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# Southern Ocean Echinoids database – An updated version of Antarctic, Sub-Antarctic and cold temperate echinoid database

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## Abstract

This database includes over 7,100 georeferenced occurrence records of sea urchins (*Echinodermata*: *Echinoidea*) obtained from samples collected in the Southern Ocean (+180°W/+180°E; -35°/-78°S) during oceanographic cruises led over 150 years, from 1872 to 2015. Echinoids are common organisms of Southern Ocean benthic communities. A total of 201 species is recorded, which display contrasting depth ranges and distribution patterns across austral provinces and bioregions. Echinoid species show various ecological traits including different nutrition and reproductive strategies. Information on taxonomy, sampling sites, and sampling sources are also made available.

Environmental descriptors that are relevant to echinoid ecology are also made available for the study area (-180°W/+180°E; -45°/-78°S) and for the following decades: 1955–1964, 1965–1974, 1975–1984, 1985–1994 and 1995–2012. They were compiled from different sources and transformed to the same grid cell resolution of 0.1° per pixel. We also provide future projections for environmental descriptors established based on the Bio-Oracle database (Tyberghein et al. 2012).

## Keywords

Echinoidea, oceanographic features, Southern Ocean, Antarctic, Sub-Antarctic

## Project description

**Project title:** Species distribution modelling of Echinoids in the Southern Ocean

**Personnel**: Salomé Fabri-Ruiz, Thomas Saucède, Bruno Danis, Bruno David

### Funding

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### Study area descriptions/ descriptors

The study area extends from the Antarctic continent in the south to 35° S latitude to the north; it comprises the sub-Polar, Antarctic, Polar Frontal, and sub-Antarctic zones. The Southern Ocean is characterized by unique oceanographic features mainly including an unusually deep continental shelf ranging from 450 m to 1000 m depth (Clarke and Johnston 2003), and the Antarctic Circumpolar Current (ACC), the strongest and largest current of the planet that flows clockwise from west to east around Antarctica (Barker and Thomas 2004) and conditions marine species dispersal (Griffiths et al. 2009). Four major marine fronts are distributed from north to south : Subtropical Front (STF), Sub-Antarctic Front (SAF), Polar Front (PF), Antarctic Divergence (AD), and separate water masses of different physical and biotic properties (Sokolov and Rintoul 2002, Roquet et al. 2009).

One of these major fronts is the Polar Front that acts as a biogeographic barrier to the dispersal of many invertebrates between sub-Antarctic and Antarctic waters (Koubbi 1993, Clarke et al. 2005).

### Design description

Nowadays, ecological niche modelling is commonly used in macroecological and biogeographic studies to enhance mapping and understanding of species distribution patterns. Models also constitute useful tools for marine area management purposes (Sánchez-Carnero et al. 2016), predicting invasive species distribution (Václavík and Meentemeyer 2012), identifying biodiversity hot spots and highlighting potential impacts of climate change on species distribution (Elith and Leathwick 2009). Extensive and consistent databases are essential to biogeographic studies to explore species distribution patterns in the Southern Ocean (De Broyer and Koubbi 2014). Reliability and robustness of distribution models are mainly conditioned by the quality and accuracy of occurrence data (Graham et al. 2007, Lobo 2008, Osborne and Leitão 2009). With this in mind, the

creation of SCAR-Marbin in 2005 (Griffiths et al. 2011) and RAMS in 2010 (De Broyer and Danis 2011) allowed the first Antarctic marine biodiversity data compilation.

Objectives of our project are to produce robust and reliable species distribution models at the scale of the Southern Ocean, an area where distribution data are very heterogeneous and sampling gaps frequent.

This requires consistent and comprehensive datasets. For this purpose, an extensive echinoid occurrence dataset was compiled, updated, and checked for accuracy. This dataset is presented here.

Taxonomic information was updated according to the most recent literature. For example, *Sterechinus bernasconiae* Larrain, 1975 is now considered a junior synonym of *Gracilechinus multidentatus* (Clark, 1925) (Saucède et al. 2015). We checked for taxonomic accuracy using the World Echinoidea database (Kroh and Mooi 2017) and experts knowledge. However, mentions of former species identifications are kept in the dataset and clearly distinguished from updated taxonomy.

The dataset includes historical data sampled in the Southern Ocean over a century and a half from the Challenger expedition to the most recent oceanographic campaigns led on the Kerguelen Plateau, in Adelie Land and around the Antarctic Peninsula (Figure 1). All compiled georeferenced locations were scanned and checked for accuracy.

*DatasetName* links the origins of occurrence records which are from academic collections (British Antarctic Survey Collection, Burgundy University collection, …), published articles, former databases (David et al. 2005, Pierrat et al. 2012) or cruise reports.

In order to quantify sampling effort, a 3° by 3° cell grid was shaped (Clarke et al. 2007) and each record of the database was assigned to a grid cell. Following Griffiths et al. (2011) sample and species numbers were both counted for each grid cell using ArcGIS v10.2 (ESRI 2011) and Microsoft Access (2013).

We also provide oceanographic features as environmental maps for physical and abiotic parameters that are relevant to echinoid ecology. Environmental data come from the World Ocean Circulation Experiment 2013 database and depth data come from ETOPO1 (Amante and Eakins 2009). Cell resolution was set up at 0.1 degree with the R 3.3.0 software. These data needed to be corrected for precise depth accuracy, which was performed using ArcGIS and following the protocol proposed by Guillaumot et al. (2016). A seafloor temperature layer was generated based on available temperatures for multiple depth layers of the water column. However, due to missing data, some values were interpolated using the nearest neighbour method with Arctoolbox (ESRI 2011).

## Sampling effort and data description

The database includes more than 7,100 georeferenced records (Figure 1). It is an updated version of the former database "Antarctic, Sub-Antarctic and cold temperate echinoid database" (Pierrat et al. 2012) that contains 1,000 additionnal records compared to Pierrat et al. 2012. This new version includes new records from the most recent oceanographic campaigns led in the Southern Ocean (e.g. POKER II, PRO-

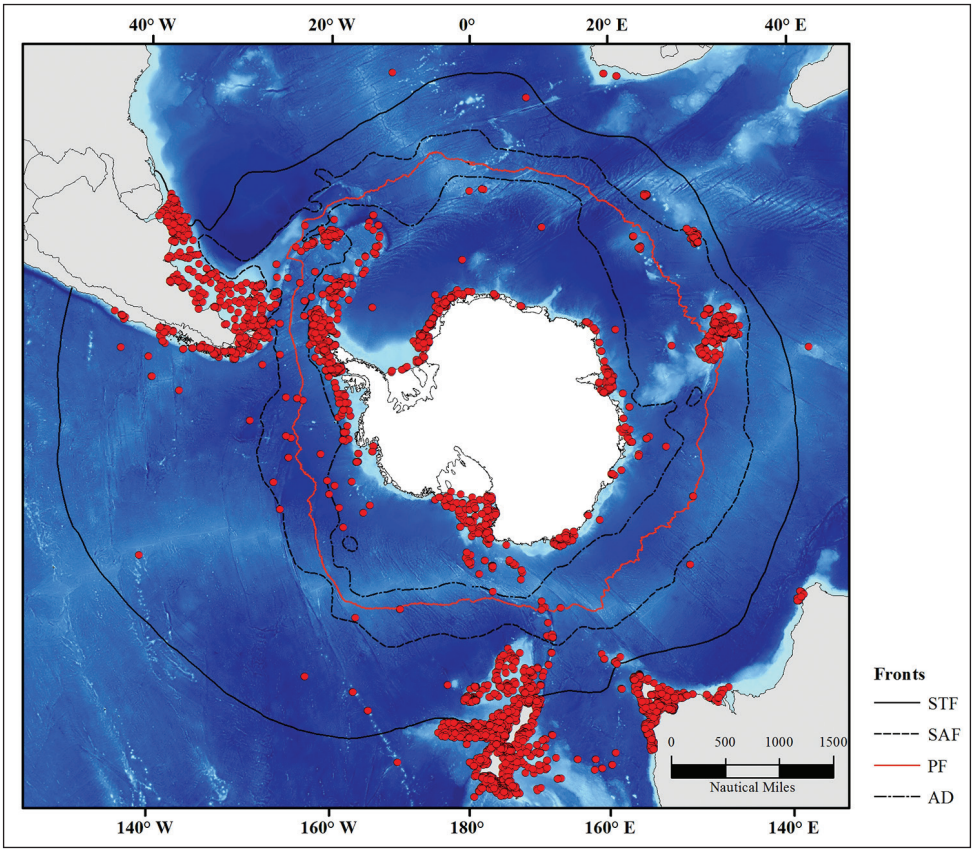


**Figure 1.** Echinoid occurrence records in the Southern Ocean with major marine fronts

TEKER, ANT-XXIX/3) and recent reviews of academic collections (e.g. Smithsonian Institution Museums). In addition, taxonomy and georeferenced positions were updated and checked for accuracy. records.

Sampling effort has long been heterogeneous in the Southern Ocean. It has been the highest along the Antarctic Peninsula and off New Zealand (>200 samples), two areas characterized by a high species number (25–30) (Figure 2a, 2b). In contrast, the number of species remains low (2–5 species) in the region of the Kerguelen Plateau while it has been intensively sampled as well (POKER 2 and PROTEKER cruises).

Our knowledge of genus and species distributions is strongly biased by the quality of sampling effort. Figure 3 highlights the link between the number of samples available and the recorded number of species and genera per grid cell.

Several areas have been little sampled including the waters close to the sea ice margin and deep oceanic basins, most records being concentrated in the first 400 meters (Figure 4) and in the vicinity of scientific stations like in the north of the Kerguelen Plateau, in Adelie Land or along the Antarctic Peninsula. Conversely, the South Kerguelen Plateau and the west of the Ross Sea have been little explored. These under-sampled parts of the Southern Ocean constitute challenging areas for future

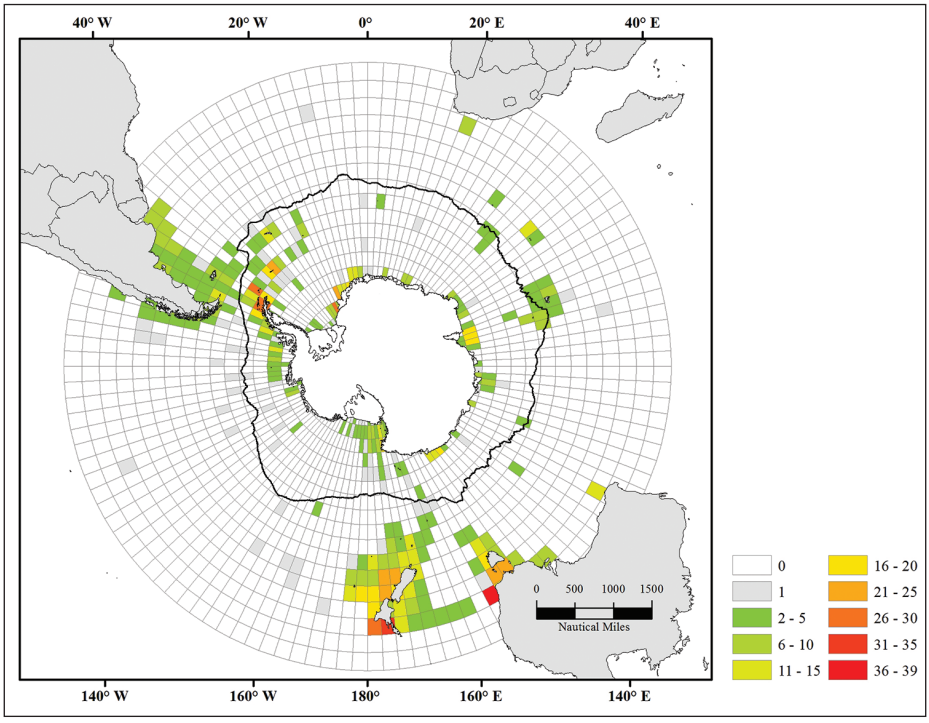


**Figure 2a.** Number of species recorded per grid cell

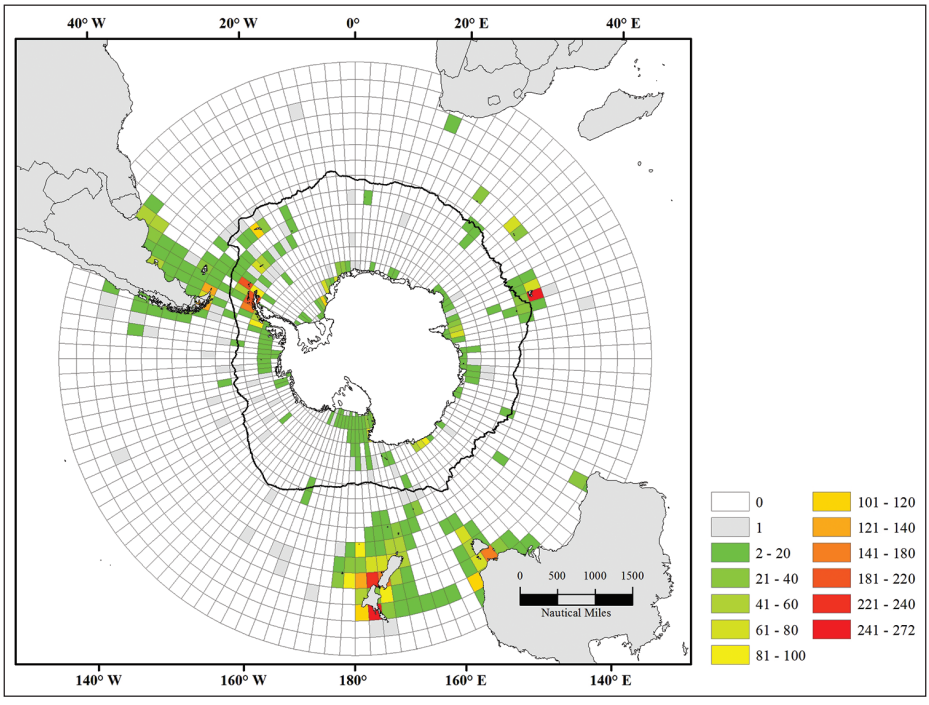


**Figure 2b.** Number of samples recorded per grid cell

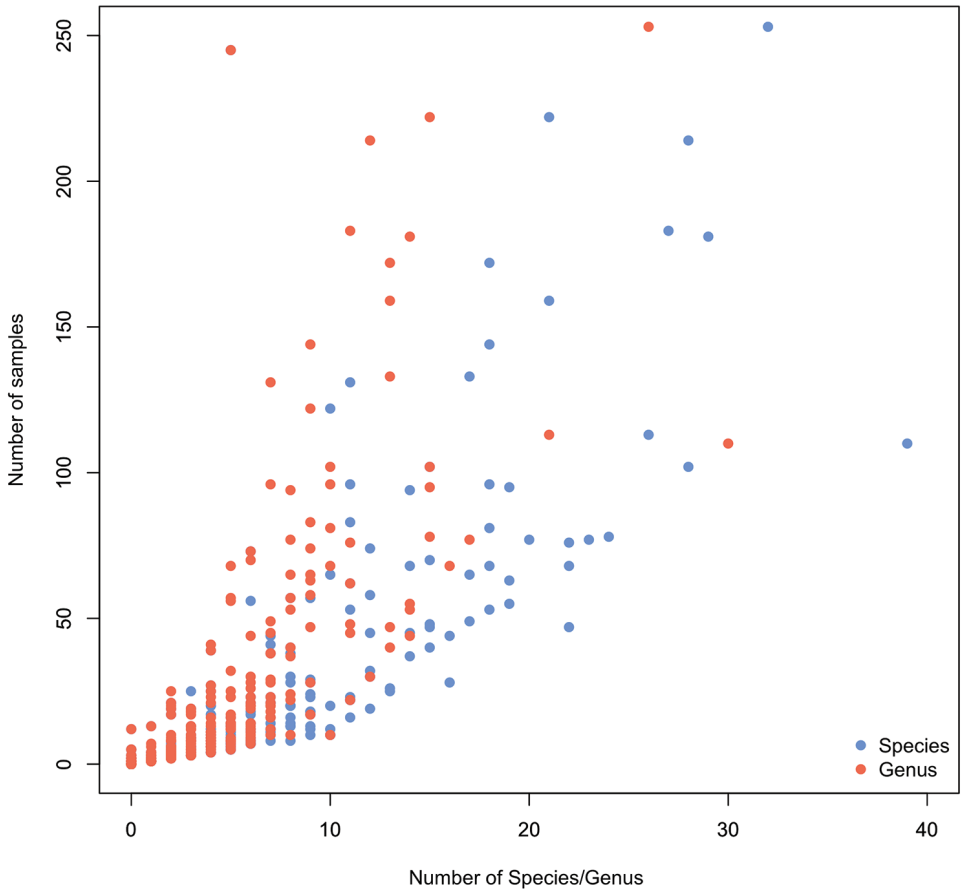


**Figure 3.** Number of recorded samples against species (blue dots) and genus (red dots) richness per grid cell in the Southern Ocean.

scientific cruises. However, new sampling technics and standardizations over the last few years improved our knowledge of the Southern Ocean biodiversity (Kaiser et al. 2013). Common tools have been developed like ecological niche modelling in order to interpolate occurrence records to under-sampled areas and allow improving our knowledge of species potential distribution areas.

## Latitudinal gradient

Main biogeographic features of Southern Ocean echinoids is a constant decrease of genus richness southward whereas species richness decreases from 35°S to 60°S, increases from 60°s to 65°S, then decreases again southward until 70°S (Figure 5). Such a pattern has already been published for Southern Ocean echinoids (Saucède et al. 2014) and herein supported by new data addition. The high number of species recorded between

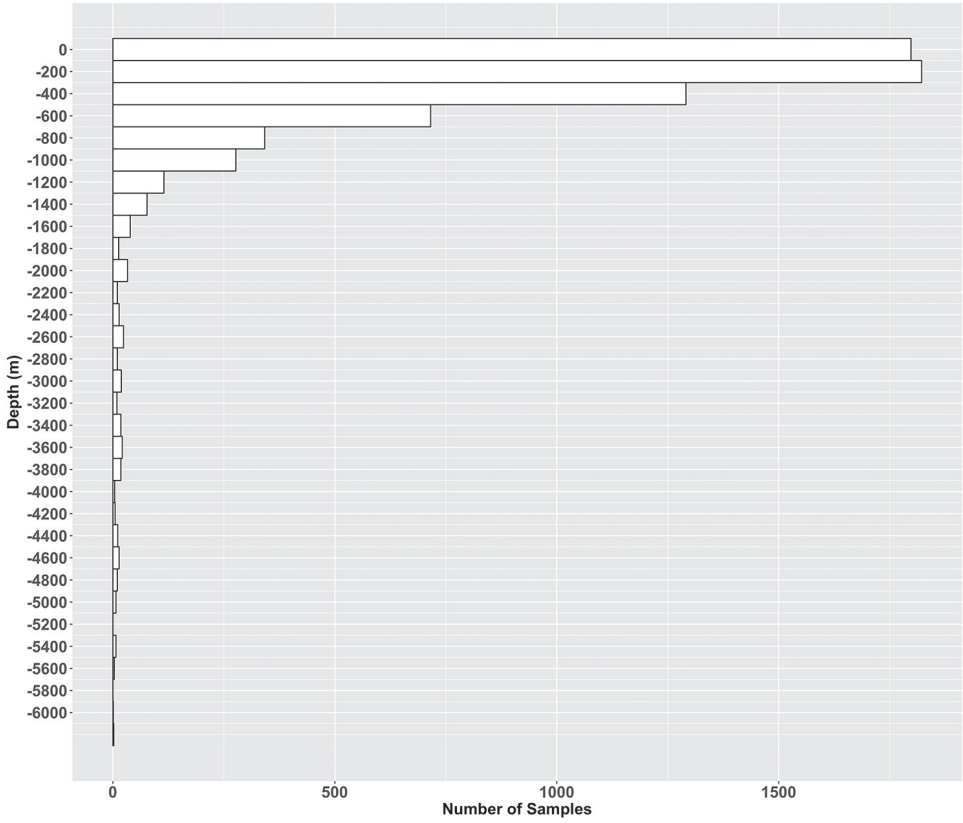


**Figure 4.** Number of occurrences according to depth (m)

60°S and 65°S could be due to the high sampling effort devoted to the region of the Antarctic Peninsula (Figure 2a–b) while conversely, sampling effort decreases southward until 70°S.

## Environmental data

Environmental data were compiled from the following sources: Smith and Sandwell 1997, Boyer et al. 2013, Douglass et al. 2014. Environmental data are provided in raster format (Fabri-Ruiz et al, 2017). Mean surface temperature, mean seafloor temperature, mean surface salinity, and their respective amplitudes (winter minus summer averages) were calculated for the following decades: [1955 to 1964], [1965 to 1974], [1975 to 1984], [1985 to 1994] and [1995 to 2012]. Future projections are provided for mean surface temperature and salinity and for different IPCC scenarios (A2, A1B, B1) (IPCC, 5$^{th}$) they were downloaded from the Bio-Oracle database (Tyberghein et al. 2012).

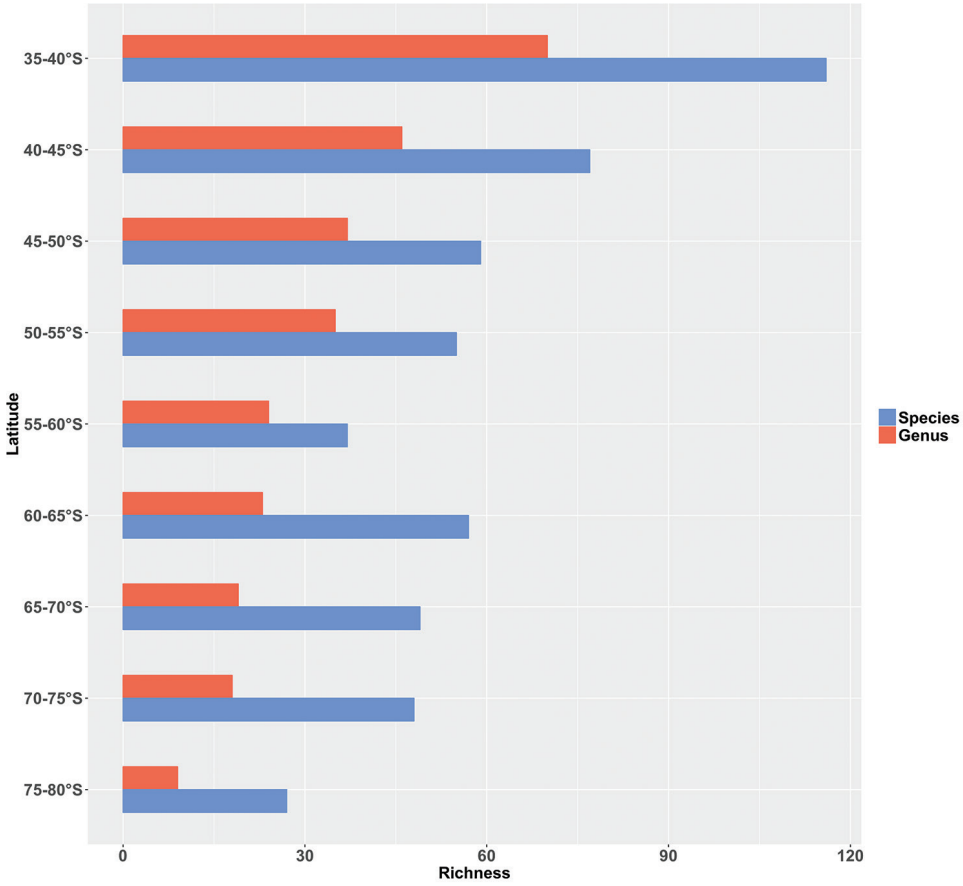


**Figure 5.** Species (blue) and genus (red) richness against latitude

## Taxonomic coverage

### General taxonomic coverage

The database includes occurrence records of all echinoid species reported in the Southern Ocean from the Antarctic continent to 35°S latitude. Echinoids are common organisms of Southern Ocean benthic communities. They have contrasting depth ranges and distribution patterns across austral provinces and bioregions, ranging from coastal areas to the abyssal zone. Echinoid species show various ecological traits including different nutrition and reproductive strategies. In total, 201 species belonging to 31 families were recorded. Many of them are endemic to the Southern Ocean.

## Taxonomic ranks

**Kingdom**: Animalia
**Phylum**: Echinodermata
**Class**: Echinoidea
**Order**: Arbacioida, Camarodonta, Cassiduloida, Cidaroida, Clypeasteroida, Diadematoida, Echinoida, Echinothurioida, Holasteroida, Neognathostomata, Pedinoida, Salenioida, Spatangoida, Temnopleuroida.
**Family**: Arbaciidae, Arachnoididae, Arbaciidae, Aspidodiadematidae, Asterostomatidae, Brissidae, Clypeasteridae, Ctenocidarinae, Cyclasterinae, Diadematidae, Echinidae, Echinolampadidae, Echinometridae, Echinothuriidae, Fibulariidae, Hemiasteridae, Kamptosomatidae, Laganidae, Loveniidae, Mellitidae, Palaeotropidae, Pedinidae, Phormosomatidae, Plexechinidae, Pourtalesiidae, Saleniidae, Schizasteridae, Spatangidae, Temnopleuridae, Toxopneustidae, Urechinidae.
**Genus:** *Abatus*, *Aceste*, *Amblypneustes*, *Ammotrophus*, *Amphipneustes*, *Anametalia*, *Antrechinus*, *Apatopygus*, *Aporocidaris*, *Araeosoma*, *Arbacia*, *Aspidodiadema*, *Austrocidaris*, *Brachysternaster*, *Brisaster*, *Brissopsis*, *Brissus*, *Caenocentrotus*, *Caenopedina*, *Calveriosoma*, *Centrostephanus*, *Ceratophysa*, *Clypeaster*, *Coelopleurus*, *Ctenocidaris*, *Cyclaster*, *Cystechinus*, *Cystocrepis*, *Delopatagus*, *Dermechinus*, *Diadema*, *Echinocardium*, *Echinocrepis*, *Echinocyamus*, *Echinolampas*, *Echinosigra*, *Echinus*, *Encope*, *Eupatagus*, *Evechinus*, *Fellaster*, *Fibularia*, *Genicopatagus*, *Goniocidaris*, *Gracilechinus*, *Gymnopatagus*, *Helgocystis*, *Heliocidaris*, *Hemiaster*, *Heterobrissus*, *Histocidaris*, *Holopneustes*, *Hygrosoma*, *Kamptosoma*, *Linopneustes*, *Loxechinus*, *Mellita*, *Microcyphus*, *Moira*, *Notocidaris*, *Ogmocidaris*, *Orechinus*, *Pachycentrotus*, *Paleotrema*, *Paramaretia*, *Peronella*, *Phormosoma*, *Phyllacanthus*, *Pilematechinus*, *Plexechinus*, *Polyechinus*, *Poriocidaris*, *Pourtalesia*, *Prionocidaris*, *Protenaster*, *Pseudechinus*, *Pseudoboletia*, *Rhopalocidaris*, *Rhynchocidaris*, *Salenia*, *Salenocidaris*, *Salmaciella*, *Solenocystis*, *Spatagocystis*, *Spatangus*, *Sperosoma*, *Sterechinus* , *Stereocidaris*, *Stylocidaris*, *Temnopleurus*, *Tetrapygus*, *Toxopneustes*, *Tripneustes*, *Tripylaster*, *Tripylus*, *Tromikosoma*, *Urechinus*.

## Spatial coverage

**General spatial coverage:** Southern Ocean
**Coordinates:** 79°0'0"S and 35°0'0"S Latitude; 180°0'0"W and 180°0'0"E Longitude

## Temporal coverage

Temporal coverage: 1872–2015

## Datasets

### Dataset occurrence description

Occurrence of echinoids in the Southern Ocean from 1872 to 2015.
**Object name:** Echinoids_occurrences_Southern_Ocean
**Character encoding:** x-MacRoman
**Format name:** Darwin Core Archive Format
**Distribution:** http://ipt.biodiversity.aq/resource?r=echinoids_occurrences_southern_ocean
**Publication of data:** 2017-06-22
**Language:** English
**Metadata language:** English
**Date of metadata creation:** 2017-06-22
**Hierarchy level:** Dataset

### Environmental parameters description

Environmental descriptors for the Southern Ocean were compiled from various sources but most of them come from the World Ocean Atlas (Boyer et al. 2013) for current parameters. Available data are mean surface temperature, mean seafloor temperature, mean surface salinity and their respective amplitudes (winter minus summer averages) were calculated for the following decades: [1955 to 1964], [1965 to 1974], [1975 to 1984], [1985 to 1994] and [1995 to 2012]. Future projections are provided for mean surface temperature and salinity and for different IPCC scenarios (A2, A1B, B1) (IPCC 5$^{th}$); they were downloaded from the Bio-Oracle database (Tyberghein et al. 2012).

**Object name:** Environmental_data_Southern_Ocean
**Format name:** Raster
**Distribution:** data.aad.gov.au/metadata/records/Environmental_data_Southern_Ocean doi:10.4225/15/5949ba54ca33c
**Publication date of data:** 2017-05-18
**Language:** English
**Metadata language:** English
**Date of metadata creation:** 2017-05-18
**Hierarchy level:** Dataset

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# On the identity of *Leptoxis taeniata* – a misapplied name for the threatened Painted Rocksnail (Cerithioidea, Pleuroceridae)

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## Abstract

The Painted Rocksnail, currently known as *Leptoxis taeniata*, is a federally threatened species native to the Mobile River basin in Alabama, USA. Presently restricted to four disjunct populations, the species is at considerable risk of extinction after a range decline of over 95% in the 20th century because of habitat alteration following impoundment of the Coosa River. Here, we reassess the identity and historical range of the Painted Rocksnail to improve communication and conservation efforts for the species. We determined that *L. taeniata* is a synonym of *L. picta* and that the name *L. taeniata* has been misapplied to the current concept of the Painted Rocksnail for which *L. coosaensis* is the oldest available name. *Leptoxis coosaensis* and *L. picta* are herein redescribed. After examination of historical material, we determined that records of the Painted Rocksnail outside the Coosa River drainage were misidentifications. Thus, we redefine the historical range of the Painted Rocksnail as restricted to the Coosa River and select tributaries above the Fall Line at Wetumpka, Alabama, rather than extending into the Alabama River as previously thought. *Leptoxis coosaensis* is in dire need of conservation, and management plans should take into consideration the revised historical range of the species.

**Keywords**
Gastropoda, snails, Pleuroceridae, nomenclature, taxonomy, Mobile River Basin

## Introduction

The Painted Rocksnail is a riverine gastropod in the family Pleuroceridae that is listed as threatened under the US Endangered Species Act (Clark 1998). It is currently restricted to four disjunct locations in the Coosa River drainage in Alabama: Choccolocco Creek, Buxahatchee Creek extending into Watson Creek, Ohatchee Creek, and Coosa River in the tailwater below Logan Martin Dam (Fig. 1). The Painted Rocksnail is characterized by a globose shell with an inflated body whorl, a large, ovate aperture, and a very low spire (Figs. 2, 3). Most individuals are prominently banded, with four reddish brown spiral bands that are usually interrupted (Figs. 2, 3); the head-foot is orange with mottled black patches and has a black band across the head and another across the middle of the snout (Fig. 4). Painted Rocksnails lay eggs in small clutches of approximately 3–5 eggs with limited organic and/or inorganic matter incorporated into egg casings (Whelan et al. 2015). Given its threatened status, an evaluation of the identity of the Painted Rocksnail was necessary to facilitate communication about the species, and to clarify its historical range, both of which are vital for management and recovery efforts (Hartfield 2005).

The scientific name of the Painted Rocksnail is *Leptoxis taeniata* (Conrad, 1834) (Conrad 1834b), originally described under the name *Anculotus taeniatus* Conrad, 1834 from the Alabama River at Claiborne. *Anculotus* is an incorrect subsequent spelling of *Anculosa* Say, 1821, which is a junior objective synonym of *Leptoxis*. Conrad characterized *L. taeniata* as, "oval, or oblong; olivaceous, with dark green spiral bands, four on the body whorl; one whorl of the spire not eroded, often longitudinally produced" (Conrad 1834b: 63). This vague description could apply to several Mobile River drainage *Leptoxis* species including *L. picta* (Conrad, 1834) (Conrad 1834a), *L. ampla* (Anthony, 1855), and the current concept of the Painted Rocksnail. In the original description, Conrad (1834b) did not provide a figure nor did he designate a holotype or indicate a repository for the type material. Baker (1964) designated a lectotype (ANSP 27620 [as "27620a"]; Fig. 5F) from a lot of five possible syntypes in the Academy of Natural Sciences of Philadelphia (ANSP) that was labeled as "Ex auct" (i.e. "from the author"). The locality was noted simply as "Alabama", rather than Alabama River or Alabama River at Claiborne as in the original description. Consequently, there could be some question as to whether these were syntypes and, thus, whether Baker's designation is valid. However, as stated, the material was received from Conrad and the ANSP has type material of other taxa named by Conrad in 1834. Although the locality information is incomplete, the original label is missing, and the display label may have included only abbreviated locality information. Thus, there is insufficient evidence to overturn Baker's lectotype designation. The collections of the Museum of Comparative Zoology at Harvard (MCZ) contain a lot of four additional paralectotypes

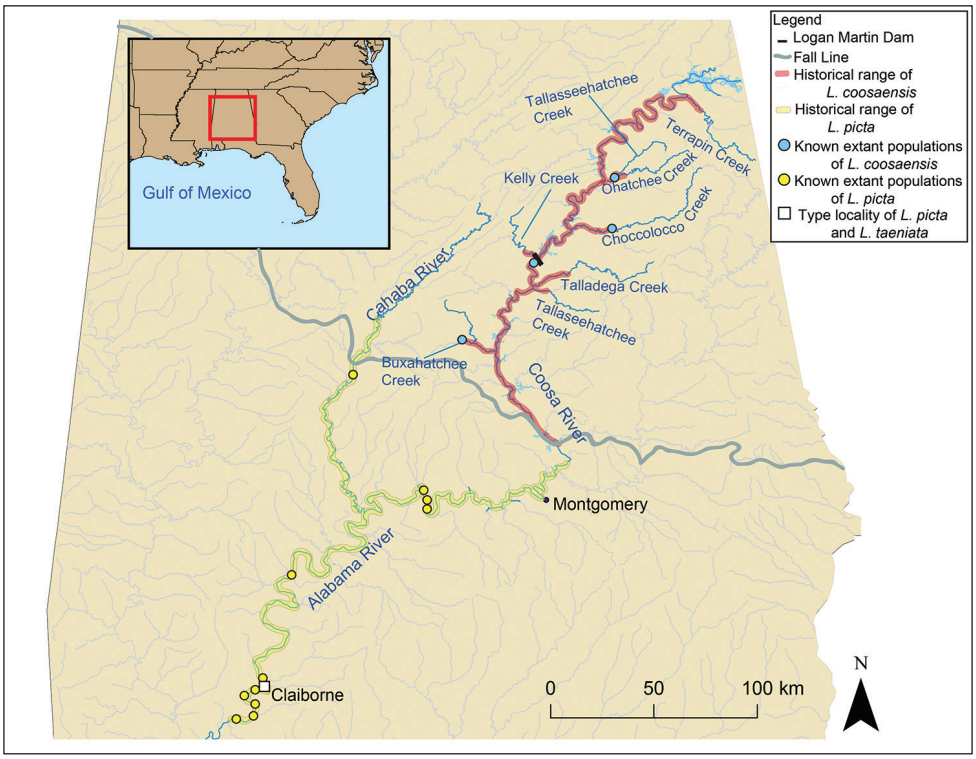


**Figure 1.** Map showing historical range of the Painted Rocksnail, *L. coosaensis*, and the Spotted Rocksnail, *L. picta*.

(MCZ 294987) bearing an original A.A. Gould label stating "Anc. taeniatus Conr. Claiborne Al. from Conrad" (Fig. 5E). We also located an uncatalogued lot of four specimens containing another possible paralectotype at the Natural History Museum in London (NHMUK; *ex* Cuming collection), again labeled with the locality simply as "Alabama" (Fig. 5D). The latter lot is accompanied by an original J.G. Anthony label, annotated in his hand, "the separate one is authentic, marked so by Conrad himself, are not the others mature forms of the same?" Although one specimen is no longer conspicuously separated from the rest, we have concluded that the smallest specimen within the lot may be the specimen referred to in Anthony's note. Although it was received from Conrad, given the ambiguity about the identity of the specimen Anthony was referring to, and the locality inconsistency, we consider it only a possible type.

Pleurocerid species display high levels of morphological variation in their shells, which can overlap between close relatives (Goodrich 1922; Whelan et al. 2015). Consequently, confidently identifying species using shells alone can be difficult, particularly for poorly localized historical material including types. As such, we carefully examined possible type material of *L. taeniata* to determine if it matches the current concept of the Painted Rocksnail. Baker's lectotype (ANSP 27620) has a less inflated body whorl and is more elongately conical than the Painted Rocksnail. The paralectotypes

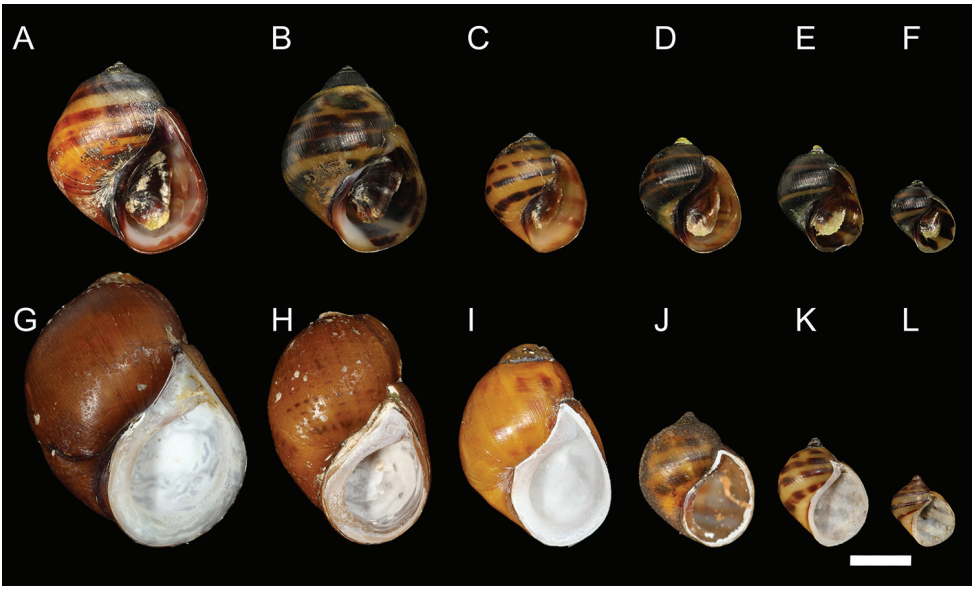


**Figure 2.** Growth series. **A–F** Adult (**A–C**) and juvenile (**D–F**) shells of the Painted Rocksnail, *Leptoxis coosaensis*. All individuals grown in captivity. **G–L** Adult (**G–I**) and juvenile (**J–L**) shells of the Spotted Rocksnail, *Leptoxis picta*. Juveniles grown in captivity. Scale bar: 4 mm.

in lot ANSP 413583 (formerly 27620) (Fig. 5G–I), particularly the adult specimens (Fig. 5G, H), are more clearly not representative of the Painted Rocksnail, indicating that the lectotype is a slightly atypical shell. The specimen labelled by Anthony at NHMUK (Fig. 5D) has a more narrowly ovate aperture than that seen in Painted Rocksnails. MCZ 294987, the one lot explicitly from the type locality (Fig. 5E), has a shell morphology that clearly does not conform to the current concept of the Painted Rocksnail (Figs. 2, 3). Its body whorl is more narrowly conical and the aperture is more narrowly ovate, rather than broadly ovate in large adults. Painted Rocksnails also usually have more impressed sutures. Overall, the possible type material of *L. taeniata* (Fig. 5) does not conform with the current concept of the Painted Rocksnail.

In addition to examining type and historical material (Fig. 5), we evaluated pleurocerid collections from the Alabama River made in the last 30 years to help determine the identity and range of the Painted Rocksnail. No modern survey has recovered the Painted Rocksnail from any location in the Alabama River (Garner et al. 2011). These surveys included over 190 hours of dive time since 1990 in the Alabama River (JT Garner, unpubl. data). Furthermore, examination of historical museum collections has failed to produce a single lot from the Alabama River that corresponds to the current concept of the Painted Rocksnail. Lots labeled as "*L. taeniata*" from the Alabama River are misidentified specimens, usually of *L. picta* (e.g. UF 82371, ANSP 65451 Fig 5L-O; also see photographs uploaded to FigShare, https://doi.org/10.6084/m9.figshare.5084272.v1); lots identified as "*L. taeniata*" from the Cahaba River drainage are also misidentified, usually of specimens of *L. ampla* (e.g. UF 81652,

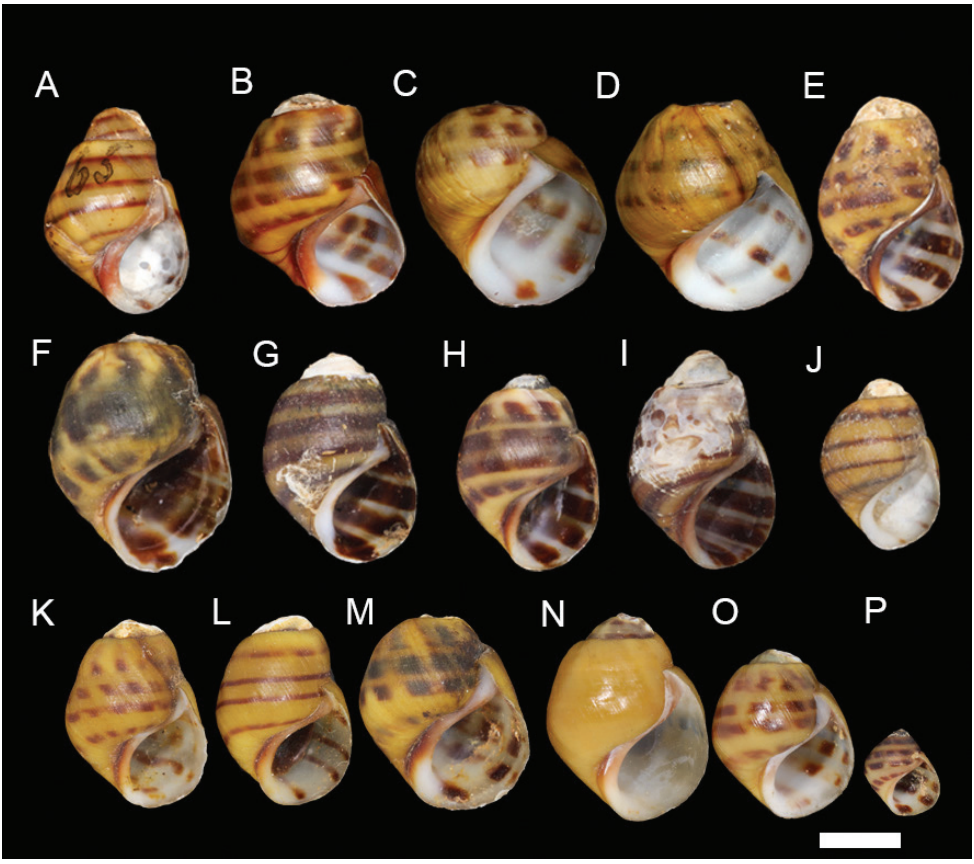


**Figure 3.** Type material of *L. coosaensis* and its synonyms and other specimens showing conchological variation seen in *L. coosaensis*. **A** USNM 121295, *Anculosa coosaensis* Lea, 1861 (lectotype) **B** UMMZ 101139, *Anculosa aldrichi* Goodrich, 1922 (holotype) **C** UMMZ 10144, *Anculosa brevispira* Goodrich, 1922 (holotype) **D** UMMZ 10145, *Anculosa choccoloccoensis*, Goodrich, 1922 (holotype) **E** USNM 1456804, *Anculosa coosaensis* Lea, 1861 (paralectotype) **F–I** USNM 121294 *Anculosa coosaensis* Lea, 1861 (paralectotypes) **J** USNM 121296 *Anculosa coosaensis* Lea, 1861 (paralectotype) **K, L** USNM 504866 "*Leptoxis taeniata*". **N–P** USNM 336408 "*Leptoxis taeniata*". Scale bar: 5 mm.

USNM 519194; see photographs uploaded to FigShare, https://doi.org/10.6084/m9.figshare.5084272.v1). Currently, the Alabama River near Claiborne hosts healthy populations of other pleurocerids including *L. picta*, *Pleurocera prasinata* (Conrad, 1834) (Conrad 1834b), and multiple *Elimia* species (Garner et al. 2011). Therefore, we doubt that the apparent failure to collect the Painted Rocksnail from its ostensive type locality for over 150 years reflects extirpation of the species at that site.

We also considered the historical range of the Painted Rocksnail as presently understood from a biogeographic perspective. The Alabama River is exclusively below the Fall Line, a major physiographic break that separates the Gulf Coastal Plain from the Appalachian Highlands (Renner 1927). No other *Leptoxis* species from the Coosa

River above the fall line has a historical range that extends into the Alabama River or Coastal Plain physiographic region. *Leptoxis ampla*, a Cahaba River endemic and the sister species to the Painted Rocksnail (Whelan et al. 2015), is also found only above the Fall Line. Therefore, if the Painted Rocksnail was historically found in both the Alabama River and the Coosa River above Wetumpka, the species would represent a significant departure from distribution patterns seen among Mobile River basin *Leptoxis* species.

Despite examining records at seven major natural history collections [ANSP, MCZ, NHMUK, University of Michigan Museum of Zoology (UMMZ), National Museum of Natural History (USNM), North Carolina Museum of Natural Sciences (NCMNS), and Florida Museum of Natural History (UF)], we have not located a single lot from the Alabama River that could conclusively be identified as the Painted Rocksnail. After careful examination of historical collections and consideration of both contemporary surveys and broad biogeographic patterns, we conclude that all possible type material of *L. taeniata* more closely resembles *L. picta* than the current concept of the Painted Rocksnail.

*Leptoxis picta* was described from the same location as *L. taeniata*. The original description of *L. picta* is sufficiently vague that it could be applied to multiple Mobile River drainage *Leptoxis* species. *Leptoxis picta* was described as, "Shell sub-oval, shoulder obtusely rounded; aperture ovate, large; columella callous above; epidermis olive, with numerous quadranglular small spots disposed in revolving lines, strongly marked in the aperture" (Conrad 1834a: 342–343). To differentiate the two species, Conrad (1834b) noted that *L. picta* often had pigmentation spots and that *L. taeniata* had dark green bands, but both patterns have been documented in both species (Figs. 2, 3, 5). Further, *Leptoxis picta* was described as inhabiting pebble bars, whereas *L. taeniata* was observed to inhabit friable calcareous banks and siliceous breccias (Conrad 1834b). We question whether any pleurocerid species could be reliably distinguished based on minor differences in habitat preference as we have often observed individuals of the same *Leptoxis* species to inhabit many different microhabitats (i.e. near the banks and in the main current, both pebble and bedrock substrates; Whelan et al. pers. obs.).

## Results

Taking all the above into consideration, we have concluded that the type material of *L. taeniata* and *L. picta* represents the same taxonomical species and that the two are synonyms. *Leptoxis picta* was described four months prior to *L. taeniata* and thus has priority under Article 23 of the International Code of Zoological Nomenclature (ICZN). Nevertheless, the current concept of the Painted Rocksnail represents a unique monophyletic clade in phylogenetic analyses of *Leptoxis* species, and possesses body coloration patterns (Fig. 4) and egg laying behavior different from that of *L. picta* (Whelan et al. 2015). Misapplication of the name *Leptoxis taeniata* to specimens from the Coosa River apparently became widespread after the publication of Tryon (1873),

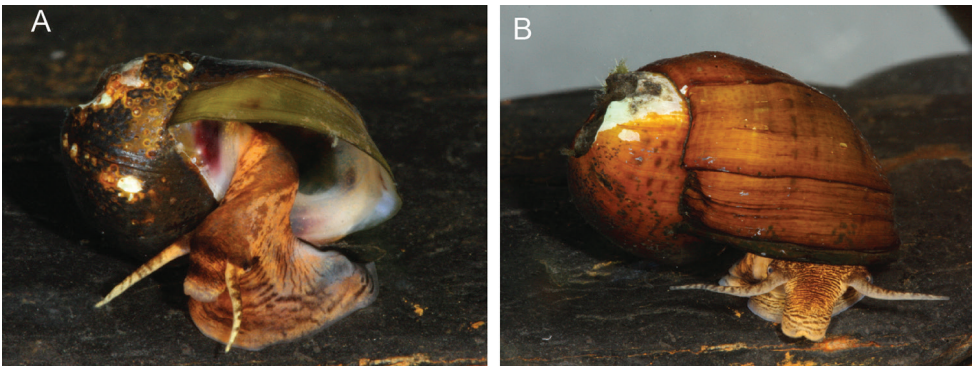


**Figure 4.** Photographs of live animals. **A** Painted Rocksnail (*L. coosaensis*) **B** Spotted Rocksnail (*L. picta*)

when *L. coosaensis* (Lea, 1861) was synonymized with *L. taeniata*. The name *taeniata* does not meet requirements of Art. 23.9.1 of the ICZN that prevailing usage must be maintained because the name has not been used as valid in 25 or more publications in the last 50 years (Burch 1982; Burch and Tottenham 1980; Dillon and Lydeard 1998; Holznagel and Lydeard 2000; Johnson et al. 2013; Lee et al. 2006; Lydeard et al. 1997; Lydeard et al. 1998; Strong and Köhler 2009; Tolley-Jordan et al. 2015; Whelan 2016; Whelan et al. 2015). In such instances, one possible course of action would be conservation of prevailing usage by designation of a neotype under Art. 75.6, which would require a request to the Commission to use its plenary powers to set aside any existing name-bearing types and select a neotype. However, the name *L. taeniata* has long been associated with an incorrect historical distribution for the Painted Rocksnail and further use of the name could perpetuate this error and create confusion for future management plans. Thus, rather than maintain prevailing usage, we here prefer to recognize the oldest available name for the Painted Rocksnail, which we have determined to be *Leptoxis coosaensis* (Lea, 1861).

## Systematics

### *Leptoxis coosaensis* (Lea, 1861)

*Anculosa coosaensis* Lea, 1861: 54; 1863a: 257–258, pl. 35, fig. 65; 1863b: 79–80, pl. 35, fig. 65. Lectotype USNM 121295 (Graf, 2001); paralectotypes USNM 1456804 (1 spm), USNM 121294 (4 spms) and USNM 121296 (1 spm) *leg*. Showalter. "Coosa River, Alabama."

*Anculosa aldrichi* Goodrich, 1922: 31, pl. 1, figs. 1, 2. Holotype UMMZ 10139, by original designation; paratype, MCZ 169987 (1 spm). "Coosa River, near mouth of Yellowleaf [*sic*, Yellow Leaf] Creek, Chilton County, Alabama."

*Anculosa brevispira* Goodrich, 1922: 35, pl. 1, fig. 6. Holotype UMMZ 10144, by original designation; paratype MCZ 169991 (1 spm). "Fort William Shoals,

Coosa River, Talladega County, Alabama"; possible paratypes UF 18202 (7 spms). "Coosa River".

*Anculosa choccoloccoensis* Goodrich, 1922: 34, pl. 1, fig. 7. Holotype UMMZ 10145, by original designation; paratype MCZ 169989 (1 spm). "Choccolocco Creek at Jackson Shoals, Talladega County, Alabama."

*Anculosa taeniata lucida* Goodrich, 1944: 42. Type material not located, potentially lost. "Coosa [River] tributaries."

Other references:

*Anculosa coosaensis*—Lea 1863: 257-258, pl. 35, fig. 65; Goodrich 1922: 28, pl. 1, figs. 13, 14.

*Leptoxis taeniata*—Haldeman 1848: 3, pl. 3 figs. 71–72; Burch and Tottenham 1980: 156, figs. 484–486; Burch 1982: 43, figs. 484–486; Lydeard et al. 1997: 117–128; Lydeard et al. 1998: 183–193; Dillon and Lydeard 1998: 113–121, fig. 2; Holznagel and Lydeard 2000: 233–257; Lee et al. 2006: 314–317; Strong and Köhler 2009: 483–502; Tolley-Jordan et al. 2015: 235–249; Whelan et al. 2015: 85–95, fig. 4; Whelan 2016: 221–226. [Not *L. taeniata* of Conrad]

*Anculosa taeniata*—Tryon 1873: 408–409, figs. 813–815. [Not *L. taeniata* of Conrad]

*Anculotus taeniatus*—Reeve 1860: pl. 6, fig. 50. [Not *L. taeniata* of Conrad]

**Other material examined.** UMMZ 10144, Coosa River at Fort Williams Shoals, Talladega County, Alabama (~33.1477°N, 86.4831°W); UMMZ 10139, Coosa River near mouth of Yellow Leaf Creek, Chilton County, Alabama (~32.9566°N, 86.5177°W); UMMZ 10145, Choccolocco Creek at Jackson Shoals, Talladega County, Alabama (~33.5450°N, 86.0896°W); MCZ 169987, Coosa River near mouth of Yellow Leaf Creek, Chilton County, Alabama (~32.9566°N, 86.5177°W); MCZ 169989, Choccolocco Creek at Jackson Shoals, Talladega County, Alabama (~33.5450°N, 86.0896°W); USNM 12068, Coosa River, Alabama; USNM 321181, Duncan's Riffle Coosa River, Chilton County (~32.8057°N, 86.4450°W; USNM 321862, Higgin's Ferry Coosa River, Chilton County, Alabama (~32.8056°N, 86.4448°W); UF 82401, Peckerwood Shoals, Talladega County, Alabama (~33.1176°N, 86.4728°W); UF 416903, Talladega Creek near Nottingham, Talladega County, Alabama (~33.3614°N, 86.2237°W); UF 82358, Coosa River at Fort Williams Shoals, Talladega County, Alabama (~33.1477°N, 86.4831°W); UF 81660, Coosa River at Fort Williams Shoals, Talladega County, Alabama (~33.1477°N, 86.4831°W); UF 82419, Coosa River at Butting Ram Shoals, Coosa County, Alabama (~32.9414°N 86.5159°W); UF 413800, Coosa River at Lonigan Shoals, St. Clair County, Alabama (~33.7627°N, 86.0447°W); UF 81085, Choccolocco Creek at Jackson Shoals, Alabama (33.5450°N, 86.0896°W); UF 18202, Coosa River; UF 416913, Coosa River at Wetumpka, Elmore County, Alabama (~32.5396°N, 86.2056°W); UF 416911, Coosa River at Wetumpka, Elmore County, Alabama (~32.5396°N, 86.2056°W); UF 416908, Waxahatchee Creek 4 mi above mouth, Shelby County, Alabama (~33.0335°N, 86.5787°W); UF 416920, Kellys [*sic*, Kelly] Creek 2 mi above mouth, St. Clair County, Alabama (~33.4348°N, 86.3539°W); UF 416897,

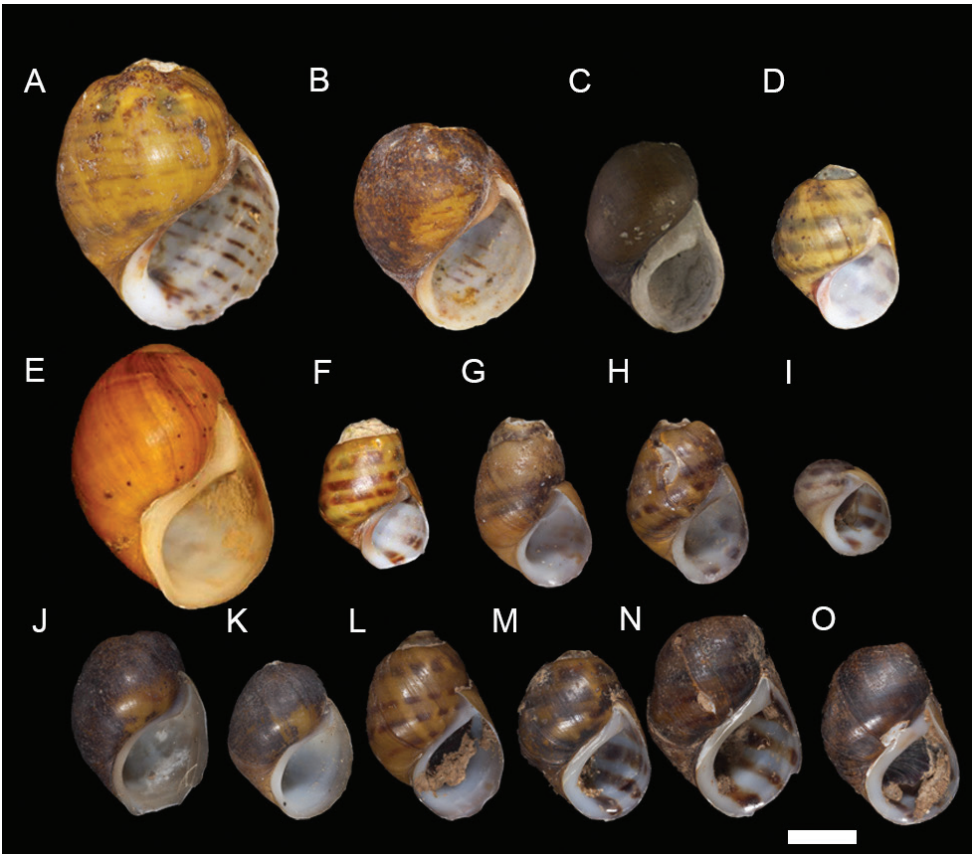


**Figure 5.** Type and topotypic material of *L. picta* and its synonym *L. taeniata*. **A, B** USNM 12074, *Anculosa picta* Conrad, 1834 (possible syntypes) **C** MCZ 294989, *Anculosa picta* Conrad, 1834 (possible syntype) **D** NHMUK uncatalogued, *Anculosa taeniata* Conrad, 1834 (possible paralectotype) **E** MCZ 294987, *Anculosa taeniata* Conrad, 1834 (paralectotype) **F** ANSP 27620, *Anculosa taeniata* Conrad, 1834 (lectotype) **G–I** ANSP 413583, *Anculosa taeniata* Conrad, 1834 (paralectotypes) **J, K** ANSP 187076, "*Leptoxis taeniata*", collected by J.H. McLellan, unknown year (topotypic). **L–O** ANSP 65451, "*Leptoxis taeniata*", collected by C.W. Johnson, 1894 (topotypic). Scale bar: 5mm.

Tallasahatchee [*sic*, Tallaseehatchee] Creek 2-3 mi east of Childersburg, Talladega County, Alabama (~33.2841°N, 86.3098°W); UF 416917, Beeswax Creek, Shelby County, Alabama; NCSM 59354, Terrapin Creek 7 mi south of Centre, Cherokee County, Alabama (~34.0639°N, 85.6146°W); NCSM 59359, Tallahatchie [*sic*, Tallasseehatchee] Creek, Calhoun County, Alabama (33.7785°N, 85.9908°W); NCSM 59358, Ohatchee Creek, Calhoun County, Alabama (33.7801°N, 85.9972°W); USNM 504866, Coosa River, near Wilsonville, Shelby County, Alabama (~33.2162°N, 86.4645°W); USNM 504867, Coosa River 1 mi from Wilsonville, Shelby County, Alabama (~33.2162°N, 86.4645°W); USNM 336408, Coosa River at The Bar, Chilton County, Alabama (~32.7976°N, 86.4348°W); USNM 1437765, Coosa River, Shelby County, Alabama (33.3744°N, 86.3550°W); USNM 1249597, Choccolocco Creek, Talladega County,

Alabama (33.5445°N, 86.0414°W); USNM 1249600, Buxahatchee Creek, Shelby County, Alabama (33.0727°N, 86.6775°W); USNM 1437762, Ohatchee Creek, Calhoun County, Alabama (33.7795°N, 86.0002°W); USNM 1437763, Ohatchee Creek, Calhoun County, Alabama (33.7795°N, 86.0002°W); USNM 1437764, Ohatchee Creek, Calhoun County, Alabama (33.7795°N, 86.0002°W).

**Diagnosis.** Shell ovate, two to four whorls, spire often reduced to obsolete but sometimes elevated with obtuse apex. Aperture large, ovate, at least half the height of body whorl. Reddish brown spiral bands typically present, usually four in number, almost always interrupted. Columella often purple. Head-foot and mantle pigmented orange, mottled with black, with one transverse black band across middle of snout and one transverse black band across middle of head. Clutches small (<6 eggs), with minimal organic and/or inorganic matter incorporated into external casings.

**Historical distribution.** Coosa River above the Fall Line from Wetumpka, Alabama, upstream to the confluence of Terrapin Creek and the Coosa River in Cherokee County, Alabama. Some large Coosa River tributaries including Choccolocco, Buxahatchee, Talladega, and Terrapin creeks.

**Current distribution.** Four disjunct populations: Choccolocco Creek, Talladega County, Alabama; Buxahatchee and Watson creeks, Shelby County, Alabama; Ohatchee Creek, Calhoun County, Alabama; Logan Martin Dam tailwaters of the Coosa River, Shelby-Talladega counties, Alabama.

**Remarks.** USNM 121295 (Fig. 3A) originates from the Lea collection, is from the published *L. coosaensis* type locality, *leg*. Showalter, and is the shell figured by Lea (1863), with the number 65 inked onto the apertural aspect of the body whorl corresponding to the figure number. Lea (1863) indicated that he had six specimens, yet there are seven specimens distributed among the three simultaneously accessioned lots now registered as USNM 121294 (Fig. 3F-I), USNM 1456804 (see photographs on FigShare, https://doi.org/10.6084/m9.figshare.5084272.v1), and USNM 121296 (Fig. 3J). Consequently, either the specimen count in Lea (1863) was in error, or one of the included shells was acquired subsequently and has no type status.

Lea (1861) described *Anculosa coosaensis* from the Coosa River and provided a brief description in Latin. In a subsequent extended description in English, Lea (1863) characterized the species as, "smooth, obtusely conical, thick, dark horn-color, very much banded; spire elevated, obtuse at the apex; sutures very much impressed; whorls four, very much constricted below the sutures, the last large; aperture rounded, white, much banded within; columella thickened, incurved, dark purple; outer lip acute and expanded." In the remarks, he commented that the aperture is more than half the length of the shell and that the bands may be interrupted. This description, as well as the type material (Fig. 3F–J) match the current concept of the Painted Rocksnail (Fig. 2). Although formerly considered a synonym of *L. taeniata*, *L. coosaensis* is the oldest available name for the Painted Rocksnail.

We have been unable to locate type material of *Anculosa taeniata lucida* Goodrich, 1944. No holotype was designated, nor was a figure provided, but based on the original description and the type locality of tributaries of the Coosa River, we conclude that this entity does not merit recognition at the subspecies level and synonymize it with *L. coosaensis*.

***Leptoxis picta* (Conrad, 1834)**

*Anculosa picta* Conrad, 1834a: 343, pl. 1, fig. 16. Possible syntype MCZ 294989 (4 spms); possible syntypes USNM 12074 (2 spms). "Alabama River" [near Claiborne].
*Anculosa taeniata* Conrad, 1834b: 63. Lectotype ANSP 27620 (Baker, 1964; as "27620a"); paralectotypes ANSP 413583 (3 spms; formerly 27620); paralectotypes MCZ 294987 (4 spms); possible paralectotype NHMUK uncatalogued (1 spm). "Alabama River at Claiborne."

Other references:
*Leptoxis picta*—Haldeman 1848: 3, figs. 74–80; Burch and Tottenham 1980: 154, fig. 476; Burch 1982: 42, fig. 476; Lydeard et al. 1997: 117–128; Dillon and Lydeard 1998: 113–121, fig. 2; Holznagel and Lydeard 2000: 233–257; Lee et al. 2006: 314–317; Strong and Köhler 2009: 483–502; Tolley-Jordan et al. 2015: 235–249; Whelan et al. 2015: 85–95, fig. 4.
*Anculosa picta*—Tryon 1873: 415–417, figs. 829–830; Goodrich 1922: 14-15, figs. 34, 35;

**Other material examined.** ANSP 120760, Alabama River, Alabama; ANSP 85033, Cahaba River, Alabama; ANSP 163024, Cahaba River, Lilly Shoals, Bibb County, Alabama (~33.1552°N, 87.0365°W); ANSP 65451, Alabama River at Claiborne, Monroe County, Alabama (31.5512°N, 87.5142°W); ANSP 187076, Alabama River at Claiborne, Monroe County, Alabama (31.5512°N, 87.5142°W); UF 81414, Alabama River at Selma, Dallas County, Alabama (~32.4049°N, 87.0190°W); UF 82371, Alabama River, Alabama; UMMZ 10175, Alabama River at Selma, Dallas County, Alabama (~32.4049°N, 87.0190°W); UMMZ39983, Alabama River at Selma, Dallas County, Alabama (~32.4049°N, 87.0190°W); UMMZ 57813, Cahaba River, 19.3 KM W of Selma, Dallas County, Alabama; USNM 507433, Alabama River at Claiborne, Monroe County, Alabama (31.5512°N, 87.5142°W ); USNM 525014, Alabama River at Claiborne, Monroe County, Alabama (31.5512°N, 87.5142°W ); USNM 121232, Alabama River at Selma, Dallas County, Alabama (~32.4049°N, 87.0190°W); USNM 519212, Alabama River at Selma, Dallas County, Alabama (~32.4049°N, 87.0190°W); USNM 1437744, Alabama River downstream of Benton boat ramp, Lowndes-Autauga Counties, Alabama (32.3214°N, 86.8215°W); USNM 1437745, Alabama River downstream of Benton boat ramp, Lowndes-Autauga Counties, Alabama (32.3214°N, 86.8215°W); USNM 1437749, Alabama River downstream of Benton boat ramp, Dallas-Autauga Counties, Alabama (32.3226°N, 86.8220°W); USNM 1437746, Alabama River at river mile 231.5, Dallas-Autauga Counties, Alabama (32.3413°N, 86.8159°W); USNM 1437750, Alabama River at river mile 70.5, Monroe County, Alabama (31.5914°N, 87.5415°W); USNM 1437758, Alabama River at river mile 223.7, Dallas-Autauga Counties, Alabama (32.4299°N, 86.8308°W); USNM 1437747, Alabama River at river mile 224.7, Dallas-Autauga Counties, Alabama (32.4210°N, 86.8337°W); USNM 1437748, Alabama River at river mile 226.5, Dallas-Autauga Counties (32.4064°N, 86.8463°W);

USNM 1437759, Alabama River at river mile 227.0, Dallas-Autauga Counties, Alabama (32.3941°N, 96.8375°W); USNM 1437753, Alabama River at river mile 46, Clarke-Monroe Counties, Alabama (31.4274°N, 87.6452°W); USNM 1437754, Alabama River at river mile 51.5, Clarke-Monroe Counties, Alabama (31.4372°N, 87.5716°W); USNM 1437756, Alabama River at river mile 54.6, Clarke-Monroe Counties, Alabama (31.4734°N, 87.5620°W); USNM 1437761, Alabama River at river mile 58.0, Clarke-Monroe Counties, Alabama (31.5051°N, 87.6125°W); USNM 1437755, Alabama River at river mile 59.7, Clarke-Monroe Counties, Alabama (31.5196°N, 87.6205°W); USNM 1437760, Alabama River at river mile 64.3, Monroe County, Alabama (31.5559°N, 87.5611°W); USNM 1437757, Alabama River at river mile 75.0, Monroe County, Alabama (31.5898°N, 87.5391°W); USNM 1437752, Alabama River at river mile 75.8, Monroe County, Alabama (31.5923°N, 87.5407°W); USNM 1437743, Alabama River at river mile 128.6, Wilcox County, Alabama (32.0409°N, 87.4118°W); USNM 1437744, Alabama River at river mile 233.0, Lowndes-Autauga Counties, Alabama (32.3214°N, 86.8215°W); USNM 1437751, Alabama River 2.4 KM downstream of US Highway 84 bridge, Monroe County, Alabama (31.5455°N, 87.5367°W).

**Diagnosis.** Shell globose, larger shells elongately globose, two to three whorls, spire reduced to obsolete. Reddish brown spiral bands typically present on smaller shells, often faded on larger shells, usually four in number, often interrupted. Headfoot and mantle pigmented orange, mottled with black. Egg clutches spiral, 10-11 eggs per clutch on average, with minimal organic and/or inorganic matter incorporated into external casings.

**Historical distribution.** Alabama River from Claiborne, Alabama, upstream to mouth of Coosa River. Coosa River below Wetumpka. Goodrich (1922) reported *L. picta* from as far upstream as bars of the Coosa River below Wetumpka; although we have not examined any lots of *L. picta* from the Coosa River, we consider this record reliable as it is below the Fall Line. In the Cahaba River, from its confluence with the Alabama River upstream to Lily Shoals in Bibb County, Alabama.

**Current distribution.** Disjunct populations in the Alabama River from river mile 46.0 in Monroe-Clarke counties, upstream to approximately river mile 231.5, near the Lowndes/Dallas county line. One recently reintroduced population in the Cahaba River at Centreville, Bibb County, Alabama (P.D. Johnson *unpublished data*.)

**Remarks.** Baker (1964) doubtfully listed ANSP "120960a?" (*sic*, error for 120760a; now ANSP 120760) as the possible "TOM" of *L. picta* from among a lot of 16 specimens. Baker stated "TOM" as meaning, "type because only one example was included in the original description, or was indicated by only one set of dimensions (of course the first) or by reference to a (cited) illustration(s) of only one shell, in the definition proper, exclusive of additional remarks." He considered use of this abbreviation as a "type by subsequent selection" (Baker 1963: 191). Consequently, his use of the abbreviation "TOM" could be a valid lectotype designation under certain circumstances. However, as Baker placed a question mark after the catalogue number indicating uncertainty that the specimen selected was the type by original measurement, he did not unambiguously select a specimen to act as the name bearing type as required by Art. 74.5 (ICZN) and

hence this does not constitute a valid lectotype designation. Furthermore, there is no evidence that the lot has any type status; it originated from the Wheatley collection via the University of Pennsylvania and there is no evidence on the labels or in the original ANSP ledger that the lot was obtained from Conrad. It is possible that Baker knew that Wheatley received material from Conrad, but the original label is not in Conrad's handwriting (G. Rosenberg, pers. comm.). Despite Conrad stating that the type material had been deposited in the ANSP, we have been unable to locate any other possible type material during several searches of the collections. USNM 12074 (Fig. 5A, B) and MCZ 294989 (Fig. 5C) both resemble the shell figured by Conrad and have labels indicating they were received from Conrad. However, both lots are accompanied by labels bearing the less-specific locality Alabama, rather than Alabama River or Alabama River at Claiborne. Moreover, as mentioned, the possible syntypes were not found in ANSP, the stated repository of the types. Consequently, it is possible that neither MCZ 294989 nor USNM 12074 are syntypical and so we refrain from designating a lectotype.

Tryon (1873) considered both *Leptoxis foremani* (Lea, 1843) and *L. flammata* (Lea, 1843) to be synonyms of *L. picta*. Burch and Tottenham (1980) restored *L. foremani* to species status, but retained *L. flammata* as a synonym of *L. picta*. Both *L. picta* and *L. foremani* are reciprocally monophyletic and valid species (Whelan et al. 2015). However, based on shell morphology we here consider *L. flammata* and *L. foremani* to be synonyms. As both were described concurrently (Lea 1843), we here take the right of First Reviser (ICZN Art. 24.2) and establish the priority of *L. foremani* over *L. flammata*, making *L. flammata* a subjective junior synonym of *L. foremani*. *Leptoxis zebra* (Anthony, 1860) was also considered by Tryon (1873) to be a synonym of *L. picta*, but the type material (MCZ 161794, see shells photographs on FigShare, https://doi.org/10.6084/m9.figshare.5084272.v1) resembles *L. foremani*. Consequently, we here consider *L. zebra* also to be a junior synonym of *L. foremani*.

## Discussion

Today, the Painted Rocksnail, i.e. *Leptoxis coosaensis*, is mostly restricted to Coosa River tributaries (Fig. 1). In tributary habitats, pleurocerids are generally smaller than main stem conspecifics. Consequently, modern specimens of wild caught individuals typically are smaller and have more eroded spires than those seen in types and some historical material. Juveniles grown in captivity with uneroded spires have four extremely compressed whorls (Fig. 2; Whelan et al. 2015). We failed to find any specimens with costae and consider the specimen with prominent costae figured in Burch and Tottenham (1980: fig. 486) as the Painted Rocksnail to be a probable misidentification, possibly of *L. showalterii*. Pleurocerids are notoriously difficult to identify, and similarities in shell morphology of *L. picta* and *L. coosaensis*, particularly of the juveniles, undoubtedly contributed to the confusion in application of an incorrect scientific name to the Painted Rocksnail for nearly 150 years (Tryon 1873).

*Leptoxis coosaensis* is listed as threatened under the Endangered Species Act as "*L. taeniata*" (Clark 1998). The species is currently restricted to four disjunct popula-

tions in the Coosa River drainage in eastern Alabama. Those in Choccolocco Creek, Ohatchee Creek, and Buxahatchee and Watson creeks appear stable, while the status of the population in the Logan Martin Dam tailwaters is unclear because of low abundance and/or difficulties associated with sampling at this location (i.e. depths that require diving in high flow and poor visibility).

Listing of this species was based, in part, on the misperception that the historical distribution included a long stretch of the Alabama River from which it had been extirpated during the 20th century (Clark 1998). Furthermore, many museum lots identified as "*L. taeniata*" represent taxonomical species different from the Painted Rocksnail, typically of *L. picta* or *L. ampla*. The latter is endemic to the Cahaba River drainage above the Fall Line in Alabama; consequently, these misidentifications have resulted in erroneous reports that the Painted Rocksnail was historically present in the Cahaba River (Burch and Tottenham 1980; Goodrich 1922; Mirachi et al. 2004). Conversely, records of *L. ampla* in the Coosa River drainage are misidentifications, typically of *L. coosaensis*. In light of our reanalysis, the historical range of *L. coosaensis* is here revised to have been restricted to the Coosa River and its tributaries, which is a considerably smaller historical range for the Painted Rocksnail than previously believed (Clark 1998). Nevertheless, the current range reduction of 90% from historical occupancy given by Clark (1998) appears to have been conservative.

The historical range of *L. coosaensis* just in the Coosa River proper is a distance of approximately 317 km (Fig. 1). Since *L. coosaensis* is now known to inhabit less than 10 km of the main stem Coosa River, its range has declined over 95% in that river alone. In addition, *Leptoxis coosaensis* is believed to be extirpated from four of the eight Coosa River tributaries from which it was known. As such, even with a redefined and reduced historical range, this species is in obvious need of continued protection. Management efforts for pleurocerid snails in the Mobile River basin have focused on habitat improvement and captive propagation and reintroduction. Reintroduction should never occur outside a species' historical range, which is one reason why clarifying the range of *L. coosaensis* is so important. Recovery efforts should include a review of historic tributaries that once supported *L. coosaensis* to determine if any sites are appropriate for reintroduction. As with most listed mollusks, establishment of additional populations contributes to species recovery, and is necessary for the possible delisting of the species (Hartfield 2005).

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RESEARCH ARTICLE

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# Biogeography and phylogenetic position of *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. from Maritime Antarctic (Nematoda, Nordiidae)

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## Abstract

The taxonomic position of the endemic Antarctic species *Enchodeloides signyensis* (Loof, 1975), **gen. n.**, **comb. n.** (= *Enchodelus signyensis* Loof, 1975) is discussed on the basis of morphological study, including SEM, morphometric data, postembryonic observations, and sequence data of 18S rDNA and the D2-D3 expansion fragments of the large subunit rDNA. A number of characters such as the cuticle and stoma structures, including the presence of moderately developed cuticularised ring around the oral aperture, peculiarities of pharynx expansion, size and position of the posterior pair of pharyngeal nuclei, a less complex uterus, and the position of a posterior ventromedian supplement show that this species differs substantially from the other members of the genus *Enchodelus*. Furthermore, both the 18S and 28S rDNA-based phylogenetic trees of the *Enchodelus* sequences available in the GenBank formed two distinct clusters with *E. signyensis* being a part of a well-supported group with species of the genus *Pungentus*; therefore, it is proposed that its taxonomic position should be reconsidered.

## Keywords

Distribution, morphology, SEM, SNPs, taxonomy, 18S and D2-D3 rDNA

## Introduction

Antarctic represents unique types of habitats – polar deserts, caused by its geological history, harsh climate conditions, and remoteness. Therefore, terrestrial Antarctic biota, including nematodes, is characterised by a very high degree of endemism and low diversity (Nielsen et al. 2011). Besides, distribution of nematodes exhibits clear biogeographical patterns regarding the two Antarctic ecozones, Continental and Maritime Antarctic (Andrássy 1998a). Order Dorylaimida Pearse, 1942 is represented in Antarctic by nineteen species (12 species described from Maritime Antarctic, 7 species, from the continental part, all but one endemics; of six genera reported from this polar region, two are endemic (Elshishka et al. 2015a). *Enchodelus signyensis* Loof, 1975 is the only representative of the genus *Enchodelus* Thorne, 1939 reported from the southern hemisphere and is an endemic for the Maritime Antarctic. This species was recorded from Signy Island (Spaull 1973) as *Enchodelus* sp. Later Loof (1975) studied Spaull's collections from some of the islands and described this species as *E. signyensis*, naming it after the type locality. Subsequently, Andrássy (1998a) presented a brief description based on a female paratype specimen. Peneva et al. (2002) provided new morphological data about this species from Livingston Island, and described the males. Here new molecular and additional morphological data is presented of adults and juveniles of this species from Livingston and King George Islands, and its taxonomic position discussed.

## Materials and methods

Samples were collected from Livingston Island by Dr. N. Chipev (IBER), Dr. R. Mecheva (IBER), D. Apostolova (Sofia University) and from King George Island by Dr. R. Zidarova (Sofia University) during the regular Bulgarian Antarctic Expeditions (2006-2016). Nematodes were extracted from soils and plant materials by a Baerman funnel method (van Bezooijen 2006) for at least 48 hours, killed by gentle heat, and fixed in 4% formalin. For light-microscopy, specimens were processed in anhydrous glycerine (Seinhorst 1959) and mounted on permanent slides. Drawings were prepared using an Olympus BX 51 compound microscope, equipped with a drawing tube. Photographs were taken using an Axio Imager.M2-Carl Zeiss compound microscope equipped with a digital camera (ProgRes C7) and specialised software (CapturePro Software 2.8). Measurements were made using an Olympus BX 41 light microscope with a drawing tube and digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA) and Digitrak 1.0f computer program (Philip Smith, John Hutton Institute, Dundee, UK).

Specimens used for SEM observations were rinsed in 0.1 M cacodylate buffer (twice for 10 min), post-fixed in 1% $OsO_4$ for 2 h, washed twice for 10 min in 0.1 M cacodylate buffer and dehydrated in an ethanol series (Mutafchiev et al. 2013), immersed in hexamethyldisilazane for 30 min and air dried. They were sputter coated with gold in a JEOL JFS 1200 and examined using a JEOL JSM 5510 microscope at 10 kV.

The locations of pharyngeal gland nuclei are given following Loof and Coomans (1970) and Andrássy (1998b).

### DNA extraction, amplification, and sequencing

Genomic DNA was extracted from two female specimens per species using a standard nematode digestion protocol (Holterman et al. 2006). The specimens used for DNA extraction, amplification, and sequencing were from King George island (*E. signyensis*) and from Rila Mountain (*Enchodelus* sp.). For further details on the procedures used for DNA extraction, amplification, and sequencing, see Nedelchev et al. (2014). Identical sequences were obtained from both individuals of the same species and have been deposited in GenBank with the following accession numbers: for the 18S rDNA KY 881720 (*E. signyensis* gen. n., comb. n.) and KY766261 (*Enchodelus* sp.) and for D2-D3 rDNA KY881719 (*E. signyensis* gen. n., comb. n.) and KY766260 (*Enchodelus* sp.).

### Sequences and phylogenetic analyses

The 18S and D2-D3 28S rDNA sequences were compared with those of other nematode species available at the GenBank sequence database using BLASTN similarity search tool. The sequences revealing the highest similarity were used for sequence and phylogenetic analyses (Meldal et al. 2007; Holterman et al. 2008; Pedram et al. 2009, 2011a; Pedram et al. 2011b; Pedram et al. 2015, etc.). Bayesian Inference (BI) algorithm implemented in MrBayes 3.2.5 was used for reconstruction of phylogenetic relationships (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012). For further details on phylogeny analyses and tree visualisation, see Lazarova et al. (2016). Based on previous studies (Holterman et al. 2006; Elshishka et al. 2015a) *Aporcelaimellus* spp. were selected as an outgroup for both phylogenies. The estimates of evolutionary divergences between sequences/species within and between groups (numbers of base differences and p-distances) were performed with MEGA7 (Kumar et al. 2016). The analyses involved nine nucleotide sequences with 790 and 1666 positions in total for D2-D3 and 18S rDNA, respectively.

## Taxon treatment

***Enchodelus signyensis* Loof, 1975**
Figs 1–6

**Material examined.** Twenty-eight females and twenty-one juveniles (J1-J4) from Livingston and King George Islands (Table 1).

**Description.** Measurements. See Table 2–4.

**Table 1.** Origin of the examined materials of *Enchodeloides signyensis* gen. n., comb. n.

| Site description | Collection year | Abbreviation |
|---|---|---|
| **King George Island (KGI)** | – | – |
| ***Fildes Peninsula*** */Moist brown soil without vegetation, surrounded by moss* | 2013 | **KGI_F** |
| **Livingston Island (LI)** | – | – |
| ***Svetilishteto*** | 2006–2007 | **LI_SV** |
| ***Playa Bulgara*** */Mosses* | 2008 | **LI_M** |
| ***Punta Hesperides*** */Soil under moss crust* | 2010 | **LI_PH** |
| ***Punta Hesperides*** */Soil* | 2016 | **LI_PH_n** |

**Table 2.** Morphometrics of *Enchodeloides signyensis* gen. n., comb. n. (females). All measurements, unless indicated otherwise, are in μm (and in the form: mean±SD (range)).

| Locality | King George Island | Livingston Island | | | |
|---|---|---|---|---|---|
| Characters | **KGI_F** | **LI_SV** | **LI_PH** | **LI_M** | **LI_PH_n** |
| n | 7 | 4 | 3 | 12 | 2 |
| L (mm) | 1.59±0.1 (1.47–1.66) | 1.45±0.05 (1.39–1.49) | 1.43; 1.51; 1.44 | 1.35±0.1 (1.20–1.45) | 1.27, 1.37 |
| a | 28.9±1.9 (26.9–32.8) | 28.1±1.2 (26.7–29.5) | 28.5; 31; 27 | 29.5±1.4 (27.6–32.4) | 26.1, 28.2 |
| b | 5.3± 0.3 (4.7–5.6) | 4.8± 0.2 (4.6–4.9) | 5; 5; 4.8 | 4.5± 0.2 (4.2–4.8) | 4.1, 4.6 |
| c | 43.7±2.1 (40.6–46.9) | 48.2±3.1 (43.8–50.7) | 49.3; 48; 44.8 | 43.7±3.9 (37–50) | 50.1, 53.2 |
| c‘ | 1.0±0.04 (1.0–1.1) | 1.0±0.1 (0.9–1.0) | 0.9; 1.0; 1.0 | 1.0±0.1 (0.8–1.1) | 0.9, 0.9 |
| V % | 50.4±0.7 (49.5–51.5) | 53.8±1.3 (52–55) | 51; 54; 53 | 54.4±1.0 (52–56) | 55, 56 |
| Lip region diameter | 14.3±0.4 (14–15) | 14.2±0.2 (14–14.4) | 14; 14; 15 | 14.1±0.7 (13–15) | 14, 13 |
| Odontostyle length | 19.9±0.8 (19–21) | 18.9±0.7 (18–19.5) | 19; 20; 20 | 19.2±0.8 (18–20) | 18, 19 |
| Odontophore length | 25.2±0.8 (24–26.5) | 26.5±0.4 (26–27) | 25; 23.5; 25 | 26.4±2.8 (22–32) | 27, 26 |
| Anterior end to guiding ring | 12.0±0.7 (11–13) | 12.6±0.3 (12–13) | 12; 11; 12 | 12.1±0.6 (11–13) | 12, 12 |
| Pharynx length | 297.8±11.4 (277–310) | 304.0±3.8 (302–308) | 283; 301; 297 | 302.2±11.8 (271–314.5) | 307, 300 |
| Pharyngeal base diameter | 51.5± 3.8 (45–55) | 46.7±2.4 (44–49) | 47; 45; 47.5 | 43.3± 2.9 (38–46.5) | 45, 45.5 |
| Mid-body diameter | 55.2±3.5 (50–60) | 51.8±1.8 (49–54) | 50; 49; 53 | 45.9±3.8 (39–51) | 48, 48.5 |
| Prerectum length | 104.2±32.2 (72–166) | – | 71 | 84.5±24.5 (62–128) | -, 75 |
| Rectum length | 36.4±3.0 (32–40) | 32, 46 | 30.5; 37; 41 | 33.4±2.0 (31–36.5) | -, 37.5 |
| Tail length | 36.4±2.1 (32–39) | 30.3±2.7 (28–34) | 29; 31.5; 32 | 31.2±3.4 (25–35) | 25, 26 |

*Female.* Habitus curved ventrally after fixation, adopting a C-shape. Cuticle consisting of four layers with different refraction, the outer two layers thinner, the second outer with stronger refraction, the inner layers thicker, especially at tail region. Cuticle 2–3 μm thick at postlabial region at the level of the guiding ring, 2–4 μm at mid-body and 4–6 μm on tail; outer layer with very fine transverse striations, innermost layer coarsely striated (Figs 1, 2). Lip region 4–5 μm high angular (following terminology adopted by Peña-Santiago (2006)), offset from the adjoining body by a constriction; about 3 times as wide as high. Based on SEM photographs (Fig. 3), perioral area high, disc-like structure with apparently four elevations surrounding oral aperture, oral aperture appearing cross-like in shape in frontal view. Labial and cephalic papillae prominent; labial papillae button-like, each surrounded by a small ring, their openings pore-like. Inner labial papillae located at distinct elevations; separated from each other, and far from oral aperture and outer labial papillae; divided from the outer labial and cephalic papillae by a circular striation (Fig. 3). Cephalic papillae button-like; outer labial and cephalic papillae below the margin of oral field. Six radial striations beginning from the oral field interrupted by inner and ending at outer labial papillae. Amphidial fovea cup-shaped, its aperture approximately half of lip region diameter, its margin curved; under SEM, the amphidial aperture with an operculum, however the presence of this structure should be confirmed with further studies. Cheilostom a truncate cone with weakly developed walls, its anteriormost part representing a moderately cuticularised perioral ring, appearing as small perioral refractive dots. Odontostyle short and slender, straight, 18–20 times as long as wide, 1.2–1.6 times lip region diameter, aperture 14–16% of its length, 1.2–1.7% of body length. Odontophore 1.2–1.6 times as long as odontostyle, with small swellings at its base. Guiding ring double, located at 0.8–1.0 times lip region diameter from anterior end. Anterior region of pharynx enlarging gradually; pharyngeal expansion 112.5–134 μm, occupying 37–45% of total pharynx length. Location of pharyngeal gland nuclei and their orifices is presented in Table 3. Distance DO-DN 14–19 μm, nuclei of dorsal and second ventrosublateral glands clearly visible, nuclei of first ventrosublateral glands in most specimens indistinct, located slightly behind the middle of the distance DN-$S_2$N (n = 1). Nuclei of dorsal glands 3.5–5 μm diameter, first and second pair ventrosublateral 1 μm and 2–3 μm, respectively. Excretory pore opposite the nerve ring with slightly cuticularised canal clearly visible at 100–112 μm from the anterior end. Cardia rounded conoid. Prerectum 1.7–4.8, rectum 0.9–1.4 times anal body diameter long. Tail bluntly conoid, 2–3% of body length, with numerous saccate bodies. Hyaline part 4–8 μm wide or 12–25% of tail length. Two pairs of caudal pores present. Both branches of female genital system equally and well-developed (in specimens of Livingston Island shorter: anterior 236.2 ± 23.3 (186–275) μm and posterior 208.2 ± 34.4 (143–259) μm long, in specimens from King George Island anterior 298.3 ± 31.9 (245–330) μm and posterior 323.1 ± 46.4 (243–361) μm long). Ovaries short, rarely reaching sphincter level; oviduct with well-developed *pars dilatata*. Sphincter well developed. Uteri tubular, thick walled, surrounded by hyaline cells along almost the whole length, anterior uterus 104–152 μm long, posterior 105–156 μm long, 2–3 times correspond-

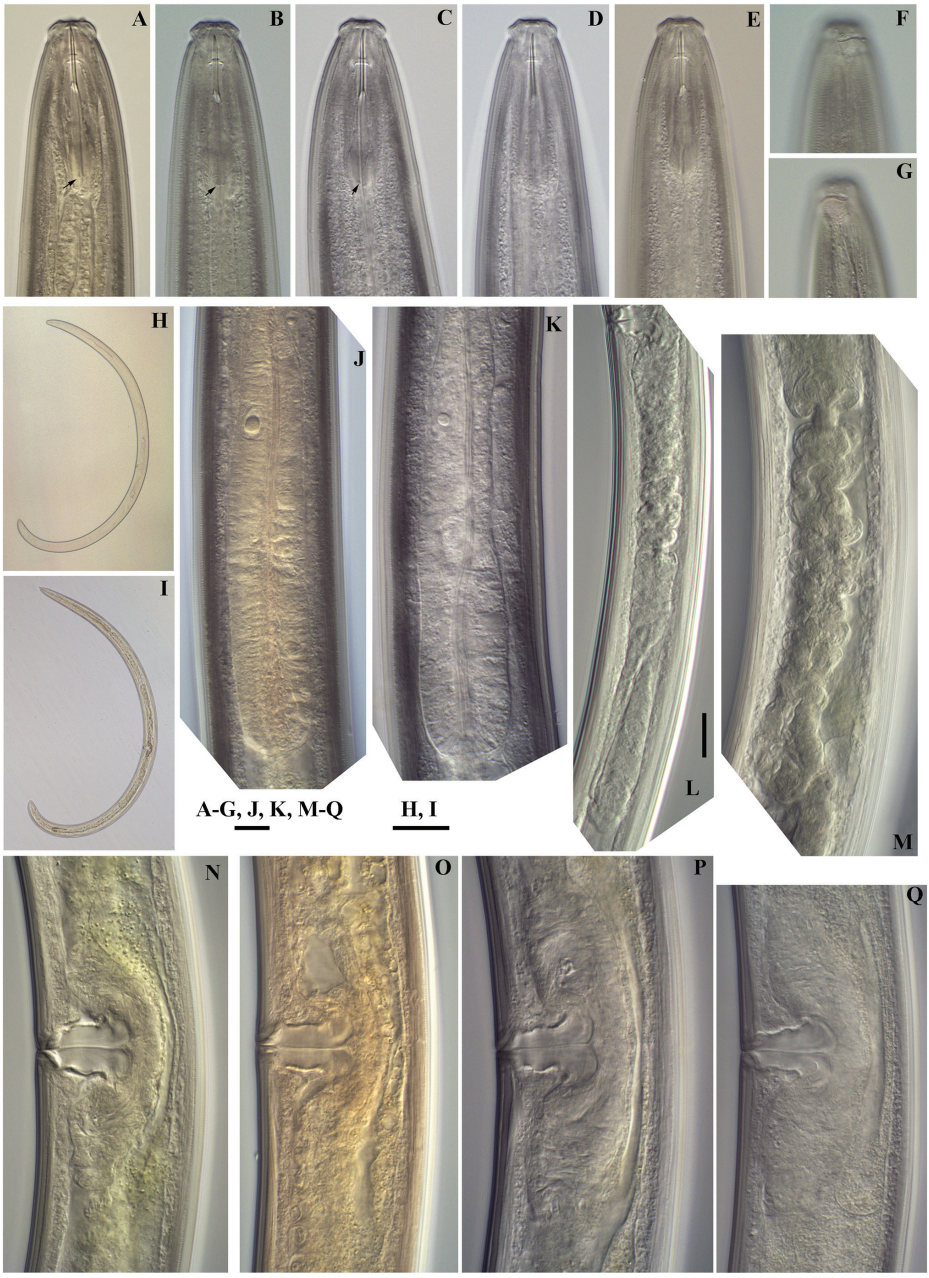


**Figure 1.** *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975). *Female*: **A–E** Anterior region (**A, B** specimens from Livingston Island **C, D, E** specimens from King George Island), black arrows indicate the minute basal swellings **F, G** Amphideal fovea (**E** specimen from Livingston Island **G** specimen from King George Island) **H, I** Entire body **J, K** Pharyngeal bulb (**J** specimen from Livingston Island **K** specimen from King George Island) **L** Posterior genital branch (specimen from Livingston Island) **M** Uterus (specimen from Livingston Island) **N–Q** Vulval regions (**N, O** specimens from Livingston Island **P, Q** specimens from King George Island). Scale bars: 10 μm (**A–G, J, K, M–Q**); 200 μm (**H, I**); 20 μm (**L**).

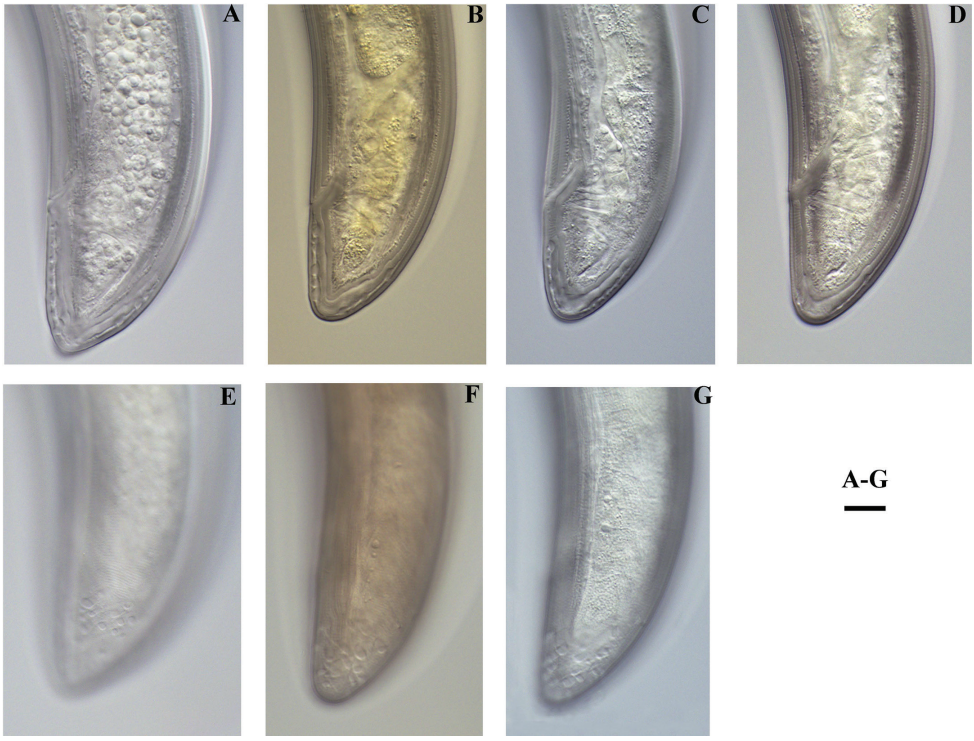


**Figure 2.** *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975). *Female*: **A–D** Tail ends (**A** specimen from King George Island; **B, C, D** specimens from Livingston Island) **E–G** Tail ends with saccate bodies (**E** specimen from King George Island **F, G** specimens from Livingston Island). Scale bar: 10 μm.

ing body diameter, not differentiated. Vulva a transverse slit. Vagina extending inwards for 54–76% of body diameter; *pars proximalis* 19.5–25×12–15 μm, *pars refringens* with two drop shaped sclerotised pieces, with combined width of 11–13 μm, *pars distalis* 4–5 μm long.

*Juveniles.* Based on morphometrics of juvenile specimens and the relationships between the lengths of their functional and replacement odontostyles and body lengths, four juvenile stages were identified (Figs 4–7). Habitus in first juvenile stage slightly ventrally curved, lip region flat, continuous with the body, genital primordium 11–12 μm long, tail conical elongated with long central peg (Figs 4–6). Tail in J2 and J3 conoid elongated in J4 bluntly conoid as in females with numerous saccate bodies on tail, c' decreasing during the successive stages to J4 and females.

*Sequences and phylogenetic analyses.* The phylogenies based on both gene regions showed that *Enchodelus* sp. and *E. signyensis* are parts of two distantly related and well-supported groups (I and II), and in both analyses, they revealed similar relationships with other dorylaimid species (Figs 8, 9). With one exception (AY593052, *E. macrodorus* (de Man, 1880) from The Netherlands), *E. signyensis*, was evolutionary close to *Pungentus* spp. (AY593050, AY593052–53 for D2-D3 28S, and AJ966501

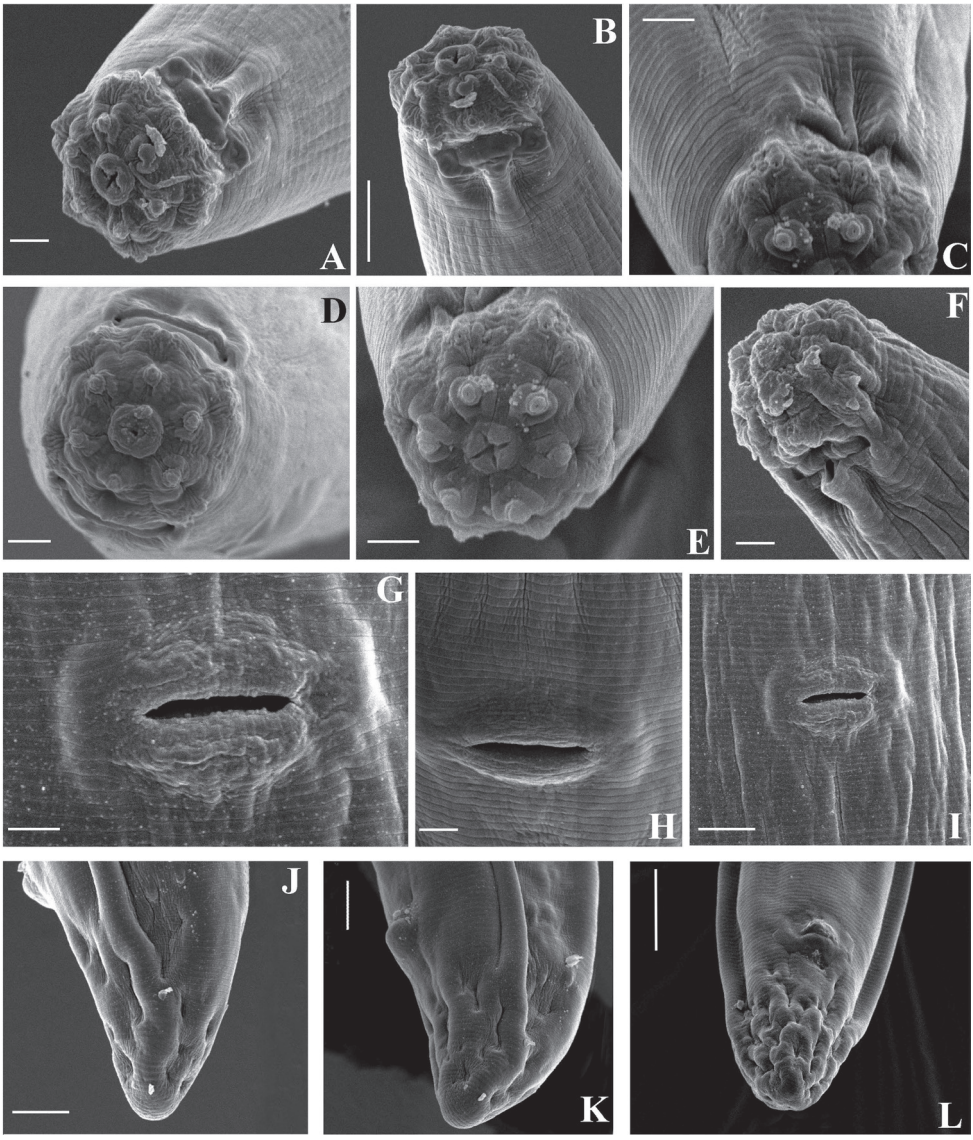


**Figure 3.** SEM micrographs. *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975). *Female*: **A, D, E** Lip region, in face view, amphid aperture **B, F** Lip region, in sublateral view **C** Cephalic and labial papillae **G–I** Vulval region **J–L** Tail ends. Scale bars: 2 μm (**A, C, D, E, F, G**); 5 μm (**B, I, J**); 10 μm (**L**).

and AY284788 for 18S rDNA) while, *Enchodelus* sp. from Bulgaria clustered with other *Enchodelus* spp. from the Netherlands and Iran being a part of well-supported clade including species of various genera (*Eudorylaimus* Andrássy, 1959, *Epidorylaimus* Andrássy, 1986, *Prodorylaimus* Andrássy, 1959 and *Crassolabium* Yeates, 1967).

The estimates of evolutionary divergences (p-distances) between D2-D3 28S rDNA sequences within and between both groups are presented in Table 5. The dis-

**Table 3.** Morphometrics of *Enchodeloides signyensis* gen. n., comb. n. (juveniles). All measurements, unless indicated otherwise, are in μm (and in the form: mean±SD (range)).

| **Locality** | **Livingston Island** | | | | | **King George Island** |
|---|---|---|---|---|---|---|
| Characters | **LI_S** | **LI_M** | | | **LI_PH_n** | **KGI** |
| Stages | J1 | J2 | J3 | J4 | J4 | J4 |
| n | 6 | 1 | 1 | 7 | 1 | 4 |
| L (mm) | 0.40±0.1 (0.37–0.42) | 0.60, 0.62 | 0,7 | 1.02±0.1 (0.93–1.13) | 1.03 | 1.23±0.1 (1.07–1.34) |
| a | 26.5±1.1 (24.7–27.9) | 27.0, 27.5 | 27 | 28.9±1.8 (26.8–31.7) | 26.9 | 28.3±1.6 (26.9–30.4) |
| b | 3.2±0.5 (2.9–4.2) | 3.6, 3.8 | 3,5 | 3.9± 0.2 (3.7–4.1) | – | 4.9, 5.2 |
| c | 13.6±0.9 (12.8–14.8) | 24.7, 26.2 | 27,6 | 36.8±2.3 (33.7–39.6) | 35.5 | 39.0±2.7 (36.5–42.8) |
| c‘ | 2.8±0.2 (2.6–3.1) | 1.5, 1.5 | 1,4 | 1.1±0.1 (1.0–1.2) | 1.1 | 1.0±0.05 (1.0–1.1) |
| Lip region diameter | 7.3±0.2 (7–7.5) | 8.5, 8 | 11 | 11.8± 0.3 (11–12) | 11 | 12.2±0.4 (12–12.5) |
| Odontostyle length | 6.6±0.4 (6–7) | 8, 8 | 11 | 14.7±0.2 (14–15) | 15 | 15.6±0.2 (15–16) |
| Replacement odontostyle length | 8.2±0.2 (8–8.3) | 11, 10 | 14 | 18.5±0.4 (18–19) | 20 | 19.3±0.9 (18–20) |
| Pharynx length | 126.2±15.9 (95–140) | 165.5, 163 | 200.5 | 258.8±14.2 (244–281) | – | 207, 249 |
| Pharyngeal base diameter | 16.0±0.3 (15.6–16.3) | 23, 23 | 27 | 35.3±2.5 (32–40) | 36 | 40.5±3.1 (37–44) |
| Mid-body diameter | 15.0±0.4 (14–15.5) | 22, 23 | 26 | 35.4±2.9 (31–40) | 38.5 | 43.4±4.3 (40–47) |
| Prerectum length | 35 | – | – | 87, 110 | – | 76, 86, 82 |
| Rectum length | 14,5 | – | – | 25.5±2.7 (21.5–28.5) | 26 | 33, 30, 35 |
| Tail length | 29.6±2.2 (27–31) | 24, 24 | 25 | 27.7±1.2 (26–30) | 29 | 31.4±2.1 (29–34) |

similarity between *E. signyensis* and other *Enchodelus* spp. is very high, varying from 16.6% to 17.1% while within **group II** the distances between sequences are between 0.8–7.1%. The dissimilarity within **group I** varies from 0.1% to 7.6% with the highest values (7.4–7.6%) estimated from pair-wise comparison of *E. signyensis* to other sequences within the group. A similar pattern was observed when 18S rDNA evolutionary divergences were analysed. Although having much lower resolution, the 18S rDNA distance of *E. signyensis* to other *Enchodelus* species available at NCBI was 2.6–2.8% (or 44–47 nucleotides). This species was the most closely related to two *Pungentus* spp. from Europe (AJ966501 and AY284788) showing 1.4–1.6% dissimilarity (or 24–26 nucleotides difference). The SNPs analyses of the parsimony-informative sites between sequences for *Enchodeloides* gen. n., *Enchodelus* and *Pungentus* Thorne & Swanger, 1936 and for both genes are given as Suppl. materials 1 and 2.

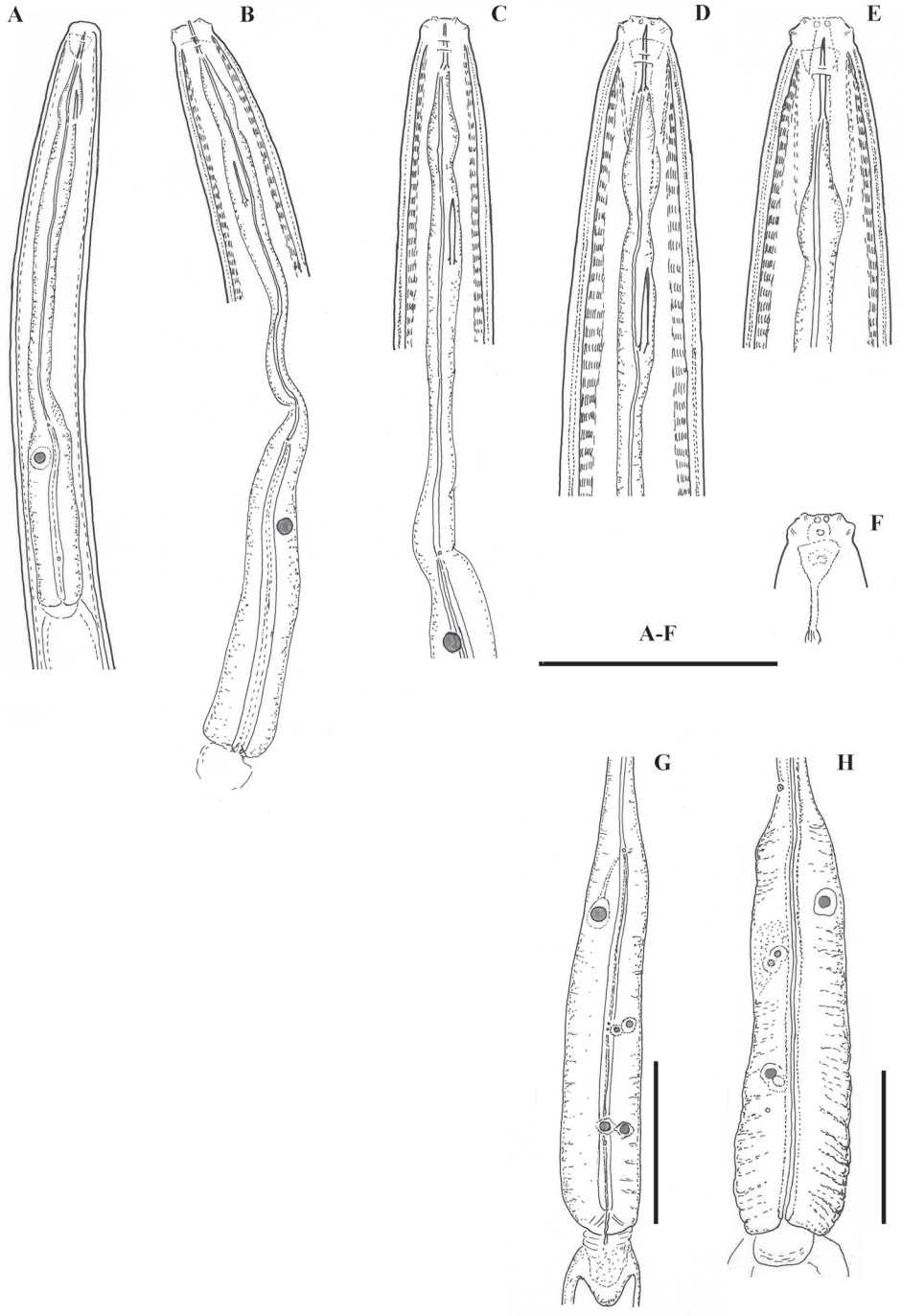


**Figure 4.** *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975). *Juveniles*: **A–D** Anterior ends (J1-J4) (specimens from Livingston Island) *Female* (specimen from Livingston Island) **E** Anterior end **F** Amphideal fovea **G** Pharyngeal bulb. *Enchodelus groenlandicus* (Ditlevsen, 1927) **H** Pharyngeal bulb. Scale bar: 50 μm.

**Table 4.** Pharyngeal characters of *Enchodeloides signyensis* gen. n., comb. n. For abbreviations see Loof & Coomans (1970) and Andrássy, 1998b.

| | LI_ | LI_PH | LI_S | KGI_F |
|---|---|---|---|---|
| DN=D | 67–70 | 69 | 72, 68 | 63–67 |
| DO | 64, 64, 62 | 63 | 66, 62 | 55–63 |
| $S_1N_1$ | – | – | 80 | – |
| $S_1N_2$ | – | – | 79 | – |
| $S_2N$ | 89–91 | 90, 91 | 92, 90 | 89–90 |
| $S_2O$ | 92 | – | 93 | 90, 91 |
| $AS_1$ | – | – | 37 | – |
| $AS_2$ | – | – | 35 | – |
| $PS_1$ | 65–71 | 70 | 71, 68 | 67–74 |
| $PS_2$ | 66–72 | 68 | 70, 69 | 67–72 |

**Table 5.** Genetic distances using D2-D3 28S rDNA sequence data (p-distances given in percents). Pairwise comparisons are based on alignment with 790 nucleotide positions (all positions containing gaps were eliminated).

| | Sequence number/species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **KY881719 *E. signyensis* gen. n., comb. n., Antarctica** | | | | | | | | | |
| **2** | AY593050 *Pungentus engadinensis* (Altherr, 1950) Altherr, 1952 | **7.6** | | | | | | | | |
| **3** | AY593052 *Pungentus silvestris* (de Man, 1912) Coomans & Geraert, 1962, 1 NL | **7.4** | 2.7 | | | | | | | |
| **4** | AY593053 *P. silvestris* 2, NL | **7.5** | 2.8 | 0.1 | | | | | | |
| **5** | AY593054 *Enchodelus macrodorus* (de Man, 1880) Thorne, 1939, NL | **7.5** | 2.8 | 0.1 | 0.3 | | | | | |
| **6** | KY766260 *Enchodelus* sp., Bulgaria | **17.1** | 17.3 | 16.0 | 16.2 | 16.2 | | | | |
| **7** | EF207240 *Enchodelus* sp., NL | **16.6** | 16.1 | 14.9 | 15.0 | 15.0 | 6.5 | | | |
| **8** | KP190119 *E. longispiculus* Guerrero, Liébanas & Peña-Santiago, 2008, Iran | **17.0** | 17.1 | 15.9 | 16.0 | 16.0 | 0.8 | 6.3 | | |
| **9** | KP190120 *Enchodelus* sp. 1, Iran | **17.1** | 17.3 | 16.0 | 16.2 | 16.2 | 1.5 | 7.1 | 1.2 | |

**Discussion.** Based on the main morphological characters, the studied populations are very similar, but specimens from King George Island differ by a somewhat longer (average 1.47–1.66 *vs* 1.20–1.51 mm), and wider body (55.2 ± 3.5 (50–60) µm *vs* 48.0±3.9 (39–54) µm), longer female genital branches (anterior 298.3±31.9 (245–330) µm and posterior 323.1 ± 46.4 (243–361) µm *vs* 236.2 ± 23.3 (186–275) µm and 208.2 ± 34.4 (143–259) µm, respectively, vulva position (V=50.4 ± 0.7 (49.5–51.5)% *vs* V=54.1±1.3 (51–56)%), and tail (32–39 *vs* 25–35 µm). The specimens examined generally agree well with data previously reported for this species (Loof 1975; Andrássy 1998a; Peneva et al. 2002), although some minor differences occurred: our populations have somewhat shorter body length (1.20–1.66 *vs* 1.37–1.88 mm) and the presence of a moderately developed cuticularised ring around the oral aperture has

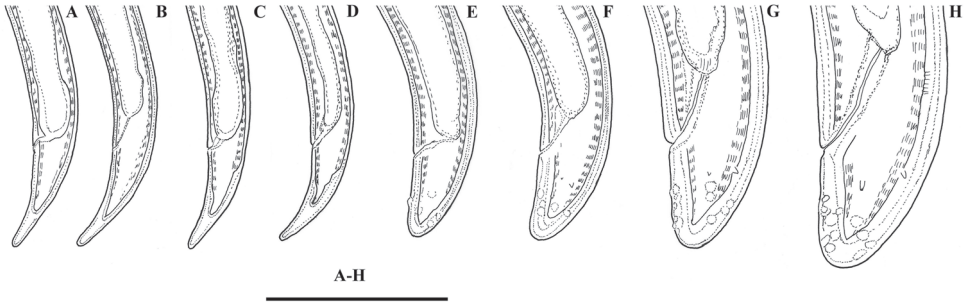


**Figure 5.** *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975). *Juveniles* (specimens from Livingston Island): **A–D** Tail ends (J1) **E–G** Tail ends (J2-J4) *Female* (specimen from Livingston Island) **H** Tail end. Scale bar: 50 μm.

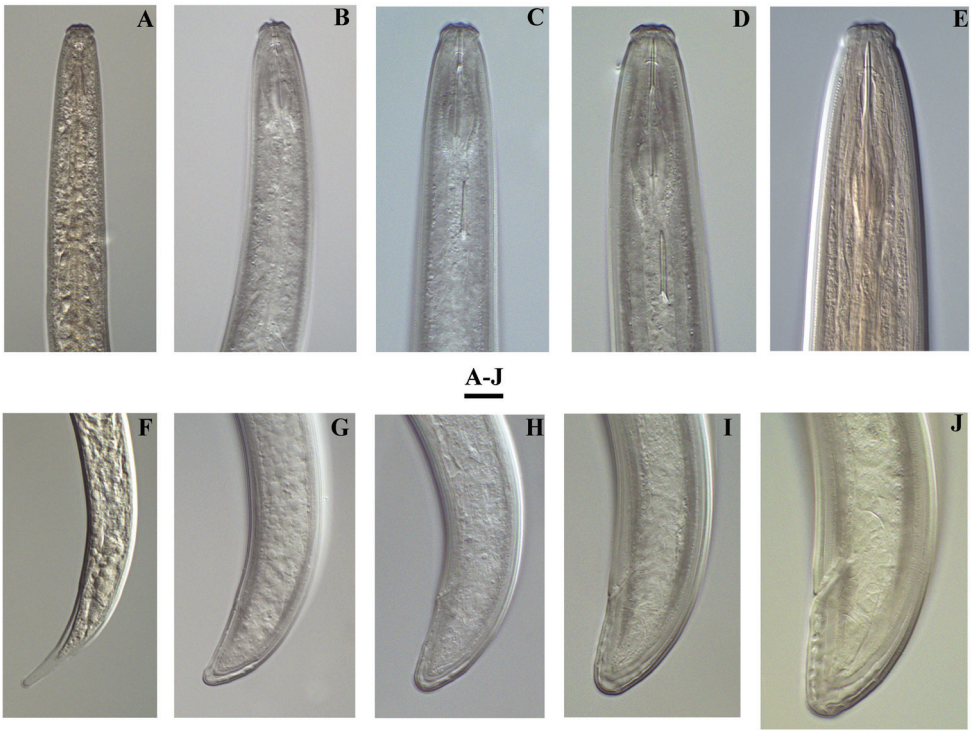


**Figure 6.** *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975). *Juveniles* (specimens from Livingston Island): **A–D** Anterior ends (J1-J4) **F–I** Tail ends (J1-J4) *Female* (specimen from Livingston Island) **E** Anterior end **J** Tail end. Scale bar: 10 μm.

not been described in those studies. Although *E. signyensis* resembles members of the genus *Enchodelus* in many respects, this structure has not been reported for any of its species. The number of morphological characters (see below), as well as molecular data, do not support the current taxonomic position of this species as a member of the genus *Enchodelus* and therefore a new genus *Enchodeloides* gen. n. is proposed.

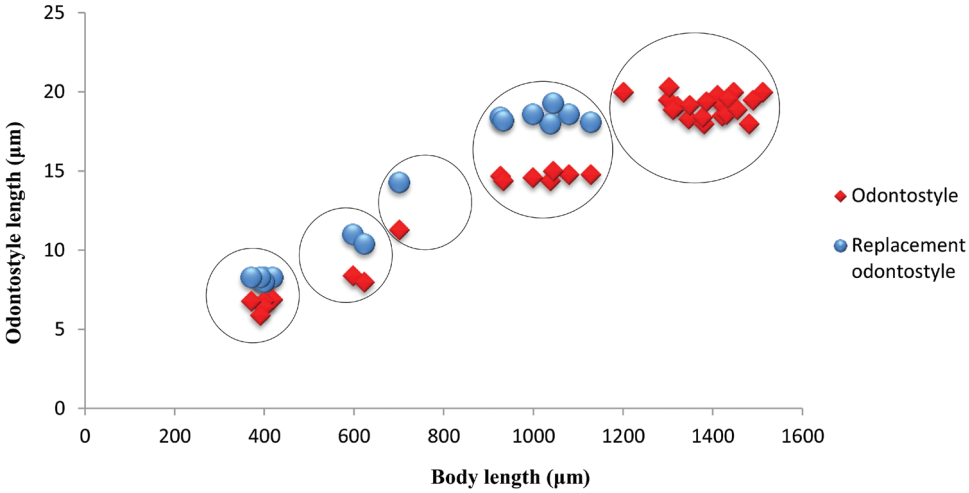


**Figure 7.** *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975). Scatter plot of the functional (○) and replacement odontostyle (◊) in relation to the body length of the juvenile stages and females.

### *Enchodeloides* gen. n.

http://zoobank.org/0AFC0BD5-CA16-4A19-9165-16CD7EE71176

**Diagnosis.** Nordiidae. Nematodes of medium size. Cuticle dorylaimoid, consisting of four layers, outer layer finely, inner layer coarsely transversally striated. Lip region angular; stoma entrance surrounded by a moderately developed cuticularised ring, appearing as small perioral refractive dots. Amphidial fovea cup-shaped, its aperture about half of lip region diameter, curved. Odontostyle short and slender, straight. Odontophore with small swellings. Guiding ring double. Anterior region of pharynx enlarging gradually into pharyngeal expansion. Posterior pair of pharyngeal nuclei smaller than dorsal nucleus, located posteriorly in pharyngeal expansion. Cardia rounded conoid. Female genital system amphidelphic. Uterus not differentiated. Vagina moderately sclerotised. Vulva a transverse slit. Males rare. Spicula stout ventrally curved. Lateral guiding pieces present. Sperm cells spindle-shaped. Supplements 2 to 4 in number preceded by an ad-cloacal pair of papillae, starting far behind the level of the spicules. Tail bluntly conoid, with numerous saccate bodies on tail. First juvenile stage with elongate conical tail with long central peg.

**Relationships.** The new genus resembles members of the subfamily Pungentinae Siddiqi, 1969, especially the genera *Enchodelus*, *Pungentella* Andrássy, 2009, *Pungentus* and *Stenodorylaimus* Álvarez-Ortega & Peña-Santiago, 2011. It differs from *Enchodelus* by having lip region with six radial striae starting from inner and ending at outer labial papillae *vs* absent (seen under SEM), four *vs* three layered cuticle, two *vs* one thicker inner layer at tail region (under light microscopy), cheilostom thin walled *vs* thick walled, a moderately developed cuticularised ring around the oral ap-

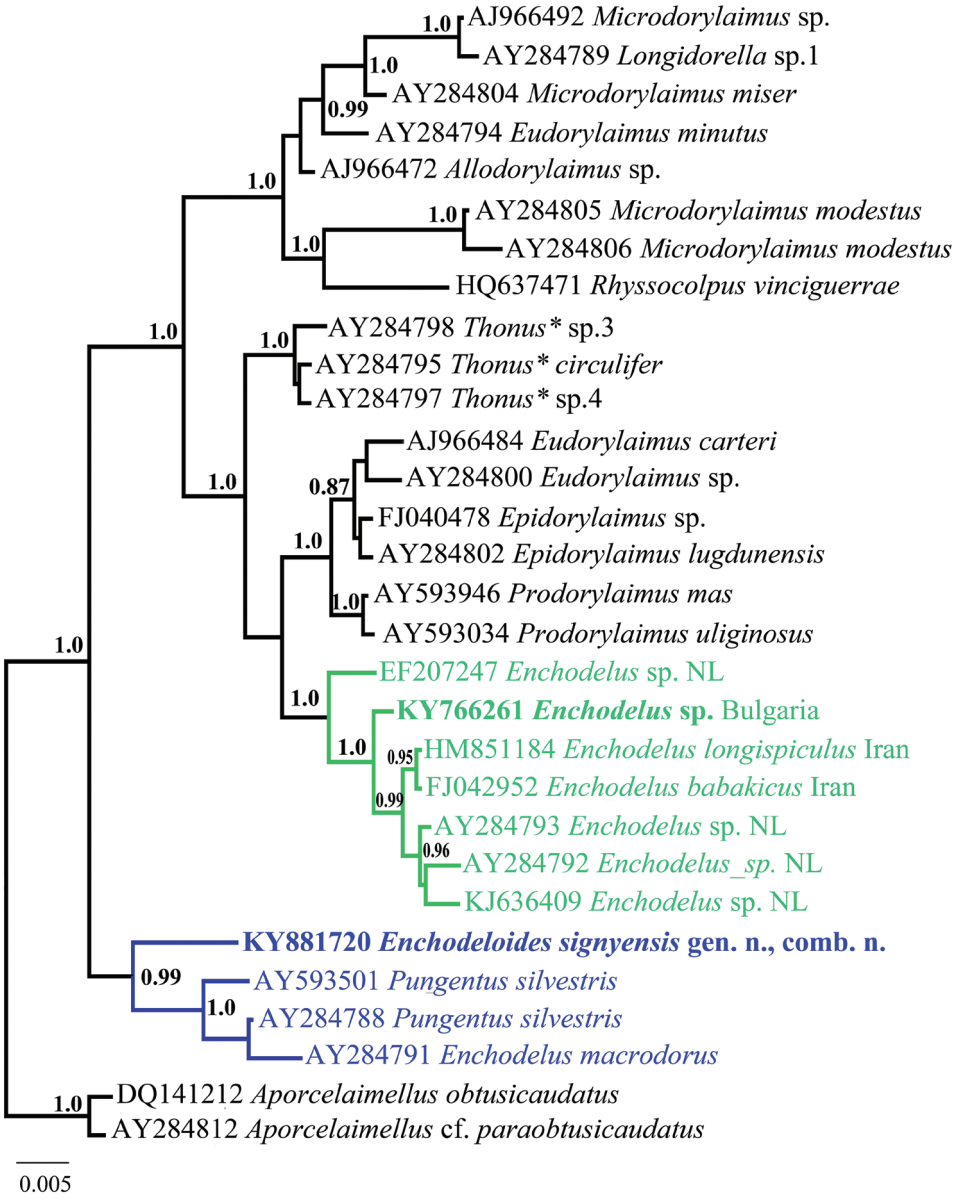


**Figure 8.** Phylogenetic relationships of *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975) based on 18S rDNA inferred from a Bayesian analysis (GTR+G model) and two *Aporcelaimellus* species used as an outgroup. * *Thonus* is currently considered a synonym of *Crassolabium* (Peña-Santiago and Ciobanu, 2008).

erture *vs* absent; less developed *vs* well developed basal swellings; a pharynx enlargement gradually expanding *vs* abruptly expanding into basal expansion (Fig. 4G, H), the posterior pair of pharyngeal nuclei generally smaller than dorsal nucleus *vs* as

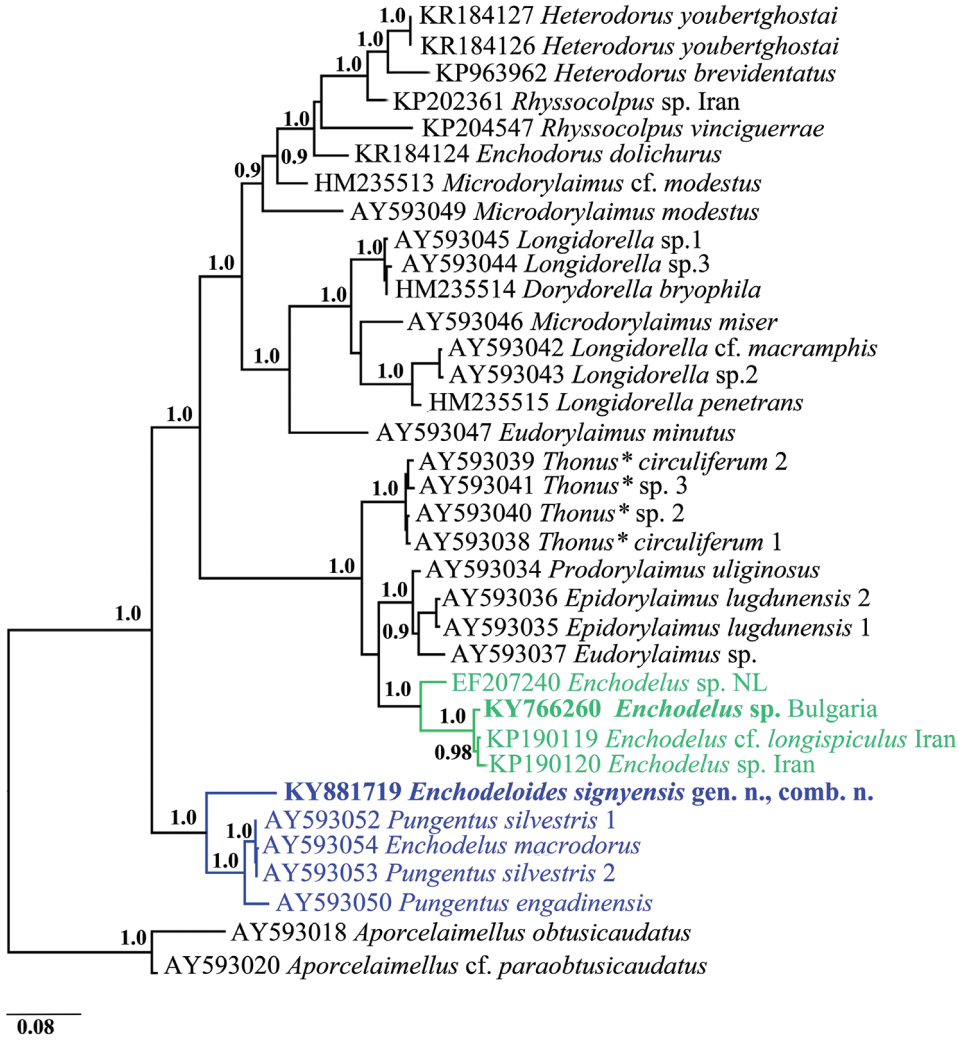


**Figure 9.** Phylogenetic relationships of *Enchodeloides signyensis* (Loof, 1975), gen. n., comb. n. (= *Enchodelus signyensis* Loof, 1975) based on 28S rDNA D2-D3 inferred from a Bayesian analysis (GTR+G model) and two *Aporcelaimellus* species used as an outgroup. * *Thonus* is currently considered a synonym of *Crassolabium* (Peña-Santiago & Ciobanu, 2008).

large as dorsal nucleus (Andrássy 2009), except for *E. macrodorus* Thorne, 1939 (Guerrero and Peña-Santiago 2007) and located more posteriorly, more than 89% *vs* 83–88% of the pharyngeal expansion (Loof and Coomans 1970); less complex uterus *vs* tripartite (bipartite in *E. distinctus* Ahmad & Jairajpuri, 1980 and *E. ponorensis* Popovici, 1995); posteriormost ventromedian supplement located at a considerable distance from the adcloacal pair and outside of the spicule range *vs* posteriormost one or two ventromedian supplements rather close to the adcloacal pair

and inside the spicule range, 2–4 *vs* 7–16 in number, and finally, all representatives of the genus *Enchodelus* have been reported only from the northern hemisphere. *Enchodeloides* gen. n. differs from *Pungentella* by having transversally striated cuticle *vs* smooth; a longer odontostyle (much longer *vs* equal to or slightly longer than lip region diam.) with a smaller aperture (up to one-sixth *vs* one-fourth to one-third its length); a moderately developed cuticularised ring *vs* four small platelets around the oral aperture and the guiding ring double *vs* simple. From *Pungentus* it differs in having a moderately developed cuticularised ring *vs* four distinct circumoral platelets around the oral aperture; a straight *vs* arcuate odontostyle; shorter odontostyle (1.2–1.6 times *vs* 2–3 times lip region diameter (Andrássy 2009a); the first pair of ventrosublateral pharyngeal gland nuclei indistinct, difficult to observe *vs* well developed; a long distance DO-DN (5–6% *vs* 2–4% (Loof and Coomans 1970)); ventromedian supplements located at a considerable distance from the adcloacal pair and outside of the spicule range *vs* posteriormost 1–4 supplements lying within the spicule range, and with *vs* without hiatus. From the genus *Stenodorylaimus* it differs by having a shorter body (L=1.2–1.9 *vs* 3.7–5.1 mm), and a slender *vs* more robust odontostyle (1.2–1.7 *vs* 0.51–0.87% of body length); a longer pharynx (b-ratio up to 6 *vs* more than 7); saccate bodies present *vs* absent; the first pair of ventrosublateral pharyngeal gland nuclei indistinct, difficult to observe *vs* well developed; ventromedian supplements spaced *vs* irregularly spaced, 2–4 *vs* 14–19 in number, and with *vs* without hiatus.

Consequently, the new combination *Enchodeloides signyensis* (Loof, 1975) is proposed to accommodate the only nordiid species occurring in Maritime Antarctic.

## Distribution

*Enchodeloides signyensis* is a widespread endemic for the Maritime Antarctic, occurring in several islands: Signy (Loof 1975; Maslen 1981; Caldwell 1981), Coronation, Elephant, Galindez, Blaiklock (Loof 1975), Alamode (Loof 1975; Maslen and Convey 2006), Dream (Shishida and Ohyama 1989), Charcot (Convey et al. 2000; Maslen and Convey 2006), Livingston (Peneva et al. 2002, 2004; Elshishka et al. 2015b), Alexander (Maslen and Convey 2006), and King George Islands (Russell et al. 2014). It has been recorded from various microhabitats, different moss and algae communities, and in association with species of higher plants, reported from Maritime Antarctic (*D. antarctica* and *C. quitensis*) (Table 6). Data from previous records and the present study show that *E. signyensis* is associated with different type of microhabitats. Like other terrestrial nematodes in extreme polar conditions, a majority of which colonise all microhabitats, this species does not show specific biotope preferences. According to Chernov et al. (2011) the major life strategy of organisms inhabiting extreme environments is the development of tolerance and plasticity, not specialisation and competitiveness, which is typical of other biomes.

**Table 6.** Distribution of *Enchodeloides signyensis* gen. n., comb. n. in Antarctic islands and habitats.

| Island | Microhabitats and plant associations | References |
|---|---|---|
| Signy | *Tortula excelsa* Card (type host)<br>*Deschampsia antarctica* Desv.<br>*Colobanthus quitensis* (Kunth) Bartl. | Loof 1975 |
| | *Polytrichastrum alpinum* (Hedwig), *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth., *Sanionia uncinata* (Hedw.), *Calliergon sarmentosum* (Wahlenb.), *Calliergidium austro-stramineum* (C. Muell.) Bartr. | Maslen 1981 |
| | *P. alpinum*, *Ch. aciphyllum*, *S. uncinata*, *C. sarmentosum*, *Cephaloziella varians* (Gottsche) Steph., soils contaminated by vertebrate, e.g. close to seabird nests | Caldwell 1981 |
| Coronation | *D. antarctica* | Loof 1975 |
| Elephant | *D. antarctica*<br>*Polytrichum* sp. | |
| Galindez | *D. antarctica* | |
| Blaiklock | *P. alpinum, Pohlia nutans* (Hedw.) | |
| Alamode | *S. uncinata* | |
| | Moss | Maslen and Convey 2006 |
| Dream | Moss mats with green algae | Shishida and Ohyama 1989 |
| Charcot | Soil, moss clumps, algae, various lichens | Convey et al. 2000 |
| | Moss, lichen and soil | Maslen and Convey 2006 |
| Livingston | *D. antarctica*, *S. uncinata*, *Sanionia georgico-uncinata* (Müll. Hal.) Ochyra & Hedenäs, *C. quitensis*, *P. alpinum*, *Bryum* sp., *Usnea* sp., *Cladonia* sp., *Polytrichum juniperinum* Hedw., *Bartramia patens* Brid. | Peneva et al. 2002 |
| | Moss; soil under moss crust; soil | Present study |
| Alexander | Moss; lichen; soil; microbial mat | Maslen and Convey 2006 |
| King George | *D. antarctica*, *C. quitensis*, *Sanionia* sp., *Syntrichia filaris* (Müll.Hal.), *Syntrichia magellanica* (Mont.) | Russell et al. 2014 |
| | Moist brown soil without vegetation, surrounded by moss | Present study |

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## Supplementary material 1

**18S Parsimoni informative sites TemporaryMEGA17**

Authors: Milka Elshishka, Stela Lazarova, Georgi Radoslavov, Peter Hristov, Vlada K. Peneva

Data type: (nucleotide)

Explanation note: 18S rDNA Phylogenetic analysis between tree Genus based on Parsimony informative nucleotide sites.



Link: https://doi.org/10.3897/zookeys.697.13770.suppl1

## Supplementary material 2

**28S Parsimoni informative sites TemporaryMEGA15**

Authors: Milka Elshishka, Stela Lazarova, Georgi Radoslavov, Peter Hristov, Vlada K. Peneva

Data type: (nucleotide)

Explanation note: 28S rDNA Phylogenetic analysis between tree Genus based on Parsimony informative nucleotide sites.



Link: https://doi.org/10.3897/zookeys.697.13770.suppl1

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# A new species of *Notodiaptomus* from the Ecuadorian Andes (Copepoda, Calanoida, Diaptomidae)

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## Abstract

*Notodiaptomus cannarensis* **sp. n.** is described from a reservoir on the Amazonian slope of the Ecuadorian Andes. The new species is unique among diaptomid calanoid copepods in the display of hypertrophied, symmetrical wing-like extensions at each side of the female composite genital somite. Furthermore, it displays a female urosome reduced to only two somites due to the incorporation of abdominal somites III and IV to the composite genital double-somite, and a male right fifth leg with the outer spine of second exopodal segment recurved and implanted proximally on margin. It differs from any other *Notodiaptomus* in the display of a large rectangular lamella on proximal segment of exopod of male right fifth leg. The species is currently known only from Mazar reservoir, a eutrophic water body placed above 2127 m a.s.l. on the River Paute (Cañar Province; southern Ecuador), where it is the most common crustacean in the water column.

**Keywords**
Crustacea, Ecuador, reservoirs, South America, zooplankton

## Introduction

The inland waters of the Neotropical region harbour representatives of at least three different calanoid copepod families, *viz*. Centropagidae Giesbrecht, 1892, Pseudodiaptomidae G.O. Sars, 1902, and Diaptomidae Baird, 1850. The Centropagidae (22 species reported thus far; Boxshall and Defaye 2008) are distributed from Patagonia to the Andes, with only three species known to occur out of those regions, in SE Brazil (Previattelli et al. 2013). The Pseudodiaptomidae appear mainly in estuaries and other shallow coastal marine habitats of reduced salinity, and include notorious examples of accidental translocation of exotic species (Andrade dos Santos et al. 2009); four native species of *Pseudodiaptomus* Herrick, 1884 have been reported so far from the region under consideration (Santos-Silva 2008). Finally, the Diaptomidae are distributed through the rest of South America except at high altitudes and latitudes, with a broad overlapping zone with the area exclusive of the Centropagidae embracing from 28°S (Santa Catarina State, Brazil) to almost 39°S in the Argentinian northern Patagonia (Bănărescu 1990; Previattelli et al. 2015). The most recent accounts (Perbiche-Neves et al. 2014) estimate in 98 the number of species of diaptomids known from the Neotropical region, distributed over 15 genera, 14 of which being endemic (Dussart and Defaye 2002). Here we describe a new species of diaptomid of the genus *Notodiaptomus* Kiefer, 1936, from the Amazonas High Andes Ecoregion (*sensu* Abell et al. 2008).

## Materials and methods

The copepods were collected in the water column of Mazar reservoir using a plankton net of 60 μm mesh size hauled from 25 m depth. Sampling was performed in the framework of the project "Comprensión de los Procesos Hidroecológicos como base para la Estimación del Caudal Ecológico en las Cuencas del Jubones y Paute", sponsored by the Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación of the Government of the Republic of Ecuador. Water in the reservoir is poorly mineralized (56-62 μS/cm) and turbid due to presence of phytoplankton (Secchi Disk depth 1.4 m), in accord to its eutrophic condition. Material was fixed *in situ* with formalin and dissected in glycerine on an excavated slide. Drawings were prepared with a camera lucida attached to an Olympus BH-2 microscope equipped with phase contrast. Terminology used in descriptions follows Huys and Boxshall (1991). Type material is deposited in the Museo Ecuatoriano de Ciencias Naturales del Instituto Nacional de Biodiversidad, Quito, Ecuador [MECN].

## Taxonomy

**Subclass COPEPODA Milne Edwards, 1830**
**Order CALANOIDA Sars, 1903**
**Family DIAPTOMIDAE Baird, 1850**
**Genus *Notodiaptomus* Kiefer, 1936, emend. Santos-Silva, Boxshall & da Rocha, 1999**

***Notodiaptomus cannarensis* sp. n.**
http://zoobank.org/4D4FEBB1-4A88-4BD8-8593-2EAD177E0956
Figs 1–5

**Material examined.** Mazar reservoir (River Paute, Cañar Province, southern Ecuador). Coordinates 2º35'53.08"S; 78º37'32.16"W. Altitude: 2127 m a.s.l. Holotype: male 1.2 mm long, preserved in formalin vial. Paratypes: Ten males and ten females, preserved in formalin vial. Holotype and paratypes registered under same registration number [MECN-SI-Cal-0001]. Collected by Verónica Ordóñez, April 2013.

**Diagnosis.** Female urosome reduced to only two somites due to incorporation of abdominal somites III and IV into composite genital double-somite; resulting composite somite with symmetrical, hypertrophied wing-like (in dorsal aspect) extensions at each side. Male right fifth leg with outer spine of second exopodal segment recurved and implanted proximally on margin.

**Etymology.** Species name refers to the Ecuadorian province where it was found (Cañar Province; southern Ecuador).

**Distribution.** Known only from Mazar reservoir, located on the River Paute (Amazon Basin, Cañar Province, southern Ecuador), 2127 m a.s.l.

**Description of adult female.** Body up to 1.4 mm long. *Prosome* 5-segmented, comprising cephalosome plus first to third free pedigerous somites, and partially-fused fourth and fifth pedigerous somites (Fig. 1B–D); epimeral plates of latter extended backwards and displaying two pointed processes at each side, oriented as figured. *Rostrum* (Fig. 1A) bifid, with paired short rostral filaments. *Urosome* (Fig. 1B–D) 2-segmented, with genital somite incorporating all abdominal somites except anal somite; resulting composite genital somite displaying pair of hypertrophied ventrolateral ovoid swellings extended backwards, each with pointed tip. Genital field not fully resolved, with paired gonoporal plates placed medially on ventral surface of composite genital somite, partially covered with short genital operculum (Fig. 1E). Caudal rami symmetrical, slightly longer than broad with setulose margins; caudal setae symmetrical, short, all plumose except dorsal seta, simple and more slender than rest; anterolateral accessory seta absent.

*Antennules* (Fig. 1F, G) symmetrical, each 25-segmented, with fusions affecting ancestral segments II-IV and XXVII-XXVIII; segmentation pattern and armature formula as follows: segment 1 (corresponding to ancestral segment I), 1 seta + aesthetasc; segment 2 (fused ancestral segments II-IV), 3 setae + aesthetasc; segment 3 (V), 1 seta + ae;

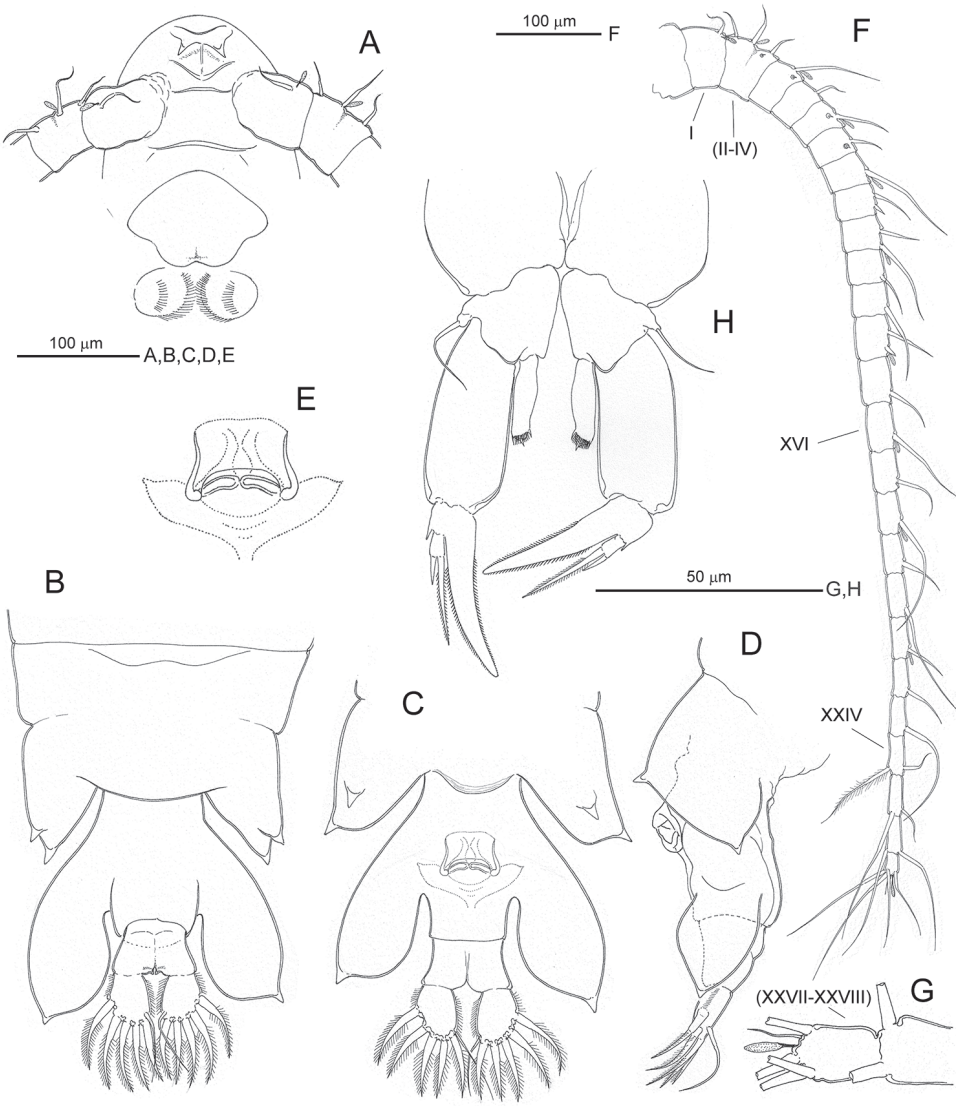


**Figure 1.** *Notodiaptomus cannarensis* sp. n., adult female. **A** anterior portion of prosome showing rostrum, insertion of antennules, labrum and paragnaths, ventral **B** last pedigerous somite plus urosome and caudal rami, dorsal **C** same, ventral **D** same, left lateral **E** inset of genital aperture **F** right antennule, ventral **G** inset of terminal segments of latter **H** fifth legs, posterior.

segment 4 (VI); 1 seta; segment 5 (VII), 1 seta + ae; segment 6 (VIII), 1 seta; segment 7 (IX), 1 seta + ae; segment 8 (X), 2 setae, of which distal most reduced; segment 9 (XI), 2 setae + ae; segments 10 (XII) and 11 (XIII), 1 seta each; segment 12 (XIV), 2 setae + ae, with distal seta reduced; segment 13 (XV), 1 seta; segment 14 (XVI), 1 seta + ae; segment 15 (XVII), 1 seta; segment 16 (XVIII), 1 seta+ ae; segments 17 (XIX) and 18 (XX), 1 seta each; segment 19 (XXI), 1 seta+ ae; segments 20 (XXII) and 21 (XXIII), 1 seta each; segments 22 (XXIV) to 24 (XXVI), 1 + 1 setae each; segment 25 (fused XXVII-XXVIII), 5 setae + ae. Sensilla present on anterodorsal surface of segments 2, 3, 5 and 6.

*Antenna* (Fig. 2D, E) biramous. Coxa with one seta on medial margin. Basis with two setae on distomedial margin. Exopod 8-segmented, setal formula 1, 3, 1, 1, 1, 1, 1, 3. Endopod 2-segmented with compound second segment bilobed; setal formula 2, 7+6 (see Fig. 2E).

*Labrum* (Fig. 1A) with concave distal margin. *Paragnaths* (Fig. 1A) globose, each with two rows of setae as figured.

*Mandible* coxal gnathobase (Fig. 3A) cutting edge 9-denticulate and with simple distal seta; innermost denticle broadly separated from rest. Palp (Fig. 3B) biramous, basis with four medial setae; exopod 5-segmented, setal formula 1, 1, 1, 1, 2; endopod 2-segmented, distal segment bilobed, with row of setules along outer margin and transverse row of setules about midway, setal formula 4, 5+5.

*Maxillule* (Fig. 3C) praecoxal arthrite with 15 armature elements ornamented and distributed as figured. Coxal epipodite with nine setae; coxal endite with four setae. Basal exite seta present; basal endites each with four setae. Exopod with six setae. Endopod 2-segmented, setal formula 4, 5.

*Maxilla* (Fig. 3D) syncoxal endites armature formula: 5 + reduced spine, 3, 3, 3. Allobasis basal endite with three setae; allobasis endopodal endite with single seta. Free endopod 3-segmented, setal formula 1, 1, 3.

*Maxilliped* (Fig. 3E) praecoxal endite with single seta. Coxal endites with 2, 3, and 4 setae, respectively; distal endite with spinulose swelling. Basis with three medial setae. Endopod 6-segmented, armature formula 2, 3, 2, 2, 1+1, 4.

*Swimming legs* 1-4 (Fig. 4) each biramous with 3-segmented rami except for 2-segmented endopod on leg 1 (Fig. 4A). Second endopodal segment of leg 2 with smooth rounded swelling ("Schmeil's organ") on posterior surface (Fig. 4B). Outer exopodal spines reduced in all limbs. Armature formula as follows:

| | Coxa | Basis | Exopod | Endopod |
|---|---|---|---|---|
| Leg 1 | 0-1 | 0-0 | I-1; 0-1; I,I,4 | 0-1; 1,2,3 |
| Legs 2 & 3 | 0-1 | 0-0 | I-1; I-1; I,I,5 | 0-1; 0-2; 2,2,3 |
| Leg 4 | 0-1 | 1-0 | I-1; I-1; I,I,5 | 0-1; 0-2; 2,2,3 |

*Fifth legs* (Fig. 1H) symmetrical, biramous, coxa and basis separate, outer basal seta simple and implanted on socle. Exopod 3-segmented, proximal segment unarmed,

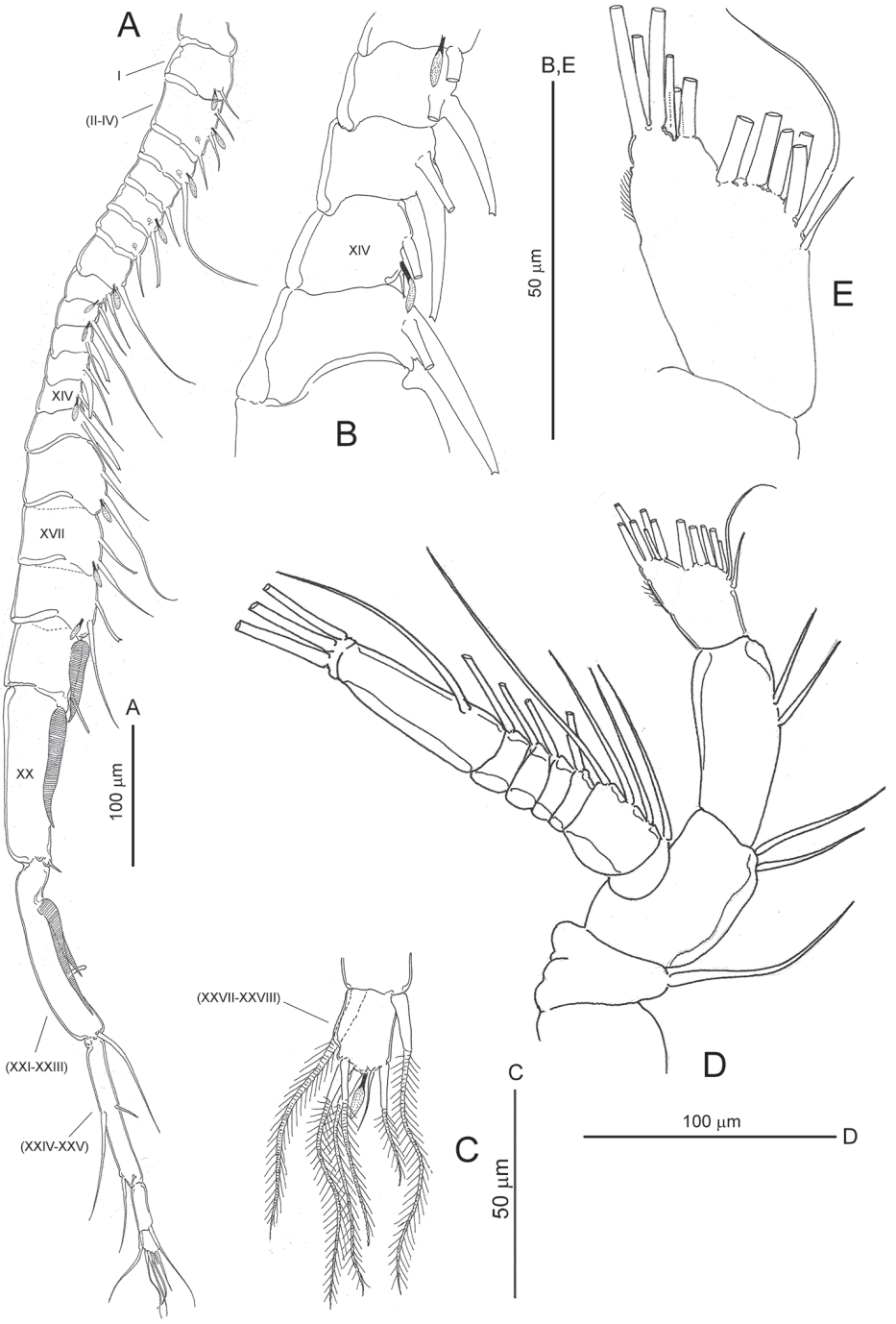


**Figure 2.** *Notodiaptomus cannarensis* sp. n. **A** male right antennule, ventral **B** detail of segments 10 to 13 of latter **C** inset of terminal segment **D** adult female right antenna, ventral **E** inset of terminal segment of latter.

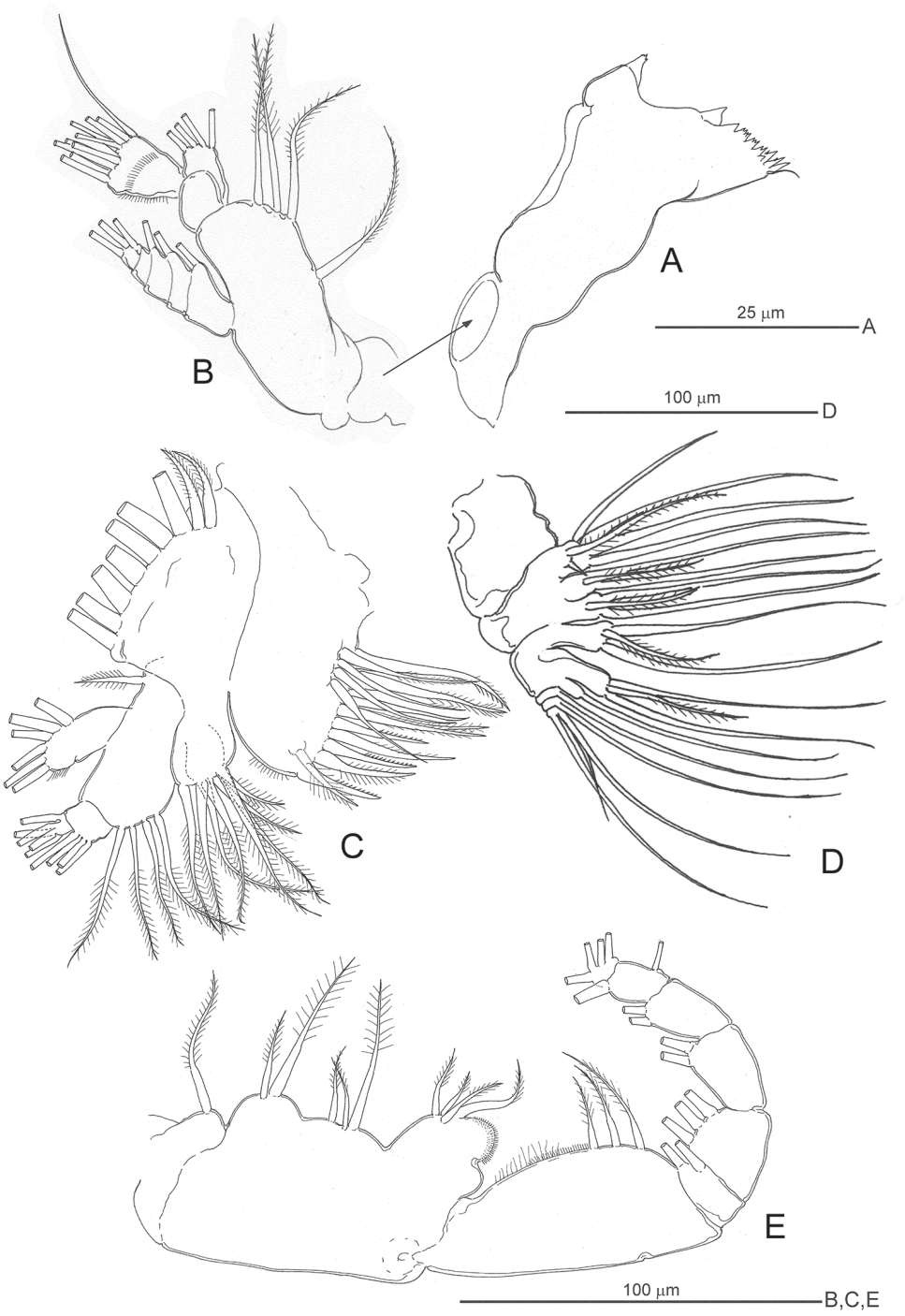


**Figure 3.** *Notodiaptomus cannarensis* sp. n., adult female. **A** mandible coxal gnathobase **B** mandibular palp **C** maxillule **D** maxilla **E** maxilliped.

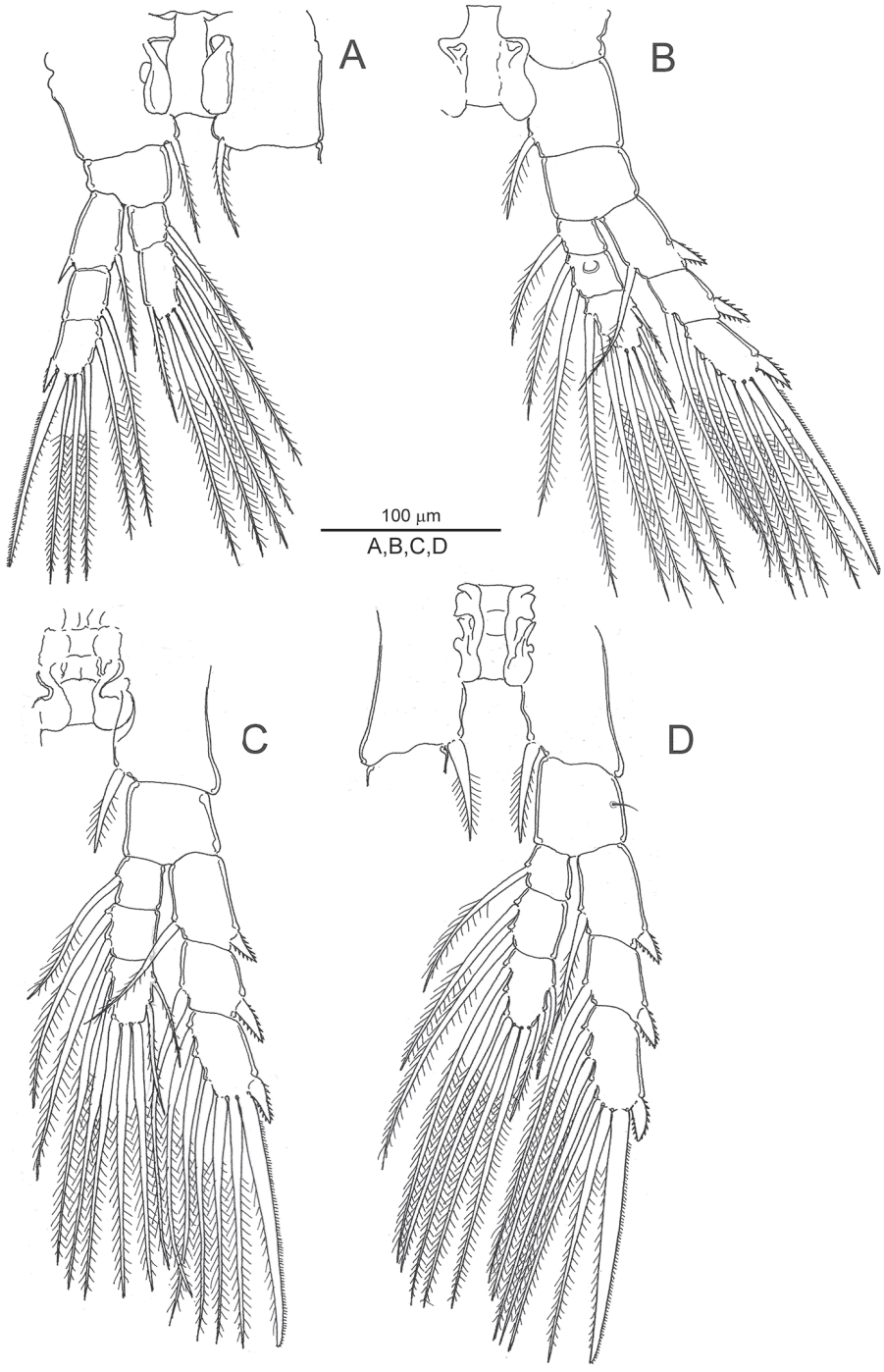


**Figure 4.** *Notodiaptomus cannarensis* sp. n., adult female. **A** left leg 1, posterior view **B** right leg 2, posterior **C** left leg 3, anterior **D** right leg 4, anterior.

longer than middle segment; distomedial angle of middle segment prolonged into stout spinous process fringed with short setules, distolateral angle with tiny smooth spinous process; distal segment reduced, with two unequal setae distally, innermost longer and setulose, but not as long as inner spinous process of middle segment–, outermost seta much shorter and smooth. Endopod unsegmented, subrectangular with three tiny spinous processes and transverse row of setules distally.

**Description of male.** Body up to 1.22 mm long. Differing from female in fifth legs, modified right antennule, asymmetrical epimeral plates of composite last prosomal somite, and segmentation and asymmetry of urosome, including caudal rami. Thus, the extensions of the epimeral plates corresponding to the partially fused fourth and fifth pedigerous somites are directed laterally instead of backwards, with the pointed processes present on each side less marked than in the female (Fig. 5A, B). The *urosome* is 5-segmented, with the genital somite asymmetrical, slightly protruding on the right side; the third abdominal somite is also asymmetrical, showing a dorsolateral hump crowned with a tiny spine on the right side. In addition, the *caudal rami* are slightly asymmetrical and comparatively more elongated than in female, with proportionally longer caudal setae except for dorsal seta, that is shorter.

*Right antennule* (Fig 2A–C) 22-segmented, geniculate, with geniculation located between segments 18 (corresponding to ancestral segment XX) and 19 (corresponding to fused ancestral segments XXI-XXIII). Other fusions involving ancestral segments II-IV, XXIV-XXV and XXVII-XXVIII. Segmentation pattern and armature formula as follows: segment 1 (ancestral I), 1 seta + aesthetasc; segment 2 (II-IV), 3 setae + ae; segment 3 (V), 1 seta + ae; segment 4 (VI), 1 seta; segment 5 (VII), 1 seta + ae; segment 6 (VIII), 1 seta; segment 7 (IX), 1 seta + ae; segment 8 (X), 2 setae, of which distal reduced, conical; segment 9 (XI), 2 setae + ae; segments 10 (XII) and 11 (XIII), each with 1 stout truncate spiniform process + 1 seta; segment 12 (XIV), 2 setae + ae, with distal seta reduced, conical; segment 13 (XV), 1 stout truncate spiniform process plus seta; segment 14 (XVI), 2 setae + ae; segments 15 (XVII) and 16 (XVIII), each with 1 slender truncate spiniform process proximally and 1 seta + ae distally; segment 17 (XIX), 1 striated hyaline seta proximally + reduced truncate spiniform process distally; segment 18 (XX), 1 striated hyaline seta proximally plus tiny seta distally; segment 19 (XXI-XXIII), 2 striated hyaline setae plus short blunt seta midway of margin plus distal seta; segment 20 (XXIV-XXV), 2 + 2 setae; segment 21 (XVI), 1 + 1 setae; segment 22 (XVII-XXVIII), 5 setae + ae. Segments 13 to 18 swollen. Anterodorsal sensilla present on each segments 2, 3, 5 and 6.

*Fifth leg* (Fig. 5C) biramous, highly asymmetrical, each with coxa fused to intercoxal sclerite. Coxa and basis separate on both sides, each with tiny posterolateral seta implanted on socle. Right leg largest, basis with simple seta on posterolateral margin; Exopod 3-segmented, proximal segment with large rectangular lamella implanted on posterior surface (see Fig. 5B, C), second segment expanded and armed with recurved spine proximally on outer margin; distal segment of exopod modified as curved claw with inner margin finely serrated distally. Endopod (Fig. 5F) unsegmented, short –not surpassing distal margin of proximal exopodal segment–, subrectangular with tiny terminal spine on each side; distal margin of segment finely denticulate. Left leg (Fig. 5D, E) basis unarmed.

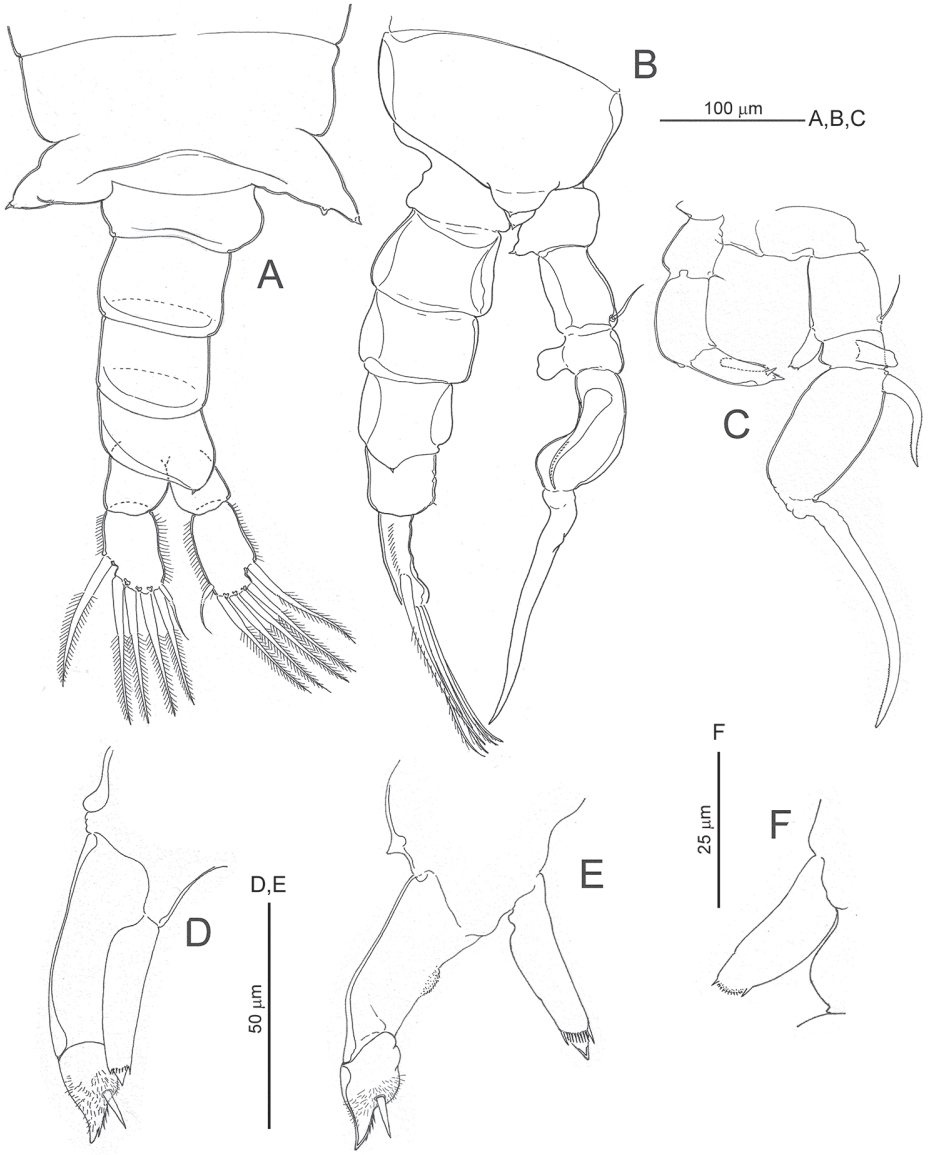


**Figure 5.** *Notodiaptomus cannarensis* sp. n., adult male. **A** last pedigerous somite plus urosome and caudal rami, dorsal **B** inset of last pedigerous somite with fifth legs attached plus urosome and caudal rami, right lateral aspect **C** fifth legs, posterior **D** and **E** different aspects of left fifth leg rami **F** inset of right fifth leg endopod.

Exopod 2-segmented, distal segment shortest, bifid with distal spine and subdistal conical robust seta, densely covered with short setules; proximal segment with micro-denticulate rounded outgrowth about midway of inner margin. Endopod unsegmented, implanted on produced distomedial angle of basis, with pointed tip wearing tiny spine subdistally at each side plus transverse row of setules in between as figured.

## Remarks

The new species described herein corresponds in almost all respects to the re-diagnosis of *Notodiaptomus* Kiefer, 1936, as presented by Santos-Silva et al. (1999) and Previatelli (2010). This is the most broadly distributed and species-rich genus of freshwater calanoids in the Neotropics, embracing currently 39 nominal species (Santos-Silva 2008).

The new taxon can be distinguished from any other representative of the genus by its female composite genital somite, which displays a hypertrophied, wing-like (in dorsal aspect) extension at each side; no other calanoid copepod is known to display such hypertrophied symmetrical extensions, although two Neotropical taxa, viz. *Tumeodiaptomus* Dussart, 1979, and some members of *Rhacodiaptomus* Kiefer, 1936 (e.g. *Rhacodiaptomus besti* Santos-Silva & Robertson, 1993, and in a lesser extent *R. insolitus* (Wright, 1927) or *R. retroflexus* Brandorff, 1973), show somewhat similar structures but with only one of the two wings hypertrophied (see Dussart 1979; Santos-Silva and Robertson 1993). Nevertheless, at least *Tumeodiaptomus* appears distantly related to *Notodiaptomus* in Previatelli's (2010) analysis of the phylogenetic relationships of Neotropical diaptomids. In addition, the female urosome reduced to only two somites, and the proximal placement of the outer spine on margin of the second exopodal segment of the male right fifth leg are also salient traits of the new taxon. Nevertheless, a 2-segmented condition of the female urosome is known also to occur in several non-Neotropical diaptomids such as in many species of *Tropodiaptomus* Kiefer, 1932, and several *Thermodiaptomus* Kiefer, 1932 and *Mixodiaptomus* Kiefer, 1932. On the other hand, males of *Tumeodiaptomus* show also the outer spine on margin of the second exopodal segment of the right fifth leg inserted proximally, but not as close to the base of the segment as in the new species.

Apart from these unique features, the new species shows a series of character states in the armature of several limbs that differ from the condition found in the type-species of the genus *N. deitersi* (Poppe, 1891) as redescribed by Santos-Silva et al. (1999). Thus, the distal segment of the antennules of both sexes displays five setae plus aesthetasc (versus 4 + ae in *N. deitersi*); there is a sensilla present on antennulary segments 2, 3, 5 and 6 (versus sensilla present only on segments 2, 3 and 5 in *N. deitersi*); segments 15 and 16 of the geniculate male antennule are devoid of pointed process (versus process present on both segments in *N. deitersi*); segments 17 and 18 are each armed with a modified hyaline seta and a short ordinary setae (versus modified hyaline seta + 2 ordinary setae present on each in *N. deitersi*); the distal endopodal segment of the mandibular palp bears ten setae (versus only nine in *N. deitersi*); the proximal en-

dopodal segment of maxilla displays four setae (versus only three in *N. deitersi*); and the distal coxal endite of maxilliped displays four setae (versus three in *N. deitersi*). Both taxa differ notoriously also in the ornamentation of the rounded outgrowth present on the medial margin of the proximal exopodal segment of male left fifth leg (microdenticulate in the new species, versus covered with long setules in *N. deitersi*); in the relative length and outline of endopod of male fifth leg (subrectangular and longer than the corresponding proximal exopodal segment in the new species, versus subtriangular and shorter than proximal exopodal segment in *N. deitersi*); and in the relative size of the distolateral spine of the second exopodal segment of the female fifth leg (shorter than the third segment in the new species, versus as long as segment in *N. deitersi*).

The new species differs also from any other *Notodiaptomus* in the display of a large rectangular lamella on proximal segment of exopod of male right fifth leg.

## Acknowledgements

This study has been supported by a grant PROMETEO from the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación of Ecuador, to the first author. We are grateful to Verónica Ordóñez, who first collected the new species and kindly offered us her material for study.

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# New species of *Rogmocrypta* Simon, 1900 from New Caledonia, with remarks on relationships and distribution (Araneae, Salticidae)

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**Abstract**

Five new species of *Rogmocrypta*: *R. karolinae* (♀), *R. koniambo* (♀), *R. patryki* (♀), *R. raveni* (♀), and *R. rollardae* (♀) are diagnosed, described, and illustrated. The definition of the genus is ammended and its distribution and relationships are discussed.

**Keywords**

distribution modelling, jumping spiders, Pacific Islands

## Introduction

The fauna of New Caledonia is often discussed in terms of Gondwanan heritage. Indeed, the island group was separated from Gondwana some 85 MYA, but later experienced multiple subductions and submergences (Cluzel et al. 2012) and, in fact it only emerged in post-Eocene (37 MYA). Consequently, New Caledonian biota, fauna, and flora should not be discussed in terms of direct *Gondwanan heritage*, but rather as the result of local radiations and colonisation from other sources (Keast and Miller 1996, Grandcolas et al. 2008, Heads 2008, 2010, 2014). The phenomenon of local radiation is also known for several salticid genera such as *Corambis* Simon, 1901, *Penionomus* Simon, 1903 and *Rhondes* Simon, 1901 (Maddison et al. 2008); all are part of the Aus-

tralasian Astioida clade and derived from Australian ancestors between 9 and 20 MYA (Bodner and Maddison 2012). The genus *Rogmocrypta* (here) with seven nominal species is also the case of radiation *in situ*.

Our initial aim is to present a complete revision of the genus; however, the lack of type material for *R. nigella* Simon, 1900 and *R. puta* Simon, 1900 limited our goals.

## Materials and methods

The material was obtained from the following collections:

**MNHN** Museum National d'Histoire Naturelle, Paris, France
**QM** Queensland Museum, Brisbane, Australia.

The examination specimen methods were as described by Żabka (1991). The drawings were made using a grid system. The photographs were taken with Nikon D5200 camera and Nikon SMZ1000 stereomicroscope, and were digitally processed with ZoomBrowser and HeliconFocus software. The dissected epigynes were digested in 10% KOH and studied under compound microscope. The actual and predicted distributional maps were generated with DIVA-GIS bio-climatic software using BIOCLIM application (Nix, 1986; Busby, 1991). Our model has been produced with 14 field records and met the requirements for the software (at least 5–10 records; Hernandez et. al. 2006). The following environmental variables were used in the analysis: annual mean temperature, mean monthly temperature range, isothermality, temperature seasonality, max temperature of warmest month, min temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, precipitation of coldest quarter.

**Abbreviations used in the text and figure legends are:**

**AEW** anterior eye width,
**AME** anterior medial eyes,
**AL** abdomen length,
**AW** abdomen width,
**cd** copulatory duct,
**CH** cephalothorax height,
**CL** cephalothorax length,
**co** copulatory opening,
**CW** cephalothorax width,
**EFL** eye field length,
**e** embolus,
**eo** endites outgrowth,
**fd** fertilisation duct,
**L** leg,
**PEW** posterior eye width,
**PLE** posterior lateral eyes,
**PME** posterior medial eyes,
**rta** retrolateral tibial apophysis,
**s** spermatheca,
**t** tegulum,
**tr** transverse ridge.

## Taxonomy

### Genus *Rogmocrypta* Simon, 1900

*Rogmocrypta* Simon, 1900: 387; 1901: 389, 445–446; Maddison et al. 2008: 52–55; Maddison 2015: 277.

**Type species.** *R. elegans* (Simon 1885) = *Chalcoscirtus elegans* Simon 1885, originally designated by Simon (1900).

**Diagnosis.** Differs from related genera by tiny or small body size. Unlike in *Lystrocteissa* (Patoleta and Gardzińska 2013, figs 9–15) the habitus is not ant-mimic (Figs 1, 7, 10, 16, 28) and much more compact than in *Corambis* (Szűts 2002, figs 1, 10–12). Male palpal embolus[1] is sabre-like (Fig. 5) and shorter than in *Penionomus* (Żabka 1988, fig. 114) and in some species of *Rhondes* (Patoleta 2016, figs 9–14). Tegulum without lobe (more or less marked in relatives). Seminal duct not meandering, tibial apophysis short (Fig. 6). Unlike in *Rhondes* (Patoleta 2016, figs 22–27). Epigyne with no central pocket (Figs 8, 14, 20, 25, 34). Copulatory ducts much shorter than in *Penionomus* (Żabka 1988, fig. 118) and not twisted (Figs 9, 15, 21, 27, 36, 43). Accessory glands not distinctive - unlike in *Corambis* (Szűts 2002, figs 4, 17) where they are long.

**Description.** Cephalothorax medium-high, longer than broad and widest at the level of coxae II; fovea in distinct depression, posterior slope steep, starting behind fovea, eye field wider than long, trapezoid (PLE<ALE). Eyes in three rows, the first row straight. Chelicerae with two promarginal teeth, retromarginal tooth 4–6-cuspidate (Figs 19, 33, 41). Endites slender and divergent, in male with lateral outgrowth (Fig. 3). Labium wider than long. Sternum longer than wide. Abdomen ovoid, longer than wide. Spinnerets short. Legs moderately long and thin. Leg formula: I–IV–II–III. Male palpal organ simple: cymbium unmodified, tegulum longer than wide, ovoid, with no lobes, embolus curved, rather thin, retrolateral tibial apophysis single (Fig. 6). Epigyne copulatory openings located close to each other (Figs 21, 27, 43) or distinctly separated (Figs 9, 15, 36), sometimes strongly sclerotised (Figs 25–27). Copulatory ducts narrow. Spermathecae C-shaped (Figs 9, 15, 36) or semicircular (Figs 21, 27, 43).

**Distribution.** According to WSC (2017) three species of *Rogmocrypta* are listed from New Caledonia (*R. elegans*), Philippines (*R. nigella* Simon, 1900) and Singapore (*R. puta* Simon, 1900). However, two latter are poorly known, their bioclimatic distributional predictions (Fig. 45) do not match *Rogmocrypta*-pattern and they probably are not congeneric. Additionally, the five species described here seem to confirm New Caledonia as the diversity and radiation centre.

**Biology.** The species treated here are litter dweller in humid forests.

**Remarks.** According to recent molecular studies (Maddison et al. 2008, Maddison 2015), *Rogmocrypta* belongs to Viciriini tribe within the Australasian Astioida clade and is closely related to other New Caledonian genera such as *Trite* Simon, 1885,

<?> The diagnosis is handicapped by the lack of males for most species

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*Penionomus* Simon, 1903 and *Lystrocteissa* Simon, 1884. However, the analysis of male genitalia here and in Maddison et al (2008: fig. 3) raises some doubts about congeneric status of *R. elegans* (we dealt with the type) and cf. *Rogmocrypta* sp. in Maddison et al. (2008): both show important differences in embolus structure and tegular lobe, which is missing in *R. elegans*. To clarify the relationships of *Rogmocrypta* it is necessary to perform molecular tests for all species ever listed in the genus. At this stage any reference to other New Caledonian genera as possible relatives can only be provisional.

### *Rogmocrypta elegans* (Simon, 1885)

Figures 1–9, 44

*Chalcoscirtus elegans* Simon, 1885: 90.
*Rogmocrypta elegans*: Simon 1901: 445–446, figs 506D–E; Prószyński 1984: 123–124.

**Material.** 1♂ holotype, 1♀ paratype, New Caledonia: Nouméa, MNHN Paris, nr 7527.

**Diagnosis.** Males abdomen with two whitish stripes (Figs 1–2), embolus curved, arising from antero-prolateral part of tegulum (Fig. 5), retrolateral tibial apophysis short and conical (Fig. 6). Epigyne copulatory openings oriented towards each other, more separated than in *R. koniambo* sp. n.

**Distribution.** Known only from the type locality (Fig. 44).

**Remark.** This is the only known and illustrated species of *Rogmocrypta* (Prószyński 1984), and it is used here for comparative purposes.

### *Rogmocrypta karolinae* sp. n.

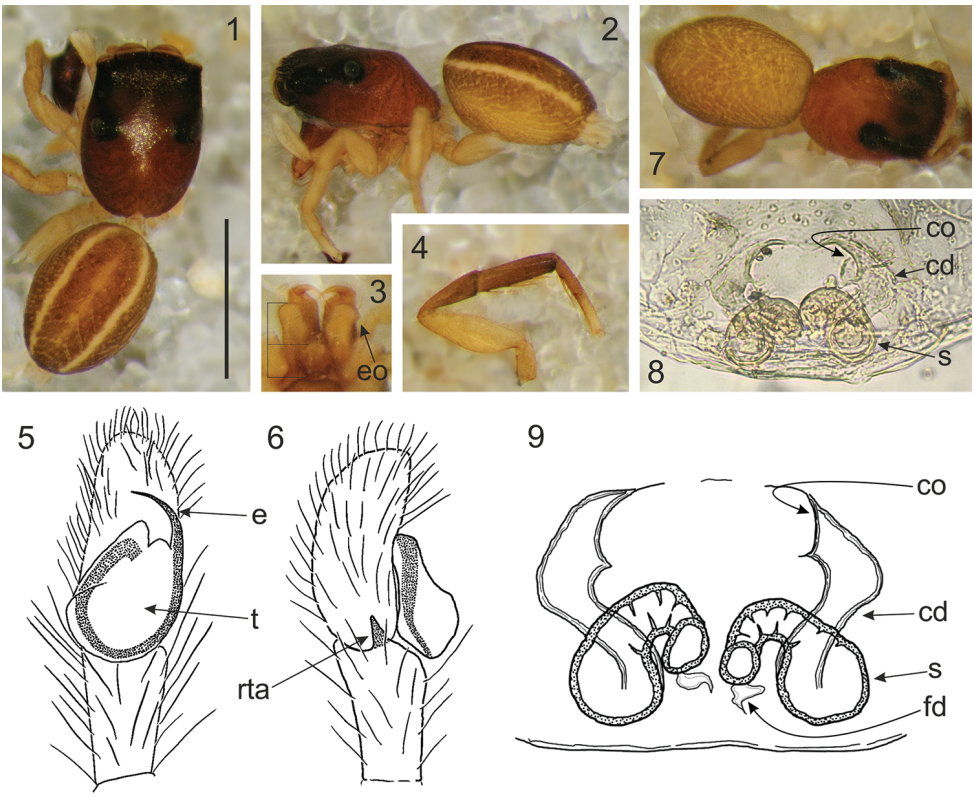
http://zoobank.org/184FCC48-B4F4-4034-B68B-68B2F3BF1D8A
Figures 10–15, 44

**Material.** 1♀ holotype, New Caledonia: Mandjélia (164°32'E, 20°24'S), 600m elev., rainforest, pitfalls, October 1992–17 February 1993, Raven R, Guillbert E, QM S44894; 1♀, paratype, same data as holotype; 3♀, New Caledonia: Mandjélia (164°32'E 20°24'S), pitfalls, 13 May–October 1992, Raven R, Guillbert E, Ingram G, QM S37722.

**Etymology.** For Karolina, daughter of Joanna Gardzińska.

**Diagnosis.** Cephalothorax and abdomen with distinctive patches of white scales (Figs 10–11). Copulatory openings closer to each other than in *R. elegans* (Fig. 15). Spermathecae horizontal (Figs 14–15).

**Description.** Female holotype. Cephalothorax brown with darker cephalic part, with patches of scales (Fig. 10). Abdomen greyish brown, with three pairs of patches covered with white scales. Spinnerets whitish. Chelicerae with single retromargin 5-cuspidate tooth. Clypeus narrow (17% of AME diameter), covered with sparse white hairs. Labium and endites brown with lighter chewing margins. Sternum and venter greyish brown. Legs light brown, tibiae and metatarsi with darker bands (Fig. 12),




**Figures 1–9.** *Rogmocrypta elegans*. **1–6** Male (holotype) **1** Dorsal view **2** Lateral view **3** Endites and labium **4** First leg **5** Right palp ventrally **6** Same, retrolaterally **7–9** Female (paratype) **7** Dorsal view **8–9** Vulva. Abbreviations: cd: copulatory duct, co: copulatory opening, e: embolus, eo: endites outgrowth, fd: fertilisation duct, rta: retrolateral tibial apophysis, s: spermatheca, t: tegulum. Scale bar: 1 mm.

metatarsi and patellae covered by white scales. Epigyne copulatory openings oriented towards each other, copulatory ducts sinuous, spermathecae C-shaped, close to each other (Fig. 15). Dimensions. CL 1.23, CW 0.95, CH 0.54, EFL 0.55, AEW 0.97, PEW 0.85, AL 1.15, AW 1.05, LI: 3.42, LII: 1.99, LIII: 1.70, LIV: 2.61.

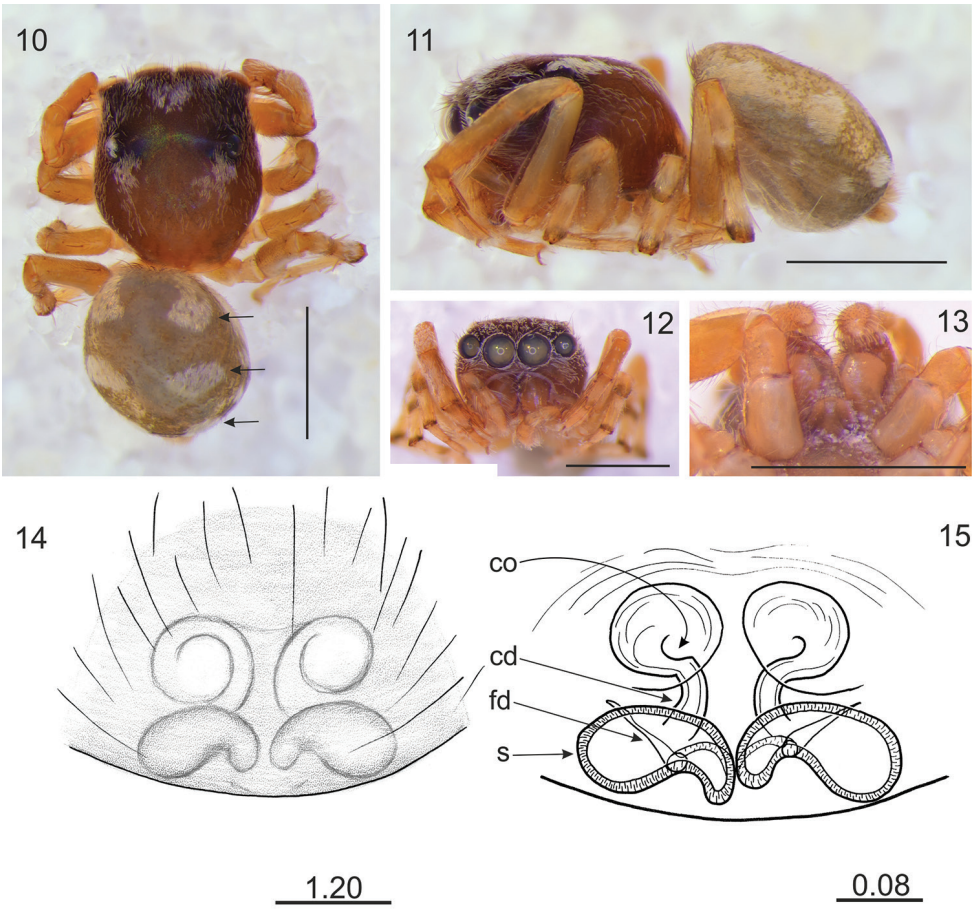
Male unknown.

**Distribution.** Known from type locality only (Fig. 44).

***Rogmocrypta koniambo* sp. n.**
http://zoobank.org/FF2E8207-FCA3-4854-8F15-BABDE1AE13AF
Figures 16–21, 44

**Material.** 1♀ holotype, New Caledonia: Mt. Koniambo (164°47'11"E, 20°59'42"S), 700m elev., forêt seche/rub, A&S Tillier, 25 March 1987, MNHN.

**Etymology.** The name refers to the type locality.



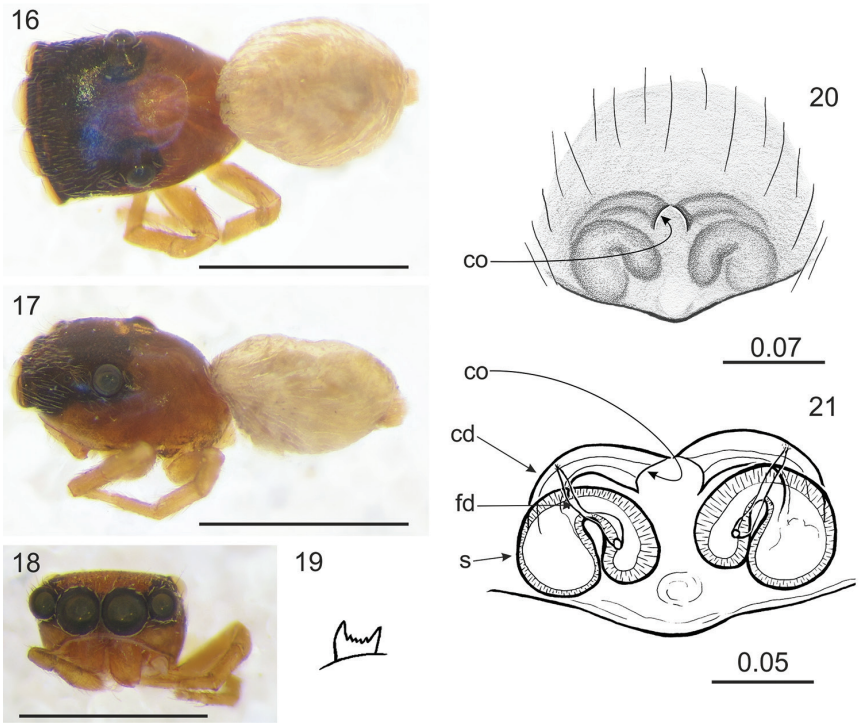

**Figures 10–15.** *Rogmocrypta karolinae* sp. n. (female holotype). **10** Dorsal view (arrows indicate patches of white scales being distinctive diagnostic characters) **11** Lateral view **12** Frontal view **13** Endites and labium **14** Epigyna **15** Vulva. Abbreviations: cd: copulatory duct, co: copulatory opening, fd: fertilisation duct, s: spermatheca. Scale bars: 1 mm (**10–13**); 1.20 mm (**14**); 0.08 mm(**15**).

**Diagnosis.** In comparison to previous species copulatory openings closer to each other and located just in front of spermathecae.

**Description.** Female holotype (in bad condition). Cephalothorax brown with darker cephalic part, covered with sparse white scales. Foveal depression well marked (Fig. 16). Abdomen ovoid, pale, covered with sparse white scales. Spinnerets whitish. Palps and legs II and III greyish brown. Other legs missing. Chelicerae short, brown, retromarginal tooth 6-cuspidate (Fig. 19). Labium brown, endites light brown with whitish chewing margins. Venter whitish. Epigyne copulatory ducts and spermathecae semicircular, the latter almost horizontal (Fig. 21). Dimensions: CL 1.06, CW 0.77, CH 0.42, EFL 0.51, AEW 0.80, PEW 0.65, AL 0.93, AW 0.74, LI and LII missing, LIII: 1.58, LIV: 1.72.

Male unknown.

**Distribution.** Known from type locality only (Fig. 44).




**Figures 16–21.** *Rogmocrypta koniambo* sp. n. (female holotype). **16** Dorsal view **17** Lateral view **18** Frontal view **19** Retromarginal tooth **20** Epigyna **21** Vulva. Abbreviations: cd: copulatory duct, co: copulatory opening, fd: fertilisation duct, s: spermatheca. Scale bars: 1 mm (**16–18**); 0.07 mm (**20**); 0.05 mm (**21**).

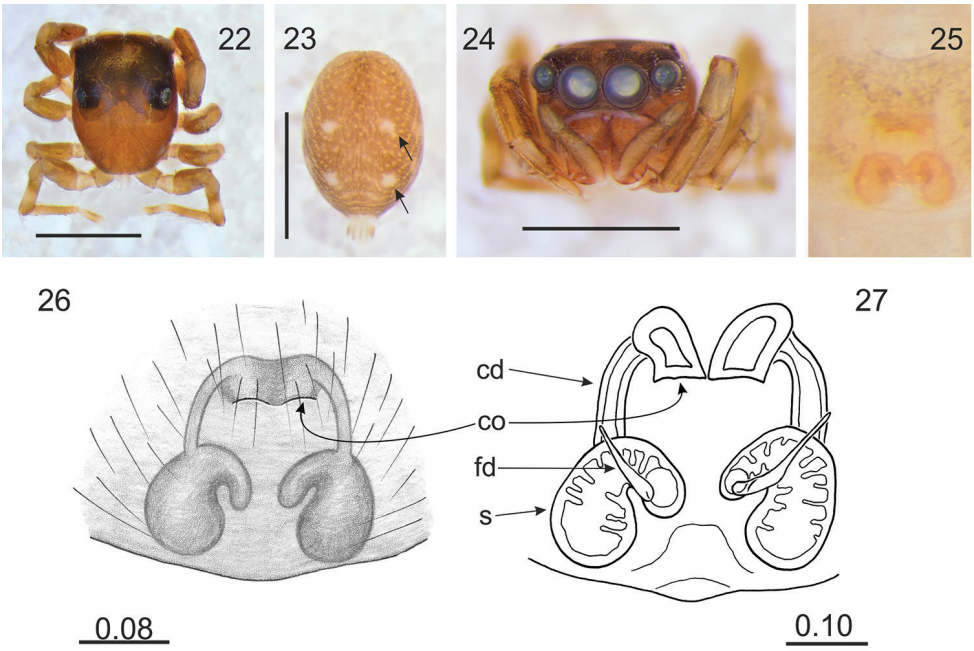
***Rogmocrypta patryki* sp. n.**
http://zoobank.org/550946C1-6C62-417A-815C-68E1E0D0C870
Figures 22–27, 44

**Material.** 1♀ holotype, New Caledonia: Mt. Oua Tilou (164°51'28"E, 20°51'57"S), arête S, 510 m elev., foret sèche, berlesate, st. 198a, 19 October 1988, A&S Tillier, Chazeau J, MNHN; 1♀ paratype same data as holotype; 1♀, New Caledonia: Mt. Panié, 450–950m, 14 May 1984, Monteith G, Cook D, QM S35668; 1♀, New Caledonia: Mt. Panié summit, 1628 m elev., 15 May 1984, Monteith G, Cook D, QM S35665; 2♀, 1 juv. New Caledonia: Mt. Oua Tilou (164°51'28"E, 20°51'57"S), arête S, 510m elev., foret sèche, berlese, st. 198a, 19 October 1988, A&S Tillier, Chazeau J, MNHN.

**Etymology.** For Patryk Patoleta, Barbara Patoleta's son.

**Diagnosis.** Abdomen with lighter chevrons and distinctive white patches (Fig. 23). Copulatory openings close to each other, orientated posteriorly, and located well anteriorly to spermathecae (Figs 25–27).

**Description.** Female holotype. Cephalothorax brown, with darker eye field and foveal depression (Fig. 22). Abdomen greyish brown, with distinctive pattern as in Fig. 23. Spinnerets light brown. Cheliceral retromarginal tooth 5-cuspidate. Clypeus




**Figures 22–27.** *Rogmocrypta patryki* sp. n. (female holotype). **22** Cephalothorax, dorsal view **23** Abdomen, dorsal view (arrows indicate white patches being distinctive diagnostic characters) **24** Frontal view **25–26** Epigyna **27** Vulva. Abbreviations: cd: copulatory duct, co: copulatory opening, fd: fertilisation duct, s: spermatheca. Scale bars: 1 mm (**22–24**); 0.08 mm (**26**); 0.10 mm (**27**).

brown, much narrower (6%) than AME diameter. Labium and endites light brown. Sternum brown. Venter whitish, with brownish spots. Legs brown, tibiae and metatarsi with darker bands (Figs 22, 24). Epigyne copulatory ducts and spermathecae semicircular, the latter in diagonal position (Figs 26–27). Dimensions. CL 1.40, CW 1.05, CH 0.65, EFL 0.63, AEW 1.00, PEW 0.95, AL 1.53, AW 1.08, LI: 3.06, LII: 2.42, LIII: 2.56, LIV: 3.37.

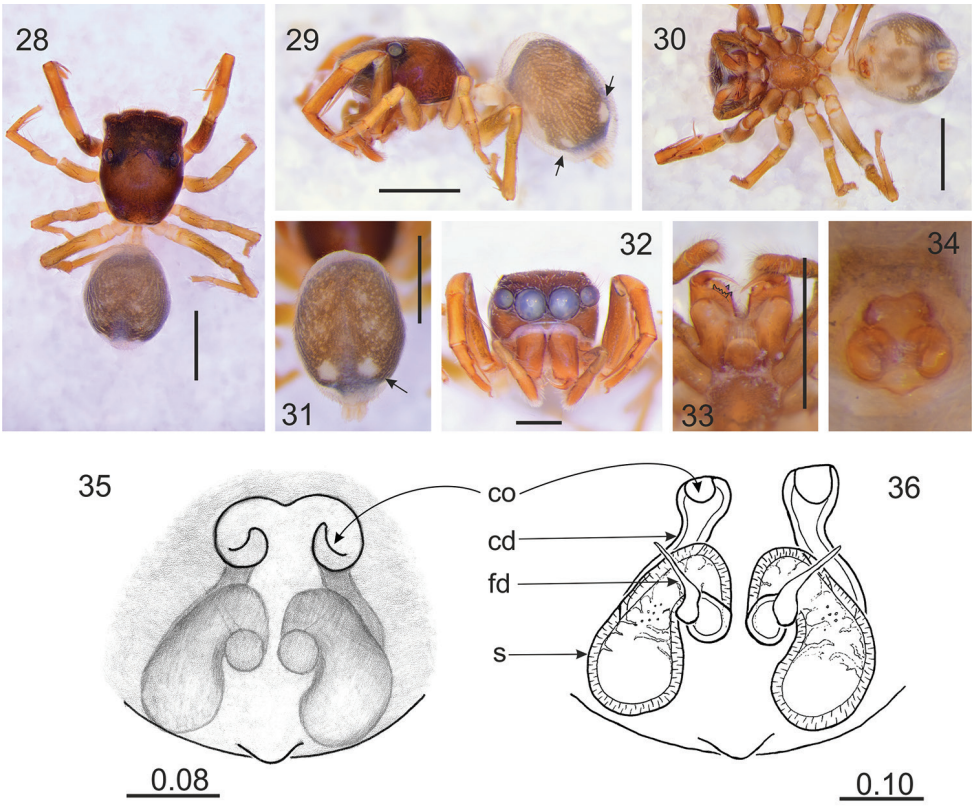
Male unknown.

**Distribution.** Known from Mt. Panié and Mt. Oua Tilou in New Caledonia (Fig. 44).

***Rogmocrypta raveni* sp. n.**
http://zoobank.org/8A020BFF-2D8F-4D8F-9EDA-510F8FB8E22C
Figures 28–36, 44

**Material.** 1♀ holotype, New Caledonia: Mt. Panié (20°35'S, 164°45'E), 400m elev., pitfalls, October 1992 – February 1993, Raven R, Guillbert E, Ingram G, QM S35759; 2♀♀ paratypes, same data as holotype.

**Etymology.** For Dr Robert Raven (Queensland Museum, Brisbane), distinguished Australian arachnologist and collector of the material studied.



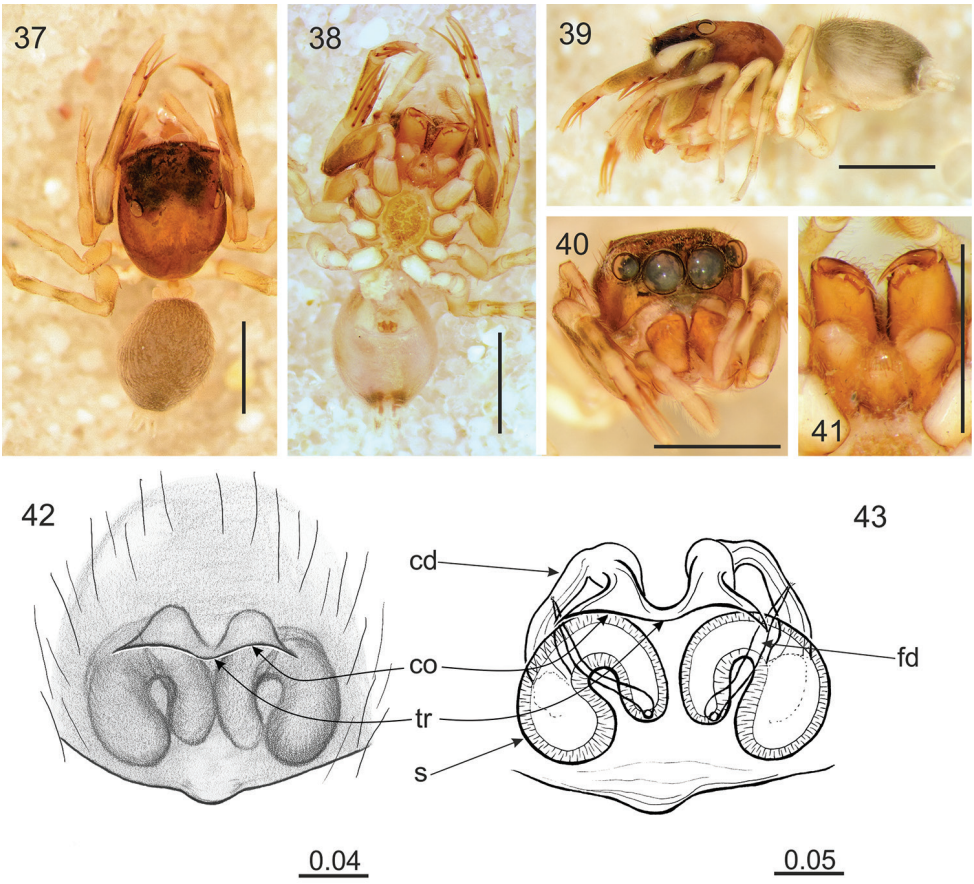

**Figures 28–36.** *Rogmocrypta raveni* sp. n. (female holotype). **28** Dorsal view **29** Lateral view **30** Ventral view **31** Abdomen dorsal view **32** Frontal view **33** Endites and labium **34–35** Epigyna **36** Vulva. (arrows in Figs **29** & **31** indicate white spots being distinctive diagnostic characters). Abbreviations: cd: copulatory duct, co: copulatory opening, fd: fertilisation duct, s: spermatheca. Scale bars: 1 mm (**28–33**); 0.08 mm (**35**); 0.10 mm (**36**).

**Diagnosis.** Abdomen with white dorsal and ventral spots (Figs 29–31). Copulatory openings oriented anteriorly (Fig. 35), copulatory ducts undulating (Fig. 36).

**Description.** Female holotype. Cephalothorax brown, with darker cephalic part, covered by sparse whitish scales. Foveal depression well marked (Fig. 28). Abdomen ovoid, grey brown with lighter pattern as in Fig. 31, covered with sparse brown hairs. Anterior spinnerets light brown, posterior ones whitish. Palps brown. Legs I brown, others lighter. Chelicerae brown, retromarginal tooth 5-cuspidate (Fig. 33). Labium and endites light brown, with lighter chewing margins. Sternum brown. Venter with white and greyish brown pattern (Fig. 30). Epigyne with copulatory openings well separated from each other and from spermathecae, the last in diagonal position, C-shaped (Figs 34–36). Dimensions. CL 1.30, CW 1.03, CH 0.60, EFL 0.60, AEW 1.00, PEW 0.95, AL 1.50, AW 1.07, LI: 4.05, LII: 2.85, LIII: 2.70, LIV: 3.15.

Male unknown.

**Distribution.** Known from type locality only (Fig. 44).




**Figures 37–43.** *Rogmocrypta rollardae* sp. n. (female holotype). **37** Dorsal view **38** Ventral view **39** Lateral view **40** Frontal view **41** Endites and labium **42** Epigyna **43** Vulva. Abbreviations: cd: copulatory duct, co: copulatory opening, fd: fertilisation duct, s: spermatheca, tr: transverse ridge. Scale bars: 1 mm (**37–41**); 0.04 mm (**42**); 0.05 mm (**43**).

### *Rogmocrypta rollardae* sp. n.

http://zoobank.org/94431F06-A47C-4CBA-936B-DE925E98083D
Figures 37–44

**Material.** 1♀ holotype, New Caledonia: Mandjélia (20°24'S, 164°32'E), 650m elev., rainforest, litter, berlesate, 12 May 1984, Monteith G, Cook D, QM S35648; 2♀ paratypes, New Caledonia: 4 km N of Col d'Amieu (21°19'48"S, 165°30'E), rainforest, litter, 300 m elev., berlesate No 640, 8 May 1984, Monteith G, Cook D, QM; 1♀, New Caledonia: Mandjélia (20°24'S, 164°32'E), 700 m elev., rainforest, litter, berlesate nr 648, 12 May 1984, Monteith G, Cook D, QM S35651; 1♀ New Caledonia: Dent de St. Vincent (166°13'02"E, 21°52'12"S), arete S, 1150 m elev., forêt-magius haut humide, berlese, 6 August 1987, A&S Tillier, Bonnet, Letocart, MNHN.

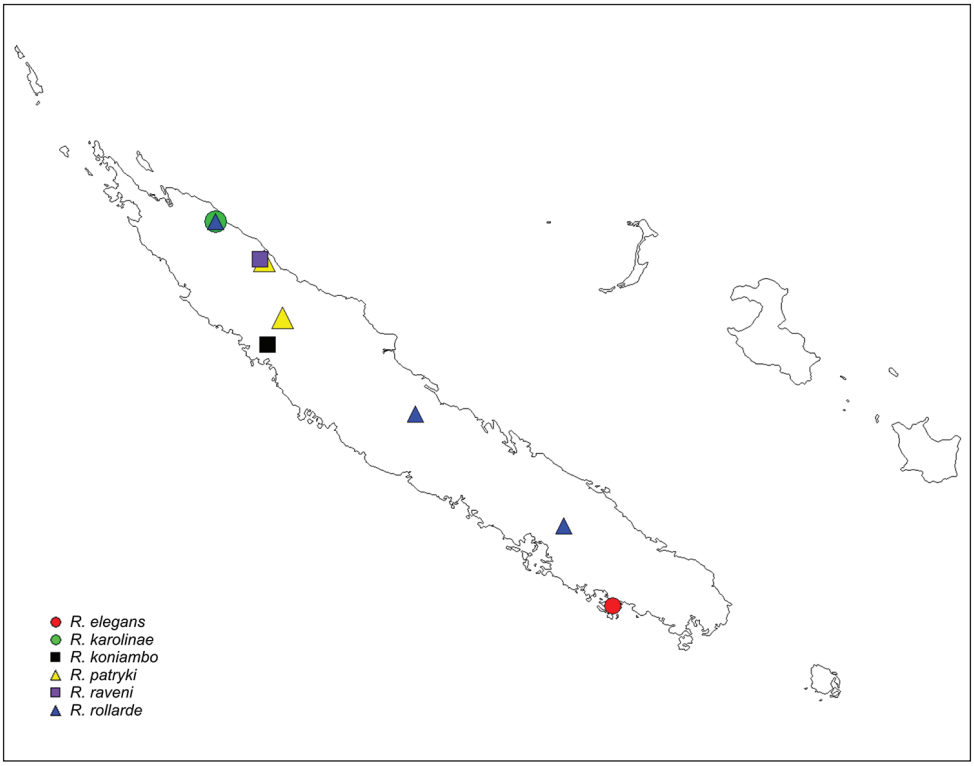



**Figure 44.** Distribution records of *Rogmocrypta*: *R. elegans* (red circle), *R. karolinae* (green circle), *R. koniambo* (black square), *R. patryki* (yellow triangle), *R. raveni* (purple square), *R. rollardae* (blue triangle).

**Etymology.** For Dr. Christine Rollard (MNHN, Paris), distinguished French arachnologist.

**Diagnosis.** Copulatory openings close to spermathecae, oriented posteriorly and joined, forming kind of transverse ridge (Figs 42–43).

**Description.** Female holotype. Cephalothorax brown, covered with sparse white scales and brown hairs. Foveal depression well marked. Abdomen brownish, with lighter chevrons (Fig. 37). Anterior spinnerets light brown, posterior ones whitish. Palps and legs brownish with darker bands. Chelicerae brown, retromarginal tooth 6-cuspidate. Labium and endites light brown with lighter chewing margins. Sternum grey brown. Venter with white and grey brown pattern (Fig. 38). Epigyne with copulatory openings strongly sclerotized and close to each other (Figs 42–43). Copulatory ducts and spermathecae semicircular, the latter in diagonal position (Fig 43). Dimensions. CL 1.82, CW 1.32, CH 0.83, EFL 0.78, AEW 1.22, PEW 1.09, AL 1.97, AW 1.45, LI: 5.24, LII: 3.64, LIII: 4.34, LIV: 4.92.

Male unknown.

**Distribution.** Known from Mandjélia, Col d'Amieu and Dent de St. Vincent in New Caledonia (Fig. 44).

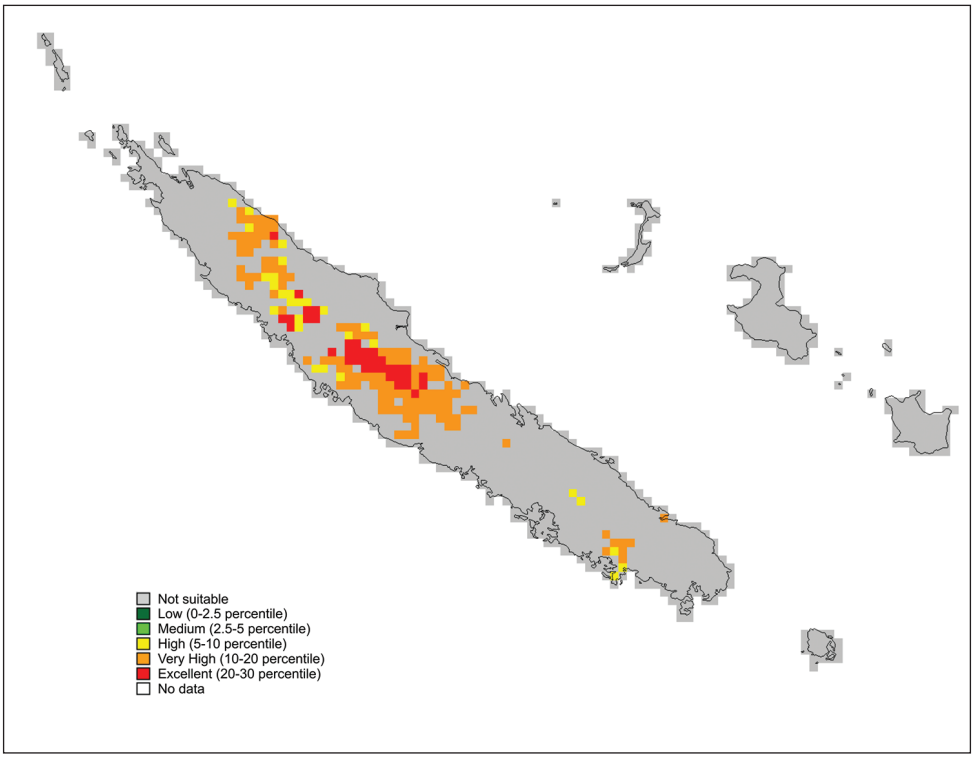



**Figure 45.** Predicted distribution of *Rogmocrypta* (14 records).

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Dr Christine Rollard (Museum National d'Histoire Naturelle, Paris) and Dr. Robert Raven (Queensland Museum, Brismane) provided specimens for study and generous help during our visits (BP & MŻ) to their institutions. Prof. Wayne Maddison (Vancouver, Canada) and Dr. Dmitri Logunov (Manchester, United Kingdom) reviewed the manuscript and provided critical comments and suggestions. The research is part of the project 18/91/S conducted in our university.

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RESEARCH ARTICLE

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# A gene-tree test of the traditional taxonomy of American deer: the importance of voucher specimens, geographic data, and dense sampling

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## Abstract

The taxonomy of American deer has been established almost entirely on the basis of morphological data and without the use of explicit phylogenetic methods; hence, phylogenetic analyses including data for all of the currently recognized species, even if based on a single gene, might improve current understanding of their taxonomy. We tested the monophyly of the morphology-defined genera and species of New World deer (Odocoileini) with phylogenetic analyses of mitochondrial DNA sequences. This is the first such test conducted using extensive geographic and taxonomic sampling. Our results do not support the

monophyly of *Mazama*, *Odocoileus*, *Pudu*, *M. americana*, *M. nemorivaga*, *Od. hemionus*, and *Od. virginianus*. *Mazama* contains species that belong to other genera. We found a novel sister-taxon relationship between *"Mazama" pandora* and a clade formed by *Od. hemionus columbianus* and *Od. h. sitkensis,* and transfer *pandora* to *Odocoileus*. The clade formed by *Od. h. columbianus* and *Od. h. sitkensis* may represent a valid species, whereas the remaining subspecies of *Od. hemionus* appear closer to *Od. virginianus*. *Pudu* (*Pudu*) *puda* was not found sister to *Pudu (Pudella) mephistophiles*. If confirmed, this result will prompt the recognition of the monotypic *Pudella* as a distinct genus. We provide evidence for the existence of an undescribed species now confused with *Mazama americana*, and identify other instances of cryptic, taxonomically unrecognized species-level diversity among populations here regarded as *Mazama temama*, "*Mazama*" *nemorivaga*, and *Hippocamelus antisensis*. Noteworthy records that substantially extend the known distributions of *M. temama* and "*M.*" *gouazoubira* are provided, and we unveil a surprising ambiguity regarding the distribution of "*M.*" *nemorivaga*, as it is described in the literature. The study of deer of the tribe Odocoileini has been hampered by the paucity of information regarding voucher specimens and the provenance of sequences deposited in GenBank. We pinpoint priorities for future systematic research on the tribe Odocoileini.

**Keywords**

Deer, Cervidae, Neotropics, Americas, Taxonomy, *Odocoileus*, *Mazama*, *Pudu*, *Hippocamelus*, phylogenetics, mDNA, CYTB

## Introduction

The tribe Odocoileini (Cervidae: Capreolinae) represents a monophyletic group encompassing all modern deer native to the New World (Americas) with the exception of the Holarctic taxa *Alces alces* (Alceini), *Cervus canadensis* (Cervini), and *Rangifer tarandus* (Rangiferini) (Price et al. 2005, Gilbert et al. 2006, Hughes et al. 2006, Agnarsson and May-Collado 2008, Decker et al. 2009, Hassanin et al. 2012, Heckeberg et al. 2016)—see Heckeberg et al. (2016) for current suprageneric taxonomy. Living Odocoileini deer have been traditionally classified in six genera (*Blastocerus*, *Hippocamelus*, *Mazama*, *Odocoileus*, *Ozotoceros*, and *Pudu*) and 16 species (Merino and Rossi 2010, Mattioli 2011; see also Gutiérrez et al. 2015), but alternative taxonomic propositions have suggested that the alpha-level diversity of the tribe might be higher (Molina and Molinari 1999, Molinari 2007, Groves and Grubb 2011). Some authors have also included *Rangifer* as a member of Odocoileini (e.g., Groves and Grubb 2011).

The native distribution of Odocoileini ranges from northern North America (Alaska, Canada) to southern South America (Patagonia), including some islands of the Caribbean Sea and the Atlantic and Pacific oceans. Collectively, members of the tribe occupy a wide variety of habitats, including desert scrub, savannas, swamps, lowland rain forests, humid-montane forests, páramo, and alpine tundra at elevations from sea level to about 4800 meters (Allen 1915, Hershkovitz 1982, Baker 1984, Méndez 1984, Brokx 1984, Medellín et al. 1998, González et al. 2002, Cronin et al. 2006, Meier and Merino 2007, Molinari 2007, Rumiz et al. 2007, Latch et al. 2009, Miranda et al. 2009, Piovezan et al. 2010, Groves and Grubb 2011, Mendes-Oliveira et al.

2011, Barrio 2013, Gutiérrez et al. 2015). By virtue of this wide ecogeographic range, Odocoileini is of great biogeographic interest.

Despite being heavily hunted animals in the Western Hemisphere and also of great public health interest (Bennett and Robinson 2000, Hurtado-Gonzales and Bodmer 2004, Angers et al. 2006, Campbell and VerCauteren 2011, Martinsen et al. 2016, Uehlinger et al. 2016), relatively little progress has been achieved in recent decades with regard to the systematics of Odocoileini deer. To date, only the genera *Mazama* (Allen 1915) and *Pudu* (Hershkovitz 1982) have been subjects of specimen-based revisionary taxonomic work, but these studies did not employ explicit phylogenetic methods. In general, the scientific community has largely followed the taxonomic arrangements recognized by 20th century authorities, predominantly E. R. Hall for North America (Hall 1981) and A. Cabrera for South America (Cabrera 1961). The uncritical acceptance of these taxonomic arrangements for decades is indefensible because the criteria, data, and methods used to construct them are largely unknown, unclear, or even incorrect (see example pointed out by Molinari [2007, p. 31]). Several recent taxonomic studies have demonstrated that the traditional taxonomy of Odocoileini deer needs to be revisited. For instance, morphometric analyses and differences in the frequency of qualitative skeletal traits in *Odocoileus virginianus* of northern South America and North America led Molina and Molinari (1999) to propose that populations from North and South America are not conspecific. These authors also demonstrated a remarkable degree of morphological variability among Venezuelan populations of *Od. virginianus*, whose taxonomy remains disputed (Moscarella et al. 2003, 2007, Molinari 2007). Another example comes from phylogenetic analyses of molecular data demonstrating that the genus *Mazama*, as traditionally understood (Allen 1915, Cabrera 1961), is polyphyletic (Gilbert et al. 2006, Duarte et al. 2008, Hassanin et al. 2012, Escobedo-Morales et al. 2016, Heckeberg et al. 2016). Unfortunately, phylogenetic studies of Odocoileini published to date have been based on limited taxonomic and/or geographic sampling—i.e., lacking taxa or using exemplars for widely distributed and highly variable taxa (e.g., species of *Odocoileus*). Nevertheless, these and other taxonomic studies, some based on karyology (e.g., Jorge and Benirschke 1977, Duarte and Jorge 2003, Cursino et al. 2014), have documented the need to revise the systematics of Odocoileini deer.

Biologically meaningful species-level taxonomies are essential for study design in evolutionary biology, and inadequate species-level classifications, such as uncritically lumping or splitting taxa in absence of appropriate evidence, can detrimentally impact species conservation (George and Mayden 2005, Gutiérrez and Helgen 2013, Heller et al. 2013, Kaiser et al. 2013, Zachos et al. 2013, Voigt et al. 2015, Gippoliti et al. 2017). Accordingly, our long-term goal is to improve all aspects related to the systematics of odocoileines. A first step is to test whether phylogenetic analyses of mtDNA sequence data support the monophyly of recognized genera and species. These analyses have the potential to identify or indicate (1) distant phylogenetic relationships and deep divergences in species or populations currently lumped into a single genus or species, respectively; and (2) close phylogenetic relationships and

shallow divergences in species or populations currently split into different genera or species, respectively. Discovering any of these conditions can help target taxa requiring closer attention by taxonomists. Such a test also affords the first assessment of phylogenetic relationships among odocoileines that is simultaneously based on data for all traditionally recognized species, relatively dense geographic sampling within their ranges, and informed by our morphological examination of relevant voucher material in most cases. Nevertheless, because phylogenetic relationships can only be convincingly inferred based on sequence data from multiple, independently inherited loci—e.g., mtDNA, nuclear introns and exons located on different chromosomes—we understand the need to avoid overinterpretations of the gene tree that resulted from our analyses. As interpreted here, our results represent a set of explicit hypotheses that will serve to guide further research.

## Methods

### Sources of material, and taxonomic and geographic sampling

Our analyses were based on 192 sequences of the mitochondrial cytochrome-*b* (*CYTB*) gene. We drew on this marker for three reasons. First, *CYTB* sequences can be obtained relatively easily from degraded DNA that is extracted from museum specimens, which is important for our study since no freshly-preserved samples were available for several targeted species or populations. Second, previous studies have shown that analyses of *CYTB* sequence data can substantially clarify the taxonomic status of mammals whose taxonomy had been predominantly studied based only on morphological and/or karyological data (Duarte et al. 2008, Helgen et al. 2009, Gutiérrez et al. 2010, 2014, 2015, Voss et al. 2013). This coding gene evolves relatively rapidly yet is stable enough to offer insights at suprageneric levels (Agnarsson and May-Collado 2008, Ge et al. 2014), and many studies employing *CYTB* alongside unlinked nuclear sequences have found compatible patterns of variation among them, indicating that *CYTB* can be useful as a first-order estimator of phylogenetic history (Velazco and Patterson 2013, Voss et al. 2014, Upham and Patterson 2015). Third, a large number of *CYTB* sequences are available from GenBank and include most of our focal species. We obtained 171 sequences from GenBank and generated the remaining 21 sequences. All but two (KY928656, KY928667) of the latter sequences were obtained from degraded DNA extracted from museum specimens, from residual soft tissue attached to skeletons, or from maxilloturbinate bones (Wisely et al. 2004) (Table 1). Use of museum specimens allowed us to obtain sequence data for (1) species for which molecular data were previously lacking (i.e., *Mazama chunyi* and *Pudu mephistophiles;* but see Heckeberg et al. 2016), and (2) populations from regions never included in any phylogeographic or phylogenetic study—e.g., from southern Central America and the Andes of Ecuador and Peru for *Odocoileus virginianus*. A study just published by Heckeberg et al. (2016) included *CYTB* data obtained from European museum specimens for *Mazama chunyi*

**Table 1. Sequenced specimens.** GB: GenBank accession number. Catalogue#: museum catalogue number. Provenance: geographic origin (name of country, larger administrative entity, and a numeric identifier that corresponds to detailed locality information presented in the Gazetteer; supplementary file 1). DNA: number assigned to DNA extracted. Year: year in which the specimen was collected. M: Sequencing method (I: Illumina; S: Sanger; see Methods).

| Species | GB | Catalogue# | Provenance | DNA | Year | M |
|---|---|---|---|---|---|---|
| *B. dichotomus* | KY928652 | FMNH 52329 | Brazil: São Paulo (3) | EEG 343 | 1941 | I |
| *M. americana* | KY928653 | AMNH 67109 | Peru: Cajamarca (10) | EEG 437 | 1924 | I |
| *M. americana* | KY928654 | USNM 443588 | Venezuela: Yaracuy (21) | EEG 636 | 1967 | I |
| *M. chunyi* | KY928655 | FMNH 79912 | Peru: Puno: Sandia (12) | EEG 297 [MTRH 293] | 1951 | S |
| *M. gouazoubira* | KY928656 | KU 155307 | Guyana: Potaro-Siparuni (8) | EEG 568 | 1997 | I |
| *M. nemorivaga* | KY928657 | AMNH 96171 | Brazil: Para (2) | EEG 470 | 1931 | I |
| *M. nemorivaga* | KY928658 | USNM 374916 | Venezuela: Bolívar (20) | EEG 628 | 1966 | I |
| *Od. pandora* | KY928659 | KU 93857 | Mexico: Campeche (13) | EEG 570 | 1963 | I |
| *M. rufina* | KY928660 [1] | FMNH 70563 [2] | Colombia: Cundinamarca (5) | EEG 326 | 1952 | I |
| *M. temama* | KY928661 | KU 82215 | Guatemala: Petén (7) | EEG 572 | 1960 | I |
| *Od. virginianus* | KY928662 | AMNH 62872 | Ecuador: Los Ríos (6) | EEG 374 | 1922 | S |
| *Od. virginianus* | KY928663 | AMNH 29453 | Nicaragua: Jinotega (16) | EEG 398 | 1909 | S |
| *Od. hemious* | KY928664 | USNM 99455 | USA: Arizona (18) | EEG 672 | 1900 | I |
| *Od. hemious* | KY928665 | USNM 249424 | USA: Alaska (17) | EEG 666 | 1930 | I |
| *Od. virginianus* | KY928666 | USNM 99351 | Mexico: Chihuahua (14) | EEG 039 | 1899 | I |
| *Od. virginianus* [3] | KY928667 | – | USA: Washington DC (19) | WTD0028 | 2010 | S |
| *Od. virginianus* | KY928668 | FMNH 78421 | Peru: Puno (11) | EEG 227 | 1950 | I |
| *Od. virginianus* | KY928669 | KU 149129 | Honduras: Cortes (9) | EEG 559 | 1955 | I |
| *Od. virginianus* | KY928670 | KU 93852 | Mexico: Yucatán (15) | EEG 562 | 1963 | S |
| *Oz. bezoarticus* | KY928671 | FMNH 28297 | Brazil: Mato Grosso (1) | EEG 354 | 1927 | I |
| *P. mephistophiles* | KY928672 | AMNH 181505 | Colombia: Cauca (4) | EEG 362 | 1958 | S |

[1] A previous study (Gutiérrez et al. 2015) generated a CYTB sequence (GenBank accession number is KR107038) for this specimen employing Sanger sequencing procedures.

[2] The museum abbreviation for this specimen has been mistakenly reported as "USNM" (see Supporting information in Gutiérrez et al. 2015).

[3] Hybrid, cross between *Od. virginianus* and *Od. hemionus* (see Discussion).

and *Pudu mephistophiles* that we could not access during the development of the present study. We independently generated and analyzed data for these species. We analyzed our sequences employing a more comprehensive geographic sampling for most Odocoileini taxa; hence, we take the opportunity to compare results from both studies and discuss the effect of geographic sampling on the resolution of the gene-trees and its impact on associated taxonomic interpretations. We deposited all sequences that we generated in GenBank, along with the museum catalogue numbers of their respective voucher specimens, tissue numbers, or both (Table 1). The geographic provenance and the names of the institutions that house voucher specimens are provided in the supplementary file 1 (see also Figures 1a, 1b, 1c for abbreviated provenance locality information and GenBank accession numbers of all analyzed sequences).

**Laboratory methods**

We employed both Sanger (following Gutiérrez et al. 2015) and massively parallel (following Hawkins et al. 2016) sequencing technologies to generate part of the analyzed sequences. In order to minimize the risk of contamination with exogenous DNA, all pre-amplification procedures—i.e., DNA extractions, and either settings of conventional PCR reactions or library preparations—based on material obtained from museum specimens were conducted in an isolated facility dedicated exclusively to work with degraded DNA (i.e., where no PCR products have ever been present). We conducted phenol/chloroform DNA extractions following Wisely et al. (2004). Samples were concentrated with Amicon (Millipore, Darmstadt, Germany) filters via centrifugation and stored in siliconized tubes with an additional 20–50 µl of 1 X TE plus 0.5% Tween 20 (Sigma) and stored at -20°C. The DNA of the single freshly preserved tissue sample was extracted in a standard DNA extraction laboratory with a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions.

We employed various combinations of primers to amplify and to sequence short *CYTB* fragments (supplementary file 2). These reactions were conducted in a six-stage touchdown protocol using a thermal cycler (MJ Research). After an incubation at 95°C for 10 min, the first stage consisted of 2 cycles of the following steps: denaturing at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 min. The subsequent stages were identical to the first stage except for lowered annealing temperatures, which were 58°C, 56°C, 54°C, and 52°C for the second, third, fourth, and fifth stages, respectively. The sixth (final) stage consisted of 40 cycles with an annealing temperature of 50°C. All PCR reactions were set in 25 µl volumes containing 0.5 U AmpliTaq Polymerase (Applied Biosystems, Foster City, CA), 1X PCR AmpliTaq Buffer, 0.2 µM each dNTP, 0.4 µM of forward and 0.4 µM of reverse primers, 1.5 µM MgCl2, 10X BSA (New England Biolabs, Ipswich, MA), and 50–250 ng of genomic DNA template. Successful amplifications were purified using ExoSAP (USB Corporation, Cleveland, OH) incubated at 37°C for 15 min followed by 80°C for 15 min. Both strands of each PCR product were cycle sequenced by subjecting them to a second amplification using a total of 10 µL sequencing reaction mixture, including 50–200 ng of PCR product, 10 pM of corresponding forward or reverse primer, 5X Big Dye Buffer (Applied Biosystems), 1/8 reaction of Big Dye version 3 (Applied Biosystems). The following conditions were used for the Dye Terminator Cycle Sequencing: 25 cycles consisting of denaturing at 96°C for 10 s, annealing at 50°C for 10 s and extension at 60°C for 4 min. The final products were cleaned using Sephadex filtration and then both the 3' and 5' strands were sequenced on a 50 cm array using the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). To compile and edit the sequences that were generated via Sanger sequencing, we employed Geneious v.7.1.5. (Biomatters; http://www.geneious.com/).

Some of the analyzed *CYTB* sequences were trimmed from 31 mitochondrial genomes (mitogenomes), 16 obtained from GenBank (generated by Hassanin et al. 2012) and 15 generated by us (following Hawkins et al. 2016). To generate these mi-

togenomes, we prepared samples for Illumina sequencing using commercially available library preparation kits (Kapa Biosystems Illumina Library Preparation Kit #KK8232, Wilmington, MA, USA). Single indexed TruSeq-style adapters were used (Faircloth and Glenn 2012) employing 50 µl of DNA extract. Minor modifications to the manufacturer's protocol (see Supplementary Materials Hawkins et al. [2016]) were made, including additional PCR cycles on degraded samples (18 cycles for degraded DNA from museum samples, and 10 for the freshly-preserved DNA sample). The success of library preparation was determined by visualization on an agarose gel. Then, the samples were purified with MagNA magnetic beads (Rohland and Reich 2012) in place of AMPure XP beads to bind DNA and remove the primer and adapter dimer. A ratio of 2.4:1 of MagNA beads to DNA was added to remove adapter dimer. DNA concentration was determined using the Nanodrop v.2.0.

We multiplexed samples in order to decrease the costs associated with library enrichments. Individual samples were multiplexed in equimolar ratios for enrichment based on Nanodrop values in conjunction with the appearance and size distribution from the agarose gel. Each multiplexed pool contained 4–10 uniquely indexed samples for a total concentration of 500 ng concentrated to 3.4 µl volume. The pools also included non-cervid samples from other projects (see Hawkins et al. 2016). We enriched each pool of samples using a probe set that was diluted 1:5, giving each multiplexed pool approximately 100 ng of probes per enrichment. The probes employed corresponded to the same array described by Hawkins et al. (2016). Each pool of libraries was incubated with the RNA probes and buffers as described in the MYcroarray protocol for 48 hours at 65°C. Following incubation, DNA was separated from the probes via magnetic beads and purified with QiaQuick PCR Purification Kits (Qiagen) following MYcroarray's enrichment protocol (version 1.3.8). Detailed protocols for MYbaits kits have been published online (http://ultraconserved.org/#protocols; http://www.mycroarray.com/pdf/MYbaits-manual.pdf). Post-enrichment pools were amplified for 25 cycles to produce a high enough concentration for gel extraction. QiaQuick Gel Extraction Kits (Qiagen) were used to size select the enriched pools for ~200–500 bp fragments and to remove residual adapter and primer dimer. Quantitative PCR was performed on enriched pools using an Illumina Library Quantification Kit (Kapa Biosystems) with two replicates of 1:1000, 1:2000, and 1:4000 dilutions for each pool. Pools were combined in equimolar ratios based on the number of samples in each pool. The samples were sequenced with paired-end chemistry and with a read length of 143 bp on a single lane of an Illumina HiSeq2500 at the Semel Institute UCLA Neurosciences Genomics Core; reads were demultiplexed at the core facility.

To assemble the mitogenomes, we first merged the forward and reverse paired reads with the program PEAR v0.9.4. (Zhang et al. 2014). Using the default settings of PEAR, we merged forward and reverse reads when they had a 10 bp or greater overlap. All sequences were screened for the presence of adapter sequences, which were removed with cutadapt v.1.4.2 (Martin 2011). We then employed PRINSEQ-lite v.0.20.4 (Schmieder and Edwards 2011) for quality filtering, trimming reads with average quality scores below 20 and exact PCR replicates (more than three identical cop-

ies). The filtered reads were then mapped to a reference sequence of the most closely related species using bwa v.7.10 (Li and Durbin 2009). The 'bwa aln' and 'samse' as well as the 'bwa mem' algorithms were tested on the degraded samples, with 'bwa aln' conducted as specified in Kircher et al. (2012). The reads corresponding to the freshly preserved tissue sample were mapped using the 'bwa mem' algorithm.

### Sequence alignment, matrix properties, and selection of partition scheme and models of nucleotide substitution

We aligned sequences using default options of MAFFT v.7.017 (Katoh and Standley 2013) as implemented in Geneious v.7.1.5. Multiple substitutions in a DNA site (i.e., saturation) compromise historical information from it; therefore, we evaluated whether our *CYTB* matrix suffered from this undesirable condition. Thus, we employed the software DAMBE version 5.3 (Xia 2013) to generate saturation plots based on the GTR-corrected genetic distances. Subsequently, we used the Bayesian Information Criterion (BIC) as implemented in PartitionFinder ver. 1.0.1. (Lanfear et al. 2012) to determine the most suitable partition scheme and best-fit models of nucleotide substitution. This analysis considered models of nucleotide substitution applicable in MrBayes and evaluated five partition schemes.

### Phylogenetic analyses

We conducted phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) as optimality criteria. For all analyses, we employed one sequence of each of the closest related taxa to the Odocoileini—*Alces alces*, *Capreolus capreolus*, and *Hydropotes inermis* (Gilbert et al. 2006, Hassanin et al. 2012)—as outgroup taxa. However, we included *Rangifer* (tribe Rangiferini) as part of the ingroup to test whether it was recovered sister to the clade formed by undisputed Odocoileini (as found in more limited previous studies). Because *Rangifer* has also been treated as a member of Odocoileini by some authors (Groves and Grubb 2011; but see Heckeberg et al. 2016), we take the opportunity to perform the same set of analyses that we are conducting for Odocoileini also for *Rangifer*. For inferring the best topology in the ML analysis, we conducted 50 independent searches in the Genetic Algorithm for Rapid Likelihood Inference (GARLI 2.0) (Zwickl 2006) applying the best-fitting model (see Results) and the default settings. The Bayesian analysis was conducted in MrBayes v. 3. 2 (Ronquist et al. 2012). The search started with a random tree, and the Markov chains were run for 100,000,000 generations; trees were sampled every 1,000 generations. Default values were kept for the "relburnin" and "burninfrac" options in MrBayes (i.e., we used the commands relburnin = yes; burninfrac = 0.25); therefore, the first 25,000,000 generations (25,000 trees) were discarded as burn-in, and posterior probability estimates of all model parameters were based on the remaining (75,000) trees. Convergence

and stationarity were assessed in the Bayesian analyses by plotting likelihood values in Tracer 1.5 (Drummond and Rambaut 2007).

To assess nodal support, we used nonparametric bootstrapping (Felsenstein 1985) for the ML analysis, and posterior probabilities for the BI analysis (Ronquist et al. 2012). The ML bootstrap analysis was performed in GARLI 2.0 using 100 pseudoreplicated data matrices, with 10 searches performed on each. Bayesian posterior probabilities were calculated simultaneously with the search for the best Bayesian topology, conducted as described earlier. Throughout the text, we refer to different degrees of nodal support for the ML bootstrap analysis using the following categories: *strong support*, for bootstrap values ≥ 75%; *moderate support*, for bootstrap values > 50% and < 75%; *negligible support* for values ≤ 50%. For the BI analysis, we refer to degrees of nodal support with two categories, *significant* or *strong* in cases in which a node's posterior probability was ≥ 0.95, and *insignificant* or *negligible* posterior probability values < 0.95.

We assessed the strength of phylogenetic evidence for species boundaries in the *CYTB* tree employing various statistics calculated via the Species Delimitation plugin (Masters et al. 2011) of Geneious v.7.1.5. This plugin allows users to assign terminals of a phylogenetic tree to putative species, which we did using traditional taxonomy of Odocoileini (see Introduction). Based on these designations and the recovered tree, Geneious' Species Delimitation plugin calculates various statistics relating to the phylogenetic exclusivity of each putative species, the probabilities of such exclusivity having arisen by chance in a random coalescent process, and the degree to which the species can be diagnosed (Masters et al. 2011). The calculated metrics are abbreviated and defined as follows (from Masters et al. 2011): *Intra*, the average pairwise tree distance among members of the focal haplogroup; *Inter*, the average pairwise tree distance between the focal haplogroup and the members of the closest haplogroup; *Intra/Inter*, the ratio of Intra to Inter; *P ID (strict)*, the mean (95% confidence interval) probability of correctly identifying an unknown member of the focal haplogroup using the criterion that it must fall within, but not sister to, the species clade in a tree; *P ID (liberal)*, the mean (95% confidence interval) probability of correctly identifying an unknown member of the putative species using the criterion that it falls within, or sister to, the species clade in a tree; *Av (MRCA-tips)*, the mean distance between the most recent common ancestor of a haplogroup and its members. We computed these statistics twice, once based on the ML tree and another based on the BI tree.

A high degree of sequence divergence is neither necessary nor sufficient for species recognition (Ferguson 2002, Dávalos and Russell 2014); however, as pointed out by Gutiérrez et al. (2010), values of sequence divergence do provide a heuristically useful basis for comparing genetic variation within and among lineages and can help identify taxa in need of closer taxonomic attention. Therefore, we report average uncorrected (p) distance and average Kimura 2-parameter-corrected (K2P) distance within and among haplogroups. Whether justified or not, the latter metric has become widely used in mammals, and therefore we report it to facilitate comparisons with values reported for other groups and by other researchers. Genetic distances were calculated using MEGA version 5.2.1 (Tamura et al. 2011).

## Results

### Alignment properties, partition schemes, and models of nucleotide substitution

The saturation plot demonstrated that the sequence data used in this study do not suffer from saturation; the number of transversions is substantially lower than the number of transitions, even at the highest values of genetic distances (supplementary file 3). The alignment contained 11% missing data. The most suitable partitioning scheme was that in which the three codon positions were analyzed together (i.e., without using subsets). The best-fit model of nucleotide substitution was the generalized time-reversible model with gamma-distributed rate heterogeneity and a proportion of invariant sites (GTR + Γ + I).

### Monophyly of traditionally recognized genera

The topologies of the two phylogenetic analyses were similar; we show only the tree resulting from the Bayesian inference analysis (BI) (Figures 1a, 1b, 1c), with nodal support for both the BI and maximum-likelihood (ML) analyses. We comment on the few instances in which results from the two analyses differ. In both analyses, the genera *Blastocerus*, *Hippocamelus*, *Ozotoceros*, and *Rangifer* were recovered as monophyletic with strong support, whereas the genera *Mazama*, *Odocoileus*, and *Pudu* were not (Figures 1a, 1b, 1c; see also column "*Focal haplogroup support*" on Tables 2 and 3). *Mazama* was recovered as polyphyletic, with *Mazama americana* (type species of the genus *Mazama*), *M. bororo, M. nana, M. pandora*, *M. rufina*, and *M. temama* showing a closer relationship to *Odocoileus* than to the other three species of *Mazama*, namely *M. chunyi*, *M. gouazoubira*, and *M. nemorivaga*, which were recovered elsewhere in the tree. These latter three species showed closer relationships to the genera *Blastocerus*, *Hippocamelus*, *Ozotoceros*, and *Pudu* than to *Odocoileus*. With regard to the genus *Odocoileus*, it was recovered as paraphyletic with respect to *M. pandora* (Figures 1a, 1b), which was recovered sister to a haplogroup containing, almost exclusively, sequences of *Od. hemionus columbianus* and *Od. h. sitkensis* (hereafter referred as the *columbianus* group; Figure 1b). However, the relationship between *M. pandora* and the *columbianus* group received negligible support in both analyses. Lastly, neither analysis supports the monophyly of the genus *Pudu* as currently recognized (Figure 1c). In the BI analysis, our only sequence of *P. mephistophiles* was part of a polytomy that included also a haplogroup formed by *M. nemorivaga* and a clade formed by haplogroups of *M. gouazoubira*, *Blastocerus*, *Hippocamelus*, *Ozotoceros*, and *Pudu puda*. In the ML analysis, this latter multi-genus clade and *P. mephistophiles* were recovered as sister groups with negligible support.

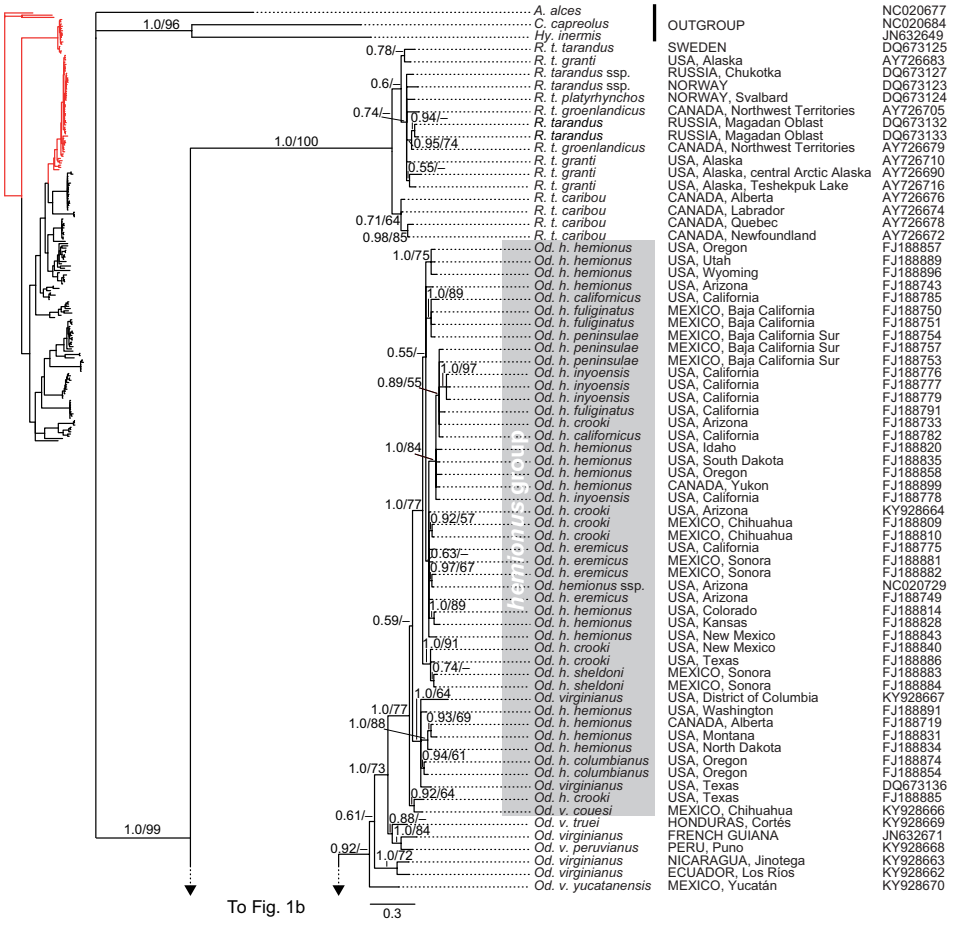


**Figure 1a.** Phylogenetic tree of cytochrome-*b* sequences of Odocoileini. This is a strict consensus topology resulting from the Bayesian inference analysis. Nodal support is indicated at each node, except where the relationship received negligible support. Posterior probabilities (from the Bayesian inference analysis) and bootstrap values (from the maximum-likelihood analysis) are indicated before and after the slashes ("/") at branches of interest (i.e., nodal support for fairly shallow relationships within intraspecific haplogroups are omitted). The scale represents substitutions per site. For each terminal, country of origin and next-largest administrative unit (state, department, province, etc.) are provided (when reported by the team that generated them; see detailed voucher and locality information in supplementary file 1 for sequences that we generated). GenBank accession numbers are indicated for each terminal.

## Monophyly of traditionally recognized species

Taxa traditionally regarded as valid species for which we included multiple samples were all recovered as monophyletic with strong support in both analyses (ML, BI), with four exceptions: *Mazama americana*, *M. nemorivaga*, *Odocoileus hemionus*, and *Od. virginianus* (Figures 1a, 1c). Two clades were identified for *M. americana*, and these clades

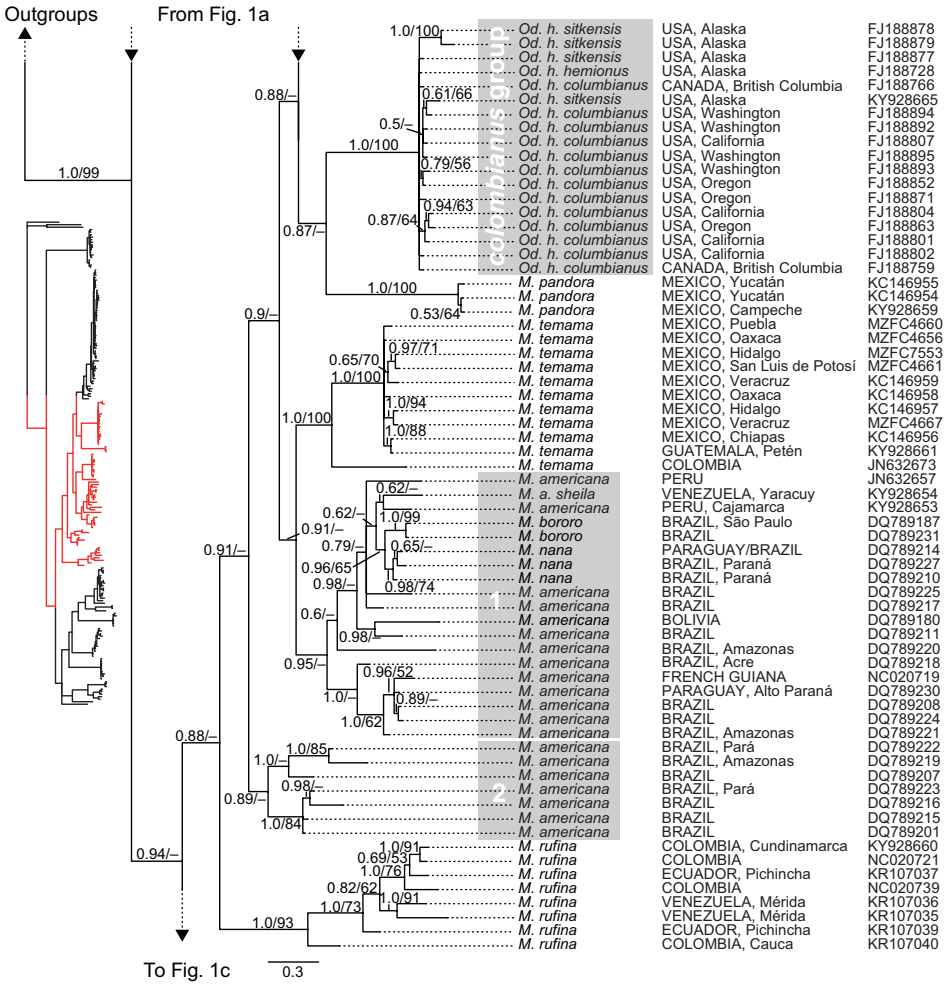


**Figure 1b.** Phylogenetic tree of cytochrome-*b* sequences of Odocoileini (continuation). This is a strict consensus topology resulting from the Bayesian inference analysis. Nodal support is indicated at each node, except where the relationship received negligible support. Posterior probabilities (from the Bayesian inference analysis) and bootstrap values (from the maximum-likelihood analysis) are indicated before and after the slashes ("/") at branches of interest (i.e., nodal support for fairly shallow relationships within intraspecific haplogroups are omitted). The scale represents substitutions per site. For each terminal, country of origin and next-largest administrative unit (state, department, province, etc.) are provided (when reported by the team that generated them; see detailed voucher and locality information in supplementary file 1 for sequences that we generated). GenBank accession numbers are indicated for each terminal.

were not sister to each other. One of these clades was formed by haplotypes from Bolivia, Brazil, French Guiana, Paraguay, Peru, and Venezuela, and a strongly supported subclade of *M. bororo* and *M. nana*; hereafter we refer to that clade as the *M. americana* group 1. The monophyly of the *M. americana* group 1 (including as members *M. bororo* and *M. nana*) received negligible and strong support in the ML and BI analyses, respectively. The

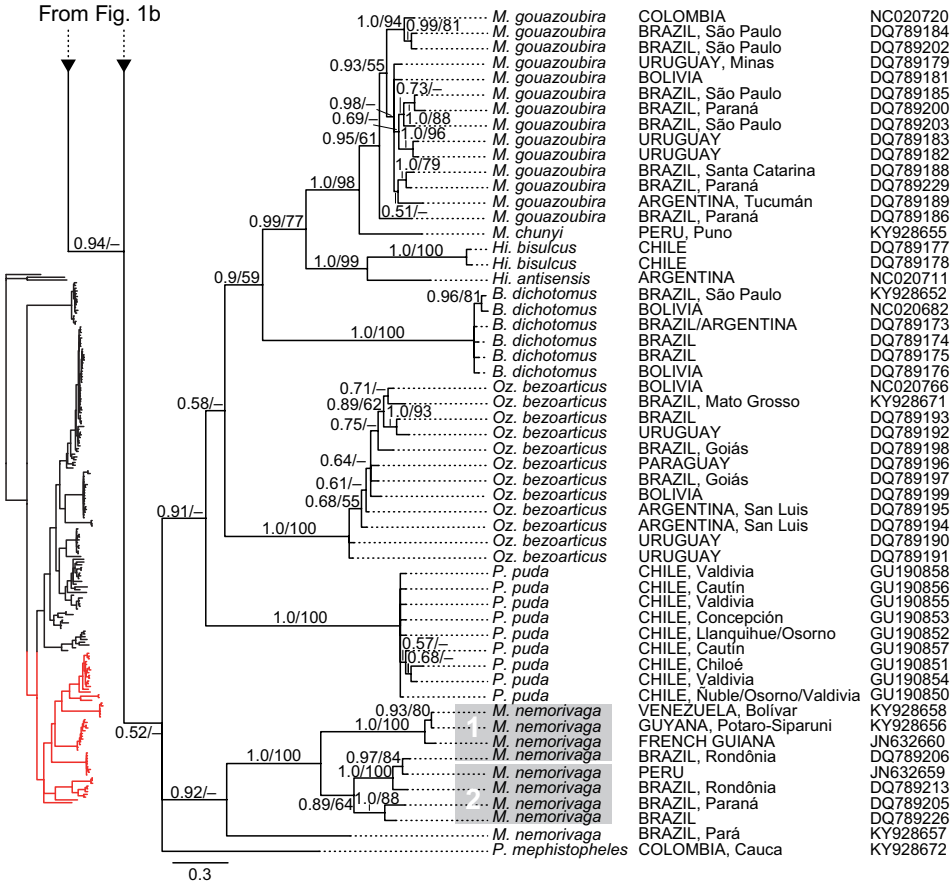


**Figure 1c.** Phylogenetic tree of cytochrome-*b* sequences of Odocoileini (continuation). This is a strict consensus topology resulting from the Bayesian inference analysis. Nodal support is indicated at each node, except where the relationship received negligible support. Posterior probabilities (from the Bayesian inference analysis) and bootstrap values (from the maximum-likelihood analysis) are indicated before and after the slashes ("/") at branches of interest (i.e., nodal support for fairly shallow relationships within intraspecific haplogroups are omitted). The scale represents substitutions per site. For each terminal, country of origin and next-largest administrative unit (state, department, province, etc.) are provided (when reported by the team that generated them; see detailed voucher and locality information in supplementary file 1 for sequences that we generated). GenBank accession numbers are indicated for each terminal.

second clade of *M. americana* included haplotypes from Amazonas, Pará, and southern states of Brazil; hereafter we refer to this clade as the *M. americana* group 2. Monophyly of the *M. americana* group 2 received negligible and moderate support in the ML and BI, respectively. *Mazama americana* group 2 was recovered as sister to a large clade containing *Odocoileus*, *M. pandora*, *M. temama*, and the *M. americana* group 1. In the case of *M. nemorivaga*, all but one sequence were recovered in a fully supported haplogroup that was sister to a single sequence of that species, but this relationship received negligible support (Figure 1c).

Neither of the traditionally recognized species of the genus *Odocoileus* were recovered as monophyletic in any of our analyses. Both analyses recovered most sequences of *Od. hemionus* in a large, strongly supported haplogroup, which also included three sequences from North American *Od. virginianus* (Figure 1a); hereafter we refer to this clade as the *hemionus* group. As mentioned earlier, both analyses also recovered most of the samples attributed to *Od. h. columbianus* and all of the samples attributed to *Od. h. sitkensis* in another fully supported haplogroup (Figure 1b). This haplogroup also included a sequence attributed to *Od. h. hemionus*, though this sample is from Alaska. This haplogroup, as previously mentioned, was found sister to *M. pandora*, albeit with negligible support. Lastly, *Od. virginianus* was not recovered as monophyletic; a few sequences of *Od. virginianus* nested within the *hemionus* group. The remaining sequences of *Od. virginianus* were recovered as closely related to the *hemionus* group, but they did not form supported haplogroups or show clear geographic patterns of relatedness.

**Gene tree-based species delimitation statistics and genetic distances**

Species delimitation statistics and genetic distances aided in identifying taxa or haplogroups of taxonomic interest. A low degree of within-haplogroup tree distance suggests that the implicated haplogroup might comprise a single species. The average within-haplogroup tree distances were 0.007 and 0.132 as calculated with the ML and BI trees, respectively. The smallest within-haplogroup tree distances corresponded to *Hippocamelus bisulcus*, *Mazama pandora*, *Blastocerus dichotomus*, and *Pudu puda*, whereas the highest within-haplogroup tree distances corresponded to the *M. americana* group 2, *M. rufina*, *M. americana* group 1, and *M. nemorivaga* (see "Intra" in Tables 2 and 3). Conversely, high tree distances between closely related haplogroups suggest that the haplogroups might not be conspecific. The average between-haplogroup tree distances were 0.115 and 1.512 as calculated with the ML and BI trees, respectively. The smallest between-haplogroup tree distances were those between the two species of *Hippocamelus*, and between *M. chunyi* and *M. gouazoubira*, whereas the highest between-haplogroup tree distances were those between the *columbianus* and *hemionus* groups of the genus *Odocoileus*, and between *M. pandora* with respect to the *columbianus* group (see "Inter" in Tables 2 and 3). Two other metrics, "P ID (strict)" and "P ID (liberal)", show probabilities of correctly identifying an unknown member of the focal haplogroup using the criteria that it must fall either within or sister to the focal haplogroup, respectively. The lower these probabilities, the less likely that the focal haplogroup represents a valid species. The mean P ID (strict) were 0.856 and 0.849 as calculated with the ML and BI trees, respectively; in both cases only four species had probabilities equal or above 0.95—*Oz. bezoarticus*, *P. puda*, the *columbianus* group, and *R. tarandus* (Tables 2 and 3). The mean values of P ID (liberal) were 0.966 and 0.963 as calculated with the ML and BI trees, respectively; in both analyses all species had probabilities equal or above 0.95, with exception of *M. americana* group 2, *M. rufina*, and *M. nemorivaga* (Tables 2 and 3). Another statistic calculated was the average distance between the most recent common ancestor of

**Table 2.** Summary statistics from the Species Delimitation plugin of Geneious for haplogroups of Rangiferini and Odocoileini deer recovered in the maximum-likelihood tree. Focal haplogroup support: bootstrap values; Intra: The average pairwise tree distance among members of the focal haplogroup; Inter: the average pairwise tree distance between the focal haplogroup and the members of the closest haplogroup; Intra/Inter: the ratio of Intra to Inter; P ID (strict): the mean (95% confidence interval) probability of correctly identifying an unknown member of the focal haplogroup using the criterion that it must fall within, but not sister to, the species clade in a tree; P ID (liberal): the mean (95% confidence interval) probability of correctly identifying an unknown member of the putative species using the criterion that it falls within, or sister to, the species clade in a tree; Av (MRCA-tips): the mean distance between the most recent common ancestor of a haplogroup and its members.

| Focal Haplogroup | Closest Haplogroup | Support | Intra | Inter | Intra/ Inter | P ID (strict) | P ID (liberal) | Av (MRCA-tips) |
|---|---|---|---|---|---|---|---|---|
| *B. dichotomus* | *M. gouazoubira* | 100 | 0.003 | 0.156 | 0.02 | 0.92 (0.80, 1.0) | 0.98 (0.87, 1.0) | 0.0025 |
| *H. antisensis* | *H. bisulcus* | NA | NA | 0.069 | NA | NA | 0.96 (0.83, 1.0) | NA |
| *H. bisulcus* | *H. antisensis* | 100 | 0.002 | 0.069 | 0.03 | 0.57 (0.43, 0.72) | 0.96 (0.81, 1.0) | 0.0011 |
| *americana* group 1 | *M. temama* | <50 | 0.050 | 0.090 | 0.56 | 0.83 (0.77, 0.88) | 0.96 (0.93, 0.98) | 0.0341 |
| *americana* group 2 | *hemionus* group | <50 | 0.036 | 0.093 | 0.39 | 0.75 (0.65, 0.86) | 0.91 (0.85, 0.97) | 0.0247 |
| *M. chunyi* | *M. gouazoubira* | NA | NA | 0.046 | NA | NA | 0.96 (0.83, 1.0) | NA |
| *M. gouazoubira* | *M. chunyi* | 61 | 0.015 | 0.046 | 0.32 | 0.87 (0.80, 0.94) | 0.96 (0.92, 1.0) | 0.0107 |
| *M. nemorivaga* | *americana* group 2 | 100 | 0.069 | 0.177 | 0.39 | 0.78 (0.70, 0.87) | 0.93 (0.88, 0.98) | 0.0749 |
| *M. pandora* | *columbianus* group | 100 | 0.002 | 0.111 | 0.02 | 0.78 (0.61, 0.96) | 1.00 (0.85, 1.0) | 0.0013 |
| *M. rufina* | *americana* group 2 | 93 | 0.041 | 0.130 | 0.32 | 0.79 (0.69, 0.90) | 0.92 (0.86, 0.99) | 0.0449 |
| *M. temama* | *americana* group 1 | 99 | 0.016 | 0.090 | 0.18 | 0.88 (0.80, 0.97) | 0.96 (0.91, 1.0) | 0.0270 |
| *hemionus* group | *americana* group 2 | <50 | 0.016 | 0.093 | 0.17 | 0.94 (0.88, 0.99) | 0.98 (0.95, 1.0) | 0.0246 |
| *columbianus* group | *hemionus* group | 100 | 0.006 | 0.097 | 0.06 | 0.97 (0.92, 1.0) | 0.99 (0.97, 1.0) | 0.0040 |
| *Oz. bezoarticus* | *M. gouazoubira* | 100 | 0.011 | 0.138 | 0.08 | 0.96 (0.89, 1.0) | 0.99 (0.95, 1.0) | 0.0111 |
| *P. mephistophiles* | *Oz. bezoarticus* | NA | NA | 0.160 | NA | NA | 0.96 (0.83, 1.0) | NA |
| *P. puda* | *Oz. bezoarticus* | 100 | 0.004 | 0.173 | 0.02 | 0.97 (0.89, 1.0) | 1.00 (0.95, 1.0) | 0.0044 |
| *R. tarandus* | *americana* group 2 | 100 | 0.010 | 0.213 | 0.05 | 0.98 (0.93, 1.0) | 1.00 (0.97, 1.0) | 0.0071 |

a focal haplogroup and the tips of its members, Av (MRCA-tips). The smaller the value of this metric, the more likely members of the focal haplogroup are conspecific. The mean Av (MRCA-tips) were 0.019 and 0.282 as calculated with the ML and BI trees, respectively; in both analyses *H. bisulcus*, *M. pandora*, and *B. dichotomus* showed the smallest Av (MRCA-tips) and *M. rufina* and *M. nemorivaga* the largest (Tables 2 and 3).

**Table 3.** Summary statistics from the Species Delimitation plugin of Geneious for haplogroups of Rangiferini and Odocoileini deer recovered in the Bayesian tree. Focal haplogroup support: posterior probability values; Intra: The average pairwise tree distance among members of the focal haplogroup; Inter: the average pairwise tree distance between the focal haplogroup and the members of the closest haplogroup; Intra/Inter: the ratio of Intra to Inter; P ID (strict): the mean (95% confidence interval) probability of correctly identifying an unknown member of the focal haplogroup using the criterion that it must fall within, but not sister to, the species clade in a tree; P ID (liberal): the mean (95% confidence interval) probability of correctly identifying an unknown member of the putative species using the criterion that it falls within, or sister to, the species clade in a tree; Av (MRCA-tips): the mean distance between the most recent common ancestor of a haplogroup and its members.

| Focal Haplogroup | Closest Haplogroup | Support | Intra | Inter | Intra/Inter | P ID (strict) | P ID (liberal) | Av (MRCA-tips) |
|---|---|---|---|---|---|---|---|---|
| *B. dichotomus* | *M. gouazoubira* | 1.00 | 0.065 | 2.014 | 0.03 | 0.91 (0.79, 1.0) | 0.98 (0.87, 1.0) | 0.0352 |
| *H. antisensis* | *H. bisulcus* | NA | NA | 0.906 | NA | NA | 0.96 (0.83, 1.0) | NA |
| *H. bisulcus* | *H. antisensis* | 1.00 | 0.047 | 0.906 | 0.05 | 0.56 (0.41, 0.71) | 0.95 (0.80, 1.0) | 0.0236 |
| *americana* group 1 | *M. temama* | 0.95 | 0.688 | 1.248 | 0.55 | 0.83 (0.78, 0.88) | 0.96 (0.93, 0.98) | 0.4722 |
| *americana* group 2 | *M. temama* | 0.89 | 0.509 | 1.334 | 0.38 | 0.76 (0.65, 0.86) | 0.91 (0.85, 0.98) | 0.3445 |
| *M. chunyi* | *M. gouazoubira* | NA | NA | 0.639 | NA | NA | 0.96 (0.83, 1.0) | NA |
| *M. gouazoubira* | *M. chunyi* | 0.95 | 0.250 | 0.639 | 0.39 | 0.85 (0.78, 0.91) | 0.95 (0.91, 1.00) | 0.1888 |
| *M. nemorivaga* | *P. mephistophiles* | 0.92 | 0.939 | 2.198 | 0.43 | 0.77 (0.68, 0.85) | 0.93 (0.87, 0.98) | 0.9906 |
| *M. pandora* | *columbianus* group | 1.00 | 0.050 | 1.437 | 0.03 | 0.77 (0.59, 0.94) | 0.99 (0.84, 1.0) | 0.0305 |
| *M. rufina* | *americana* group 2 | 1.00 | 0.585 | 1.794 | 0.33 | 0.79 (0.68, 0.89) | 0.92 (0.86, 0.98) | 0.6342 |
| *M. temama* | *americana* group 1 | 1.00 | 0.239 | 1.248 | 0.19 | 0.88 (0.79, 0.96) | 0.96 (0.91, 1.0) | 0.3774 |
| *hemionus* group | *americana* group 2 | 0.92 | 0.270 | 1.391 | 0.19 | 0.93 (0.87, 0.98) | 0.98 (0.95, 1.0) | 0.4257 |
| *columbianus* group | *hemionus* group | 1.00 | 0.117 | 1.416 | 0.08 | 0.96 (0.91, 1.0) | 0.99 (0.96, 1.0) | 0.0617 |
| *Oz. bezoarticus* | *M. gouazoubira* | 1.00 | 0.190 | 1.885 | NA | 0.95 (0.88, 1.0) | 0.98 (0.94, 1.0) | 0.1755 |
| *P. mephistophiles* | *americana* group 2 | NA | NA | 1.921 | 0.00 | NA | 0.96 (0.83, 1.0) | NA |
| *P. puda* | *Oz. bezoarticus* | 1.00 | 0.084 | 2.063 | 0.04 | 0.96 (0.88, 1.0) | 1.00 (0.94, 1.0) | 0.0454 |
| *R. tarandus* | *americana* group 2 | 1.00 | 0.179 | 2.658 | 0.07 | 0.97 (0.92, 1.0) | 0.99 (0.96, 1.0) | 0.1416 |

Mean uncorrected sequence divergence within species-level haplogroups—provisionally treating the *hemionus* group, the *columbianus* group, the *americana* group 1, and the *americana* group 2 as if each represented an individual species-level haplogroup—ranges from 0.0 to 3.6% (Table 4). However, sequence divergences across the basal split within some species are considerably higher than these average within-group values. In particular, Central American sequences of *Mazama temama* differ from the

**Table 4.** Matrix of genetic distances (percent sequence divergence) within and among recovered haplogroups of Rangiferini deer. Average uncorrected (p) distances among conspecific sequences are arrayed along the diagonal, interspecific p distances are below the diagonal, and Kimura two-parameter (K2P) distances are above the diagonal. No genetic distances were calculated within species for which we only had a single sequence available; however, we duplicated each of these sequences to allow for calculations of interspecific p-distances. The following names apply to haplogroups (as recovered in our phylogenetic analyses) rather than to species: *Mazama americana* 1, *M. americana* 2, *hemionus* group, and the *columbianus* group.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1. *Blastocerus dichotomus*** | **0.3** | 4.4 | 7.6 | 9.8 | 10.3 | 7.1 | 7.2 | 6.9 | 10.9 | 8.0 | 10.0 | 8.4 | 9.0 | 8.2 | 5.2 | 14.6 | 9.5 |
| **2. *Hippocamelus antisensis*** | 4.2 | — | 3.0 | 8.8 | 9.3 | 2.5 | 2.6 | 5.8 | 9.9 | 7.3 | 8.8 | 7.4 | 8.0 | 8.3 | 4.2 | 12.5 | 6.6 |
| **3. *Hippocamelus bisulcus*** | 7.1 | 2.9 | **0.8** | 8.3 | 9.9 | 3.8 | 5.8 | 5.9 | 11.4 | 5.6 | 8.3 | 10.1 | 9.5 | 7.8 | 7.4 | 10.2 | 5.6 |
| **4. *Mazama americana* 1** | 9.1 | 8.2 | 7.7 | **2.8** | 3.7 | 7.2 | 9.1 | 8.9 | 4.3 | 4.8 | 3.3 | 6.6 | 4.8 | 10.3 | 7.8 | 10.1 | 6.9 |
| **5. *Mazama americana* 2** | 9.5 | 8.5 | 9.1 | 3.8 | **3.2** | 7.2 | 8.8 | 9.0 | 4.3 | 5.2 | 3.9 | 6.7 | 5.8 | 10.6 | 8.3 | 11.1 | 8.6 |
| **6. *Mazama chunyi*** | 6.7 | 2.5 | 3.7 | 6.7 | 6.8 | – | 1.8 | 6.3 | 7.0 | 4.7 | 6.0 | 7.5 | 7.1 | 9.3 | 7.0 | 11.7 | 7.6 |
| **7. *Mazama gouazoubira*** | 6.8 | 2.6 | 5.4 | 8.4 | 8.1 | 1.8 | **0.5** | 7.1 | 9.0 | 6.5 | 8.0 | 7.7 | 9.1 | 11.4 | 7.1 | 13.4 | 9.6 |
| **8. *Mazama nemorivaga*** | 6.5 | 5.5 | 5.6 | 8.2 | 8.3 | 5.9 | 6.6 | **3.6** | 9.6 | 5.3 | 8.0 | 7.8 | 9.4 | 8.1 | 6.6 | 12.2 | 9.2 |
| **9. *Mazama pandora*** | 10.0 | 9.0 | 10.2 | 4.1 | 4.1 | 6.6 | 8.3 | 8.8 | **0.0** | 6.3 | 5.2 | 7.5 | 6.2 | 13.3 | 8.8 | 12.9 | 9.7 |
| **10. *Mazama rufina*** | 7.5 | 6.8 | 5.3 | 4.6 | 5.0 | 4.5 | 6.1 | 5.1 | 5.9 | **1.5** | 3.7 | 6.7 | 6.4 | 8.3 | 6.3 | 8.4 | 6.7 |
| **11. *Mazama temama*** | 9.3 | 8.2 | 7.8 | 3.2 | 3.8 | 5.7 | 7.5 | 7.5 | 5.0 | 3.6 | **0.7** | 5.6 | 4.6 | 8.7 | 8.0 | 9.9 | 6.8 |
| **12. *columbianus* group** | 7.9 | 7.0 | 9.2 | 6.2 | 6.3 | 7.0 | 7.2 | 7.2 | 6.9 | 6.3 | 5.4 | **2.2** | 6.6 | 12.3 | 6.3 | 11.9 | 9.1 |
| **13. *hemionus* group** | 8.4 | 7.5 | 8.7 | 4.5 | 5.5 | 6.7 | 8.4 | 8.7 | 5.8 | 6.0 | 4.4 | 6.2 | **0.2** | 11.3 | 7.0 | 11.0 | 7.9 |
| **14. *Ozotoceros bezoarticus*** | 7.7 | 7.7 | 7.2 | 9.5 | 9.7 | 8.5 | 10.3 | 7.5 | 11.8 | 7.7 | 8.0 | 11.1 | 10.3 | **0.7** | 9.2 | 15.2 | 7.8 |
| **15. *Pudu mephistophiles*** | 5.1 | 4.1 | 7.0 | 7.3 | 7.7 | 6.6 | 6.7 | 6.2 | 8.2 | 5.9 | 7.5 | 6.0 | 6.7 | 8.5 | — | 10.4 | 5.8 |
| **16. *Pudu puda*** | 13.1 | 11.3 | 9.4 | 9.4 | 10.2 | 10.7 | 12.0 | 11.1 | 11.7 | 7.9 | 9.2 | 10.8 | 10.1 | 13.5 | 9.7 | **0.4** | 9.8 |
| **17. *Rangifer tarandus*** | 8.7 | 6.1 | 5.3 | 6.5 | 7.9 | 7.1 | 8.7 | 8.4 | 8.9 | 6.2 | 6.4 | 8.3 | 7.4 | 7.3 | 5.5 | 9.1 | **0.8** |

single available Colombian sample of that species by 5.0%, and the lone sequence of *M. nemorivaga* from the state of Pará, in northern Brazil, differs from all other sequences of that species by 8.3%. Although not a basal split within *M. nemorivaga*, it is noteworthy that *M. nemorivaga* group 1 (from the Guiana Shield) and *M. nemorivaga* group 2 (from Brazil and Peru) differ from one another by 5.9%. Average interspecific divergences within three consistently recovered sister-species pairs (*Hippocamelus antisensis* + *H. bisulcus*, *M. chunyi* + *M. gouazoubira*, and *M. pandora* + *Od. columbianus* group) range from 1.8% to 6.2% (Table 4). The sister-species pair formed by *M. bororo* and *M. nana* was embedded within the diversity of the *M. americana* group 1; the level of divergence between these two species (*bororo* and *nana*) was only 1.3%.

## Discussion

### Polyphyly and phylogenetics of the genus *Mazama*

Based on data from all nine currently recognized species of *Mazama* (Gutiérrez et al. 2015), we confirm the findings by previous authors (Gilbert et al. 2006, Duarte et al. 2008, Hassanin et al. 2012, Escobedo-Morales et al. 2016, Heckeberg et al. 2016) that the genus, as traditionally understood (Allen 1915, Cabrera 1961), is polyphyletic. In the only comprehensive revisionary work published for *Mazama*, Allen (1915) stated that the main characteristics that distinguish the genus *Mazama* from other deer genera are: short, unbranching (spike-like) antlers in males (but note that males of the genus *Pudu* also possesses spike-like antlers); small, slightly expanded bullae in comparison with those of *Odocoileus* and *Blastocerus*; flat and usually nearly straight upper borders of the orbits; slight over-hang of the frontals over the postorbital fossa; overall small size and the red coloration of most of its species; Allen also acknowledged the existence of a group of *Mazama* with brown coloration (Allen 1915). Clearly, our results and those from previous studies, one of them based on multi-locus data, demonstrate that this morphological characterization of *Mazama* does not diagnose a natural group (Gilbert et al. 2006, Escobedo-Morales et al. 2016). Logically, either some of these morphological characteristics resulted from convergent evolution, or they represent plesiomorphies inherited from an ancestor shared by many of these deer. Ancient hybridization, incomplete lineage sorting, or both, often explain lack of monophyly in recently originated clades when limited sequence data are analyzed (particularly mitochondrial DNA data); however, species traditionally classified into the genus *Mazama* are so widely distributed throughout the recovered tree that it seems unlikely that these phenomena explain the observed, rampant polyphyly.

The tribe Odocoileini is divided into two major clades for which subtribe-level names have recently been proposed (Heckeberg et al. 2016). The subtribe Odocoileina contains taxa from temperate, subtropical, and tropical regions of the Americas, whereas the subtribe Blastocerina contains taxa exclusively from subtropical and tropical regions of South America (see Figures 1a, 1b, 1c). In our analyses, both subtribes were recovered with poor nodal support, but their monophyly has been supported by previous studies

(e.g., Gilbert et al. 2006, Hassanin et al. 2012). *Mazama*, as traditionally understood, is represented by species in both subtribes. In our analyses, the Odocoileina included all of the species of *Mazama* with red (reddish) pelage, i.e., *M. americana*, *M. bororo*, *M. nana*, *M. rufina*, *M. temama*; one *Mazama* species with brown (brownish/grayish) pelage coloration, *M. pandora*; and the genus *Odocoileus*. The remaining three species of *Mazama* with brown (i.e., brownish or grayish) pelage coloration (i.e., *M. chunyi*, *M. gouazoubira*, *M. nemorivaga*) were recovered in Blastocerina, which also includes the genera *Blastocerus*, *Hippocamelus*, *Ozotoceros*, and *Pudu*. These findings confirm, with more comprehensive sampling, those from two recent mtDNA-based studies (Escobedo-Morales et al. 2016, Heckeberg et al. 2016). Results from these studies clearly call into question the validity and usefulness of the terms "red clade", "red brocket species group", "gray clade", "gray brocket species group", "brown group", all of which have been previously applied to groups (e.g., by Allen 1915, Duarte et al. 2008, Escobedo-Morales et al. 2016) whose respective monophyly has never been supported. These terms based on pelage coloration are highly misleading. For example, the term "gray clade" erroneously implies that all of the species now allocated within the subtribe Blastocerina possess predominantly gray pelage coloration, but almost half of the species in this subtribe lack such coloration (*Blastocerus dichotomus*, *Mazama chunyi*, *Ozotoceros bezoarticus*, *Pudu mephistophiles*; Hershkovitz 1959, 1982, Jackson 1987, Rumiz et al. 2007, Miranda et al. 2009), and, more importantly, species of "*Mazama*" with gray pelage were not recovered as a monophyletic group in either our analyses or those of previous studies (Gilbert et al. 2006, Duarte et al. 2008, Escobedo-Morales et al. 2016).

## Phylogenetic relationships and taxonomy of species traditionally classified as *Mazama*

Our results have implications for the alpha-level taxonomy of *Mazama*. Phylogenetic analyses based on *CYTB* data by Duarte et al. (2008) recovered *M. americana* in two distinct haplogroups, one of which also included terminal branches that they identified as *M. bororo* and *M. nana*. In that study, however, these haplogroups formed part of a polytomy together with *Odocoileus* and a sequence of "*Mazama* sp." Subsequently, based on partial sequences of both the *CYTB* gene and the mitochondrial control-region (D-LOOP), Abril et al. (2010) recovered two monophyletic haplogroups within *M. americana*. Despite the lack of resolution in the results obtained by Duarte et al. (2008), Abril et al. (2010) assumed the monophyly of *M. americana* by the composition of their ingroup (i.e., not including other odocoileines), and, therefore, the topology they obtained could not evaluate whether *M. americana* represents a single species. However, more recent studies employing *CYTB* sequence data from multiple species of Odocoileini have shown *M. americana* to be polyphyletic (Escobedo-Morales et al. 2016, Heckeberg et al. 2016). Based on more comprehensive sampling, our results confirm the polyphyly of *M. americana* (as currently understood) and provide novel insights regarding the possible taxonomic identity and geographic distribution of at

least two species currently lumped within *M. americana*. Before discussing this topic, we clarify that comparisons of the *CYTB* sequences generated by Abril et al. (2010) with respect to those analyzed by us indicated that the two haplogroups obtained by the former group of researchers match our *M. americana* groups 1 and 2. Because the name *M. americana* is based on the type locality of Cayenne, French Guiana (see Allen 1915), our *M. americana* group 1, which included a sequence (accession number NC020719; Figure 1b) from Barrage de Petite-Saut (Alexandre Hassanin *in litt.*), northern French Guiana, located at only ca. 80 km E Cayenne, likely corresponds to *M. americana sensu stricto*. Further work is necessary to determine whether *M. americana* group 1 truly corresponds to *M. americana sensu stricto*. If confirmed, then the sequence data herein analyzed, and that produced by Abril et al. (2010), would document the presence of *M. americana sensu stricto* in French Guiana, Bolivia, Brazil (states of Acre, Pará, Paraná, Rondônia, and São Paulo), Paraguay (department of Alto Paraná), Peru (Region of Cajamarca), and Venezuela (state of Yaracuy). The provenance localities of other analyzed samples of *M. americana* group 1 are unknown (see *Caveats*). Further taxonomic work is also necessary to confirm that *M. americana* group 2 is not conspecific with *M. americana sensu stricto* and, if so, assign to it a species name. Our analysis documents this lineage (provisionally referred to as "*M. americana* group 2" or "*M. americana* 2") in the states of Amazonas and Pará in northern Brazil. In addition, a sequence that matches our *M. americana* group 2 was generated by Abril et al. (2010) from a karyotyped individual born in captivity (in "Criadouro Santarém") in the Brazilian state of Pará, but of unknown geographic origin. Previous research focused on Brazilian populations of *M. americana sensu lato* has shown the existence of at least six distinct karyotypes in different regions of that country, and inter-cytotype crosses in captivity demonstrated reproductive isolation among the most geographically-distant cytotypes (Cursino et al. 2014). The results from our phylogenetic analyses are congruent with these karyological and reproductive observations, and confirm that more than a single species is currently lumped under *M. americana sensu lato*. To date, the only study that has examined the morphological variation of *Mazama americana sensu lato* in a large portion of its distribution is the unpublished master thesis of Dr. Rogério V. Rossi (Rossi 2000). Based on morphometric analyses of Brazilian samples, Rossi found that specimens from littoral areas of southeastern Brazil (from Santa Catarina to São Paulo states) are slightly differentiated from those obtained from populations to the interior of that country. Whether a correspondence exists between these two morphologically distinguished groups and the clades identified in the present study remains to be addressed.

Reconciling current phylogenetic information for *Mazama bororo* and *M. nana* with their taxonomic status as valid species presents a conundrum. The existence of two species of small brockets in southern South America has been noted in the scientific literature since the first half of the 19th century (Lesson 1842, Goeldi 1893, Lydekker 1898, 1915, Miranda-Ribeiro 1919). These deer are currently referred to as *M. bororo*, known from remnants of Atlantic Forest in southeastern São Paulo and eastern Paraná and Santa Catarina, Brazil (Duarte and Jorge 2003, Vogliotti and Duarte 2009,

Duarte et al. 2016), and as *M. nana*, known from Atlantic Forest habitat in southern São Paulo, Paraná, Santa Catarina, and northern Río Grande do Sul, Brazil (Rossi 2000, Vivo et al. 2011, Duarte et al. 2012). Records of *M. nana* also exist from the Alto Paraná and Itapúa departments of Paraguay (Gamarra de Fox and Martin 1996) and the Misiones province of Argentina (Di Bitetti et al. 2008). No agreement about their taxonomic status was reached until recently, when they were recognized as valid species on the basis of chromosomal differences between them and with *M. americana sensu lato* (Duarte and Jorge 2003, Abril and Duarte 2008). Reported karyotypes for these species include the following diploid and fundamental numbers (2n/FN): *Mazama bororo*: 32–34/46 (Duarte and Jorge 2003); *M. nana*: 36/56, 37/59, 38/60 (Duarte and Jorge 2003), 36–39/58 (Abril and Duarte 2008); *M americana* group 1: 50/54; *M americana* group 2: 42/49, 43/48, 49/56, 51/56 (Duarte and Jorge 2003). Additional karyotypes reported for *M. americana sensu lato* lacking *CYTB* sequences are available—and hence not assigned to group 1 or 2—include the following 2n/FNs: 42/46, 43/46, 44/46, 44/48, 45/48, 50/54, 52/56, 53/56 (Abril et al. 2010). These data and a study that involved crosses in captivity to assess hybrids' fertility have demonstrated that: (1) *Mazama bororo* is not a hybrid between *M. nana* and *M. americana*, and is unable to produce fertile hybrids with either of these species (Duarte and Jorge 2003); and (2) *M. americana* groups 1 and 2 are reproductively isolated (Cursino et al. 2014). Based on these findings, phylogenetic analyses based on a relatively fast-evolving gene would be expected to recover *M. bororo*, *M. nana*, and *M. americana* as independent lineages; however, the former two species were recovered nested within *M. americana* group 1. For species in this complex, future systematic efforts should concentrate in three areas. First, to investigate the phylogeographic structure of populations in the *M. americana* group 1, which implicitly requires assessing the phylogenetic position of *M. bororo* and *M. nana*, based on sequence data from multiple unlinked loci, including nuclear DNA segments with faster mutation rates than the *CYTB* gene to resolve finer-scale relationships. This approach would concomitantly enable assessment of the potential role of hybridization, incomplete lineage sorting, or both as causal explanations for the topology obtained in our analyses (see above). Second, the specific mechanisms responsible for the remarkable karyological variation observed in this group need further investigation, as do their implications for speciation. Although important advances have been made unveiling the chromosomal variation in this group (e.g., Duarte and Jorge 1996, 2003, Abril and Duarte 2008, Cursino et al. 2014), much remains to be done, including investigating the possible role of B chromosomes—which are able to create even intra-individual karyological variation (Abril and Duarte 2008, Abril et al. 2010)—on speciation (if any). The mechanisms that have been postulated to explain the chromosomal variability of *Mazama americana sensu lato* need to be revisited because *M. americana sensu lato* is not monophyletic, as previously (and implicitly) assumed (by Abril et al. 2010, Cursino et al. 2014). Third, a morphological assessment of differences among natural groups (identifiable by molecular and karyological criteria) should be conducted in search of diagnostic traits. Preliminary analyses of linear measurements taken on craniodental and external traits allow unambiguous

discrimination between *M. americana sensu lato* and *M. nana*, but not between the former and *M. bororo* (Rossi 2000). This is likely an artifact created by the fact that such comparisons were conducted assuming that populations of *M. americana sensu lato* comprised a single species, inflating its apparent variability. Similar comparisons between *M. bororo* and *M. nana* permitted unambiguous discrimination between both of these species (Rossi 2000; but see Duarte et al. 2008).

Our results offer novel phylogenetic information with respect to *Mazama pandora*, a species endemic to the Península de Yucatán. A recent study based on mtDNA (Escobedo-Morales et al. 2016) recovered *M. pandora* as a monophyletic haplogroup sister to *Odocoileus virginianus*, the only species of *Odocoileus* analyzed in that study. Another study reanalyzed these and additional data and found *M. pandora* sister to a clade composed by a handful of sequences of *Od. virginianus* and *Od. hemionus* of unspecified geographic origin; the two species of *Odocoileus* were found intermixed with each other within a poorly supported monophyletic group (Heckeberg et al. 2016). Our more comprehensive sampling identified a novel sister-taxon relationship between *M. pandora* and the *columbianus* group—the latter is a clade formed by most *Odocoileus h. columbianus* samples and all samples of *Od. h. sitkensis*, and a sample of *Od. h. hemionus,* whose inclusion in this clade might be a consequence of hybridization. Given the traditional assignment of *pandora* to the genus *Mazama* (Allen 1915, Medellín et al. 1998), its nested position within *Odocoileus* was unexpected. However, the overall morphological appearance of *M. pandora* somewhat resembles that of the genus *Odocoileus* (Figure 2); the species has grayish pelage, and divergent antlers larger than other species classified in *Mazama*. It is expected that future work will unveil morphological synapomorphies between species of *Odocoileus* and *pandora*. The sister relationship between *pandora* and the *columbianus* group also suggests that the biogeographic history of these deer is complex, but this topic requires robust phylogenetic inference, enabling ancestral area reconstructions and proper molecular dating. However, discussing the nomenclatural implications of the close relationship between *pandora* and the genus *Odocoileus* is necessary, especially after Escobedo-Morales et al. (2016) advocated allocating species of *Odocoileus* into the genus *Mazama*. Such an action, which has been contemplated by a few modern authors (Haltenorth 1963, Grubb 2000, Groves and Grubb 2011), would increase congruence between available phylogenetic information and the taxonomic nomenclature of Odocoileini but diminish efficiency in communication of scientific information. Allocating species currently treated as *Odocoileus* within *Mazama* would unnecessarily (see below) disrupt the association between the name *Odocoileus* and at least two—and perhaps more (Molina and Molinari 1999, Molinari 2007)—species epithets and the names of numerous subspecies (between 48 and 71) (Baker 1984, Brokx 1984, Méndez 1984, Smith 1991). This action would pose difficulties for retrieval of data and bibliography from repositories, such as GenBank and the Global Biodiversity Information Facility, and search engines, such as Google Scholar and the Web of Science, respectively. This is not a trivial matter because, given the importance of *Odocoileus* in aspects raging from public health to landscape ecology, massive amounts of data are associated with the name *Odocoileus*, whose North American members are among the most studied ungulates worldwide. A more suit-


**Figure 2.** Overall morphological appearance of "*M.*" *pandora* (panels **A–C**) and that of the genus *Odocoileus* (panels **D–F**). Notice the grayish pelage and divergent antlers larger than in other species currently classified in *Mazama*. "*M.*" *pandora*, panels **A** and **C** individuals kept in captivity at the Parque Zoológico del Bicentenario Animaya, Mérida, Yucatán, Mexico (photographs by Luis A. Escobedo-Morales)—provenance unknown; panel **B** individual kept in captivity in Tekax, Yucatán, Mexico (photograph by Rosa María González Marín)—provenance unknown. *Odocoileus virginianus* (see proposals by Molina and Molinari 1999 and Molinari 2007); panels **D** and **E** Monteredondo, Parque Nacional Chingaza, ca. 47 km (by road) E Bogota, Cundinamarca, Colombia (photographs by Aideé Vargas-Espinoza and Irene Aconcha, respectively); panel **F** Laguna de Mucubají, Parque Nacional Sierra Nevada, Mérida, Venezuela (photograph by Rodrigo Díaz Lupanow).

able solution to the current incongruence between the phylogenetic information available and the nomenclature of these deer would be to restrict the use of the name *Mazama* to the clade containing *M. temama* and the *Mazama americana* group 1; to allocate *M. pandora* to the genus *Odocoileus,* and to recognize *M. rufina* and the *M. americana* group 2 as belonging to two separate genera, other than *Mazama*. Disrupting association between the genus and species epithets for "*Mazama*" *pandora*, "*Mazama*" *rufina* and taxa within the *M. americana* group 2 is both unavoidable—because of the polyphyletic nature of *Mazama* (as currently understood)—and less problematic for scientific communication because these species are far less studied than those of *Odocoileus*. This solution would reconcile the available phylogenetic information with the taxonomy of the group while minimizing nomenclatural instability. Similar considerations and actions have been recently employed to preserve binomial stability in various mammalian groups, including opossums (Giarla et al. 2010, Voss et al. 2014, Díaz-Nieto and Voss 2016, Pavan and Voss 2016), rodents (Teta et al. 2016), and primates (Garbino 2015, Gutiérrez and Marinho-Filnho 2017). A third alternative would be to retain *pandora* in *Mazama* until data from independently inherited loci become available. However, no analytical evidence, of any sort, supports a close relationship between *pandora* and *M. americana,* the type species of the genus. Although analyses of data from a single gene offer incomplete bases for taxonomic revisions, they represent an improvement when the traditional taxonomy in question is based on no evidence whatsoever. In those cases, dogmatically preserving the traditional taxonomy would essentially translate into imposing beliefs while ignoring data. The transferral of *pandora* to an already-described genus, *Odocoileus*, seems a sensible and justifiable provisional action, considering not only the phylogenetic evidence here presented but also resemblance in external morphology between *pandora* and species of *Odocoileus* (Figure 2). By contrast, allocating presumed clades currently regarded as *Mazama* sensu lato into different genera would involve either the description of new genera or the recognition of available generic names which are currently treated as junior synonymies, without sufficient consideration of morphological traits that might support such actions. These nomenclatural improvements should be carried out once a robust multi-locus phylogeny becomes available and should be coupled with morphological diagnoses of the genera to be proposed.

Besides confirming the monophyly of *Mazama temama* (Escobedo-Morales et al. 2016), we provide evidence that this species occurs in South America, or that populations in Colombia perhaps represent a currently unrecognized species. Previously, *M. temama* had been regarded as a Central American endemic, ranging from southeastern Mexico to Panama (Allen 1915). However, some authors speculated that the species could also range into northern Colombia, but provided no evidence or explanation (Bello-Gutiérrez et al. 2010). In our analyses, a sequence (GenBank accession number JN632673) from Parque Nacional Chingaza, near Bogotá, Cundinamarca, Colombia (Manuel Ruiz-García *in litt.*), previously assigned to *Odocoileus virginianus* (Hassanin et al. 2012), was recovered as sister to a haplogroup containing sequences of *M. temama* (Figure 1b). Because this latter haplogroup comprised sequences obtained from samples that were correctly identified via examination of voucher specimens (see Escobe-

do-Morales et al. 2016), we herein re-identify this Colombian sample as *M. temama*. Our finding of the species in Colombia is congruent with unpublished morphometric data obtained by EEG, KMH, and JEM. In their recent study, Escobedo-Morales et al. (2016) retained the identity of sequence JN632673 as *Od. virginianus* (a procedure also followed by Heckeberg et al. 2016) but noted that it could have resulted from misidentification, contamination, or hybridization with other species of *Mazama*, or that it might represent an unnamed species. Our results cannot reject that this sequence belongs to an currently unrecognized species because the sequence divergence existing between sequence JN632673 (from Colombia) and the Central American haplogroup of *M. temama* is (ca. 5.0%) substantially higher than divergences known between sister species pairs of Odocoileini deer (all below 3%; see Results). Hence, our assignment of sequence JN632673 to *M. temama* should be regarded as provisional; further work should explore the possibility that two species might be currently lumped within *M. temama*.

Three species traditionally regarded as members of the genus *Mazama* were recovered within Blastocerina, the subtribe endemic to South America. One of them, *M. chunyi*, has only been incorporated twice in phylogenetic assessments (herein and in the just-published study by Heckeberg et al. 2016), and in each case based on a single *CYTB* sequence (obtained from different specimens). *Mazama chunyi* was found sister to *M. gouazoubira*, which was recovered in a monophyletic haplogroup (with strong and moderate support in the BI and ML analyses, respectively). Thus, pending confirmation via analyses of additional molecular data, it is likely that *M. chunyi* and *M. gouazoubira* represent a sister-species pair: one member is restricted to montane habitats of the Bolivian and Peruvian Andes (*M. chunyi*) and the other is widely distributed in lowland habitats of South America (*M. gouazoubira*). If this result is corroborated, then both species should be assigned to a genus other than *Mazama* (which is based on *Mazama americana* and likely applies to *Mazama americana* group 1, see above). Even if further analyses do not confirm their sister-taxon relationship, both species need to be transferred to a genus other than *Mazama* because they share a most recent common ancestor with members of the subtribe Blastocerina, not with the type species of *Mazama*, which belongs to the subtribe Odocoileina. We note that the genus-group name *Nanelaphus* Fitzinger, 1873, with type species *N. namby* Fitzinger (= *M. gouazoubira*), may be available for this clade (Lydekker 1898, Allen 1915).

We recovered two principal reciprocally monophyletic haplogroups within *Mazama nemorivaga:* one (*M. nemorivaga* 1) formed exclusively by samples from the northern portion of the species' range—i.e., from the Venezuelan state of Bolivar, the Guyanean region of Potaro-Siparuni, an unknown locality from French Guiana, and the Brazilian state of Rondônia—and the other (*M. nemorivaga* 2) formed by samples from two unknown localities (one from Brazil and another from Peru) and from the Brazilian states of Pará, Paraná, and Rondônia. The monophyly of these haplogroups received either moderate or strong support. *Mazama nemorivaga* was recovered in our analyses as an isolated lineage divergent from other South American lineages of *Mazama*, including the *M. gouazoubira-M. chunyi* clade, with which it has been taxonomically associated for most of its past taxonomic history (e.g., Miranda-Ribeiro 1919, Cabrera 1961; but see Allen

1915, Rossi 2000). Further research is needed to confirm its relationships and distinctness, but our results suggest it may require genus-level recognition within the Blastocerina. We note that the generic-level name *Passalites* Gloger, 1841, with type species *Cervus nemorivagus* Cuvier, 1817 (= *M. nemorivaga*), is available for this clade (Palmer 1904).

We found evidence that suggests that habitat association in *Mazama gouazoubira* and *M. nemorivaga* might have impacted their phylogeographic structure in contrasting ways. Despite the wide distribution of *M. gouazoubira*, which apparently ranges from Colombia (see below) to Argentina, we found shallow phylogeographic relationships among analyzed populations of this species (Figures 1a, 1b, 1c). This pattern might be explained by the tolerance of this species to a wide range of environmental conditions, as suggested by its occurrence across dry, wet, forested and open habitat types (Black and Vogliotti 2008, Black-Décima et al. 2010, Duarte et al. 2012). Wide environmental tolerance might have enabled historical connectivity among populations and gene flow. Conversely, in *M. nemorivaga*, a species that seems to be predominantly associated with tropical and subtropical broadleaf moist forest habitats (as described by Olson et al. 2001; Rossi and Duarte 2016), we found substantially deeper phylogeographic pattering. This pattern might be a consequence of past expansion and contractions of wet forest habitats isolating populations. Such expansions and contractions of forest habitats are thought to have triggered vicariance events that shaped the phylogeographic structure observed in species closely associated to either wet forest- or dry forest habitat types (Gutiérrez et al. 2014).

Our analyses also yielded new insights regarding the distribution of "*Mazama" gouazoubira*. Given that a Colombian sample of "*M." gouazoubira* (GenBank accession number JN632658 [curated version number NC_020720]; Hassanin et al. 2012), obtained from an live individual from northern Bolívar department (Manuel Ruiz-Garcia, *in litt.*), was recovered nested within a haplogroup containing all other samples of that species, our results demonstrate that the northern limit of the species' distribution is not the southern margin of the Amazon basin, as recently argued (Black and Vogliotti 2008, Black-Décima et al. 2010, Duarte et al. 2012). The Colombian sample extends the distribution of *M. gouazoubira* at least ca. 1000 km to the north of literature records of the species in northwestern Bolivia (Black and Vogliotti 2008, Black-Décima et al. 2010, Duarte et al. 2012)—this distance is a rough estimate as we were not able to obtain detailed locality information for this sample (see Hassanin et al. 2012).

We take the opportunity to comment on ambiguities that have prevailed in the literature with regard to the distribution of *Mazama nemorivaga*. Important discrepancies exist among published distribution maps for this species. For example, Duarte et al. (2012) depicted a distributional range for the species that includes the Amazonian region and the Guianas, the eastern slopes of the Ecuadorian and Peruvian Andes, the southern half of the Andean cordilleras of Colombia, the Sierra de Santa Marta and lowlands in northern Colombia, and the Lago de Maracaibo basin and the Península de Paraguaná in northwestern Venezuela. However, Rossi and Duarte (2016) omitted the Colombian Andes from their range map for this species, but included the entire Ven-

ezuelan mainland with exception of the Andean cordilleras, the Península de Paraguaná, and the northern half of the La Guajira department of Colombia. These differences seem to have resulted from attempts to combine records of *M. nemorivaga* from Amazonia and the Guianas with alleged records of that species from other regions. A modern revisionary work evaluating the taxonomy of brockets in northern South America is indispensable to achieve reliable knowledge on the distribution of *M. nemorivaga* and determine which of the populations in northwestern South America, if any, correspond to *M. nemorivaga*, whose type locality is Cayenne, French Guiana (Allen 1915).

## Monophyly and phylogenetics of the genus *Odocoileus*

Our results do not support the monophyly of the genus *Odocoileus* as traditionally understood because the node shared by all samples of *Odocoileus* received negligible support in both analyses and, more importantly, because *Mazama pandora* was found embedded within *Odocoileus* (as previously discussed). Because of the apparent recent origin of *Odocoileus*, it is likely that recovering the genus and its species as monophyletic groups would require examination of DNA segments with higher mutations rates than that of the *CYTB* gene. In fact, we conducted preliminary analyses (not shown) of sequence data from the mitochondrial control region (D-loop) and *CYTB* generated for a previous study on the phylogeography of *Od. hemionus* (Latch et al. 2009) and found that, when analyzed alone, the *CYTB* data failed to provide an adequately supported topology. By contrast, D-loop sequences analyzed in combination with the *CYTB* data yielded a more structured tree and with better nodal support (similar to that shown in figure 2 of Latch 2009).

## Phylogenetic relationships and taxonomy of species of *Odocoileus*

Our results do not support the monophyly of either of the species traditionally recognized within the genus *Odocoileus*, i.e., *Od. virginianus* and *Od. hemionus*. Two explanations are likely. First, as mentioned above, the substitution rate of *CYTB* appears too low to allow adequate resolution of relationships as recent as these. In other words, incomplete lineage sorting might be responsible for the observed lack of monophyly in these taxa. Second, the observed lack of monophyly in these species is a partial consequence of hybridization between them, a phenomenon that has been widely documented (Carr et al. 1986, Stubblefield et al. 1986, Cronin et al. 1988, Key and Boe 1992, Cathey et al. 1998, Hornbeck and Mahoney 2000, Bradley et al. 2003). Hybridization between *Od. hemionus* and *Od. virginianus*, or among their respective subspecies (e.g., Hopken et al. 2015), seems to occur not only along contact zones of their native ranges, but also in areas to which they have been translocated for commercial purposes. For instance, a free-ranging deer in natural areas within Washington

DC (the National Zoo, Smithsonian Institution), with external characteristics matching *Od. virginianus*, had a *CYTB* haplotype (KY928667) that places it within the *hemionus* group in our gene tree. This is a sign of hybridization between both species in the state of Virginia, where individuals of *Od. hemionus* were translocated decades ago (Linzey 1998). Hybridization can also explain other instances in which nominal taxa were not recovered in monophyletic groups. For example, although most samples of black-tailed deer (*Od. hemionus columbianus* and *Od. h. sitkensis*) form a clade, two sequences attributed to *Od. h. columbianus* from Alaska were not recovered within this clade. These two sequences were recovered within the *hemionus* group which can be attributed to hybridization between *Od. h. columbianus* and other subspecies of *Od. hemionus* (Latch et al. 2009, 2011 and references therein). Similarly, hybridization may also explain why a sequence attributed to *O. h. hemionus* from Alaska was recovered within the *columbianus* group.

The traditional classification of species of *Odocoileus* is incongruent with the phylogenetic information currently available for them. Our results suggest (1) that the *columbianus* and *sitkensis* lineages, currently treated as subspecies of *Od. hemionus,* form a clade that is more closely related to *Od. pandora* than to *Od. hemionus*; and that (2) *Od. hemionus* appears more closely related to *Od. virginianus* (even to *Od. virginianus* from South America!) than to its putative subspecies *columbianus* or *sitkensis*. In agreement with this possibility, the level of uncorrected genetic divergence, calculated with *CYTB* sequence data, between the *hemionus* and the *columbianus* groups (6.2%) greatly exceeds mean levels of divergences within species (and species-like lineages) of Odocoileini and Rangiferini (all below 3.6%, Table 4). Surprisingly in view of their importance to North American hunters, no phylogenetic study using nuclear sequence data from mule deer, white-tailed deer, and black-tailed deer have been conducted to date. If further analyses based on sequence data obtained from independently inherited loci confirm the topology obtained from mtDNA, then reconciling taxonomy with phylogenetics would require elevating *columbianus* and *sitkensis* to species rank (see Future Directions). However, such further analyses based on multiple loci are likely to produce an alternative topology, for example by recovering all lineages of mule deer, white-tailed deer, and black-tailed deer as a monophyletic group and with *pandora* sister to it. Under this plausible scenario, and for the sake of binomial stability, which has important implications for scientific communication (see discussion on this topic by Gutiérrez and Marinho-Filho 2017), we transfer *pandora* to the genus *Odocoileus*, in congruence with the close relationship and overall similarity it shares with other members of *Odocoileus*. Regardless of which of these alternative topologies will be favored by additional analyses, dense geographic sampling is necessary to produce a suitable taxonomic classification with respect to lineages currently treated as members of *Od. hemionus* and *Od. virginianus*. This is particularly important due to the tremendous morphological variation documented among (even geographically close) populations of Neotropical white-tailed deer and the possibility that they might not be conspecific (as proposed by Molina and Molinari 1999, and Molinari 2007).

**Monophyly and phylogenetic relationships of the remaining Blastocerina**

According to the traditional taxonomy of Odocoileini deer, the recently described subtribe Blastocerina contains four species-poor genera, *Hippocamelus* and *Pudu* containing two species each, and the monotypic *Blastocerus* and *Ozotoceros*. Our analyses supported the monophyly of *Hippocamelus* and *H. bisulcus.* In addition, none of our tree- or genetic-distance metrics suggests the existence of additional unrecognized species within this genus. The single analyzed sequence of *H. antisensis* did not nest within the haplogroup of any other species. Nevertheless, our sampling for this genus was poor; additional studies might reveal higher diversity within the two traditionally recognized species of *Hippocamelus*. In fact, the recent study by Heckeberg et al. (2016) analyzed the same sequences that we analyzed and two additional sequences of *Hippocamelus antisensis* (of unknown geographic precedence). These authors recovered these additional sequences (hereafter referred to as *H. antisensis* lineage 2) as sister to *Ozotoceros*. A third sequence of that species analyzed by these authors (which we analyzed; hereafter referred to as *H. antisensis* lineage 1) was recovered as sister to *H. bisulcus*. Therefore, their results challenge the monophyly of both the genus *Hippocamelus* and *H. antisensis* (Heckeberg et al. 2016), and suggest that an unrecognized species related to *Ozotoceros* might exist among populations currently assigned to *H. antisensis*. Nevertheless, ancient hybridization and incomplete lineage sorting remain as alternative causal explanations for these results; these possibilities need to be tested with data from unlinked loci.

Our results support the monophyly of both *Blastocerus* and *Ozotoceros*, and none of our tree- or genetic-distance metrics suggest the possible existence of currently unrecognized species within sampled populations currently referred to as *Blastocerus dichotomus* or *Ozotoceros bezoarticus*. These results agree with results from previous studies (González et al. 1998, 2002, Márquez et al. 2006, Duarte et al. 2008). Both of our phylogenetic analyses (BI, ML) recovered *Blastocerus* sister to a clade containing "*Mazama*" *gouazoubira*, "*Mazama*" *chunyi*, and the genus *Hippocamelus* (*H. bisulcus* + *H. antisensis* lineage 1); however, this relationship received insignificant support in the BI analysis and modest support in the ML analysis. That phylogenetic position for *Blastocerus* agrees with that recovered by Heckeberg et al. (2016) from *CYTB* data, but disagrees with the topology obtained by Hassanin et al. (2012) from complete mitochondrial genomes, who recovered *Blastocerus* sister to "*Mazama*" *nemorivaga*. Duarte et al. (2008) found *Blastocerus* sister to a clade formed by *H. bisulcus* and "*Mazama*" *gouazoubira*. A likely explanation for this difference is that these authors used different optimality criteria than the ones that we used. The tree presented by Duarte et al. (2008) seems to have been produced by a neighbor-joining analysis (a phenetic technique) (showing bootstrap values from that analysis and from a Maximum-Parsimony analysis), whereas our analyses were based on Bayesian and Maximum-Likelihood optimality criteria. Duarte et al. (2008) also mentioned that an unreported Bayesian inference analysis they conducted yielded a similar topology to those of their other two analyses. Differences in the taxon sampling used by Duarte et al. (2008) and Hassanin et al. (2012)

with respect to our taxon sampling might also help explain the differences between their topologies and ours. Similar factors could also explain disagreement between our results and those from previous studies with regard to the phylogenetic position of *Ozotoceros*. Albeit with negligible support, our analyses recovered *Ozotoceros* as sister to a clade formed by *Blastocerus*, *Hippocamelus*, "*Mazama*" *gouazoubira*, and "*Mazama*" *chunyi*. Both Duarte et al. (2008) and Heckeberg et al. (2016) found *Ozotoceros* sister to a sequence representing *Hippocamelus antisensis* lineage 2, whereas Hassanin et al. (2012) recovered *Ozotoceros* sister to a clade formed by "*Mazama*" *gouazoubira* and *Hippocamelus antisensis* lineage 1.

A case deserving close attention concerns the monophyly (or lack thereof) of the genus *Pudu*. According to the traditional taxonomy, *Pudu* contains two species, *P.* (*Pudu*) *puda* and *P.* (*Pudella*) *mephistophiles* (Hershkovitz 1982). The former occurs in Argentina and Chile, at elevations from sea level up to 1700 meters, whereas the latter occurs in the Andes of Colombia, Ecuador, and Peru at elevations between 1700 and 4000 meters (Hershkovitz 1982, Cronin et al. 2006, Meier et al. 2007, Escamilo et al. 2010, Jiménez 2010). Our results do not support the monophyly of the genus as traditionally recognized. *Pudu puda*, which is the type species of the genus, was recovered sister to a clade including "*Mazama*" *gouazoubira*, "*Mazama*" *chunyi*, *Hippocamelus* (*H. bisulcus* + *H. antisensis* lineage 1), *Blastocerus*, and *Ozotoceros*—this position was recovered in the best tree resulting from the ML analysis and in the consensus tree resulting from the BI analyses, but in both cases with negligible support. This large putative clade (including all the taxa just mentioned) was recovered sister to *P. mephistophiles* in the ML analysis, but with negligible nodal support. The BI analysis recovered *P. mephistophiles* in a polytomy at the base of the subtribe Blastocerina. This polytomy contained two additional branches, one leading to "*Mazama*" *nemorivaga* and another containing all other members of Blastocerina. The recent study by Heckeberg et al. (2016) analyzed multiple partial *CYTB* sequences of *P. mephistophiles*; these authors conducted various analyses, but recovered the species in various positions, including: *P. mephistophiles* as sister to all other Blastocerina (as in our ML analysis); as sister to Odocoileini and Rangiferini; and in an unresolved position with other Odocoileini clades and Rangiferini. However, Heckeberg et al. (2016) also analyzed a sequence labeled as *P. mephistophiles* (by Hassanin et al. 2012), overlooking the observation already made by Gutiérrez et al. (2015), who demonstrated that this sequence actually corresponds to "*Mazama*" *rufina*. Despite the ambiguity regarding the position of *P. mephistophiles*, *P. puda* was consistently recovered in our analyses and in those by Heckeberg et al. (2016) as being more closely related to Blastocerina other than *P. mephistophiles*. This fact suggests the possibility that the genus *Pudu*, as traditionally defined, is not monophyletic. If confirmed by future studies, the monotypic *Pudella* (Thomas 1913), which is currently treated as a subgenus of *Pudu*, would warrant genus rank. According to Hershkovitz (Hershkovitz 1982; see also Brooke 1874, 1878), the union of the cuboideonavicular and external and middle cuneiform tarsal bones into a single bone (Figure 3) is the only osteological characteristic shared by *P. puda* and *P. mephistophiles* that consistently separates them from

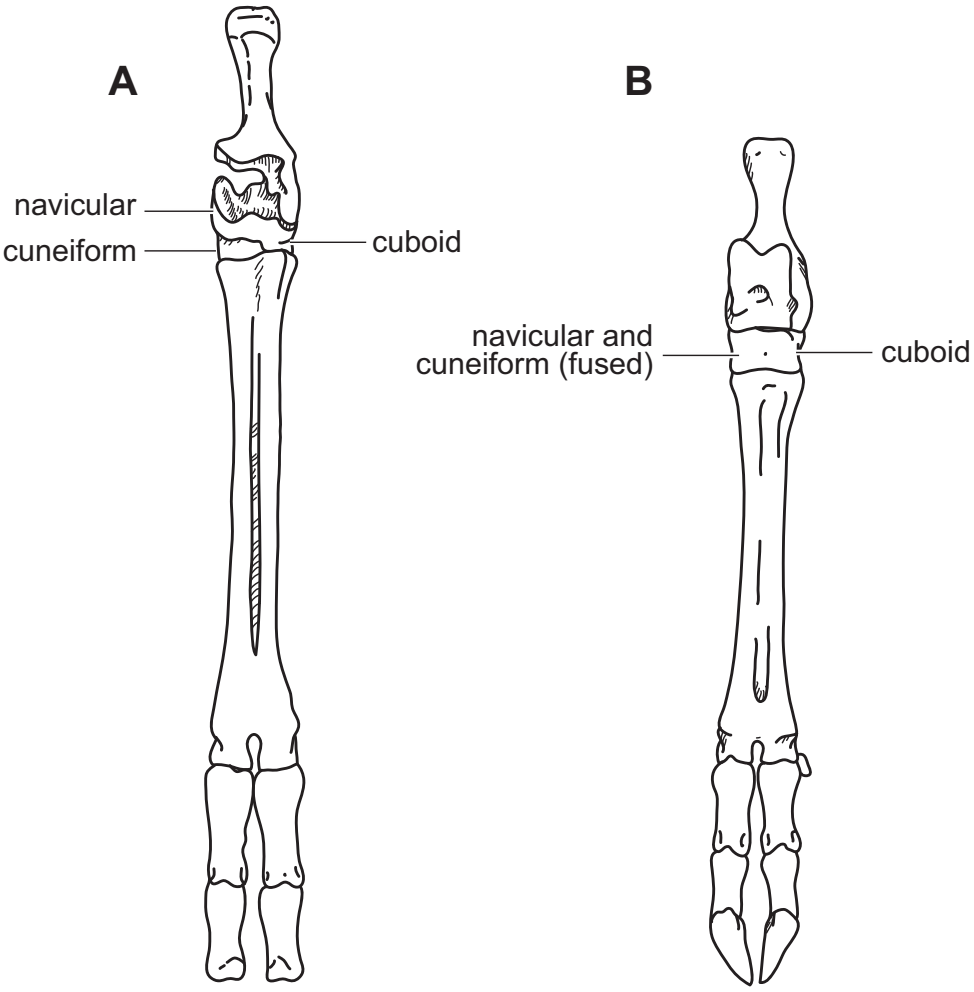


**Figure 3.** Hind foot bones of *Mazama rufina* (**A**) and *Pudu puda* (**B**) *sensu* Hershkovitz (1982). According to Hershkovitz (1982; see also Brooke 1874, 1878), the union of the cuboideonavicular and external and middle cuneiform tarsal bones into a single bone in *Pudu* is the only osteological characteristic shared by *P. puda* and *P. mephistophiles* that consistently separates them from all other living deer, with exception of the genera *Elaphodus* and *Muntiacus*.

all other living deer, except from the distantly related Asiatic genera *Elaphodus* and *Muntiacus* (Gilbert et al. 2006, Hassanin et al. 2012). Could this anatomical similarity between *P. puda* and *P. mephistophiles* be the result of evolutionary convergence rather than a trait inherited from a recent common ancestor shared between these two species? Convergence could also explain other similarities between these species, like their small sizes and spike-like antlers, among others (see Hershkovitz 1982). Evolutionary convergence in morphological appearance has misguided supraspecific classifications of deer before, most spectacularly in the case of the genus "*Mazama*" *sensu*

*lato* (as traditionally understood) (see findings of molecular studies based on data from either mDNA, nDNA, or both: Gilbert et al. 2006, Duarte et al. 2008, Hassanin et al. 2012, Escobedo-Morales et al. 2016, Heckeberg et al. 2016, the present study). Regardless of these issues concerning supraspecific classification, our results and those by Heckeberg et al. (2016), support the species-level monophyly of both *P. puda* and *P. mephistophiles*. Both of our phylogenetic analyses recovered *P. puda* in a single strongly supported haplogroup, and none of our analyses recovered the single analyzed sequence of *P. mephistophiles* embedded within another species' haplogroups. None of our tree- or genetic-distance metrics suggest the existence of species-level diversity currently unrecognized among their populations.

## A word on the genus *Rangifer*

Because we employed dense taxonomic and geographic sampling for Odocoileini deer, we sought to test if our approach confirmed the monophyly of this tribe and therefore included *Rangifer* as part of our ingroup. *Rangifer,* which is currently placed within the subtribe Rangiferini (Heckeberg et al. 2016), has been recovered sister to the Odocoileini in previous studies that were based on limited sampling for both Odocoileini and Rangiferini (Gilbert et al. 2006, Hassanin et al. 2012). We were also able to test, for the first time, the monophyly of *Rangifer* with dense sampling of both *Rangifer* and various Odocoileini. Our results were not controversial, as both of our phylogenetic analyses provided strong support to the monophyly of the genus *Rangifer* and it was found sister to a clade formed by all Odocoileini—this Odocoileini clade was recovered in both analyses, albeit with negligible support in both cases.

## Caveats

Three main caveats affect the present study and, more generally, have hampered progress towards a suitable taxonomy for Odocoileini deer. First, the scarcity of freshly preserved tissue samples for Neotropical deer has restricted many studies to Sanger sequencing technologies and mitochondrial DNA, and in most cases only partial sequences of one or two genes are used. At present, *CYTB* is the only gene sampled broadly enough to support the geographic and taxonomic scope of the present study. Our new *CYTB* sequences filled some geographic and taxonomic gaps pre-existing on GenBank, but not all of them, and particularly for widely distributed taxa (e.g., *Odocoileus virginianus* and *Mazama americana*), data are still missing from large and biogeographically interesting portions of their ranges.

Secondly, the use of sequence data from a single locus is an obvious limitation. Because the mode of inheritance of mitochondrial DNA is matrilineal, our use of *CYTB* sequences allows inference only of matrilineal relationships among sampled populations, which might be contradicted when sequence data from additional loci

become available. Nevertheless, because female philopatry is rampant in mammals, matrilineal relationships are useful to identify priority regions and taxa in phylogenetic comparison. Moreover, previous studies based on *CYTB* sequence data have regularly improved the classification of tropical mammalian groups (e.g., Patterson and Velazco 2008, Solari et al. 2009, Gutiérrez et al. 2010, 2015, Voss et al. 2013, Moratelli et al. 2016, 2017, Molinari et al. 2017, the present study) whose decades-old classifications had been based on assessments of morphological similarity. Many of these classifications, including that of Odocoileini deer, were proposed at times predating the implementation of phylogenetic, or even statistical, analyses in taxonomic research.

Third, many of the sequences available from GenBank are not associated with voucher specimens, lack geographic data, or both. This is likely due to the fact that many colleagues that generated these data are not taxonomists—but ecologists, wildlife managers, conservation biologists, and researchers working on public health issues—and they did not need to report such data for their particular research goals. Unfortunately, in many instances, it has not been reported whether voucher specimens are available and, if so, basic information associated with these specimens (e.g., institution in which they are housed, catalogue numbers, criteria used to assign taxonomic identifications) have not been provided. Similarly, geographic provenances of samples used to generate sequence data are rarely reported and, when reported, often limited to names of country and large administrative entities (e.g., state, department, etc.). Moreover, some Neotropical members of the tribe Odocoileini are rare, subject to intense pressure by humans (e.g., due to hunting and habitat loss), or both, which has hindered, in some countries, obtaining permits to collect specimens for research. To circumvent this difficulty, researchers have sometimes resorted to using samples obtained from animals kept in captivity. Often, zoos do not maintain detailed records of the provenance of animals they keep. The ambiguities resulting from all the aforementioned factors compromise the use of such samples (and derived sequences) from certain types of analyses (e.g., ancestral area reconstructions); even when they can be used, these issues often limit the interpretations that could otherwise be made. Examples of the latter type of problem are some of the sequences that we analyzed and that represent new and noteworthy distributional records—e.g., the apparent first record of *Mazama temama* for South America and Colombia; the apparent first record of "*Mazama*" *nemorivaga* for northwestern South America and Colombia—unfortunately, no detailed information about their provenance were published by the research teams that generated these sequences (see discussion above).

## Future directions

Our results suggest that future systematic studies on Odocoileini deer should prioritize assessments of the taxonomic status of populations historically assigned to widely distributed taxa—e.g., species of *Odocoileus* and *Mazama americana*. *Odocoileus vir-*

*ginianus* shows great morphological variability. Regional patterns of this high morphological variability have led authors to propose that multiple species exist among populations traditionally referred to *Od. virginianus* (Molina and Molinari 1999, Molinari 2007). A study based on phylogenetic information and adequate sampling from North, Central, and South America has yet to be conducted to evaluate these proposals. Similarly, efforts based on mtDNA sequences (including the present report) and karyology have advanced our understanding of the variability of *M. americana* and documented the existence of an undescribed species among populations traditionally referred to this taxon. This species needs to be described, a process that necessarily requires both testing our hypothesis that *M. americana* group 1 likely corresponds to *M. americana sensu stricto* and solving the current incongruence between phylogenetics and the taxonomy of *M. bororo* and *M. nana*. Other cases in which available phylogenetic information identified the likely existence of undescribed species are those of *Hippocamelus antisensis*, whose populations have been recovered in two lineages that are not sister to each other (Heckeberg et al. 2016), and South American populations provisionally assigned to "*Mazama" temama* (the present study). A single sequence of "*Mazama*" *temama* is known from this region, but it is from an unknown locality in Colombia. This sequence is highly divergent from a clade formed by Central American populations of "*Mazama*" *temama*. Future fieldwork in northwestern South America and study of specimens housed at museums, particularly those in Colombia and Venezuela, might provide additional samples of this likely undescribed taxon.

Clearly, substantial species-level taxonomic work is yet to be done. As the scientific community advances tackling the many taxonomic issues of cervid species, researchers should keep in mind that, despite the conservation status of some of these deer and the implicit difficulty to obtaining collecting permits for research, especially in the Neotropics, new species and subspecies should only be described when preserved museum specimens are available to document new names (see Ceríaco et al. 2016, Gutiérrez and Pine 2017, Dubois 2017 and references therein, Pine and Gutiérrez [in press]; contra Donegan 2008, Marshall and Evenhuis 2015). In addition, and also to avoid obstructing scientific progress, upcoming studies should provide sufficient information regarding voucher specimen availability and detailed information regarding the provenance of samples from which they have obtained data; unfortunately, this is not customary.

The current supraspecific taxonomy of Odocoileini deer does not closely align with the information currently available regarding their phylogenetic relationships (Gilbert et al. 2006, Duarte et al. 2008, Hassanin et al. 2012, Escobedo-Morales et al. 2016, Heckeberg et al. 2016, the present study). Further phylogenetic analyses and morphology-based revisionary work is required. The use of massively-parallel sequencing technologies and the unprecedented potential to generate large amounts of DNA data from museum specimens offers the most promising approach to solve this incongruence; however, museum work should also be conducted to enable proper characterization and diagnoses of generic names to be assigned to clades. Efforts to generate a more robust phylogeny will also provide a basis for biogeographic studies on Odocoileini deer. Such studies will be of great interest for understanding aspects of the Great American Biotic

Interchange and other major events in the deep history of the American continents. Results presented in this study suggest that some long-lived notions about areas of origin and number and direction of dispersal events of Odocoileini deer are erroneous, but correcting them will require meaningful estimates of times since divergences and ancestral area reconstructions.

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## Supplementary materials 1–3

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# The cockroach genus *Sorineuchora* Caudell, 1927 from China (Blattodea, Ectobiidae, Pseudophyllodromiinae)

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## Abstract

In this paper, three new species (*S. bimaculata* **sp. n.**, *S. viridis* **sp. n.**, and *S. hispida* **sp. n.**) and five known species, *S. formosana* (Matsumura, 1913), *S. nigra* (Shiraki, 1908), *S. shanensis* (Princis, 1950), *S. bivitta* (Bey-Bienko, 1969), and *S. undulata* (Bey-Bienko, 1958), are described and illustrated. *Sorineuchora undulata* was previously synonymized with *S. nigra*, and is now reinstated as a valid species. A key to the males of *Sorineuchora* from China is provided.

## Keywords

Blattellidae, distribution, key, new species, *Sorineuchora*

## Introduction

The cockroach genus *Sorineuchora* was established by Caudell (1927), and synonymized with *Chorisoneura* Brunner von Wattenwyl, 1865 by Hebard (1929). However, comparing *Sorineuchora* with *Chorisoneura*, Brujning (1948) pointed out there are obvious differences in the hind-wing venation and apical triangle. Subsequently, Asahina (1978) discussed the interspecific relationships of *Sorineuchora* and considered *S. formosana* (Matsumura, 1913) and *S. setshuana* (Bey-Bienko, 1958) to be closely related to *S. lativitrea* (Walker, 1868) and *S. nigra* (Shiraki, 1908), respectively. At the same time, the seven other known species were not treated. Later, Roth (1998) revised

*Sorineuchora* and recorded eleven species worldwide, of which nine species were from China including the four species mentioned above in Asahina (1978). Liu and Zhu (2001), who recorded *Sorineuchora* species under *Chorisoneura*, synonymized *S. setshuana* and *S. undulata* with *S. nigra* without giving any details. Recently, Che et al. (2017) showed that the subfamily Pseudophyllodromiinae was a polyphyletic group, and *S. nigra* and *S. bivitta* (Bey-Bienko, 1969) formed monophyletic groups. Wang et al. (2017) indicated that *Balta* and *Sorineuchora* are more closely related to each other than either is to *Allacta*, *Shelfordina*, or *Latiblattella*.

Recently, specimens deposited in Southwest University and Hebei University were examined, and eight species of *Sorineuchora* identified from China including five known and three new species. Because of the lack of specimens and male description of *S. punctipennis* (Princis, 1950), the species is not included in the key, only recorded as information under the remarks of *S. undulata*. These cockroaches were mostly attracted by light at night (Fig. 10A–B), but were also found on vegetation such as leaves (Fig. 10C) and flowers (Fig. 10D).

## Materials and methods

Male genital segments were macerated in 10% NaOH for one hour, and rinsed with distilled water, then stored in glycerine for dissection and observation. Line drawings were made with a Motic K400 stereomicroscope. Habitus photos were taken with a Canon 50D plus a Canon EF100mm f/2.8L Macro IS USM lens, and stacked with Helicon Focus software. The map was made with Natural Earth (http://www.naturalearthdata.com). All photos and images were edited with Adobe Photoshop CS6.

COI sequence (KY349518 and KY349519) of *S. nigra* was downloaded from GenBank to compare with COI sequence of the exceptional female specimen (Fig. 10C) (Accession number: MF612149).

Morphological terminology mainly follows Roth (2003), and wing venation and genitalia terms are according to Li and Wang (2015) and McKittrick (1964), respectively. The vein abbreviations in this article are listed as below following Li and Wang (2015):

**CuA** cubitus anterior
**M** media
**R** radius
**RA** radius anterior
**RP** radius posterior
**Sc** subcosta

Specimens examined are deposited in the following collections. **IESWU** Institute of Entomology, Southwest University (西南大学昆虫研究所), Beibei, Chongqing, China; **MHBU** Museum of Hebei University (河北大学博物馆), Baoding, Hebei, China.

## Taxonomy

### Genus *Sorineuchora* Caudell, 1927

**Type species.** *Sorineuchora javanica* Caudell, 1927.

**Diagnosis.** (Partly after Roth (1998)). Fifth segment of maxillary palpus longer than the fourth. Pronotum subelliptical. Front femur with a row of small piliform spinules and two large distal spines (Type $C_2$); proximal four tarsomeres with tarsal pulvilli, tarsal claws simple, asymmetrical, of different size. Tegmina and wings fully developed extending beyond end of abdomen, hind-wing R with oblique branches, M distinct, CuA with one to three branches, apical triangle or appendicular field present, sometimes subobsolete (*S. javanica* and *S. viridis* sp. n.). Abdominal terga of male unspecialized. Supra-anal plate symmetrical, hind margin convexly rounded; paraprocts simple, sheet-like. Subgenital plate with subsymmetrical hind margin. Phallomere L1 consisting of several irregular sclerites. Genital hook on the right side (the diagnosis of subfamily).

According to Roth (1998) there is a close relationship among *Chorisoneura* Brunner von Wattenwyl, 1865, *Chorisoneurodes* Princis, 1962, *Chorisoserrata* Roth, 1998 and *Sorineuchora* Caudell, 1927. Roth (1998) differentiated *Sorineuchora* from *Chorisoneura* and *Chorisoneurodes* by the unspecialized terga in *Sorineuchora*. *Sorineuchora* also has the following traits that differentiate it from *Chorisoserrata*: asymmetrical tarsal claws; interocular vertex not truncate, the fourth maxillary palpomere not longer than the fifth; and antero-ventral margin of forefemur with two apical spines.

Many similar morphological traits exist among *Balta* Tepper, 1893 and *Sorineuchora*, such as proximal four tarsomeres with tarsal pulvilli, tarsal claws asymmetrical and unspecialized, and abdominal terga of male unspecialized. According to the maximum likelihood COI tree in Che et al. (2017) and the combined data (12SrRNA, 16SrRNA, COII, 28SrRNA and H3) tree in Wang et al. (2017), there is a close relationship between *Sorineuchora* and *Balta*. *Sorineuchora* can be distinguished from *Balta* by the following characters: bodies of the former are generally less wide in dorsal view, in the former the fourth maxillary palpomere is not longer than the fifth, and smaller V shaped incision of the hind margin of the subgenital plate. Further study is needed to distinguish the two.

**Remarks.** Species of *Sorineuchora* have strikingly variable morphology. The body coloration ranges from pale green to black (Figs 10, 11, 12); the markings on the pronotal disk vary greatly; the shape of their styli is either cylindrical (Figs 2F, 4E, 6H, 8E) or conical (Figs 3E, 5G, 7H, 9E); the shape of sclerites of L2vm is highly variable, some are filamentary, and some are rod-like. Given this variation, the genus *Sorineuchora* might be not monophyletic, revision based on characters of the type specimen of the genus or molecular data is needed.

**Distribution.** Oriental and Palaearctic regions.

**Key to the males of *Sorineuchora* from China**

1 Body light-colored, yellow or pale green (Figs 10A, 11A–D, 12E–F) ..... **2**
– Body color comparatively dark (Figs 10B–D, 11F–K, 12A–D, 12G–J) ..... **3**
2 Uniformly yellowish white (dried specimen) (Fig. 12E–F) or light green (alive) (Fig. 10A) ..... ***S. viridis* sp. n.**
– Body yellowish brown or straw-yellow ..... **8**
3 Pronotal disk black, with white or yellow symmetrical stripes, and vertex dark with a pair of white or yellow stripes (Figs 4B, 10D, 11H) ..... ***S. shanensis***
– Pronotal disk without stripes, or some with stripes unlike those above ..... **4**
4 Tegmina yellowish brown with four dark spots on the radius and many black dots on veins (Figs 5D, 11J) ..... ***S. undulata***
– Tegmina without spots like those above ..... **5**
5 Vertex with two round yellowish brown spots on the middle (Fig. 7C) ..... ..... ***S. bimaculata* sp. n.**
– Vertex without spots or with spots unlike those above ..... **6**
6 Vertex with a white stripe or a rudimentary dark stripe or without stripes ... **7**
– Vertex with two black stripes, the regions between them yellow (Figs 6B, 12B) ..... ***S. bivitta***
7 Pronotal disk dark with a rudimentary dark stripe or without stripes ..... ***S. nigra***
– Pronotal disk brown, with a yellowish brown, longitudinal stripe (Figs 9B, 12G) ..... ***S. hispida* sp. n.**
8 Subgenital plate with an incision slightly to the left of the middle. Left stylus bent out toward the left apically and pointed and is longer than the right stylus ..... ***S. pallens***[1]
– Subgenital plate with an incision medially. Left and right styli are similar and cylindrical (Fig. 2F) ..... **9**
9 L2vm apex with one branch, and R3 lying under the L2vm (Fig. 2H) ..... ..... ***S. formosana***
– L2vm without branch and R3 (Roth, 1998, fig. 37) ..... ***S. lativitrea***

[1]From Bey-Bienko (1969). *Sorineuchora pallens* is not described in the current paper because no specimens were examined.

### *Sorineuchora formosana* (Matsumura, 1913)

Figs 2, 11A–B

*Chorisoneura formosana* Matsumura, 1913: 14, pl. 2, fig. 13 (♀); Asahina 1978: 235 (♂♀).
*Theganopteryx formosana* (Matsumura): Shiraki 1931: 209 (♂♀).
*Sorineuchora formosana* (Matsumura): Roth 1998: 15 (♂♀).

**Material examined (all deposited in IESWU). Yunnan:** 1 male, Xishuangbanna, Tropical Botanical Garden, 593 m, 12 November 2009, Guo Tang leg.; 1 male, Meng-

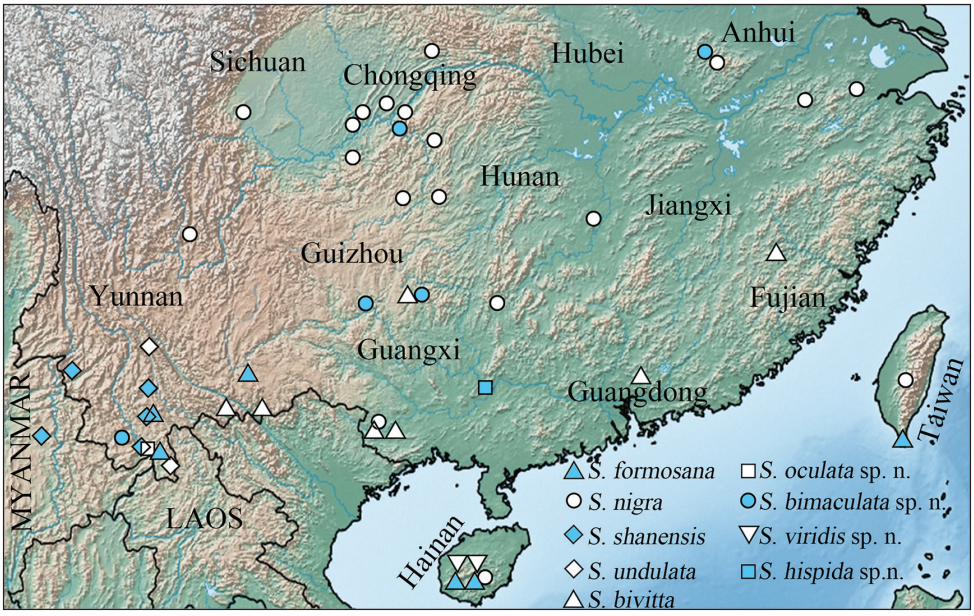


**Figure 1.** Known occurrences of *Sorineuchora* in China and Myanmar.

zi, Lvshuihe first hydroelectric station, 470 m, 19 April 2009, Wei-Wei Zhang leg.; 1 male, Simao, 01 May 2012, Li-Chao Tian leg.; 1 female 1 male, Xishuangbanna, National Nature Reserve, 736 m, 18 August 2012, Guo Zheng leg.; 1 male, Xishuangbanna, Mengla, 1200–1400 m, 10 May 1958, Chun-Pei Hong leg. **Hainan:** 1 male, Tongzha, 07 June 1963, Ya-Lin Zhang leg.; 1 male, Ledong, Mt. Jianfengling, 1050 m, 06–09 December 2007, Wei-Wei Zhang leg.

**Diagnosis.** CuA with one complete branch, between CuA and its branch existing two or three cross veins (Fig. 2D); L2vm rod-like, bifurcate; R3 shaped like a slender curved filament, lying under the L2vm; a setose membrane on the right side (Fig. 2H). Using these traits, *S. formosana* can be distinguished from its congeneric species.

**Supplement to the description provided in Roth (1998: 15–16).**

**Measurements** (mm). Body length without cerci: male 6.8–8.3, female 7.6–8.8; overall length including tegmen: male 8.9–10.4, female 8.9–10.5; pronotum length × width: male 1.85 × 3.1, female 1.95 × 3.4; tegmen length: male 7.2–8.7, female 7.6–8.1.

**Male.** Body small, yellowish brown. Vertex slightly brown, frons yellowish white. Ocellar area yellowish white. Maxillary palpi yellowish white. Tegmina yellowish brown, veins and radial field yellowish white. Abdomen and legs yellow. Interocular space slightly narrower than distance between antennal sockets. Pronotum subelliptical, anterior and posterior margins nearly truncate.

**Distribution.** China (Taiwan, Hainan, Yunnan).

**Remarks.** Based on the illustrations of wings and subgenital plate in Asahina (1978, figs 8, 16, 17) and the subgenital plate and genitalia in Roth (1998, fig. 40), we identified our materials as *S. formosana*. Asahina (1978) noted that *S. formosana* allied to *S. lativitrea* from Southeast Asia. However, the differences between *S. formosana*

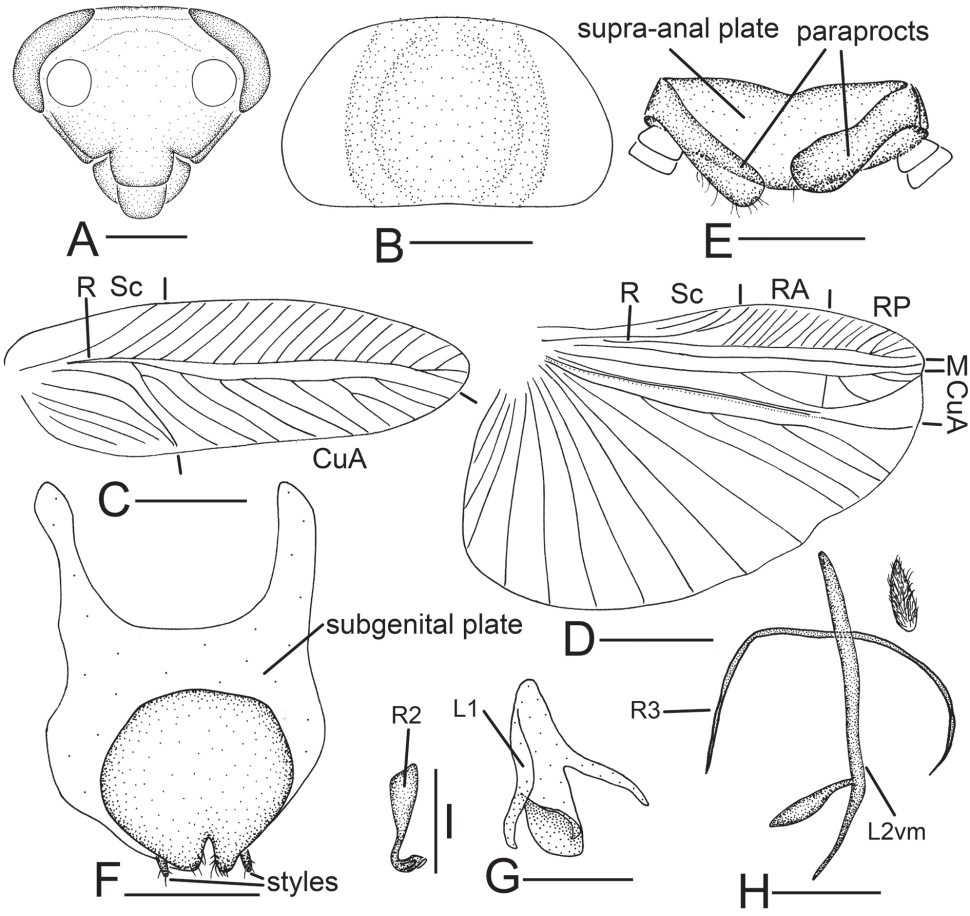


**Figure 2.** *Sorineuchora formosana* (Matsumura, 1913) male from China, Yunnan, Xishuangbanna, Mengla. **A** head, frontal view **B** pronotum **C** tegmen **D** hind wing (the dotted line indicates wing fold) **E** supra-anal plate, ventral view **F** subgenital plate, dorsal view **G** phallomere L1 **H** phallomere L2vm and R3 **I** phallomere R2. Scale bars: 0.5 mm (**A, E–I**), 1.0 mm (**B**), 2.0 mm (**C, D**).

(Fig. 11A–B) and the holotype of *S. lativitrea* (Fig. 11C–D) (size and color) make us suspect of the supposed relationship.

***Sorineuchora nigra* (Shiraki, 1908)**
Figs 3, 10B–C, 11F–G

*Chorisoneura nigra* Shiraki, 1908: 109 (♂); Matsumura 1913: 8; Karny 1915: 63; Hanitsch 1927: 42; Asahina 1991: 71.

*Lupparia nigra* (Shiraki): Shiraki 1931: 197; 1950: 59; Matsumura 1931: 1376; Asahina 1955: 204.

*Balta nigra* (Shiraki): Princis 1969: 978; 1971: 1143.
*Sorineuchora nigra*: Roth 1998: 16 (♂).
*Chorisoneura setshuana* Bey-Bienko, 1958: 680, 689, fig. 11 (♂♀); Liu and Zhu 2001 (synonymy).

**Material examined (all deposited in IESWU). Chongqing:** 1 male, Changshou, Munanyuan, 450m, 09 June 1994, Wen-Zhu Li leg.; 1 male, Wanzhou, 1200m, 10 July 1993, Jian Yao leg.; 6 males, Fengdu, Shiping, 610m, 02–03 June 1994, You-Wei Zhang leg.; 3 males, Mt. Jinyunshan, 800m, 13 June 1994, You-Wei Zhang leg.; 6 males, Mt. Bishan, Qinglong Lake, 10 June 2006, You-Wei Zhang leg.; 1 male, Youyang, Banxi, Sandaigou, 500m, 22 May 2007, Wei-Wei Zhang leg.; 1 male, Jiangjin, Mt. Simianshan, 15 July 2007, Wei-Wei Zhang leg; 1female, Jiangjin, Mt. Simianshan, 05 June 2014, Xin-Ran Li (= Conlin McCat) leg. **Hubei:** 3 males, Mt. Dabieshan, Taohuachong, 604m, 27 June 2014, Yan Shi and Xin-Ran Li (= Conlin McCat) leg. **Sichuan:** 1 male, Huili, 2200m, 29 July 1974, collector unknown; 1 male, Mt. Emei, Qinyinge Temple, 800–1000m, 30 May 1957, You-Cai Yu leg.; 4 males, Mt. Emei, Baoguosi Temple, 550–750m, 23–24 May 1957, Fu-Xing Zhu leg. **Guangxi:** 1 male, Longzhou, Nonggang, 20 May 1985, Wei-Hua Li and Jing-Hong Zhang leg.; 1 male, Longzhou, Nonggang. 29 June 2015, light trapping, Lu Qiu and Qi-Kun Bai leg.; **Zhejiang:** 1 male, Mt. Tianmushan, 26 June 1957, Kun-Ji Yang leg. **Hunan:** 1 male, Hengyang, Mt. Hengshan, Mojingtai, 11 May 1983, Wei-Hua Li leg. **Anhui:** 1 male, Huangshan, Tangkou, Fuxi, 10 July 2014, Xin-Ran Li (= Conlin McCat) and Jian-Yue Qiu leg. **Hainan:** 1 male, Mt. Wuzhishan, 18 May 2014, Shun-Hua Gui, Xin-Ran Li (= Conlin McCat) leg. **Guizhou:** 3 males, Leishan, Mt. Leigongshan, Xiaodanjiang, 750m, 02 June 2005, Zai-Hua Yang leg.; 2 males, Tongren, Mt. Fanjingshan, 1200m, 02 June 2002, Qiong-Zhang Song leg.

**Diagnosis.** Body is black or blackish brown without evident stripes (Fig. 11F–G); L2vm pre-apex with a curved spine-like process, the process apex with several small spines or without (Fig. 3G–H) and ventrally with R3 whose sclerite becomes filamentous and curves to the left (Fig. 3G). Using these traits, *S. nigra* can be distinguished from its congeneric species.

**Supplement to the description provided in Roth (1998: 16–17).**

**Measurements** (mm). Body length without cerci: male 7.6–8.4, female 7.1–8.8; overall length including tegmen: male 9.6–11.0, female 9.5–9.8; pronotum length × width: male 2.05 × 3.1, female 2.0 × 3.0; tegmen length: male 7.3–8.5, female 7.1–8.2.

**Male.** Body small, black, some individuals blackish brown. Vertex black with a rudimentary dark stripe or without stripes; frons black, or vertex and upper half of frons yellowish brown, lower half brown. Pronotal disk dark brown or black, lateral and hind margins hyaline. Interocular space slightly narrower than the distance between antennal sockets. Pronotum subelliptical, anterior and posterior margins nearly truncate. Subgenital plate with a pair of stout styli, the apex slightly pointing outward. L1 consisting of several irregular seta-free sclerites (Fig. 3F); L2vm pre-apex with a curved spine-like process, the process apex with several small spines or without (Fig. 3G–H).

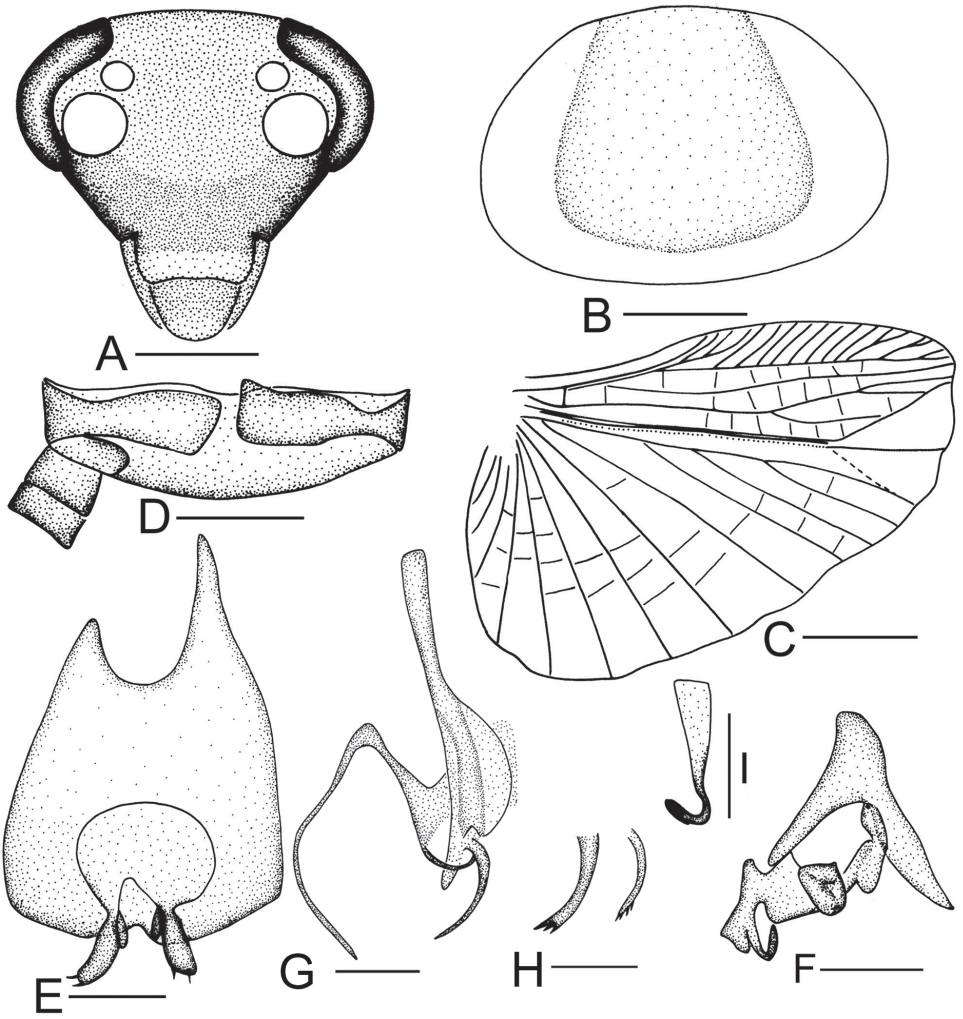


**Figure 3.** *Sorineuchora nigra* (Shiraki, 1908). **A–G, I** male from China, Chongqing, Wanzhou **H** male from China, Chongqing, Mt. Jinyunshan and China, Chongqing, Mt. Bishan Qinglong Lake **A** head, frontal view **B** pronotum **C** hind wing (the dotted line indicates wing fold) **D** supra-anal plate, ventral view **E** subgenital plate, dorsal view **F** phallomere L1 **G** phallomere L2vm and R3 **H** phallomere L2vm **I** phallomere R2. Scale bars: 0.5mm (**A, D–I**), 1.0 mm (**B**), 2.0 mm (**C**).

**Female.** Some individuals are similar to the male in color and habitus, but supra-anal plate symmetrical with hind margin rounded and subgenital symmetrical with hind margin rounded and slightly concave medially. Some individuals do vary distinctly in body color (Fig. 10C) (body brownish red). Head brownish yellow, vertex with a yellowish brown stripe. Clypeus yellowish brown. Wing veins white, legs brown, trochanter yellowish brown, abdominal brown, posterior and lateral margins milk white. We analyzed COI gene sequences of the exceptional female specimen

(MF612149), and female specimen (KY349518), which is similar to male in color and male specimen (KY349519) using MEGA7 (Kumar et al. 2016), the similarity was 98.5% (MF612149 and KY349518), 99.4% (MF612149 and KY349519) and 99.1% (KY349518 and KY349519), respectively.

**Distribution.** China (Taiwan, Chongqing, Hubei, Sichuan, Guangxi, Guizhou, Zhejiang, Hunan, Anhui, Hainan), Japan.

**Remarks.** Roth (1998) noted that *S. nigra* and *S. setshuana* might prove to be synonyms by comparing figs 10 and 18 in Asahina (1978). Liu and Zhu (2001) synonymized *S. setshuana* and *S. undulata* with *S. nigra* without giving any details. Based on examining specimens kept in IESWU and the descriptions of *S. undulata* by Bey-Bienko (1958), there are many differences between *S. nigra* and *S. undulata* in coloration, the details of tegmina and male subgenital plate (Figs 3E, 5G, 11F–G, 11J–K). Therefore, *S. undulata* is not a synonym of *S. nigra*.

***Sorineuchora shanensis* (Princis, 1950)**
Figs 4, 10D, 11H–I

*Sorineuchora nigra* Princis, 1950: 208, fig. 4 (♂♀).
*Sorineuchora shanensis* (Princis): Roth, 1998: 17, figs 44–48 (♂♀).

**Material examined (all from Yunnan, deposited in IESWU).** 1 male, Pu'er, Simao, 04 July 2004, Xiang-Rong Xu leg.; 2 females 2 males, Xishuangbanna, Mengyang, 800m, 06 June 1991, Ying-Lun Wang and Run-Gang Tian leg.; 3 males, Pu'er, Simao, Meizihu, 19 July 2009, Zong-Qing Wang leg.; 1 male, Pu'er, Simao, Meizihu, 22 May 2016, Lu Qiu, Zhi-Wei Qiu leg.; 2 females 3 males, Lincang, Nansan, 1010m, 08 July 2007, Li-Jun Cai leg.; 1 female 1 male, Menglun, 30–31 July 2009, Zong-Qing Wang leg.; 1 female, Pu'er, Xiaoheijiang, 24 July 2009, Zong-Qing Wang leg.; 2 females 1 male, Pu'er, Yixiangzhen, Cilincun, 02 May 2013, Zong-Qing Wang leg.

**Diagnosis.** Vertex dark with a pair of white or yellow transverse stripes; pronotal disk black, with symmetrical white or yellow markings (Figs 4B, 10D, 11H); L2vm with one branch (Fig. 4G); tegmen dark, veins white or dark (Figs 10D, 11H) Using these traits, *S. shanensis* can be distinguished from its congeneric species.

**Supplement to the description provided in Roth (1998: 17–19).**

**Measurements** (mm). Body length without cerci: male 4.9–5.4, 5.8–6.5; overall length including tegmen: male 7.5–8.5, female 7.0–8.5; pronotum length × width: male 1.85 × 2.6, female 1.75 × 2.55; tegmen length: male 5.4–6.0, female 5.2–6.5.

**Male.** Body small, dark. Vertex dark with a pair of white or yellow transverse stripes. Clypeus reddish brown. Antennae with first six basal antennomeres black, the rest brown. Pronotal disk black, with symmetrical white or yellow markings (Figs 4B, 10D, 11H), lateral margins hyaline. Tegmen dark, veins white or dark (Figs 10D, 11H). Pronotum subelliptical, posterior margin nearly truncate. Supra-anal

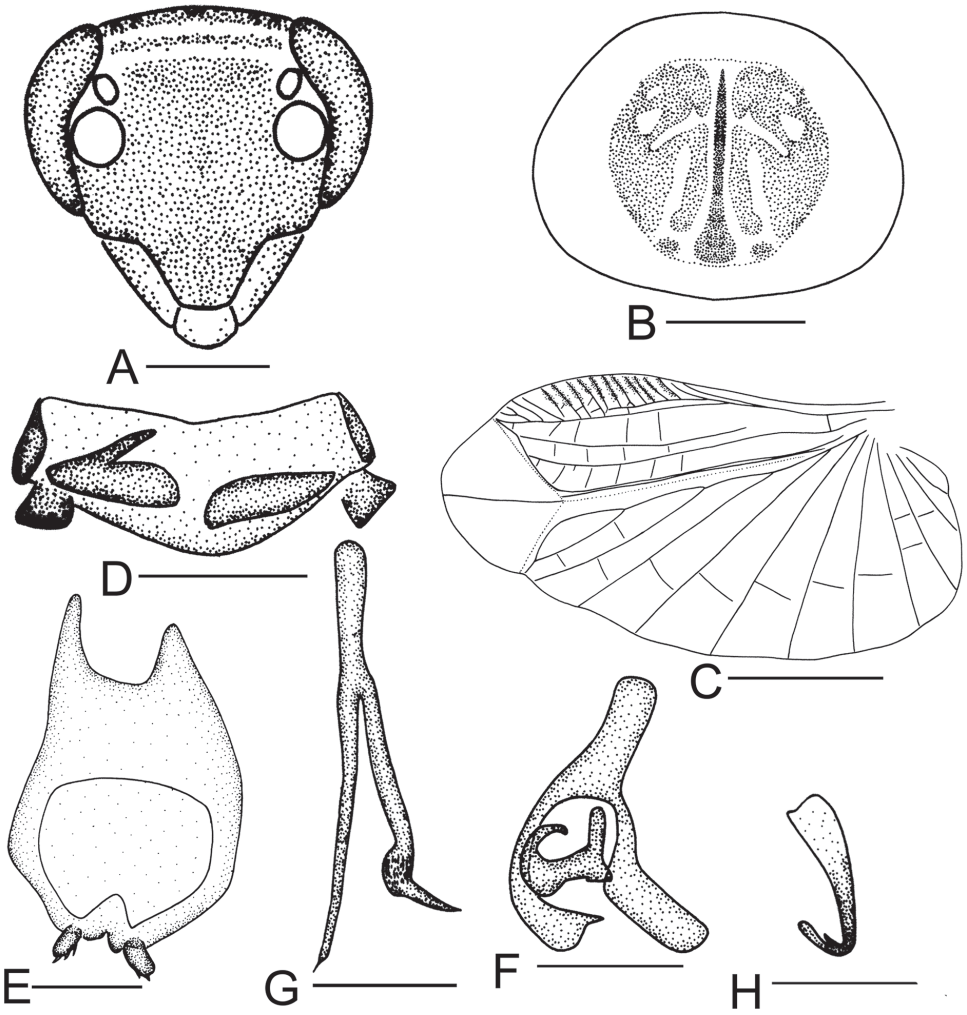


**Figure 4.** *Sorineuchora shanensis* (Princis, 1950) male from China, Yunnan, Pu'er, Simao. **A** head, frontal view **B** pronotum **C** hind wing (the dotted line indicates wing fold) **D** supra-anal plate, ventral view **E** subgenital plate, dorsal view **F** phallomere L1 **G** phallomere L2vm and R3 **H** phallomere R2. Scale bars: 0.5mm (**A, D–H**), 1.0 mm (**B**), 2.0 mm (**C**).

plate with hind margin convex, or some individuals with hind margin weakly concave, paraprocts slightly dissimilar and sheet-like (Fig. 4D). The styli with small spines at preapical and inner sides (Fig. 4E). L1 consisting of several irregular seta-free sclerites (Fig. 4F).

**Distribution.** China (Yunnan); Myanmar.

**Remarks.** According to the stripes on the vertex (Fig. 11I), the markings on the pronotal disk (Figs 4B, 11H) and the color of vein of the tegmen (Figs 10D, 11H), this species is easily recognized.

***Sorineuchora undulata* (Bey-Bienko, 1958)**
Figs 5, 11J–K

*Chorisoneura undulata* Bey-Bienko, 1958: 680, 689 (♂).
*Sorineuchora undulata* (Bey-Bienko): Roth 1998: 21 (♂).

**Material examined (all deposited in IESUW).** 1 male, China, Yunnan, Xishuangbanna, Wangtianshu, 23 May 2016, Zhi-Wei Qiu and Lu Qiu leg.

**Diagnosis.** On the frons between the ocelli with the V shaped blotch (Fig. 5A); tegmina yellowish brown with four dark spots on the radius and many black dots on veins (Fig. 5D); L1 with setae on the right apex; L2vm with its middle inflated, the apex with two branches, L2d setose, R3 right pre-apex lying under the L2vm (Fig. 5I). Using these traits, *S. undulata* can be distinguished from its congeneric species.

**Supplement to the description provided in Roth (1998).**

**Measurements** (mm). Body length without cerci: 8.9; overall length including tegmen: male 10.8; pronotum length × width: male 2.2 × 4.1; tegmen length: male 9.0.

**Male.** Tegmina yellowish brown with four dark spots on the radius and many black dots on veins (Figs 5D, 11J). Interocular space slightly narrower than the distance between antennal sockets. Paraprocts sheet-like and the left with a branch (Fig. 5F). L1 consisting of several irregular sclerites, the right apex with setae (Fig. 5H); L2vm with its middle inflated, the apex with two branches, L2d setose, R3 right pre-apex lying under the L2vm (Fig. 5I); hooked phallomere (R2) on the right side, with a preapical incision.

**Distribution.** China (Yunnan).

**Remarks.** The dots on tegmina of *S. undulata* resemble that of *S. punctipennis*, it differs in having longer body, shorter tegmina, and a strong wavy and bent CuA of the hind wing (Fig. 5E).

***Sorineuchora bivitta* (Bey-Bienko, 1969)**
Figs 6, 12A–B

*Chorisoneura bivitta* Bey-Bienko, 1969: 838, fig. 17 (♂).
*Sorineuchora bivitta*: Roth 1998: 20 (♂).

**Material examined. Deposited in IESWU:** 1 male, Yunnan, Hekou, Nanxi, Huayudong Forest Park, 20–21 April 2009, Wei-Wei Zhang leg.; 2 males, Guizhou, Maolan, Yongkang, 25–28 May 1998, Qiong-Zhang Song leg.; 1 male, Guizhou, Wangmo, 06 June 1982, Ping-Zhang Feng leg.; 1 male, Fujian, Sanming, Shaxian, 23 May 1977, Qing-Dong Luo leg.; 1 male, Guangxi, Longzhou, 31 May 1997, Mao-Fa Yang leg.; 1 male, Guangxi, Chongzuo, Banli National Nature Reserve, 174m, 31 May 2009, Wei-Wei Zhang leg.; 1 male, Guangxi, Hechi, Mt. Daqingshan, 14 May 1963, Si-Kong Liu leg.; 1 male, Hainan, 25 May 1997, Mao-Fa Yang leg. **Deposited in MHBU:** 1 male, China, Guangdong, Huizhou, Mt. Nankunshan, 25 July 2010, Hao-Yu Liu leg.

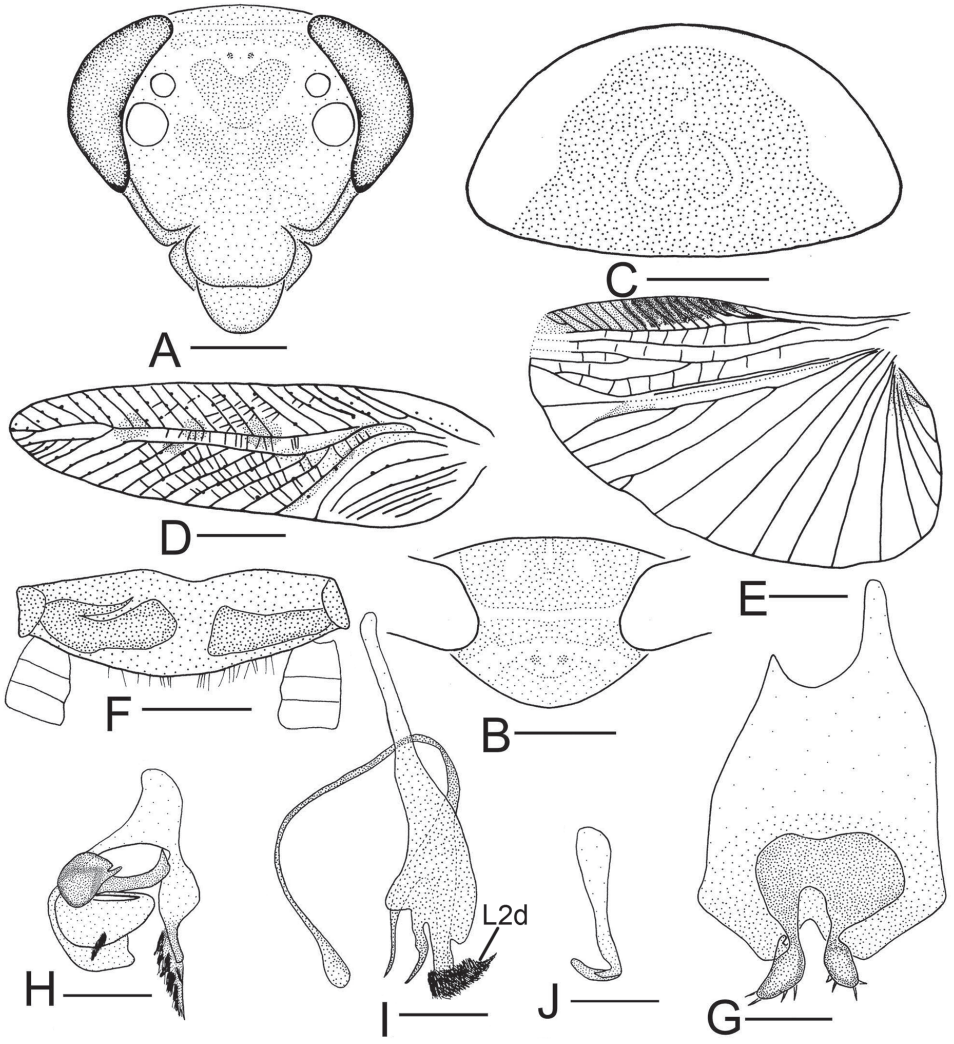


**Figure 5.** *Sorineuchora undulata* (Bey-Bienko, 1958) male from China, Yunnan, Xishuangbanna, Wangtianshu. **A** head, frontal view **B** vertex **C** pronotum **D** tegmen **E** hind wing (the dotted line indicates wing fold) **F** supra-anal plate, ventral view **G** subgenital plate, dorsal view **H** phallomere L1 **I** phallomere L2vm and R3 **J** phallomere R2. Scale bars: 0.5 mm (**A, B, F–J**), 1.0 mm (**C**), 2.0 mm (**D, E**).

**Diagnosis.** Vertex with two black stripes, the regions between them yellow (Figs 6B, 12B); L2vm with inflated apex and the left with filamentous sclerite whose apex inflated (Fig. 6J). Using these traits, *S. bivitta* can be distinguished from its congeneric species.

**Supplement to the description provided in Roth (1998: 20–21).**

**Measurements** (mm). Body length without cerci: male 6.5–7.9, female 6.5–7.8; overall length including tegmen: male 9.3–10.5, female 9.5–11.0; pronotum length × width: male 1.95 × 3.05, female 1.95 × 2.95; tegmen length: male 7.9–9.0, female 8.2–9.0.

**Male.** In some individuals, the coloration of the pronotal disk is blackish brown without stripes (Fig. 6D), and in other individuals, the pronotal disk has a circular

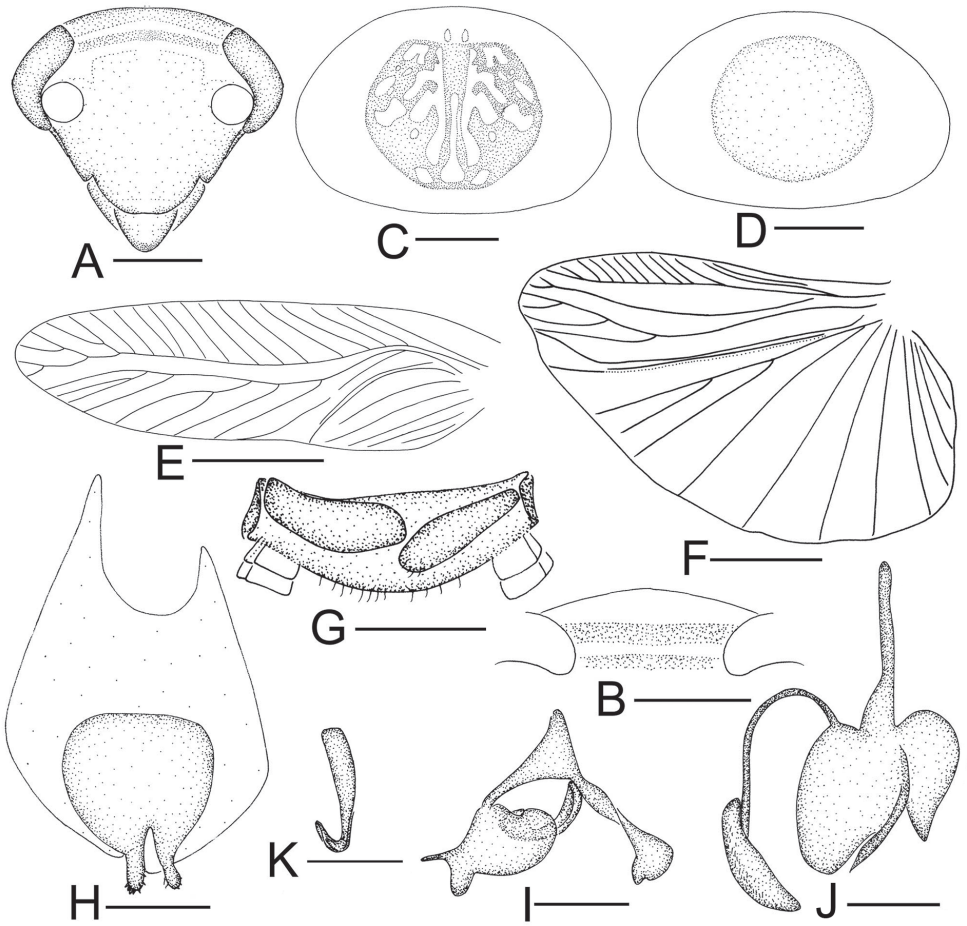


**Figure 6.** *Sorineuchora bivitta* (Bey-Bienko, 1969). **A–C, E–K** male from China, Yunnan, Hekou, Nanxi, Huayudong Forest Park **D** male from China, Guangxi, Longzhou **A** head, frontal view **B** vertex **C–D** pronotum **E** tegmen **F** hind wing (the dotted line indicates wing fold) **G** supra-anal plate, ventral view **H** subgenital plate, dorsal view **I** phallomere L1 **J** phallomere L2vm and R3 **K** phallomere R2. Scale bars: 0.5mm (**A–B, G–K**), 1.0 mm (**C, D**), 2.0 mm (**E, F**).

dark brownish spot and dense markings consisting of black spots and longitudinal and oblique stripes (Fig. 6C). Abdomen dark red-brown. Legs black-brown. Cerci apex yellowish brown. Interocular space as wide as or narrower than the distance between antennal sockets. Fifth segment of maxillary palpus longer than the fourth. Pronotum subelliptical, anterior and posterior margins nearly truncate. Tegmina and wings fully developed extending beyond end of abdomen, the former with oblique CuA. Hind-wing radial field narrow, R with oblique branches of which some apical ones bifurcated, M bent, without branches or with a small branch at the apex, CuA with three complete branches. Front femur Type $C_2$, pulvilli on four proximal tarsomeres, tarsal claws asymmetrical, arolia present. Abdominal terga unspecialized. L1 consisting of several irregular seta-free sclerites (Fig. 6I); L2vm with inflated apex and the left with filamentous

sclerite whose apex inflated (Fig. 6J); hooked phallomere (R2) on the right side with a preapical incision.

**Distribution.** China (Yunnan, Guizhou, Fujian, Guangxi, Hainan, Guangdong).

**Remarks.** The color of *S. bivitta* resembles that of *S. bimaculata* sp. n. (Fig. 12A–D), but the former is easily distinguished from the latter by the markings on the vertex (Figs 6B, 7C) and the shape of styli (Figs 6H, 7H).

### *Sorineuchora bimaculata* sp. n.

http://zoobank.org/65DEB5F4-2CCC-4042-B5D0-6DBDD6C192B7
Figs 7, 12C–D

**Type material. Holotype** male (IESWU), China, Guizhou, Luodian, June 1981, unknown leg. **Paratypes** (deposited in IESWU). 1 male, Guizhou, Maolan, Xiaoqikong, 30 May 1998, Qiong-Zhang Song leg.; 1 male, Yunnan, Xishuangbanna, Meng'a, 1050–1080m, 20 June 1958, Shu-Yong Wang leg.; 1 male, Chongqing, Wulong, Wanfeng, 800m, 7 July 1989, Long-Long Yang leg.; 1 male, Hubei, Luotian, Mt. Dabieshan, 01–02 July 2014, Yan Shi and Xin-Ran Li (= Conlin McCat) leg.

**Diagnosis.** Upper half of vertex brown, with two round yellowish brown spots in the middle (Fig. 7C); a pair of styli have three to six small spines at the apex and inner margins; L2vm the middle inflated, pre-apex curved and apex acute, R3 arched and filament, the apex inflated (Fig. 7J). Using these traits, the new species can be distinguished from its congeneric species.

**Description.** Measurements (mm). **Holotype**, body length without cerci: 7.6, overall length including tegmen: 8.8; pronotum length × width: 1.9 × 2.7; tegmen length: 7.4. **Paratypes**, body length without cerci: 7.0–7.8; overall length including tegmen: 9.0–11.0; pronotum length × width: 1.75 × 2.8; tegmen length: 8.0–9.0.

**Male.** Body small, dark brown. Upper half of vertex brown, with two round yellowish brown spots in the middle (Fig. 7C), lower half reddish brown, with a black transverse stripe. Frons brown to yellowish brown and without a stripe (Fig. 7A), or with a bent light brown stripe (Fig. 7B). Pronotum yellowish brown without stripes, or brown with a longitudinal light brown stripe, lateral margins hyaline. Tegmen reddish brown. Abdomen brown, lateral and hind margins light. Legs yellowish brown, the coxa brown.

Interocular space as wide as, or wider than, the distance between ocelli, and narrower than the distance between antennal sockets. Fifth segment of maxillary palpus longer than the fourth. Pronotum subelliptical, posterior margin truncate. Tegmina and wings fully developed extending beyond end of abdomen, the former with oblique CuA. Hind-wing R with oblique branches, M without branch, CuA with one branch, apical triangle evident. Front femur Type $C_2$, pulvilli on four proximal tarsomeres, tarsal claws asymmetrical, arolia present. Abdominal terga unspecialized.

Supra-anal plate short and symmetrical, paraprocts similar and sheet-like (Fig. 7G). Subgenital plate with subsymmetrical hind margin, a pair of styli which have three to six small spines at the apex and inner margins, situated almost in the middle of hind

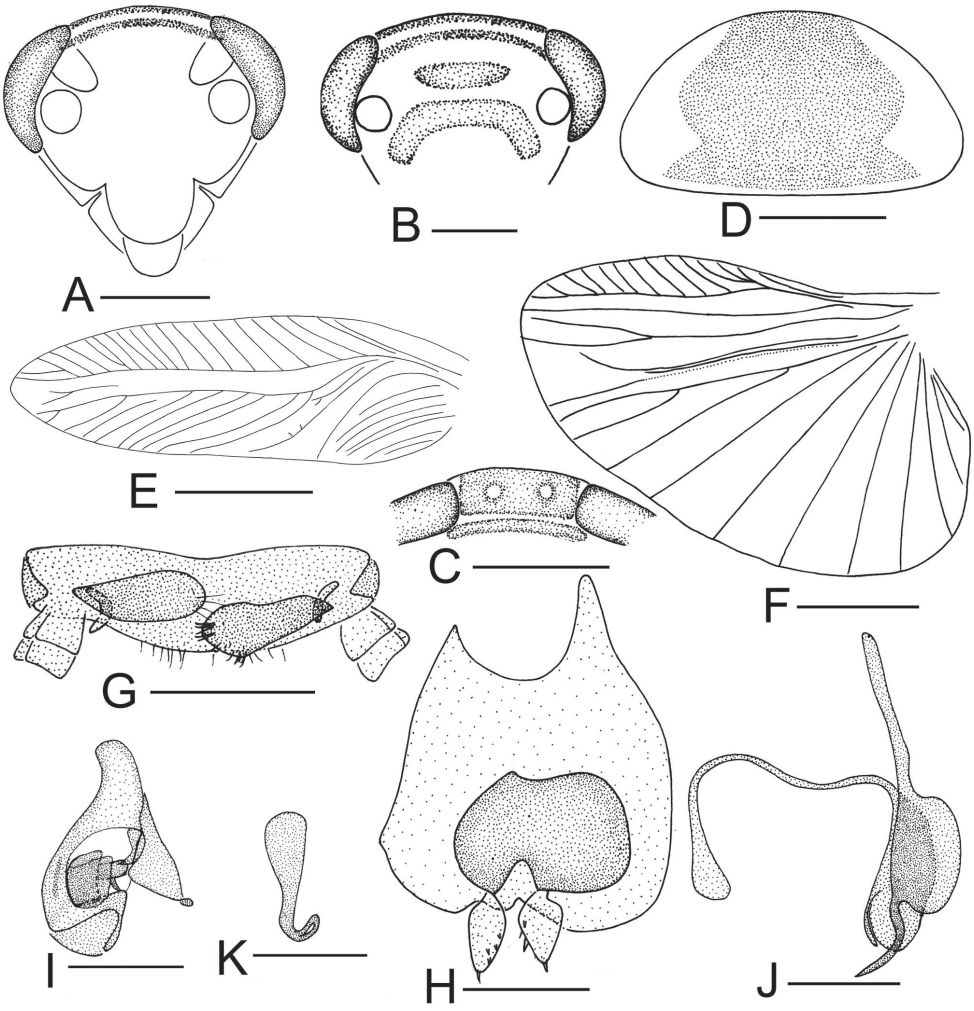


**Figure 7.** *Sorineuchora bimaculata* sp. n. **A** Paratype, male from China, Chongqing, Wulong, Wanfeng **B–K** Holotype **A–B** heads, frontal view **C** vertex **D** pronotum **E** tegmen **F** hind wing (the dotted line indicates wing fold) **G** supra-anal plate, ventral view **H** subgenital plate, dorsal view **I** phallomere L1 **J** phallomere L2vm and R3 **K** phallomere R2. Scale bars: 0.5 mm (**A–C, G–K**), 1.0 mm (**D**), 2.0 mm (**E, F**).

margin, the interstylar margin slightly concave (Fig. 7H). L1 consisting of several irregular seta-free sclerites (Fig. 7I); middle of L2vm inflated, pre-apex curved and apex acute; R3 arched and filament, the apex inflated (Fig. 7J); hooked phallomere (R2) on the right side, with a preapical incision.

**Female.** Unknown.

**Distribution.** China (Guizhou; Yunnan; Hubei; Chongqing).

**Etymology.** Latin word *bimaculata* refers to the two round yellowish brown spots on vertex.

**Remarks.** See remarks under the *S. bivitta*.

***Sorineuchora viridis* sp. n.**
http://zoobank.org/107BBC3B-8716-4737-8F45-CCAB558F4FD3
Figs 8, 10A, 12E–F

**Type material. Holotype** male (IESWU), China, Hainan, Mt. Bawangling. 13 April 2016, light trapping, Jian-Yue Qiu leg. **Paratypes** (all from Hainan, deposited in MHBU). 1 male, Mt. Bawangling, 11–12 May 2007, Yi-Bin Ba and Jun-Tong Lang leg.; 3 males, Baisha, Nankai, 450m, 25–26 June 2008, Yi-Bin Ba and Jun-Tong Lang leg.

**Diagnosis.** The color of the insects is green when they are alive (Fig. 10A), but it will become pale green or pale yellow when dried or kept in alcohol (Fig. 12E–F); vertex with three dark spots and a dark transverse stripe (Fig. 8A); Tegmina with white dots on the veins (Figs 10A, 12E); appendicular field almost disappearing (Fig. 8D); L1 with black setae on the right apex (Fig. 8F); L2vm rod-like, connected with R3 by sclerite (Fig. 8G). Using these traits, the new species can be distinguished from its congeneric species.

**Description.** Measurements (mm). **Holotype**, body length without cerci: 7.1; overall length including tegmen: 9.8; pronotum length × width: 2.0 × 3.1; tegmen length, 8.5. **Paratypes**, body length without cerci: 6.7–7.7; overall length including tegmen: 9.4–11.2; pronotum length × width: 2.35 × 3.05; tegmen length, 8.0–9.0.

**Male.** Body small, light green when alive (Fig. 10A), but it will turn pale yellow or pale green when dried or kept in alcohol (Fig. 12E–F). The morphological description here is with the specimen dried.

Vertex with three dark spots, on the frons between the ocelli with a narrow dark transverse tripe (Fig. 8A). Maxillary palpi yellowish white, antennae yellow. Pronotum hyaline. Tegmina and wings hyaline. The former's veins light with scattered white dots (Fig. 12E–F). Abdomen and legs yellowish white.

Interocular space as wide as or slightly narrower than the space between antennal sockets. Fifth segment of maxillary palpus longer than the fourth. Pronotum subelliptical, anterior and posterior margins nearly truncate. Tegmina and wings fully developed extending beyond end of abdomen, the former with oblique CuA. Hind-wing R with oblique branches, M without branches, CuA with three branches, appendicular field almost disappearing. Front femur Type $C_2$, pulvilli on four proximal tarsomeres, tarsal claws asymmetrical, arolia present. Abdominal terga unspecialized.

Supra-anal plate with hind margin rounded, paraprocts simple. Subgenital plate with subsymmetrical hind margin, a pair of styli with small setae, hind margin medially deflexed forming a short, longitudinal keel-like ridge, interstylar margin almost straight when flattened (Fig. 8E). L1 consisting of several irregular sclerites, the right apex with black setae (Fig. 8F); L2vm rod-like, connected with R3 whose apex has many setae by a sclerite (Fig. 8G); hooked phallomere (R2) on the right side, with a preapical incision.

**Female.** Unknown

**Distribution.** China (Hainan).

**Etymology.** Latin word *viridis*, meaning green, refers to the color of this species when alive.

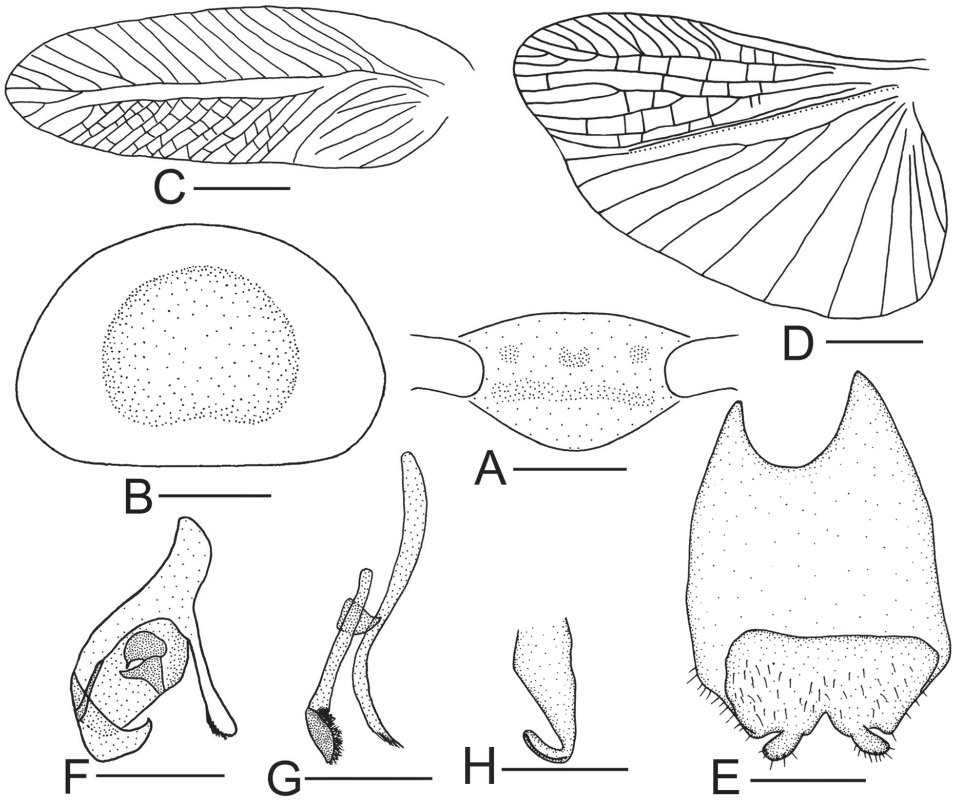


**Figure 8.** *Sorineuchora viridis* sp. n. holotype. **A** vertex **B** pronotum **C** tegmen **D** hind wing (the dotted line indicates wing fold) **E** subgenital plate, dorsal view **F** phallomere L1 **G** phallomere L2vm and R3 **H** phallomere R2. Scale bars: 0.5 mm (**A, E–H**), 1.0 mm (**B**), 2.0 mm (**C, D**).

**Remarks.** *Sorineuchora viridis* sp. n. is similar to *S. javanica* (Caudell, 1927) in color (when faded) and subobsolete apical triangle. But *S. viridis* sp. n. differs from *S. javanica* in details of vertex, dots on the tegmina, and median and left phallomeres.

***Sorineuchora hispida* sp. n.**
http://zoobank.org/A8A46CC5-E282-4835-BA18-F0773A672219
Figs 9, 12G–J

**Type material. Holotype** male (IESWU), China, Guangxi, Guiping, Longtan Park, 30 May–02 June 2014, light trapping, Shun-Hua Gui leg. **Paratypes.** 1 female, 3 males, same data as holotype.

**Diagnosis.** Pronotal disk brown, with a yellowish brown, longitudinal stripe (Figs 9B, 12G); paraprocts similar, sheet-like, with a branch (Fig. 9D); the left apex of R3 with many setae (Fig. 9G). Using these traits, the new species can be distinguished from its congeneric species.

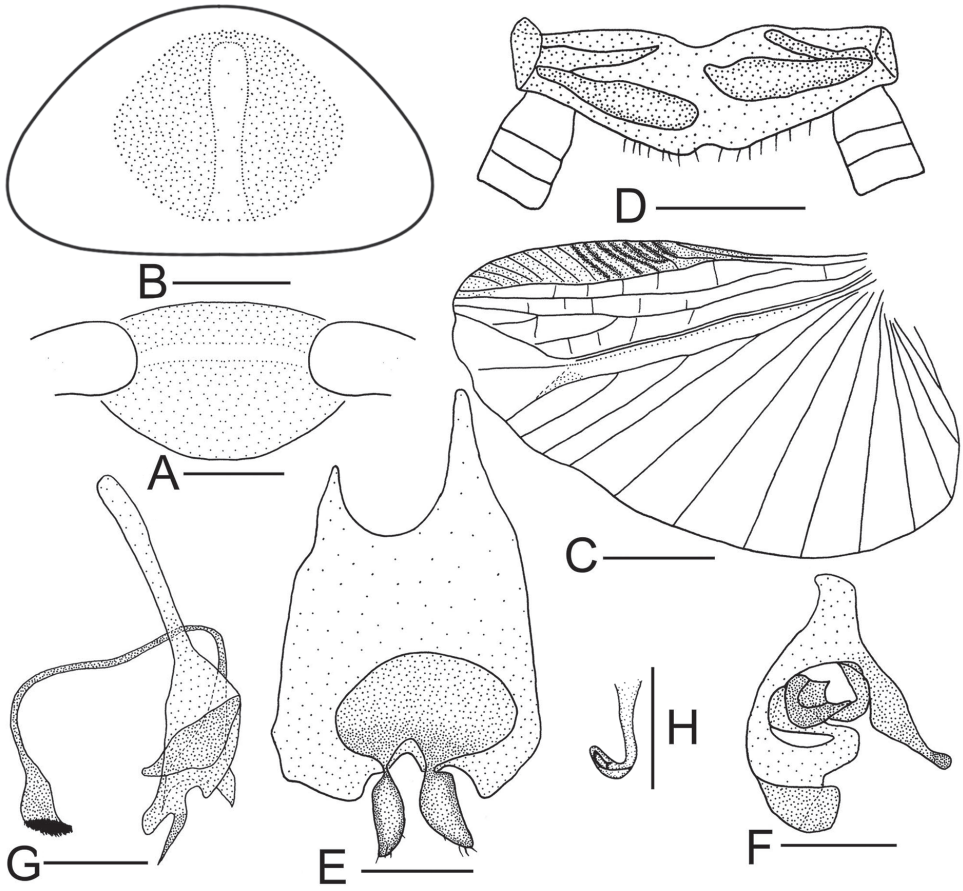


**Figure 9.** *Sorineuchora hispida* sp. n. holotype. **A** vertex **B** pronotum **C** hind wing (the dotted line indicates wing fold) **D** supra-anal plate, ventral view **E** subgenital plate, dorsal view **F** phallomere L1 **G** phallomere L2vm and R3 **H** phallomere R2. Scale bars: 0.5 mm (**A, D–H**), 1.0 mm (**B**), 2.0 mm (**C**).

**Description.** Measurements (mm). **Holotype,** male, body length without cerci: 7.0; overall length including tegmen: 8.6; pronotum length × width: 1.7 × 2.7; tegmen length: 7.5. **Paratypes,** body length without cerci: male 6.4–7.6, female 6.5; overall length including tegmen: male 8.8–9.2, female 9.1; pronotum length × width, male 2.05 × 2.75, female 1.7 × 2.8; tegmen length, male 7.5–7.6, female 7.8.

**Male.** Body small, brown. Lower half vertex yellowish brown, with one white transverse stripe (Fig. 9A). Antennae with first three basal antennomeres light yellow, the rest brown. Pronotal disk brown, with a yellowish brown, longitudinal stripe (Figs 9B, 12G), lateral margins hyaline. Hind-wing radial field brown. Legs brownish yellow. Abdomen black brown, the hind margins light.

Interocular space as wide as the distance between antennal sockets. Fifth segment of maxillary palpus longer than the fourth. Pronotum subelliptical, posterior margin truncate. Tegmina and wings fully developed, extending beyond end of abdomen. Hind-wing RA and RP parallel and inflated, M without branches, CuA

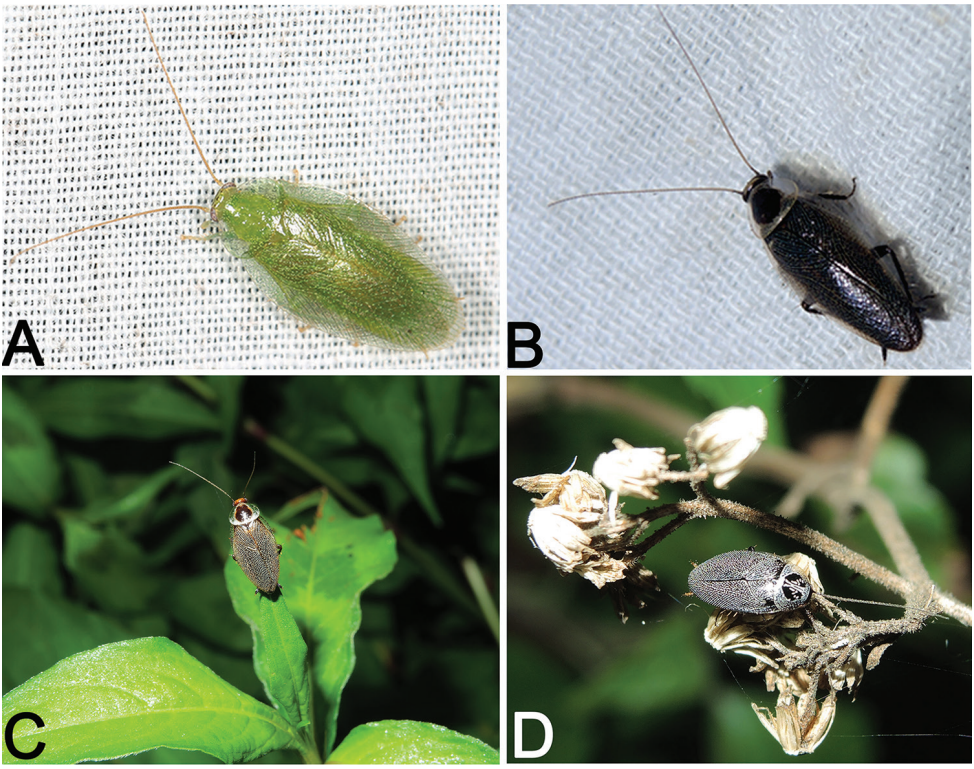


**Figure 10.** *Sorineuchora viridis* sp. n., *Sorineuchora nigra* and *Sorineuchora shanensis* in the wild. **A** *S. viridis* sp. n. holotype, photographed by Ling-Xiao Chang **B** *S. nigra* male from China, Guangxi, Longzhou, Nonggang. 29 June 2015, light trapping, photographed by Lu Qiu **C** *S. nigra* female from China, Chongqing, Jiangjin, Mt. Simianshan, 05 June 2014, photographed by Xin-Ran Li (= Conlin McCat) **D** *S. shanensis* male from China, Yunnan, Pu'er, Simao, Meizihu, 22 May 2015, photographed by Lu Qiu.

with two branches, apical triangle evident. Front femur Type $C_2$, pulvilli on four proximal tarsomeres, tarsal claws asymmetrical, arolia present. Abdominal terga unspecialized.

Supra-anal plate with hind margin rounded and weakly concave medially, lateral margins oblique, paraprocts similar, sheet-like, with a branch respectively (Fig. 9D). Subgenital plate with subsymmetrical hind margin, a pair of styli similar, both apexes with several asymmetrically distributed spines (Fig. 9E). L1 consisting of several irregular seta-free sclerites (Fig. 9F); L2vm with the middle inflated, apex thin and acute, the left apex of R3 with many seta (Fig. 9G); hooked phallomere (R2) on the right side, with a preapical incision.

**Female.** Similar to the male, but the pronotum with longitudinal and oblique markings, and subgenital plate with hind margin truncate.

**Distribution.** China (Guangxi).

**Etymology.** Latin word *hispida* means rough, shaggy, hairy, referring to the left apex of R3 with many setae.

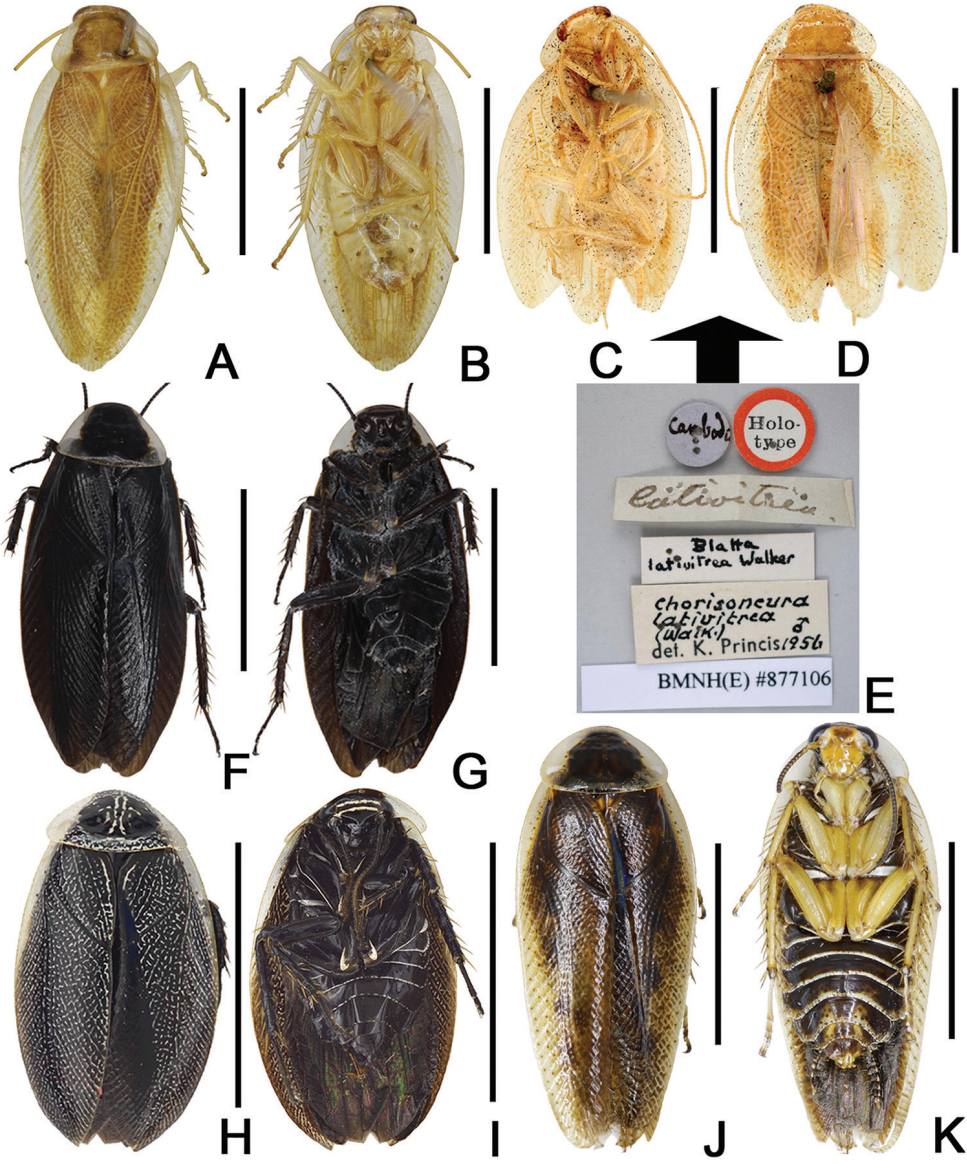


**Figure 11.** Habitus. **A, B** *S. formosana* (Matsumura, 1913) male from China, Hainan, Ledong, Mt. Jianfengling, 1050m, dorsal and ventral views **C, D, E** (labels) *S. lativitrea* (Walker, 1868) (to compare with *S. formosana*) holotype (copyright Natural History Museum, London), dorsal and ventral views **F, G** *S. nigra* (Shiraki, 1908) male from China, Hubei, Mt. Dabieshan, Taohuachong, dorsal and ventral views **H, I** *S. shanensis* (Princis, 1950) male from China, Yunnan, Lincang, Nansan, dorsal and ventral views **J, K** *S. undulata* (Bey-Bienko, 1958) male from China, Yunnan, Xishuangbanna, Wangtianshu, dorsal and ventral view. Scale bars: 5 mm.

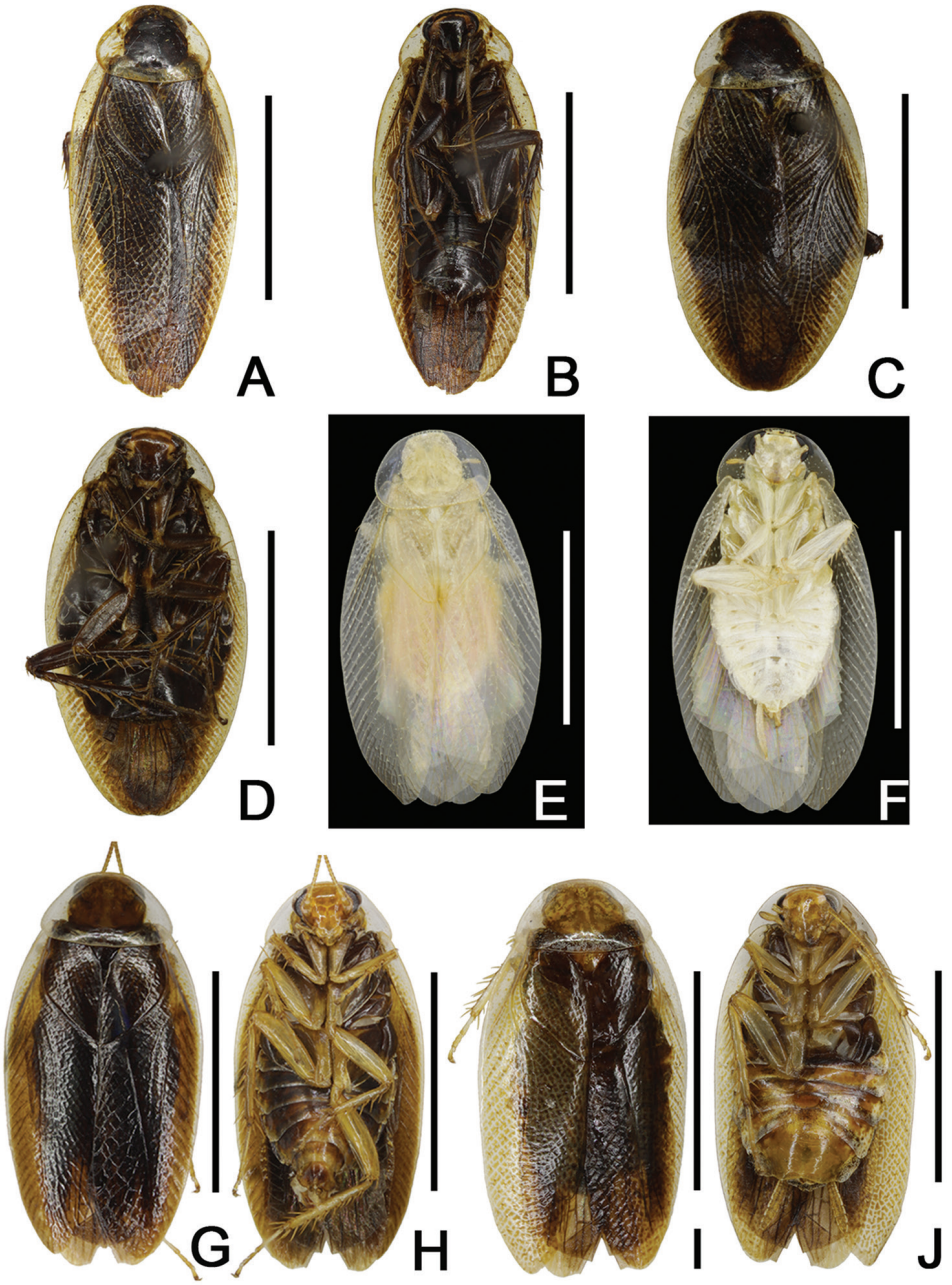


**Figure 12.** Habitus. **A, B** *S. bivitta* (Bey-Bienko, 1969) male from China, Guangxi, Hechi, Mt. Daqingshan, dorsal and ventral views **C, D** *S. bimaculata* sp. n. paratypes, male from China, Chongqing, Wulong, Wanfeng, dorsal and ventral views **E, F** *S. viridis* sp. n. holotype, dorsal and ventral views **G–J** *S. hispida* sp. n. **G–H** male paratypes, dorsal and ventral views **I, J** female paratypes, dorsal and ventral view. Scale bars: 5 mm.

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