: 133 (biology). **syn. n.**
Gonioctena (*Gonioctena*) *janovskii*: L. N. Medvedev and Zaytsev 1978: 119 (larva); L. N. Medvedev 1982: 91, 179, 252 (incl. larva); Lopatin et al. 2004: 122; Kippenberg 2010: 433; Warchałowski 2010: 558.

Type material. *Phytodecta norvegicus*: Syntypes 2♂♂, 2♀♀ (NMPC), Rundhaug M. elv, A. Strand; 1♂ (NHRS), Rundhaug M. elv, A. Strand // Paratypus // *Phytodecta norvegicus* A. Strand // NHRS-JLKB 000023154.

Phytodecta charitonowi: Holotype probably in MZHF.

Gonioctena janovskii: Holotype ♂ (LMC), Holotypus // 29.VI.1971, Mongolian People's Republic, Central Aimak, Tereldzhin gol forestry, on *Salix* leaves, V. Yanovsky leg. Paratypes: 1♂ (LMC), same data as holotype; 1♂, 1♀ (LMC), same data as holotype except 23.VI.1971.

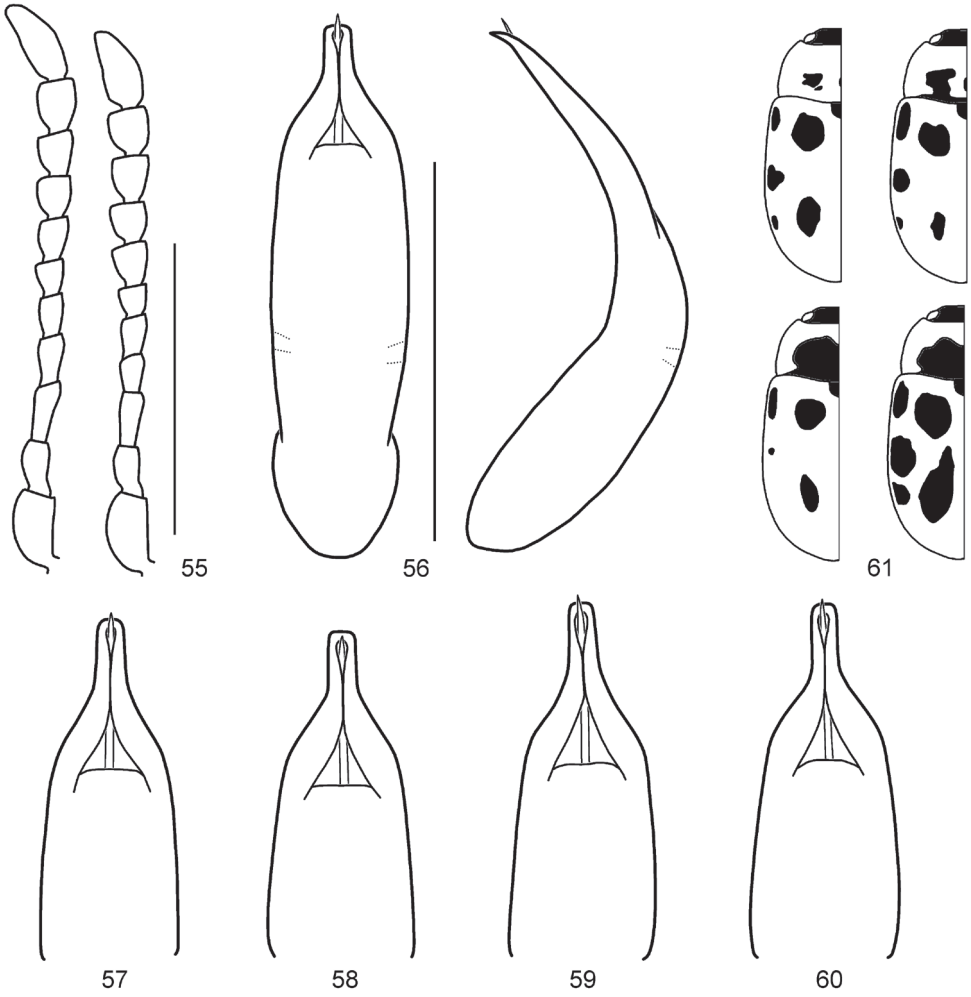
Other material. Finland: 2♂♂, 2♀♀ (ZMUC), Fennia, Ob Rovaniemi, Piaa, 21.6.1915, Hakan Lindberg; 2♂♂ (SDEI), Lapponia, Leonhard leg. **Sweden:** 1♂ (ZMUC), Nb. Storsien, 22.6.1981, G. Gillerfors // *norvegicus* // Ex coll. Viggo Mahler. **Russia:** 1♂, 1♀ (ABC), Altai Mts., environs of Bayas lake, 51°17'N, 87°56'E, 1700 m, 14.VIII.1993, M. Savitsky leg.; 1♂ (ABC), Komi Republic, Intinsky Distr., Paga-ty lake, 25.VI.2007, A.A. Kolesnikova leg.; 1♂ (AWC), RUSSIA, SE Tuva, Khorummnug-Taiga Mts., Ailyg-Kai River Valley, subalpine, on *Salix*, 15.VI.1999, Yu. Mikhailov leg. // *Gonioctena janovskii* L. Medvedev, M. Bergeal det. 2000; 1♂ (ZIN), Russia, Sverdlovsk Oblast or Tyumen Oblast, Manya River basin, forest Urals, 16–19.VI.1927, Lyapin and Flerov leg.; 1♂ (TLMF), Russia, E-Sayan, Sibir, 54 km w Mondy 1900m, 13.VII.2012, leg. S. Floßmann. **Mongolia:** 3♂♂, 2♀♀ (NMPC), Mong. bor. BOGDO-UL, 11.VIII.66, Dlabola, in litt. loc. 39.

Diagnosis. *Gonioctena norvegica* differs in having antennae much shorter than half length of body in male, not reaching elytral humeri in female, aedeagus rather thick in dorsal view and strongly curved in lateral view.

Redescription. Measurements in mm (n = 5): length of body: 4.30–5.50 (mean 4.98); width of body: 2.35–3.40 (mean 3.00); height of body: 1.60–2.30 (mean 1.98); width of head: 1.30–1.50 (mean 1.42); interocular distance: 0.95–1.10 (mean 1.03); width of apex of pronotum: 1.47–1.72 (mean 1.62); width of base of pronotum: 2.07–2.78 (mean 2.46); maximum width of pronotum: 2.10–2.80 (mean 2.48); length of pronotum along midline: 1.15–1.35 (mean 1.24); length of elytra along suture: 2.95–4.20 (mean 3.65).

Body oblong oval and moderately convex (Fig. 9). Head black. Mandibles black, with dark reddish brown band near apex. Maxillary palps blackish brown, with apical palpomere black. Antennae yellowish brown or reddish brown, generally with last 4–6 antennomeres darkened. Pronotum reddish brown, with small or large black markings (Fig. 61). Scutellum black. Elytra reddish brown, with 4–5 pairs of black spots. Venter black, with hypomera and apical margin of last abdominal ventrite reddish brown. Legs black, with tibiae reddish brown except base and inner margin and tarsi dark brown or reddish brown.

Head. Vertex weakly convex, covered with moderately dense punctures. Frontal suture V-shaped, coronal suture weak or absent. Frons flat, strongly depressed at anterior margin, covered with dense punctures. Clypeus narrow and trapezoidal. Anterior margin of labrum distinctly concave. Mandibles with 2 sharp apical teeth and a deep excavation for apical maxillary palpomere at outer side. Maxillary palps 4-segmented,



Figures 55–61. *Gonioctena norvegica*. **55** Antenna (♂, ♀) **56–57** Aedeagus (Rundhaug, Norway) **58** Aedeagus (Bogd Uul, Mongolia) **59** Aedeagus (Manya River, Russia) **60** Aedeagus (Intinsky, Komi Rupublic, Russia) **61** Color variation. Scale bars = 1.0 mm.

with apical palpomere distinctly widened, truncate apically in male; slightly widened in female. Antennae in male reaching elytral humeri; antennomere 1 robust; antennomere 2 shorter than 3; antennomere 3 longer than 4; antennomeres 7–11 each distinctly longer than wide; antennomere 11 longest, about 2.26 times as long as wide (Fig. 55). Antennae in female reaching pronotal base; antennomere 11 about 2.05 times as long as wide.

Pronotum. Lateral sides widest at or near base, roundly moderately narrowed anteriorly, anterior angles strongly produced (Fig. 10). Anterior and lateral margins bordered, lateral margins invisible in dorsal view. Trichobothria present on posterior angles. Disc covered with moderately dense punctures; lateral sides covered with much

Figure 62. Distribution of *Gonioctena norvegica* based on specimens in the Palaearctic region.

coarser punctures, becoming larger toward base, partially confluent near basal margin; interspaces covered with fine and sparse punctures. Scutellum slightly wider than long, narrowed posteriorly.

Elytra. Lateral sides moderately widened posteriorly, widest beyond middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with 11 regular rows of large punctures, including a short scutellar row; sometimes punctures rather irregular between 6th and 8th striae in apical half; interspaces shagreened, covered with fine and sparse punctures. Epipleura wholly visible in lateral view. Hind wings well developed.

Venter. Hypomera weakly rugose, with a few punctures near anterolateral corners of prosternum. Prosternum covered with coarse and dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with sparse punctures. Metasternum covered with small and sparse punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with dense punctures bearing short setae.

Legs. Moderately robust. Tibiae widened apically, with a tooth-like projection. Fore legs with tarsomere 1 strongly enlarged, distinctly wider than 3 in male; very slightly narrower than 3 in female. Tarsal claws appendiculate.

Genitalia. Aedeagus rather thick, with short apical process in dorsal view; strongly curved, with apical process pointed and slightly bent downward at apex in lateral view (Figs 56–60). Spermatheca absent.

Distribution. Finland, Norway, Sweden, Russia (North European Territory, West Siberia), Mongolia (Fig. 62).

Host plant. Salicaceae: *Salix* spp. (L. N. Medvedev and Voronova 1976, L. N. Medvedev and Zaytsev 1978, L. N. Medvedev and Roginskaya 1988).

Remarks. The shape of aedeagus slightly varies geographically (Fig. 62). After examining the type and other specimens from the Palaearctic region, we conclude that *G. janovskii* from Mongolia should be synonymized with *G. norvegica*. Medvedev and Korotyaev (1980) synonymized *G. norvegica* with *G. affinis* (= *G. nivosa*), however *G. norvegica* differs in having shorter antennae and thicker aedeagus compared with those of *G. nivosa*, as previously mentioned by Silfverberg (1994b). Kippenberg (2010) treated *G. charitonowi* as a synonym of *G. norvegica*, however the illustration of the aedeagus of *G. charitonowi* looks quite different from that of *G. norvegica*. It should be re-examined to confirm its taxonomic status.

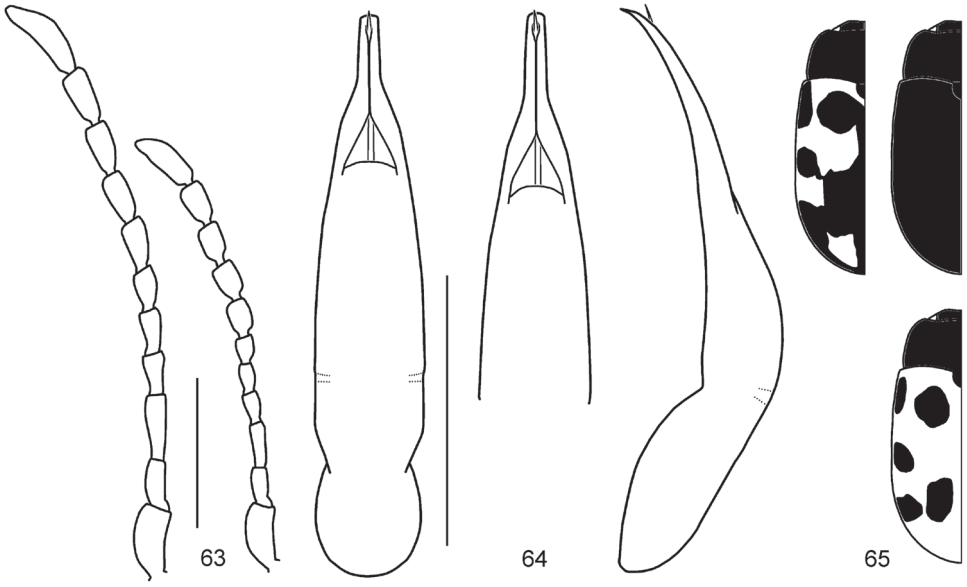
Gonioctena (*Gonioctena*) *springlovae* (Bechyně, 1948)

Figs 11–12, 16, 63–65

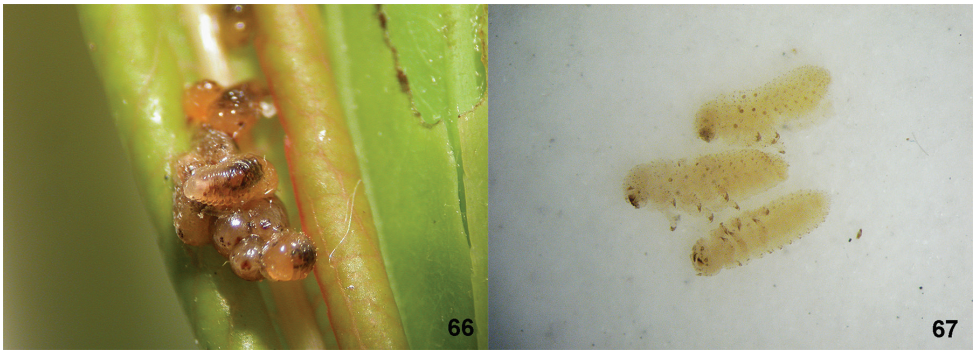
Phytodecta springlovae Bechyně, 1948: 115, 116 (type locality: Japonia, Kioto).
Gonioctena (*Gonioctena*) *springlovae*: Chûjô and Kimoto 1960: 5, 1961: 153; Gressitt and Kimoto 1963: 358, 362; Kimoto 1963: 17, 1964: 280, 282; Takizawa 1976: 454 (larva, pupa, biology); L. N. Medvedev 1992: 575 (as synonym of *G. affinis*); Kimoto and Takizawa 1994: 139, 229, 302, 452, 498 (incl. larva and pupa); V. L. Medvedev 2004: 41; Takizawa 2007: 38, 42; Kippenberg 2010: 434.
Gonioctena springlovae: Takizawa 1971: 173; Cox 1996: 146 (pupa); Kudo and Hasegawa 2003: 729 (biology); Takahashi 2012: 289.
Phytodecta gracilicornis?: Jacoby 1885: 210 (misidentification).

Type material. Holotype: ♂ (NMPC), Japon, Kioto // TYPUS // *Phytodecta* TYPE *springlovae* n. sp. 1945 Det. J. Bechyně. // Coll. Achard Mus. Pragense // Mus. Nat. Pragae Inv. 18 960 // *Gonioctena* (*Gonioctena*) *springlovae* (Bechyně) Det. S. GE 2004.

Other material. Japan (Hokkaido): 1♂ (NMPC), Japon, Kioto // TYPUS // *Ph. springlovae* ab. *graduata* n. ab. TYPE 1945 Det. J. Bechyně. // *Gonioctena* (*Gonioctena*) *springlovae* (Bechyně) Det. S. GE 2004; 1♂ (NMPC), Japon, Kioto // coll. Achard Mus. Pragense // TYPUS // *Ph. springlovae* ab. *graduata* n. ab. PARATYPE 1945 Det. J. Bechyně. // Mus. Nat. Pragae Inv. 18 961 // *Gonioctena* (*Gonioctena*) *springlovae* (Bechyně) Det. S. GE 2004; 3♂♂ (JBC), Japan, Hokkaido, Eniwa Mt. 30 km S from Sapporo, Shikotsu-Toya N.P. 500 m, 6.VII.1997, lgt. V. Kostal; 1♀ (BMNH), Japan, G. Lewis. 1910-320. // Chiuzenji; 5♂♂, 1♀ (HCC), Japan, Hokkaido, Sapporo, Kannon-zawa, 29.V.1995, S. Kudo; 1♂, 1♀ (HCC), Japan, Hokkaido, Sapporo, Jozankei, 29.VIII.2011, H. Suenaga leg.; 1♂ (SEHU), Jozankei, Hokkaido, 22.VII.1955, M. Konishi; 1♂, 1♀ (SEHU), Hidaka, Hokkaido, 1955, S. Watanabe; 1♂ (AWC), JAPAN, Takahiro Parking Area, Bifuka, Hokkaido, 4.VII.2002, Y. Komiya lgt. **Russia (Sakhalin):** 1♂ (NHMB), Sakhalin, riv. Naiba, VIII.1991; 2♂♂ (BMNH), Russia,



Figures 63–65. *Gonioctena springlovae*. **63** Antenna (♂, ♀) **64** Aedeagus **65** Color variation. Scale bars = 1.0 mm.



Figures 66–67. Ovoviviparous species. **66** Newly laid larvae of *G. gracilicornis* **67** Larvae dissected from a female of *G. nivosa*.

Saghalien, Central Expt. Sta.; 1♂ (SEHU), Saghalien, 16.VII.1933, Uchida, Okada, Sawamoto & Hoye legs; 4♂♂ (ZIN), Russia, Sakhalin Oblast, Sakhalin Island, Holmsky pass (approx. 47°N, 142°E), 1.VII.1982, Smirnov leg.

Diagnosis. See diagnosis of *Gonioctena gracilicornis*.

Redescription. Measurements in mm (n = 5): length of body: 5.70–6.20 (mean 6.00); width of body: 3.20–3.60 (mean 3.40); height of body: 2.30–2.50 (mean 2.42); width of head: 1.60–1.75 (mean 1.66); interocular distance: 1.10–1.20 (mean 1.13); width of apex of pronotum: 1.87–2.05 (mean 1.95); width of base of pronotum: 2.70–3.05 (mean 2.84); maximum width of pronotum: 2.72–3.07 (mean 2.87); length of

pronotum along midline: 1.35–1.50 (mean 1.41); length of elytra along suture: 4.10–4.60 (mean 4.42).

Body oblong oval and moderately convex (Fig. 11). Coloration variable. Head black, with dark reddish brown band near apex of mandibles. Antennomeres 1–5 yellowish brown, generally darkened, 6–7 dark brown to blackish brown, 8–11 black. Pronotum entirely black. Scutellum black. Elytra reddish brown or yellowish brown, with 5 pairs of black spots, generally connected with each other, rarely elytra entirely black (Fig. 65). Venter black, with lateral margins of last abdominal ventrite reddish brown. Legs black, with tarsi blackish brown, sometimes tibiae largely dark brown to reddish brown.

Head. Vertex weakly convex, covered with sparse punctures, becoming coarser and denser toward sides. Frontal suture V-shaped, coronal suture absent. Frons flat, strongly depressed anteriorly, covered with moderately dense punctures. Clypeus narrow and trapezoidal. Anterior margin of labrum distinctly concave. Mandibles with 2 sharp apical teeth and a deep excavation for apical maxillary palpomere at outer side. Maxillary palps 4-segmented, with apical palpomere distinctly widened, truncate apically in male; slightly widened in female. Antennae in male longer than half length of body; antennomere 1 robust; antennomere 2 shorter than 3; antennomere 3 longer than 4; antennomeres 7–11 elongate; antennomere 11 longest, about 3.95 times as long as wide (Fig. 63). Antennae in female reaching elytral humeri; antennomere 11 about 2.58 times as long as wide.

Pronotum. Lateral sides widest near base, feebly rounded, slightly narrowed anteriorly, anterior angles strongly produced (Fig. 12). Anterior and lateral margins bordered, lateral margins well visible in dorsal view. Trichobothria present on posterior angles. Disc covered with sparse punctures; lateral sides covered with much coarser and denser punctures, becoming larger toward base, partially confluent near basal margin; interspaces covered with fine and sparse punctures. Scutellum variable in length, as long as wide, longer than wide or wider than long.

Elytra. Lateral sides slightly widened posteriorly, widest beyond middle, thence roundly narrowed posteriorly. Humeral calli well developed. Disc covered with 11 regular rows of large punctures, including a short scutellar row; interspaces shagreened, covered with fine and sparse punctures. Epipleura wholly visible in lateral view. Hind wings well developed.

Venter. Hypomera weakly rugose, with dense punctures on anterior side. Prosternum covered with coarse and dense punctures bearing long setae; prosternal process enlarged apically, bordered laterally, with sparse punctures. Metasternum covered with small and sparse punctures in median region, large and dense punctures in lateral region. Abdominal ventrites covered with dense punctures bearing short setae.

Legs. Moderately robust. Tibiae widened apically, with a tooth-like projection. Fore legs with tarsomere 1 enlarged, slightly wider than 3 in male; distinctly narrower than 3 in female. Tarsal claws appendiculate.

Genitalia. Aedeagus thin, distinctly narrowed apically, with apical process thin in dorsal view; moderately curved, with apical process pointed and slightly bent downward at apex in lateral view (Fig. 64). Spermatheca absent.

Distribution. Russia (Sakhalin), Japan (Hokkaido) (Fig. 16).

Host plant. Salicaceae: *Populus* spp., *Salix* spp. (Chûjô and Kimoto 1961, Kimoto 1964); *Salix* spp. (Takizawa 1976, Kudo and Hasegawa 2003); *Populus maximowiczii*, *Salix* spp. (Takizawa 2007).

Remarks. *Gonioctena springlovae* is restricted to Hokkaido and Sakhalin, whereas its closely related species *G. gracilicornis* is widely distributed in the Northeastern Palearctic region. The distributions of these two species overlap only in southern Sakhalin (Fig. 16). The type locality "Kioto [= Kyoto in Honshu]" is probably in error. As Chûjô and Kimoto (1960) mentioned, no single specimen has been collected again in Honshu whereas many specimens have been collected in Hokkaido. This species is ovoviviparous (Takizawa 1976, Kimoto and Takizawa 1994, Kudo and Hasegawa 2003).

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DATA PAPER

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PCR primers for 30 novel gene regions in the nuclear genomes of Lepidoptera

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Abstract

We report primer pairs for 30 new gene regions in the nuclear genomes of Lepidoptera that can be amplified using a standard PCR protocol. The new primers were tested across diverse Lepidoptera, including nonditrysians and a wide selection of ditrysians. These new gene regions give a total of 11,043 bp of DNA sequence data and they show similar variability to traditionally used nuclear gene regions in studies of Lepidoptera. We feel that a PCR-based approach still has its place in molecular systematic studies of Lepidoptera, particularly at the intrafamilial level, and our new set of primers now provides a route to generating phylogenomic datasets using traditional methods.

Keywords

Molecular systematics, Lepidoptera, phylogenomics, phylogenetics

Introduction

Post-Sanger sequencing technologies have opened up vast possibilities for acquiring molecular data for inferring phylogenetic relationships among taxa using 100s to 1000s of loci (Lemmon and Lemmon 2013), from whole genome sequences (e.g. Jarvis et al. 2014), to whole transcriptome sequences (e.g. Misof et al. 2014), to the targeted capture of conserved regions in genomes (e.g. Prum et al. 2015). However,

these approaches require high quality DNA or RNA extracted from samples that are fresh or have been stored appropriately. Unfortunately, this quality requirement fails to capitalize on the wealth of material collected and cataloged in museums around the world. Recognizing this, many researchers are currently attempting to develop protocols to allow the extraction and sequencing of large amounts of DNA from old museum samples (e.g. Timmermans et al. 2016), but these methods are primarily limited to mitochondrial DNA.

For the past two decades, the standard protocol in insect molecular systematics has been to extract genomic DNA from one or two legs of dried individuals, often several years old, generally yielding very low concentrations of DNA. Today, millions of such genomic DNA extracts exist, each taken from suboptimally stored specimens, generated by individual researchers and large facilities such as the Canadian Centre for DNA Barcoding. These extracts have been used to PCR amplify specific gene regions, followed by Sanger sequencing. This standard approach has traditionally been restricted to fewer than 10 gene regions due to the lack of universal primers for more regions. Given this extensive DNA resource and the inability of the aforementioned methods to be easily applied to them, here we present an approach for using these extracts in the pursuit of phylogenomic insights.

As DNA sequencing technologies continue to evolve, the molecular systematist must judiciously choose which tools are best suited to the questions they wish to address. While genome scale data are certainly useful, such data are expensive, difficult to analyze and ultimately only a small fraction is utilized. Perhaps most importantly, such large scale datasets are likely only necessary for resolving deeper evolutionary events, such as the relationships among orders of insects (Misof et al. 2014) or superfamilies of e.g. Lepidoptera (Bazinet et al. 2013; Kawahara and Breinholt 2014). In contrast, datasets on the order of tens of genes have been highly useful for resolving relationships at the intrafamilial level, as has been repeatedly shown for e.g. lepidopteran families (Wahlberg et al. 2009, 2014; Kaila et al. 2011; Kawahara et al. 2011; Sihvonen et al. 2011; Zahiri et al. 2011, 2012; Zwick et al. 2011; Regier et al. 2012a, 2012b; Rota and Wahlberg 2012; Sohn et al. 2013). Thus, datasets generated with PCR-based methods have been and continue to be very insightful. However, in many such studies, some nodes are poorly supported with the scale of data available and more sequence data is needed. But, while it would be very interesting to sequence e.g. transcriptomes for the same species sampled, financial and practical constraints preclude such attempts. Rather, what is most likely to help resolve many of these ambiguities in a cost effective and timely fashion are more high quality loci that can be amplified with PCR across a range of DNA quality.

Whole genome sequences can now be used to search for suitable gene regions for primer design (e.g. Wahlberg and Wheat 2008). Such suitable gene regions are considered to be protein-coding genes that are single copy and have an exon that is longer than 500 bp. Long exons are needed as intron lengths can vary thousand fold between taxa, sometimes even between close relatives (Zhang and Hewitt 2003). Protein-coding genes are also preferred for inferring phylogenetic relationships as their alignments are generally unambiguous and conserved regions can be found for primer design.

Here we design and test PCR primers for long exon regions of single copy, protein-coding genes across Lepidoptera based on publicly available whole genome sequences of the order. The new gene regions are shown to be phylogenetically informative for Lepidoptera and can be used to complement the eight gene regions that have become standard in Lepidoptera phylogenetics (Wahlberg and Wheat 2008).

Material and methods

Single copy, protein-coding genes with exons longer than 500 bp were found while manually curating the set of genes listed in Misof et al. (2014) that were pulled out of eight publicly available Lepidoptera genomes using *tblastn*: *Bombyx mori* (NCBI accession GCA_000151625), *Plutella xylostella* (GCA_000330985), *Manduca sexta* (GCA_000262585), *Danaus plexippus* (GCA_000235995), *Heliconius melpomene* (GCA_000313835), *Melitaea cinxia* (GCA_000716385), *Chilo suppressalis* (GCA_000636095), and *Spodoptera frugiperda* (GCA_000753635). Sequences from all eight genomes were then used to design universal primers. We used the Python library primer-designer v0.2.0 (Peña 2015) to submit batches of FASTA alignments to the website primer4clades (Contreras-Moreira et al. 2009) in order to retrieve candidate primers for each gene sequence. Primer selection was based on high quality and amplicon length between 200 and 500 bp.

As in Wahlberg and Wheat (2008), universal tails were added to all primers to facilitate sequencing. Primers were aliquoted to a standard concentration of 10 μM for use. Primers were tested on a set of 24 species of Lepidoptera that represent major lineages within the order (Table 1). The DNA extracts of these specimens were previously used in the study by Mutanen et al. (2010). They mainly come from small amounts of tissue (such as legs) preserved in 100% EtOH (details of preservation and extraction methods can be found in Mutanen et al. 2010). The PCR reactions for all samples were done using the MyTaq™ HS Red Mix (Bioline) in a final volume of 12.5 μl per sample. For each reaction we used 4 μl of MQ-H_2O, 6.25 μl of 2x MyTaq HS Red Mix, 0.625 μl of both forward and reverse primers and 1 μl of extracted DNA. All primers were tested with a standard thermal cycling profile of 95 °C for 7 minutes, then 40 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 2 minutes, with a final extension period of 72 °C for 10 minutes. A standard cycling profile was chosen to simplify procedures and allow for large scale testing. No optimization of PCR reactions was attempted, as the goal was to find primers that work under exactly the same conditions, allowing the efficient processing of large numbers of samples in the laboratory without having to keep track of specific protocols for specific primer pairs. Success of PCR was visualized on agarose gels and successful PCR products were cleaned enzymatically and sent to Macrogen Europe (Amsterdam) for Sanger sequencing.

Sequences were trimmed of primer sequences and aligned by eye with reference to amino acid sequence in BioEdit 7 (Hall 1999) using the sequence from *Bombyx mori* as a reference for each gene. Aligned sequences were stored and curated using VoSeq (Peña and Malm 2012).

Table 1. Taxa used to test the primers for amplifying the new gene regions. The last column summarizes the number of new gene regions sequenced for each specimen. See Suppl. material 1 for information about which gene regions were successful.

Voucher code	Family	Genus	Species	Number of new genes sequenced
MM00058	Micropterigidae	*Micropterix*	*aureatella*	11
MM00867	Nepticulidae	*Ectoedemia*	*occultella*	18
MM00943	Tischeriidae	*Tischeria*	*ekebladella*	18
MM02175	Psychidae	*Taleporia*	*tubulosa*	22
MM00030	Gracillariidae	*Gracillaria*	*syringella*	26
MM00306	Yponomeutidae	*Yponomeuta*	*evonymellus*	27
MM00510	Tortricidae	*Tortrix*	*viridana*	22
MM00014	Schreckensteiniidae	*Schreckensteinia*	*festaliella*	26
MM02524	Epermeniidae	*Epermenia*	*illigerella*	24
MM03096	Pterophoridae	*Stenoptilia*	*veronicae*	22
MM00913	Alucitidae	*Alucita*	*hexadactyla*	19
MM03941	Choreutidae	*Choreutis*	*pariana*	21
MM00021	Urodidae	*Wockia*	*asperipunctella*	17
MM00116	Cossidae	*Cossus*	*cossus*	28
MM00125	Sesiidae	*Synanthedon*	*scoliaeformis*	29
MM00312	Zygaenidae	*Adscita*	*statices*	26
MM00034	Hesperiidae	*Pyrgus*	*malvae*	24
MM00042	Elachistidae	*Ethmia*	*pusiella*	25
MM00051	Pyralidae	*Pyralis*	*farinalis*	24
MM00027	Drepanidae	*Thyatira*	*batis*	28
MM00032	Geometridae	*Cyclophora*	*punctaria*	26
MM00394	Endromidae	*Endromis*	*versicolora*	29
MM01170	Noctuidae	*Apamea*	*crenata*	27
MM02696	Lasiocampidae	*Poecilocampa*	*populi*	24

Results

We selected a total of 48 gene regions (see Supplementary material for alignments) for primer design, of which 30 successfully amplified (Suppl. material 1 and 2) with the designed primers (Table 3). Only two gene regions were successfully amplified from all 24 test samples (ArgKin and DDX23), but the majority were successfully amplified from 20 or more samples (Table 2). The least successful gene region was LeuZip, which was sequenced from only 9 samples. No samples amplified all 30 gene regions (Table 1); the average number of successful gene regions was about 23. The least successful sample was *Micropterix* (11 out of 30 gene regions sequenced), which is not surprising as the primer design was based on ditrysian species, while *Micropterix* is likely to be the sister group to all the rest of Lepidoptera (Kristensen et al. 2015; Regier et al. 2015). The new gene regions give a total of 11,043 bp of data. The average amplicon length is 368 bp (ranging from 178 to 729 bp).

Table 2. Basic information about the new gene regions amplified and sequenced in this study, along with the traditional eight genes used in many previous studies for comparison.

Gene name	Length (bp)	Number of specimens successful	Variable (%)	Pars. Inf. (%)	Conserved (%)	Freq. A (%)	Freq. T (%)	Freq. C (%)	Freq. G (%)	GeneID from *Bombyx* genome
AFG3a	336	22	39.3	37.5	60.7	28.0	27.7	20.2	24.1	BGIBMGA010088
AFG3b	300	11	47.3	39.7	52.7	34.9	20.9	20.7	23.6	BGIBMGA010088
ANK13C	330	20	49.1	38.8	50.9	33.0	28.5	16.4	22.2	BGIBMGA007536
ArgK	388	24	44.6	33.5	55.4	22.9	19.0	32.1	26.1	BGIBMGA005812
Ca-ATPase	444	23	37.2	30.2	62.8	24.9	21.0	30.1	24.0	BGIBMGA000408
Ca2	410	18	44.9	38.5	55.1	33.2	23.6	18.5	24.7	BGIBMGA006603
chitinase	405	18	47.2	40.5	52.8	25.7	27.4	23.8	23.2	BGIBMGA008709
Cullin5	327	22	48.3	41.0	51.7	33.4	28.7	17.5	20.5	BGIBMGA011511
CycY	375	18	39.7	35.5	60.3	29.9	31.4	17.2	21.6	BGIBMGA005969
DDX23	303	24	46.9	43.2	53.1	40.4	22.6	13.8	23.2	BGIBMGA003429
Exp1	729	15	43.6	35.8	56.4	31.4	28.2	19.5	21.0	BGIBMGA010657
FCF1	173	17	49.7	42.8	50.3	32.4	27.7	16.2	23.7	BGIBMGA010318
GLYP	384	14	52.3	44.0	47.7	27.2	24.8	25.1	22.9	BGIBMGA010361
KRR1	283	16	47.0	39.2	53.0	35.4	26.4	18.0	20.2	BGIBMGA005381
LeuZip	372	9	49.5	35.8	50.5	36.4	24.8	18.0	20.9	BGIBMGA003300
MK6	255	20	52.2	45.1	47.8	32.8	28.1	18.6	20.6	BGIBMGA005641
MMP41	285	21	56.5	48.1	43.5	31.1	30.6	19.7	18.6	BGIBMGA007574
MPP2	330	21	44.9	40.3	55.2	29.0	29.4	22.6	19.1	BGIBMGA008312
NC	573	15	48.9	39.6	51.1	32.2	29.1	17.0	21.7	BGIBMGA005035
Nex9	420	21	60.5	47.4	39.5	33.1	24.8	19.0	23.2	BGIBMGA001032
PolII	360	22	43.9	39.4	56.1	30.1	25.3	19.7	24.8	BGIBMGA004994
ProSup	432	22	58.8	47.5	41.2	25.6	27.8	21.0	25.6	BGIBMGA004645
PSb	366	23	54.4	45.9	45.6	24.8	23.9	26.7	24.7	BGIBMGA000201
SARAH	381	16	56.4	44.9	43.6	29.2	27.8	23.3	19.7	BGIBMGA011095
Ssu72	249	23	55.0	48.2	45.0	36.0	28.1	16.0	19.9	BGIBMGA000925

Gene name	Length (bp)	Number of specimens successful	Variable (%)	Pars. Inf. (%)	Conserved (%)	Freq. A (%)	Freq. T (%)	Freq. C (%)	Freq. G (%)	GeneID from *Bombyx* genome
TIF3Cb	324	13	50.6	40.1	49.4	24.7	22.1	28.9	24.3	BGIBMGA012851
TIF6	336	18	50.0	42.6	50.0	24.4	21.4	25.5	28.8	BGIBMGA009830
UDPG6DH	405	21	49.1	41.0	50.9	30.1	27.4	20.9	21.6	BGIBMGA012188
VPS4	432	15	40.7	35.4	59.3	28.9	28.9	20.1	22.1	BGIBMGA005930
WD40	339	21	42.5	38.6	57.5	30.1	31.4	19.3	19.2	BGIBMGA006243
Genes from Wahlberg and Wheat (2008) for comparison										
CAD	826	24	52.4	42.7	47.6	35.9	28.3	14.6	21.2	
COI	1476	23	44.4	33.0	55.6	31.1	40.0	14.9	14.0	
EF1a	1047	21	34.9	27.2	65.1	25.4	23.0	27.6	24.0	
GAPDH	691	12	38.9	30.8	61.1	23.6	25.8	27.3	23.3	
IDH	722	23	48.2	41.1	51.8	31.2	27.1	19.8	21.9	
MDH	407	23	47.9	41.3	52.1	27.4	25.8	22.7	24.1	
RpS5	603	20	38.5	34.3	61.5	25.4	24.9	24.4	25.3	
wingless	400	20	58.5	48.5	41.5	21.7	18.3	28.9	31.0	

Table 3. Primers for 30 new gene regions with universal tails (T7promoter-TAATACGACTCAC-TATAGGG to forward primers and T3-ATTAACCCTCACTAAAGGG to reverse primers) attached to the 5' end. F = Forward, R = Reverse. Gene names from Table 2.

Gene	Primer
AFG3a_F	TAATACGACTCACTATAGGGTGTGAAGAAGCTAAGatwgaratyatggartt
AFG3a_R	ATTAACCCTCACTAAAGGGTGTTGTTGTATTAAAAccrtccatytchac
AFG3b_F	TAATACGACTCACTATAGGGTGCTCAAGACGACCtdaaraaratmac
AFG3b_R	ATTAACCCTCACTAAAGGGCCTGTACCTTCCACGaaytcytcrtamgt
ANK13C_F	TAATACGACTCACTATAGGGCAAATACAAAATTTTTATATGGAAytdaartgggaytt
ANK13C_R	ATTAACCCTCACTAAAGGGGCAACTGTTTCTTTTCTAtcytcwcgraadatcca
ArgK_F	TAATACGACTCACTATAGGGyGAyCCsATCATyGAGGACTACCA
ArgK_R	ATTAACCCTCACTAAAGGGAGrTGGTCCTCCTCrTTGCACCAvAC
Ca2_F	TAATACGACTCACTATAGGGAAACAGTGGACtgyttgaaraarttcaayg
Ca2_R	ATTAACCCTCACTAAAGGGGGTGTGTTGTCGATGaaraayttrtgraa
Ca-ATPase_F	TAATACGACTCACTATAGGGGAAtacgarccbgaaatgggwaargt
Ca-ATPase_R	ATTAACCCTCACTAAAGGGcdccrtgrgcggggtcgttraagtg
chitinase_F	TAATACGACTCACTATAGGGGGTGGGTGCTtayttygtngaatgggg
chitinase_R	ATTAACCCTCACTAAAGGGTGTCCACAccrtcraaraayttcca
Cullin5_F	TAATACGACTCACTATAGGGTGTTAGTTAAAGATGCTTTTATGgaygaycchmg
Cullin5_R	ATTAACCCTCACTAAAGGGTCTTAACCATTCAaccatrtcytcttcyttytc
CycY_F	TAATACGACTCACTATAGGGgattatgayaartataatccwgaacayaaaca
CycY_R	ATTAACCCTCACTAAAGGGcattgcytcyaatttytgtgcyctttcytt
DDX23_F	TAATACGACTCACTATAGGGACAAAAGATAAAGAACGTgargargargchat
DDX23_R	ATTAACCCTCACTAAAGGGTGATCTTTTTTCAgaccartghckrtcatccca
Exp1_F	TAATACGACTCACTATAGGGgthaataaaytdtttgaattyatgcatga
Exp1_R	ATTAACCCTCACTAAAGGGggrtaytcttcaaartctttrttdatcat
FCF1_F	TAATACGACTCACTATAGGGACTGGACATCGtdcarartatgatggayt
FCF1_R	ATTAACCCTCACTAAAGGGTTGTAGCCACGATGtarcayttrtgytg
GLYP_F	TAATACGACTCACTATAGGGACTGCGACAAGAAtayttyatgtgygcbgc
GLYP_R	ATTAACCCTCACTAAAGGGTTCACTCGTTTTTTCACCTtcytcytcdat
KRR1_F	TAATACGACTCACTATAGGGaatgcktggrctatgaaratwcc
KRR1_R	ATTAACCCTCACTAAAGGGtdataatrtcrcatccwatttcrtc
LeuZip_F	TAATACGACTCACTATAGGGTGCCTGTCACAAaaygaytggaaryt
LeuZip_R	ATTAACCCTCACTAAAGGGTTTGACCAGGGTTTttdgcrtarttraa
MK6_F	TAATACGACTCACTATAGGGTTAGAGAAGGTGATgtntggathtgyatgga
MK6_R	ATTAACCCTCACTAAAGGGTTCTTTCTGGTGCCATGtanggyttrca
MMP41_F	TAATACGACTCACTATAGGGGAAAACTGGGGTGCTAAagtdtayttyaaya
MMP41_R	ATTAACCCTCACTAAAGGGTCACTTTGtttttrttytchccaaawgtcat
MPP2_F	TAATACGACTCACTATAGGGCACTTCCGAATCccdtggttycartaycc
MPP2_R	ATTAACCCTCACTAAAGGGCCACAGCAGCTGTGtaytcyttdccraa
NC_F	TAATACGACTCACTATAGGGgatgaagaaaaycchaaraarttytt
NC_R	ATTAACCCTCACTAAAGGGacwatdgaccartggaarttcatdgc
Nex9_F	TAATACGACTCACTATAGGGTGCAACTGCAAgartttgtngaytggatg
Nex9_R	ATTAACCCTCACTAAAGGGCCCAGTCGTATTTAggytgbtcntcatacat
PolII_F	TAATACGACTCACTATAGGGCTGAAACACCTACAatggcbathgaytgggt
PolII_R	ATTAACCCTCACTAAAGGGGCTGTAGGGTTCCATttdgcrtgytcytt
ProSup_F	TAATACGACTCACTATAGGGGACAACAATCGACtggcayccnaayaa

Gene	Primer
ProSup_R	ATTAACCCTCACTAAAGGGCTGTCCAGTgactggaayttyttcatdgc
PSb_F	TAATACGACTCACTATAGGGGCTGGGAGCTACTggvtgytggtgygaya
PSb_R	ATTAACCCTCACTAAAGGGAGATGCAGTCTCCAGTGTAGatrtcdckytc
SARAH_F	TAATACGACTCACTATAGGGGAAGATGGTATGCCTAATAtwcaycchaayat
SARAH_R	ATTAACCCTCACTAAAGGGGTTCACCTTCTTCACGAggytcccadccna
Ssu72_F	TAATACGACTCACTATAGGGCAGCTGACAGACCTaaytgttaygarttygg
Ssu72_R	ATTAACCCTCACTAAAGGGCCGATTGTAGCTTCTtcrtgrttrtcytg
TIF3Cb_F	TAATACGACTCACTATAGGGGAAAAATCGACCACCTGtaytayaarttyga
TIF3Cb_R	ATTAACCCTCACTAAAGGGGCCAGCAGTTCTTTAggyttnccvgtcatca
TIF6_F	TAATACGACTCACTATAGGGCTGTGCGAGTGcarttygaraayaataa
TIF6_R	ATTAACCCTCACTAAAGGGTGTGTCAGCCAGGatytcytchgtrtc
UDPG6DH_F	TAATACGACTCACTATAGGGCAGGAACTGTGTtgggtvtaygarcaytg
UDPG6DH_R	ATTAACCCTCACTAAAGGGTCTTGTGTCGCCTgtrttyttyttraa
WD40_F	TAATACGACTCACTATAGGGGATCCACTTCACAcaygcyaaraayac
WD40_R	ATTAACCCTCACTAAAGGGCCTgtccartcacaytcyttytcttg
VPS4_F	TAATACGACTCACTATAGGGTGATTCTGATGATCCAGAAaaraaraaryt
VPS4_R	ATTAACCCTCACTAAAGGGCATCCATATCAttvccdacaccttgcatytg

The variability in the new gene regions appears to be similar to the widely used nuclear gene regions reported in Wahlberg and Wheat (2008) (Table 2). The base content across most of the fragments is fairly even, with some of them having a small AT bias (e.g., Exp1, CycY, and TIF3Cb), but none having a larger percentage than for example CAD, one of the widely used gene regions (Wahlberg and Wheat 2008), which has 64.1% of As and Ts. The number of parsimony informative sites ranges from 30.2% in Ca-ATPase to a little over 48% in MMP41 and Ssu72. For comparison, the range of parsimony informative sites in the Wahlberg and Wheat (2008) gene regions is almost the same, from 27.2% (EF1alpha) to 48.5% (wingless).

Discussion

We report here primers for 30 new nuclear gene regions that can be used to complement existing molecular data for Lepidoptera systematics. Our primers were designed to amplify gene regions across the entire taxonomic array of Lepidoptera and to work on relatively degraded material by amplifying less than 500 bp segments of the genome. Many of these primers are being used successfully in our laboratory for projects on e.g. the nymphalid subfamily Limenitidinae (Dhungel and Wahlberg in prep.), the families Geometridae (Brehm et al. in prep.), Choreutidae (Rota et al. in prep.), Limacodidae (Dupont et al. in prep.) and Riodinidae (Seraphim et al. in prep.). The phylogenetic utility of the used gene regions will be reported in more detail in the forthcoming papers: in summary, they are providing similar resolution as the standard gene regions reported in Wahlberg and Wheat (2008) in preliminary maximum likelihood analyses with RAxML that have been conducted.

We would like to stress that the gene regions described here should be seen as complementary to the standard gene regions (Wahlberg and Wheat 2008) and could be used in the event that more data is needed. Potential users should consult Suppl. material 1 to see which primers worked for taxa they are interested in. Based on our experiences in the laboratory, we would recommend that researchers consider using primers for AFG3a, ArgKin, Ca-ATPase, DDX23, MMP41, MPP2, Nex9, PolII, ProSup, PSb, SSU72, UDPG6DH, and WD40, as these tend to amplify consistently, especially across Ditrysia.

More specifically, it seems that several fragments are not very suitable for nonditrysians (none of the three exemplars that we used amplified AFG3b, CHITINASE, KRR1, NC, SARAH, VPS4) and the utility of several other fragments for these groups needs to be further tested (Ca2, GLYP, MPP2, NEX9, POLII, TIF3CB, and UDPG6DH amplified in only one of the nonditrysians tested). On the other hand, 21 fragments amplified in four or more of the six exemplars of Macroheterocera (the exceptions being LeuZip, which amplified in only one of them, and TIF3Cb and VPS4, which amplified in three out of six). The situation is more complex across the lower ditrysians and apoditrysians, which can be expected since these groups are quite divergent (Mutanen et al. 2010; Regier et al. 2013). For these groups our recommendation is to try CHITINASE and MK6 in addition to the above-mentioned fragments that appear to work across Lepidoptera.

In this study, we have used traditional Sanger sequencing to acquire the DNA sequences. However, almost all of the amplicons are short enough to be multiplexed and sequenced on a NextGen sequencing platform, such as Illumina. The advantages would be quick generation of a large number of sequences for a large number of samples. On the other hand, many systematists do not have access to NextGen sequencers, or the bioinformatics knowhow to process the raw data into useable formats, in which case the traditional PCR-based Sanger sequencing approach is still appropriate.

The approach we have used is highly conservative, as we sought to find primer pairs that work under standard conditions. It would thus be possible to design primers for the 18 gene regions that did not work under our strict criteria, but would work under different conditions. It is also possible to design primers that would amplify a longer segment of DNA, although such primer pairs would require fresh samples with little degradation of genomic DNA. It would also be possible to find more gene regions with exon lengths more than 500 bp, although a PCR-based approach becomes less and less efficient as the number of reactions grows. It is quite likely that datasets comprising up to 20 gene regions are sufficient for most phylogenetic studies within families (Zwick et al. 2011). For more difficult phylogenetic problems, NextGen sequencing approaches are recommended.

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Supplementary material 1

Table S1

Authors: Niklas Wahlberg, Carlos Peña, Milla Ahola, Christopher W. Wheat, Jadranka Rota

Data type: NCBI accession numbers

Explanation note: Details of the success of sequencing of the new gene regions. GenBank accession number indicates successful sequencing, dash indicates unsuccessful amplification.

Supplementary material 2

Sequences used for designing primers

Authors: Niklas Wahlberg, Carlos Peña, Milla Ahola, Christopher W. Wheat, Jadranka Rota

Data type: Reference sequences

Explanation note: A zip-file containing reference sequences for all 48 gene regions used for designing primers.

ZooKeys 596: 143–156 (2016)
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RESEARCH ARTICLE

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Varicus lacerta, a new species of goby (Teleostei, Gobiidae, Gobiosomatini, *Nes* subgroup) from a mesophotic reef in the southern Caribbean

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Abstract

We describe a new species of goby, *Varicus lacerta* **sp. n.**, which was collected from a mesophotic reef at Curacao, southern Caribbean. The new species is the tenth species of *Varicus*, all of which occur below traditional SCUBA depths in the wider Caribbean area. Its placement in the genus *Varicus* is supported by a molecular phylogenetic analysis of three nuclear genes and the mitochondrial gene cytochrome *b*. In addition, the new species has one anal-fin pterygiophore inserted anterior to the first haemal spine, which distinguishes *Varicus* species from most species in the closely related and morphologically similar genus *Psilotris*. *Varicus lacerta* **sp. n.** is distinguished from all other named species of *Varicus* by the absence of scales, having highly branched, feather-like pelvic-fin rays, and in its live coloration. We provide the cytochrome c oxidase I DNA barcode of the holotype and compare color patterns of all species of *Varicus* and *Psilotris* for which color photographs or illustrations are available. This study is one of several recent studies demonstrating the utility of manned submersibles in exploring the diversity of poorly studied but species-rich deep-reef habitats.

Keywords

Systematics, molecular phylogeny, deep reefs, submersible, Curaçao, *Psilotris*

Introduction

Operating out of Substation Curaçao (www.substation-curacao.com), the Smithsonian Institution's Deep Reef Observation Project (DROP) uses the manned submersible *Curasub* to capture tropical marine fishes and invertebrates at depths up to 300 m, providing new information on the fauna that inhabits poorly studied deep-reef ecosystems. DROP's exploratory submersible diving in the southern Caribbean has led to the discovery of a cache of undescribed fish biodiversity, some of which has been recently described (Van Tassell et al. 2012; Baldwin and Johnson 2013; Baldwin and Robertson 2013, 2014, 2015; Tornabene et al. 2016). Many of the new species belong to the Gobiidae, most notably the tribe Gobiosomatini (Van Tassell et al. 2012; Tornabene et al. 2016). This tribe comprises the American seven-spined gobies, a taxonomically and ecologically diverse clade of fishes that is endemic to the western Atlantic and eastern Pacific Oceans. A repeated pattern of rapid speciation via microhabitat specialization in this tribe has resulted in the Gobiosomatini becoming a model group for the study of adaptive radiation in the marine environment (Rüber et al. 2003; Taylor and Hellberg 2005; Tornabene et al. in press). One of the most ecologically and taxonomically diverse clades within the Gobiosomatini is the *Nes* subgroup, which comprises 39 species in 11 genera that inhabit a wide variety of marine habitats (Tornabene et al. 2016). Within the *Nes* subgroup, three genera have species described from mesophotic reefs below 50 m: *Pinnichthys* Gilmore, Van Tassell & Tornabene, 2016, with four species, all from deep reefs; *Psilotris* Ginsburg, 1953, with six species, one from deep reefs; and *Varicus* Robins & Böhlke, 1961 with nine species prior to this study, all from deep reefs (Tornabene et al. 2016). Here we describe a tenth deep-reef species of *Varicus* based on a single specimen that was collected at 129-147 m from Curaçao.

Materials and methods

The new species was collected using the *Curasub* manned submersible. The sub has two hydraulic arms, one equipped with a suction hose and the other with a quinaldine-ejection system used to anaesthetize fishes. Specimens collected with the suction hose are deposited into a vented acrylic cylinder attached to the outside of the sub. The captured holotype was brought to the surface alive, where it was photographed and tissue sampled prior to fixation in 10% buffered formalin and subsequent storage in 75% ethanol.

Tissue from the holotype was stored in saturated salt-DMSO (dimethyl sulfoxide) buffer (Seutin et al. 1991). DNA extraction and cytochrome *c* oxidase subunit I (COI) DNA barcoding were performed as outlined by Weigt et al. (2012). To confirm the phylogenetic placement of the new species we also sequenced the mitochondrial gene cytochrome *b* and the nuclear genes Rag1, sreb2, and zic1. Following nomenclature of Chakrabarty et al. (2013), new sequences here constitute genseq-1 COI, cytb, Rag1, sreb2, and zic1. Primers and PCR conditions for amplifying these four loci were identical to those used in Agoretta et al. (2013). Sequences generated here were aligned with

Gobiosomatini sequences from Tornabene et al. (2016) in *Geneious v. 9* (Biomatters, Ltd., Auckland). Substitution model choice and partitioning scheme were assessed using PartitionFinder (Lanfear et al. 2012). Phylogeny was inferred using Bayesian inference in the program MrBayes ver. 3.2, using two Metropolis-coupled Markov Chain Monte Carlo (MCMC) runs, each with four chains. The analysis was run for 10 million generations sampling trees and parameters every 1000 generations. Burn-in, convergence and mixing were assessed using Tracer (Rambaut and Drummond 2007) and by visually inspecting consensus trees from both runs.

All measurements were taken with digital calipers to the nearest 0.1 mm. Vertebral counts and pterygiophore patterns were taken from digital radiographs. Dorsal pterygiophore formula is that of Birdsong et al. (1988), and head pore terminology follows Akihito et al. (1988). Sensory papillae are described following the terminology of Sanzo (1911), with the exception that the interorbital series follows terminology described by Tornabene et al. (2016). All other morphological characters are as defined by Böhlke and Robins (1968) as modified by Van Tassell et al. (2012), who like many authors, differentiate the unsegmented spine from the segmented rays of the second dorsal, anal, and pelvic fins using the roman numeral 'I' for the spine followed by Arabic numbers for the soft rays. The holotype was deposited at the National Museum of Natural History, Smithsonian Institution (USNM).

Results

***Varicus lacerta* sp. n.**
http://zoobank.org/77FB8CDB-9B22-4F33-B76F-262C5606665F
Godzilla Goby
Figs 1–5

Type locality. Curaçao, southern Caribbean.

Holotype. USNM 434796, male, 36.2 mm SL, Curasub submersible, sta. CURASUB15-24, southern Caribbean, Curaçao, east of downline off Substation Curacao dock, near 12.083 N, 68.899 W, 129-143 m, quinaldine, 24 September 2015, Carole C. Baldwin, Darryl Felder, Bruce Brandt and Jennifer Felder.

DNA barcode of holotype. ATAAAGATATTGGCACCCTCTATTTGATCTTCGGCGCCTGAGCTGGCATAGTCGGCACTGCTCTAAGCCTTCTTATTCGGGCAGAGCTAAGCCAACCTGGCGCCCTTTTAGGGGATGACCAGATCTACAACGTGATCGTTACTGCCCACGCCTTCGTAATAATCTTCTTTATAGTAATACCCGTCATGATTGGGGGCTTTGGGAACTGGCTCGTCCCTCTTATGATTGGGGCCCCCGATATGGCCTTTCCCCGAATAAATAACATAAGCTTCTGACTCCTCCCCCCCTCTTTCCTCCTGCTCTTAGCCTCCTCCGGCGTTGAAGCAGGCGCTGGCACAGGGTGAACCGTATACCCCCCCCTAGCCGGAAACCTCGCCCACGCCGGGGCCTCTGTTGATTTAACAATTTTTTCCCTCCACTTAGCAG-

GCATTTCCTCAATCCTAGGAGCCATTAACTTTATTACCACCATCCT-
CAACATAAAGCCCCCAGCAATCTCGCAATATCAAACCCCCCTTTTT-
GTATGGGCCGTGCTAATTACGGCTGTTCTTCTATTACTCTCCCT-
GCCCGTCCTAGCTGCAGGAATTACAATACTTCTTACCGATCGTAAC-
CTAAATACAACCTTTTTTGACCCCGCAGGAGGGGGAGACCCCATTCTC-
TACCAACACCTCTTCTGATTCTT

Generic placement. In addition to molecular characters supporting the phylogenetic placement (Fig. 1), the following morphological characters support the inclusion of the new species in *Varicus*: first dorsal spines VII; dorsal-fin pterygiophore formula 3-221110; vertebrae 11+16; hypurals 1-2 and 3-4 partially fused; one anal-fin pterygiophore inserted anterior to first haemal spine; anal-fin rays I,9 or fewer (I,7 in *V. lacerta*); head pores absent; transverse papillae rows 5i and 5s connected as a single continuous row; pelvic fins completely separate, lacking both anterior frenum and membrane connecting bases of innermost pelvic-fin rays; fifth pelvic-fin ray unbranched.

Diagnosis. Second dorsal fin I,9; anal fin I,7; pectoral fin 18; no scales; cephalic papillae rows 5*s* and 5*i* connected, forming a single row; pelvic rays 1-4 highly branched and feather-like; one anal-fin pterygiophore inserted anterior to first haemal spine; body with five broad, indistinct, dark vertical bands washed with bright yellow in life; pelvic, pectoral and anal fins yellow-orange in life, dorsal, anal, and caudal fins yellow with faint orange tint.

Description. General shape: body robust, widest and deepest at head, trunk tapering in width and depth posteriorly, dorsal head profile gradually sloping from dorsum to lips.

Median and paired fins: first dorsal fin VII, second spine longest, tips of spines projecting from fin membrane; second dorsal fin I,9, last ray branched to the base; anal fin I,7, last ray branched to the base; pectoral fin 18/18, fin extending posteriorly to vertical through anus; pelvic fins I,5, fins well separated, lacking both anterior frenum and membrane connecting bases of innermost rays; 4th pelvic-fin ray longest, extending posteriorly to anus; rays 1–4 connected by a thin membrane, each ray with one primary bifurcation followed by numerous thin branches off main branch that are united by a continuous membrane to the tip of the ray, giving each ray a feather-like appearance; 5th ray unbranched and 60–70% the length of 4th ray; caudal fin rounded, branched caudal-fin rays 15, segmented caudal-fin rays 17.

Squamation: no scales on head and trunk.

Head: jaw terminal, angled approximately 40 degrees from horizontal axis of body, extending posteriorly to a vertical at anterior end of pupil; anterior nares elongate narrow tubes; posterior nares inconspicuous openings covered by a short flap; no cephalic lateralis pores on head or preopercle; eyes large, dorsolateral, extending slightly above head profile; interorbital space narrow; operculum opening slightly wider than width of pectoral-fin base; teeth in upper jaw in two rows, outer row enlarged, canine-like, recurved, and evenly spaced, extending along most of premaxilla; inner rows smaller, more numerous, and more tightly spaced; teeth in lower jaw in three rows, outermost and innermost rows slightly enlarged, middle row smaller and more numerous; tongue truncate, tip with very slight indentation.

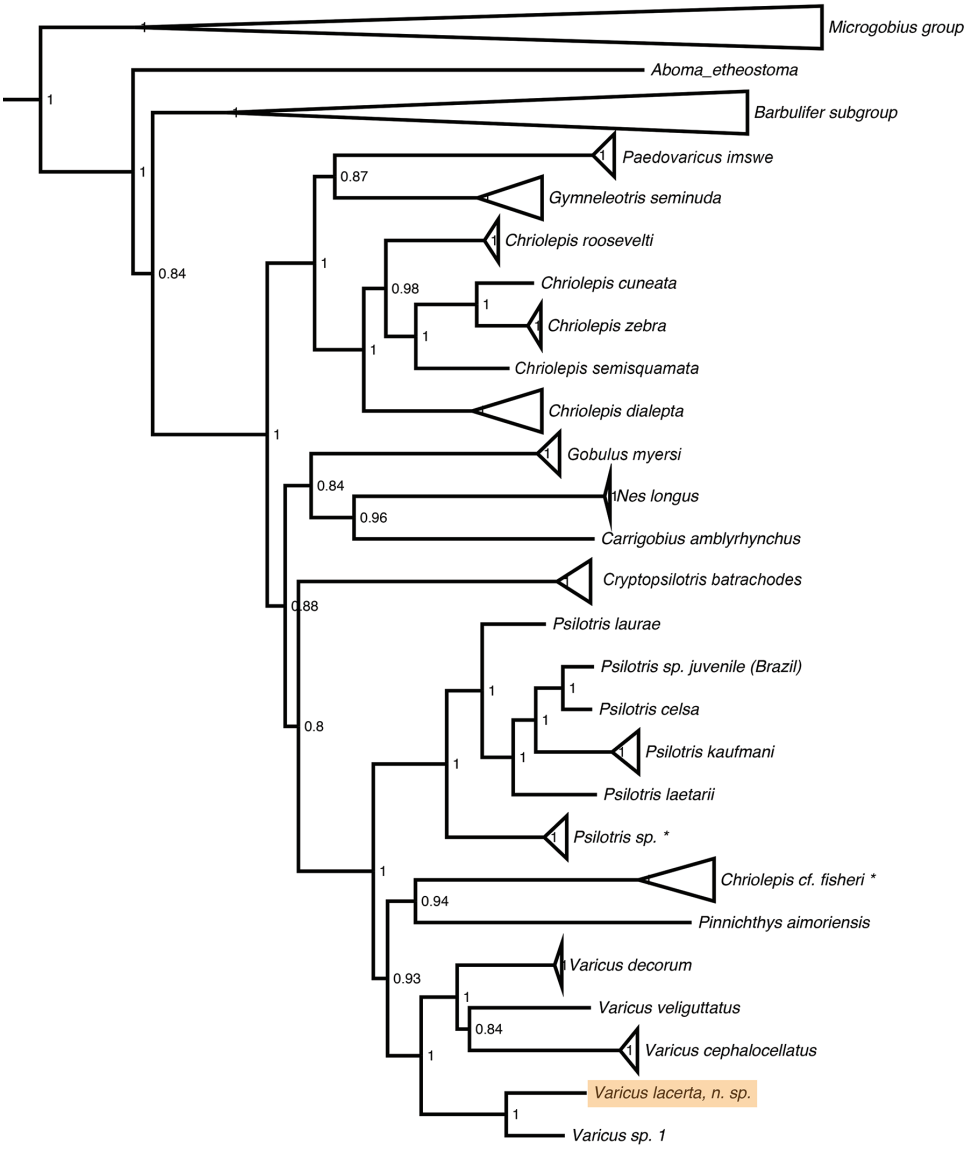

Figure 1. Molecular phylogeny of the Gobiosomatini based on three nuclear genes and one mitochondrial gene. Support values are Bayesian posterior probabilities. * indicates species that may be *Chriolepis fisheri*, see Tornabene et al. (2016) for more information.

Morphometrics (% SL): head length 33.1; eye diameter 9.4; interorbital 2.6; snout length 8; upper-jaw length 12.4; predorsal length 40.1; body depth at origin of first dorsal 19.1; body depth at anal-fin origin 15.2; body depth at caudal peduncle 10.2; caudal-peduncle length 21.1; pectoral-fin length 24.0; pelvic-fin length 26.0; caudal-fin length 27.1.

Genitalia: male with short, conical, pointed papilla, wide at base and tapering distally to a point, no melanophores present; female unknown.

Color in life (Figs 2, 3): Ground color pale grey, head and body spangled with tiny silver and black dots, upper two-thirds of head and upper half of body with yellow tint that is more visible when fish photographed against white vs. black background (Figs 2 and 3, upper panel); breast, lower portions of head and opercle, chest, and lower portion of belly pale pinkish white.

Head with areas of bright yellow pigment heavily speckled with black dots on snout, along upper lip, as an irregular blotch over most of opercle, in a broad band across nape, and as two irregular bars below the eye, one beneath center of eye and extending to rear corner of mouth, the other running obliquely back from posteroventral corner of eye to lower corner of preopercle; iris greenish yellow, heavily speckled with silver and black dots; a thin silvery-white inner ring around pupil.

Body with four broad yellow bars heavily speckled with black dots, one on upper half of body under first dorsal fin; second and third extending from dorsal midline nearly to ventral midline, second positioned under anterior half of second dorsal fin and third under posterior corner of second dorsal and anterior half of caudal peduncle; fourth and narrowest bar covering most of posterior end of caudal peduncle and extending onto base of caudal fin; first three body bars (and bar across the nape) appearing as double bars due to irregular pale blotches in centers; interspaces between first three body bars with small, black-speckled yellow blotches and short, thin yellow bars; pale areas on head and trunk with silver, iridescent markings that are most conspicuous along mid-flank in the photograph of the live fish (Fig. 2)

First dorsal fin yellow with fine yellow and orange dots on the inner two-thirds of fin, gradually replaced with silvery white dots on membranes of outer one fourth of fin; second dorsal fin similarly colored, but with silvery speckling predominating on outer one-third of fin. Basal three-quarters of caudal fin yellow, spangled with orange (mainly) and whitish dots; outer one-quarter of fin with rays gray and membranes translucent with heavy silver-white speckling, rear edge of fin with darker grey pigment suffused with orange. Anal fin orange, strongly so distally in live fish and basally in freshly dead fish (Figs 2, 3, respectively); outer half of fin membranes heavily speckled with dark brown dots; fin rays with yellow tint distally. Pectoral-fin base white, heavily spangled with silver dots, a large, black-speckled yellow blotch on upper corner and a similar, smaller, more diffuse yellow blotch on lower corner; rays pink basally, orange-red speckled with silver centrally, fading to pink distally; sparse silver spangles scattered over fin. Pelvic fins pale, washed with pinkish-orange speckling.

Color in preservation (Fig. 4): Ground color yellowish pale, snout and mouth pale gray; various dark marks present in live fish visible in preserved fish as concentrations of dark brown dots: two indistinct short dark bars under eye: dark blotch on nape; four dark bars on body and at end of caudal peduncle; dark blotches at top and bottom corners of pectoral base.

Sensory papillae (Fig. 5): sensory papillae well developed, with notably elongate papillae on nape, snout, cheek, and ventral surface of head, giving head a hairy or

Figure 2. *Varicus lacerta* sp. n., holotype, USNM 434796, 36.2 mm SL, male, live. Photo by Barry Brown.

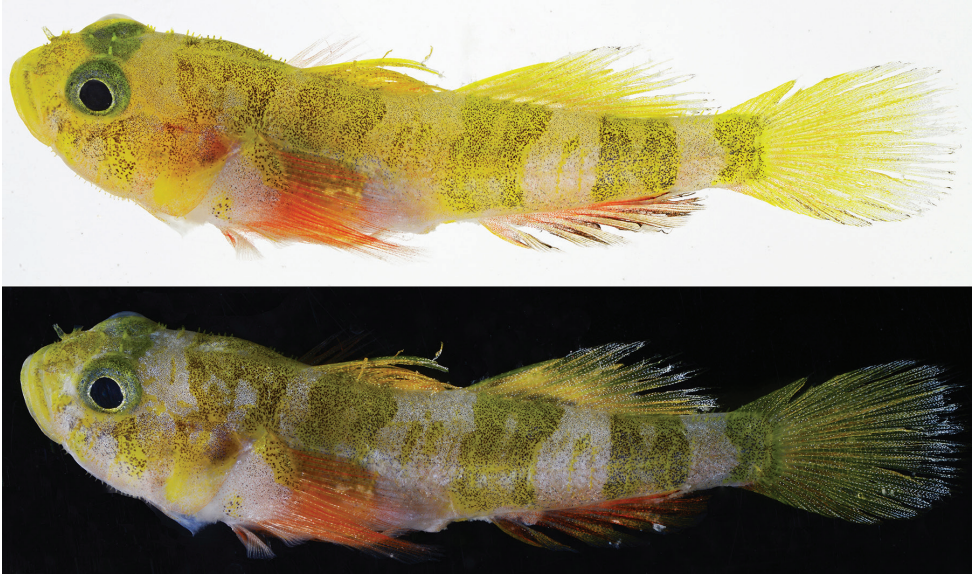

Figure 3. *Varicus lacerta* sp. n., holotype, USNM 434796, prior to preservation. Photos by Carole Baldwin and Ross Robertson.

Figure 4. *Varicus lacerta* sp. n., holotype, USNM 434796, preserved. Photo by Sandra Raredon.

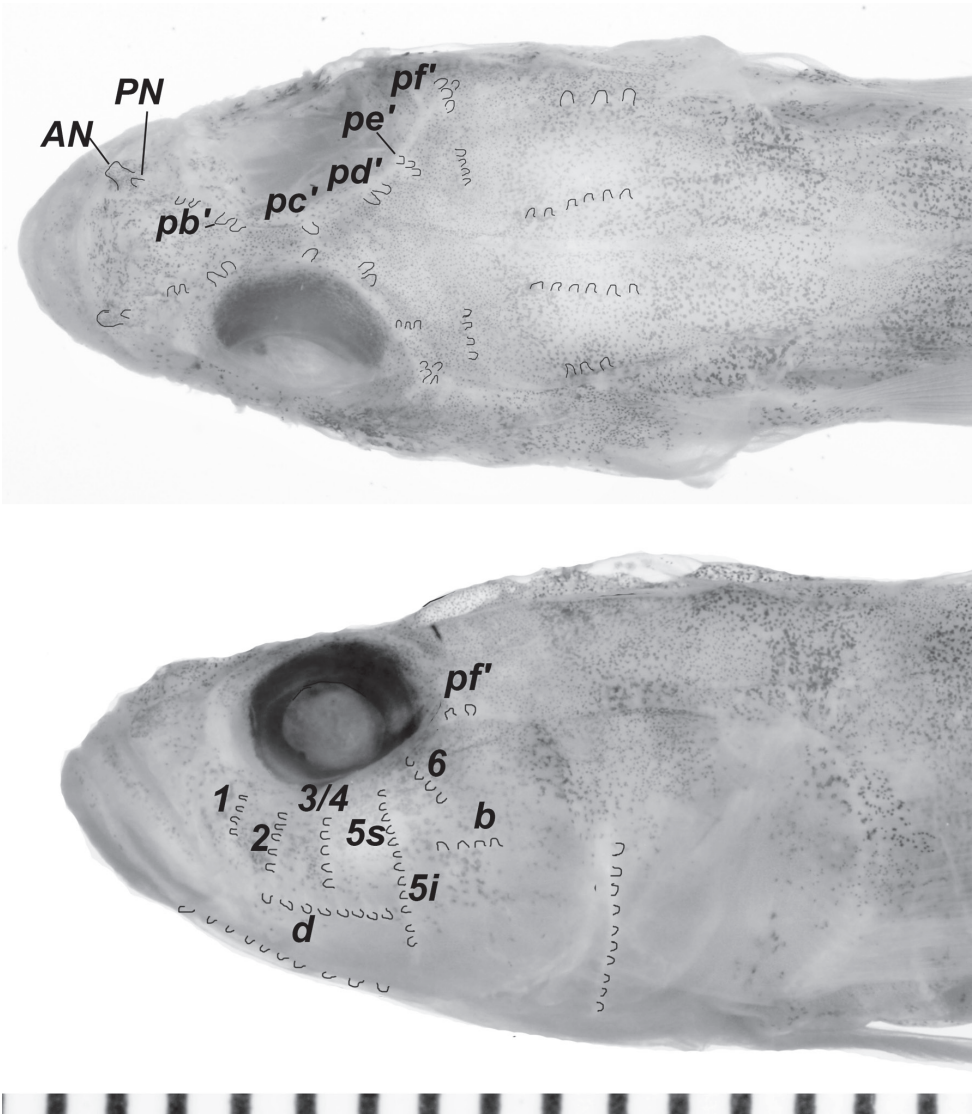

Figure 5. Sensory papillae pattern of *Varicus lacerta* sp. n. Scale-bar increments are millimeters. Photos by Sandra Raredon. Individual papillae are outlined in black for emphasis.

spikey appearance (visible in Figs 2 and 3, less obvious in preservation); a series of 5 transverse papillae rows on side of head; transverse papillae rows 5i and 5s united as a single continuous row positioned anterior to row *b*, continuing ventrally below row *d*; interorbital papillae series well developed, each side of the interorbital possessing 2 *pb'* papillae, 1 *pc'* papilla, 2 *pd'* papillae, 3 *pe'* papillae, and a cluster of 3-4 *pf'* papillae.

Vertebral skeleton: dorsal pterygiophore formula 3-221110; one anal-fin pterygiophore inserted anterior to first haemal spine; second neural spine expanded and slightly

spatulate at tip; hypurals 1–2 fused with hypurals 3–4 along approximately one-half of their length; 27 vertebrae, 11 precaudal, 16 caudal.

Habitat. The only known specimen was collected at 129–143 m. Quinaldine was dispersed around a yellow sponge (~20 cm tall) tentatively identified from videos by Allen Collins (National Marine Fisheries Service) as *Dactylocalyx pumiceus*, situated on a rocky outcropping along the deep-reef slope. After approximately 20 seconds the stunned fish emerged from a space in the rocky substrate at the base of the sponge and was captured. It is unclear whether the fish was originally in direct association with the sponge itself or was instead sheltering in spaces within the rock. Video of the capture taken from a high-definition video camera mounted on the outside of the *Curasub* is available online (https://youtu.be/UvxJEi-vER0). Subsequent collections targeting similar sponges and rocky substrates within this depth range at the type locality have not yielded additional specimens.

Distribution. Known only from the type location in Curaçao.

Etymology. The specific epithet 'lacerta' (Latin for 'lizard') is in reference to the reptilian or saurian appearance of this species, as indicated by its bright yellow and orange coloration, green eyes, disproportionately large head possessing raised ridges of papilla, and multiple rows of recurved canine teeth in each jaw. The common name Godzilla goby (gobio Godzilla in Spanish) refers to the radioactive reptilian monster from the sea that appeared in Japanese science-fiction films as Gojira, renamed Godzilla in subsequent English-language films.

Discussion and comparisons

The molecular phylogeny (Fig. 1) shows the new species nested within the genus *Varicus*, where it is recovered as sister to an undescribed species *Varicus* sp. 1 from Curaçao. This undescribed species is represented by a single specimen in poor condition that also lacks body scales, but is readily distinguishable from *V. lacerta* based on live coloration (see below). *Varicus lacerta* is easily distinguished from all described congeners by the absence of scales on the body and the presence of highly branched, feather-like pelvic-fin rays 1-4. *Varicus decorum* Van Tassell, Baldwin & Tornabene, 2016, lacks scales on most of the body, but possesses a pair of small, ctenoid scales on each side of the caudal peduncle near the base of the caudal fin, which are absent in *V. lacerta*. Live coloration also easily distinguishes *V. lacerta* from all other species of *Varicus* for which the live color pattern is known (Fig. 6). While *V. lacerta* has a color pattern of indistinct broad dark bars on a yellowish body, in five other *Varicus* species (*V. cephalocellatus* Gilmore, Van Tassell & Baldwin, 2016, *V. decorum*, *V. nigritus* Gilmore Van Tasell & Baldwin, 2016, *Varicus* sp. 1, and *V. veliguttatus* Van Tassell, Baldwin & Gilmore, 2016) the color pattern comprises blotches and spots of yellow or black on a white body. *Varicus lacerta* differs from *V. adamsi* Gilmore, Van Tassell & Tornabene, 2016, and *V. vespa* Hastings & Bortone (1981), in having indistinct broad dark bars on a yellowish body and yellow median fins vs narrow yellow bars on a white body and white median fins with black edges in *V. adamsi*, and narrow

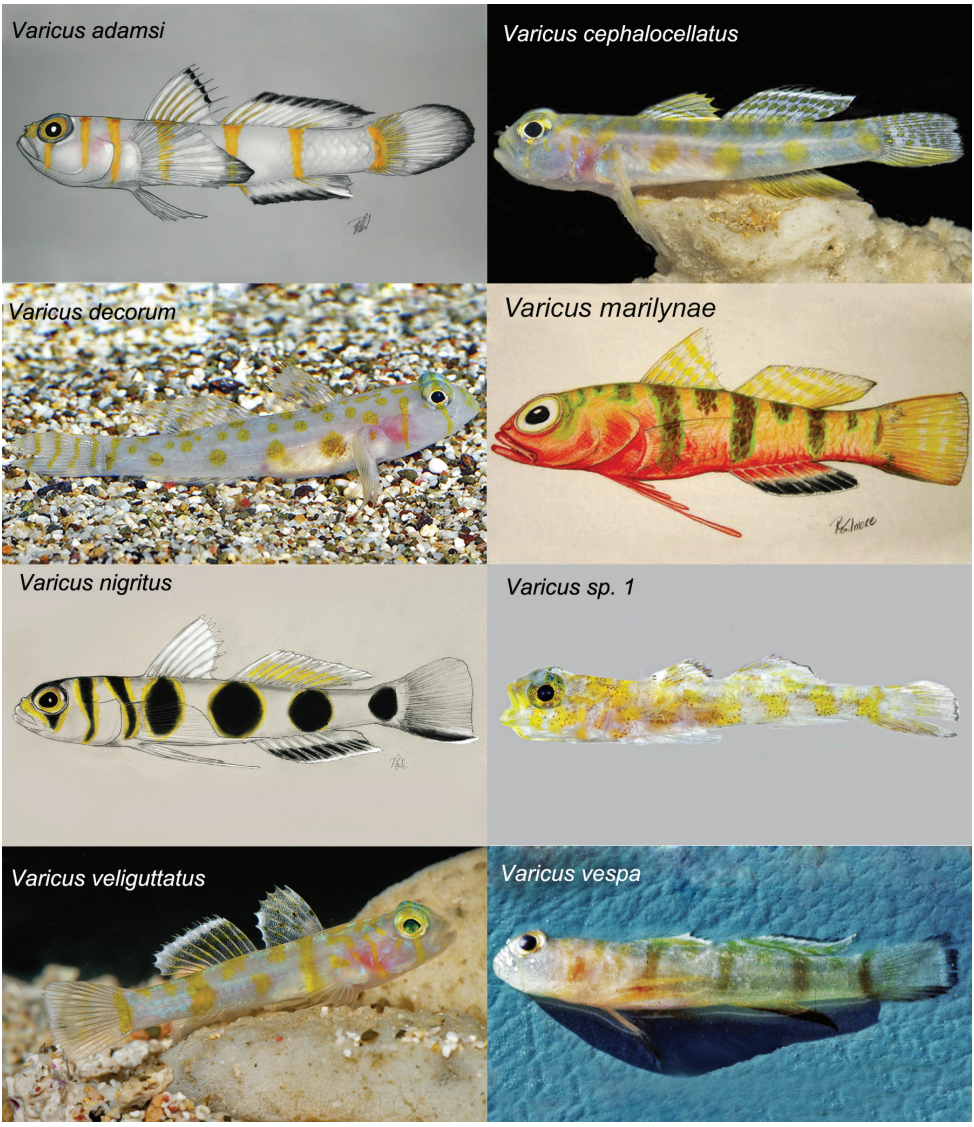

Figure 6. Coloration of species of *Varicus*. All illustrations by R. Grant Gilmore. Photographs by Barry Brown, Ross Robertson and Carole Baldwin (*Varicus sp.* 1), and the crew of the R/V Bellows (for *V. vespa*). Photos of *V. bucca* and *V. benthonis* not available.

brown bars on a white body and white fins with black borders in *V. vespa*. While *V. lacerta* has a yellowish body with indistinct broad dark bars, uniformly yellow dorsal and caudal fins, and a yellow anal fin accentuated with dark orange, in *V. marilynae* Gilmore, 1979 the body is yellow above, reddish orange below, the body bars are narrow, green-edged and dark brown, the dorsal and tail fins have narrow yellow stripes and bars, and the anal fin is red with a black border.

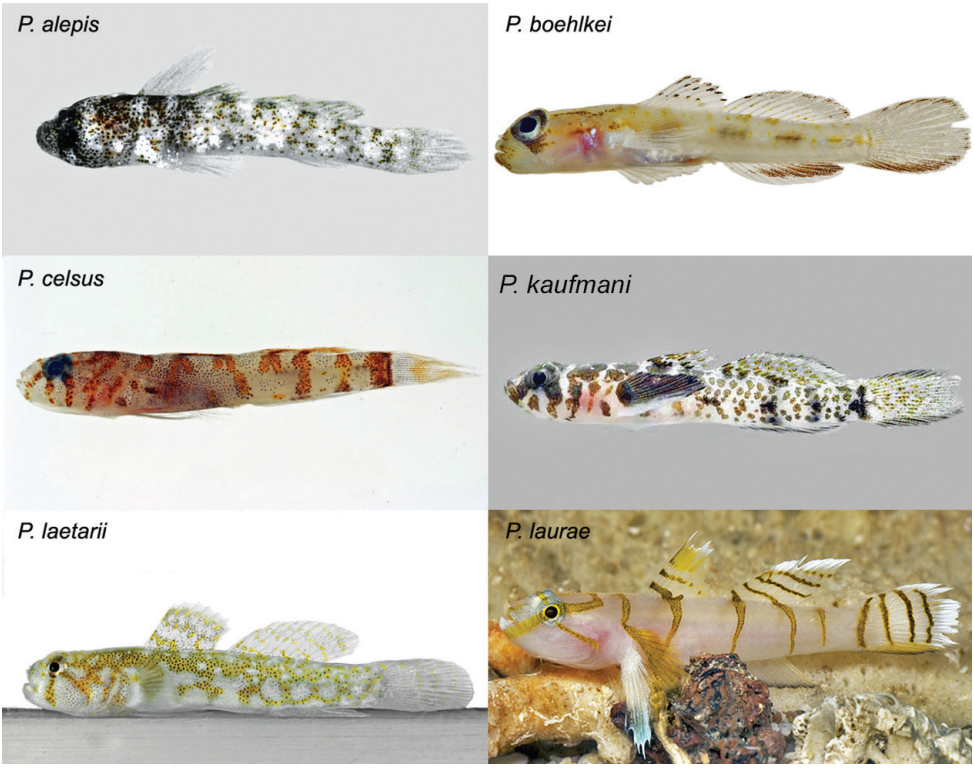

Figure 7. Coloration of species of *Psilotris*. Photos by Luiz Rocha, Jeffrey Williams, Ross Robertson, Barry Brown, and James Van Tassell.

The absence of scales and the presence of highly branched pelvic rays without fleshy tips make this species superficially similar to species of *Psilotris*. No single morphological character unambiguously distinguishes *Varicus* from *Psilotris*, but the most consistent morphological feature thus far is the presence of a single anal-fin pterygiophore inserted before the first haemal spine in *Varicus* versus two in all but one species of *Psilotris*. *Psilotris laurae* Van Tassell, Tornabene & Baldwin, 2016, has a single pterygiophore anterior to the haemal spine, and it is the only known deep-reef species of *Psilotris*. The relationship between anal-fin pterygiophore pattern and habitat depth warrants further investigation.

Despite the morphological similarities between *V. lacerta* and species of *Psilotris*, the new species is easily distinguished by live coloration (Fig. 7). Only three members of *Psilotris* have discrete body bars: *P. alepis* Ginsburg, 1953, with irregular grey bars on a white body speckled with black and brown; *P. celsa* Böhlke, 1963, with irregular narrow orange bars on a white body and head; and *P. laurae*, with narrow, strongly defined dark-yellow bars on a white head and body. *Psilotris boehlkei* Greenfield, 1993, *P. kaufmani* Greenfield, Findley & Johnson, 1993, and *P. laetarii* Van Tassell & Young, 2016, lack bars. *Varicus lacerta* can also be distinguished from *P. boehlkei*, *P. celsa*, *P. kaufmani* and *P. laurae* by having I,7 anal-fin rays (vs. > I,7; Table 1), and from

Table 1. Meristic and papillae characters for *Varicus* and *Psilotris*. AP = anal pterygiophores inserted anterior to haemal spine.

Species	Second dorsal	Anal	Pectoral	AP	Papillae rows 5i/5s	Body Scales	Basicaudal Scales
Varicus adamsi	I,9	I,7–8	18	1	connected	present	present
Varicus benthonis	I,8	I,7	16	1	separate	present	present
Varicus bucca	I,9	I,7–8	16–19	1	connected	present	present
Varicus cephalocellatus	I,10	I,9	19–20	1	variable	present	present
Varicus decorum	I,9	I,7–8	17	1	connected	absent	present
***Varicus lacerta* sp. n.**	**I,9**	**I,7**	**18**	**1**	**connected**	**absent**	**absent**
Varicus marilynae	I,8	I,7	16–18	1	connected	present	present
Varicus nigritus	I,9	I,8	17	1	connected	present	present
Varicus veliguttatus	I,8	I,6–7	17–19	1	connected	present	present
Varicus vespa	I,9	I,7 (rarely I,6 or I,8)	15–17	1	separate	present	present
Psilotris alepis	I,9 (rarely I,8)	I,7–8	15	2	separate	absent	absent
Psilotris boehlkei	I,9–10	I,9	16–18	2	separate	absent	absent
Psilotris celsa	I,9–10 (rarely I,8)	I,9–10 (rarely I,8)	16–17	2	connected	absent	absent
Psilotris kaufmani	I,10 (rarely I,9)	I,10 (rarely I,9)	16–19	2	connected	absent	absent
Psilotris laetarii	I,9–10	I,7–8	15–17	2	connected	absent	absent
Psilotris laurae	I,9	I,8	18	1	connected	absent	absent

P. alepis, *P. celsa*, and *P. laetarii* in having 18 pectoral-fin rays (vs. <18; Table 1). The connection of papillae rows 5i and 5s further distinguishes *V. lacerta* from *P. alepis* and *P. boehlkei*, in which rows 5i and 5s are separate.

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