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The *Dermacentor* (Acari, Ixodida, Ixodidae) of Mexico: hosts, geographical distribution and new records

Carmen Guzmán-Cornejo[1], Richard G. Robbins[2], Alberto A. Guglielmone[3],
Griselda Montiel-Parra[4], Gerardo Rivas[1], Tila María Pérez[4]

1 *Laboratorio de Acarología, Facultad de Ciencias, Universidad Nacional Autónoma de México, C. P. 04510, D.F. México* **2** *Armed Forces Pest Management Board, Office of the Assistant Secretary of Defense for Energy, Installations and Environment, Silver Spring, MD 20910-1202, U.S.A.* **3** *Instituto Nacional de Tecnología Agropecuaria, CC 22, C.P. 2300 Rafaela, Santa Fe, Argentina* **4** *Colección Nacional de Ácaros, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, C. P. 04510, D.F. México*

Corresponding author: *Carmen Guzmán-Cornejo* (carguzmancornejo@gmail.com)

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Abstract

Distribution and host data from published literature and previously unpublished collection records are provided for all nine species of the Holarctic tick genus *Dermacentor* that are known to occur in Mexico, as well as two species that may occur there. Parasite-host and host-parasite lists are presented, together with a gazetteer of collection localities and their geographical coordinates.

Keywords

Dermacentor, ticks, hosts, distribution, Mexico

Introduction

The genus *Dermacentor* Koch, 1844 is a largely Holarctic group of ticks that may be characterized as follows: eyes and festoons present, basis capituli sub-rectangular, palps short and thick, and scutum usually ornate. Most species are three-host parasites of mammals, although two Mexican species, *Dermacentor albipictus* (Packard) and *Dermacentor nitens* Neumann, are one-host ticks. Adults of three-host species usually feed on medium-sized to large mammals, whereas immatures feed on small mammals. This group includes species that are important vectors of microorganisms causing disease in humans and domestic and wild animals (Cooley 1938, Yunker et al. 1986, Durden and Beati 2014).

In the Western Hemisphere, the genus *Dermacentor* currently comprises 14 species, if *Dermacentor kamshadalus* Neumann and *Dermacentor panamensis* Apanaskevich and Bermúdez are included (Guglielmone et al. 2010, Apanaskevich 2013, Apanaskevich and Bermúdez 2013). Collection records for Mexican *Dermacentor* species date to the first half of the 20th century. Hoffmann (1962) and Hoffmann and López-Campos (2000) recognized nine species in this country: *Dermacentor albipictus*, *Dermacentor dissimilis* Cooley, *Dermacentor halli* McIntosh, *Dermacentor hunteri* Bishopp, *Dermacentor imitans* Warburton, *Dermacentor nitens* (formerly classified as both *Anocentor nitens* (Neumann) and *Otocentor nitens* (Neumann)), *Dermacentor occidentalis* Marx, *Dermacentor parumapertus* Neumann, and *Dermacentor variabilis* (Say). Chavarría (1941) stated that *Dermacentor andersoni* occurs in Mexico, but Hoffmann (1962) believed that *D. andersoni* is not an established Mexican species. Recently, nymphs of *D. andersoni* were recorded by Gordillo-Pérez et al. (2009) from vegetation in Tamaulipas, but this determination also requires confirmation. A second problematic Mexican species is *Dermacentor latus* Cooley, which was recorded by Cruz-Aldán et al. (2006) and is among the most poorly studied members of this genus (Apanaskevich and Bermúdez 2013). The presence or absence of both *D. andersoni* and *D. latus* in Mexico will have to be determined before our inventory of Mexican *Dermacentor* can be considered complete.

Material and methods

Bibliographic searches were conducted, using an array of public and proprietary databases (Biological Abstracts, BioOne, Biosis, CAB Abstracts, ISI Web of Knowledge), to locate published references to the species of *Dermacentor* that have been reported from Mexico. We then searched the Colección Nacional de Ácaros database (CNAC) (Biota version 1.6.1) to locate any unpublished collection records of Mexican *Dermacentor*. This work is divided into four sections. The first section is a parasite-host list organized alphabetically by tick species and Mexican state. Published tick collection records are presented in the following order: state (capitalized and in **boldface**), collection locality, host species, and reference(s). Where information is unavailable, we denote this as "ND" (Not Determined). For new records, we cite the number and sex or stage(s) (♀ = female, ♂ = male, N = nymph(s), L = larva(e)), locality, date, host name, and CNAC accession number. The second section is a host-parasite list, where hosts and their respective parasites are presented in alphabetical order. Mammalian names have been updated to accord with those of Wilson and Reeder (2005) and Ceballos (2014). The third section is a gazetteer of collection localities and their geographical coordinates. Where coordinates are not available for a specific locality, we reference the coordinates for the nearest municipality. The last section is a map, constructed using the program ArcGIS 9.3 (ESRI 2008), showing the distribution of *Dermacentor* species in Mexico (Fig. 3).

Results

This work summarizes collection data for 11 *Dermacentor* species known or thought to occur in 31 of Mexico's 32 federal entities. Mammals belonging to five orders are known to be parasitized by Mexican *Dermacentor*. Although records are provided here for *D. andersoni* and *D. latus*, it remains unclear whether these two species occur in the country.

Parasite-Host List

Dermacentor albipictus (Packard, 1869)

Figs 1A, 2A

Records. ND: east coast of Mexico, horses, asses, mules (Bishopp and Trembley 1945) (referenced as *D. nigrolineatus*); ND, ND (Vargas 1955); ND, cattle (Becklund 1968) (referenced as *D. nigrolineatus*). **AGUASCALIENTES:** Asientos, cattle (Hoffmann 1962); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **BAJA CALIFORNIA:** Unidad de Manejo y Conservación de Vida Silvestre (UMA) "El Tepi," Sierra San Pedro Mártir, *Odocoileus hemionus fuliginatus* (Contreras et al. 2007). **CAMPECHE:** ND, ND (Hoffmann 1962); ND, horses, deer, cattle (Hoffmann and López-Campos 2000).**CHIAPAS:** Loma Bonita, Selva Lacandona, *Odocoileus virginianus* (Romero-Castañón et al. 2008); Flor de Marqués, Selva Lacandona, *Odocoileus virginianus* (Romero-Castañón et al. 2008); Flor de Marqués, Selva Lacandona, *Mazama americana* (Romero-Castañón et al. 2008); ND, horse (Guglielmone et al. 1990).**CHIHUAHUA:** ND, ND (Woodham et al. 1983). **COAHUILA:** Ocampo, cattle, horses (Chavarría 1941); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **DISTRITO FEDERAL:** Mexico City, horse (Keirans 1985). **DURANGO:** ND, cattle (Hoffmann 1962); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **ESTADO DE MÉXICO:** Huehuetoca, cattle, horses (Chavarría 1941); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **GUERRERO:** Arroyo, Taxco, ND (Hoffmann 1962) (CNAC002100). ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **GUANAJUATO:** ND, ND (Woodham et al. 1983). **HIDALGO:** Hacienda del Astillero, Huichapan, cattle (Hoffmann 1962) (CNAC002102); Sayula, cattle (Hoffmann 1962); Calcali (probably Calnali), ND

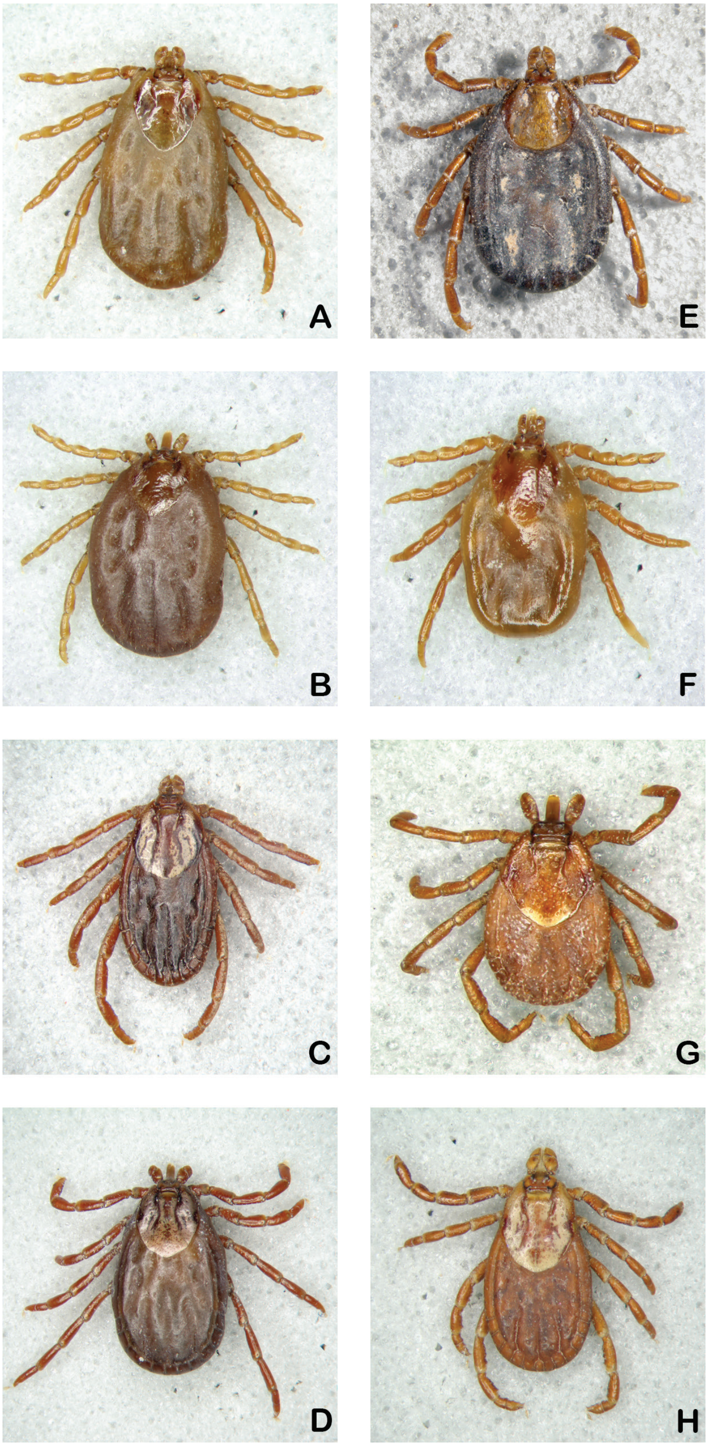

Figure 1. Females. **A** *D. albipictus* **B** *D. dissimilis*, **C** *D. halli* **D** *D. hunteri* **E** *D. imitans* **F** *D. nitens* **G** *D. parumapertus* **H** *D. variabilis*.

(Hoffmann 1962) (CNAC002101); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **JALISCO:** ND, ND (Woodham et al. 1983). **MICHOACÁN:** ND, cattle, horses (Chavarría 1941); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **NAYARIT:** ND, ND (Woodham et al. 1983). **NUEVO LEÓN:** Sierra de San Antonio Peña Nevada, *Liomys irroratus*, *Peromyscus boylii*, *Peromyscus maniculatus* (Tijerina-Medina et al. 2006). **QUERÉTARO:** ND, ND (Woodham et al. 1983). **PUEBLA:** ND, cattle, horses (Chavarría 1941); ND, horses, deer, cattle (Hoffmann and López-Campos 2000); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **QUINTANA ROO:** ND, ND (Woodham et al. 1983) (referenced as *D. nigrolineatus*). **SAN LUIS POTOSÍ:** ND, ND (Woodham et al. 1983). **SONORA:** ND, ND (Woodham et al. 1983). **TABASCO:** ND, ND (Hoffmann 1962); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **TAMAULIPAS:** ND, ND (Woodham et al. 1983). **VERACRUZ:** ND, cattle, horses (Chavarría 1941); Jilotepec, cattle (Hoffmann 1962); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **YUCATÁN:** ND, cattle, horses (Chavarría 1941); Temax, ND (Hoffmann 1962); ND, horses, deer, cattle (Hoffmann and López-Campos 2000). **ZACATECAS:** ND, ND (Woodham et al. 1983).

Notes. Romero-Castañón et al. (2008) claim that Mexico is a new locality record for *D. albipictus*, but this species had earlier been recorded from this country by Hoffmann (1962).

New records. COAHUILA: 6♀, 3♂, Baca de Huachi, 2-II-1975, cattle (CNAC002105). **VERACRUZ:** 1♀, Jilotepec (CNAC002106).

Notes. Baca de Huachi probably refers to Bacadéhuachi; however, this locality is located in Sonora State.

Dermacentor andersoni Stiles, 1908

Records. ND: ND, ND (Vargas, 1955). **CHIAPAS:** Selva Lacandona, *Bos taurus* (Romero-Castañón et al. 2008) (Doubtful record). **CHIHUAHUA:** Ciudad Juárez, sheep (Chavarría 1941). **TAMAULIPAS:** ND, vegetation (Gordillo-Pérez et al. 2009).

Dermacentor dissimilis Cooley, 1947

Figs 1B, 2B

Records. ND: ND, ND (Vargas 1955). **CHIAPAS:** Las Margaritas, about 45 km southeast of Comitán, horses (Cooley 1947); Unión Fronteriza (probably Unión Juárez), horses (Hoffmann 1962) (CNAC002127); ND, horses (Hoffmann and López-Campos 2000). **GUERRERO:** ND, ND (Woodham et al. 1983). **HIDALGO:** ND, ND (Woodham et al. 1983). **MICHOACÁN:** ND, ND (Woodham et al. 1983). **NUEVO LEÓN:** ND, ND (Woodham et al. 1983). **OAXACA:** Teotila, Cuicatlán (probably a

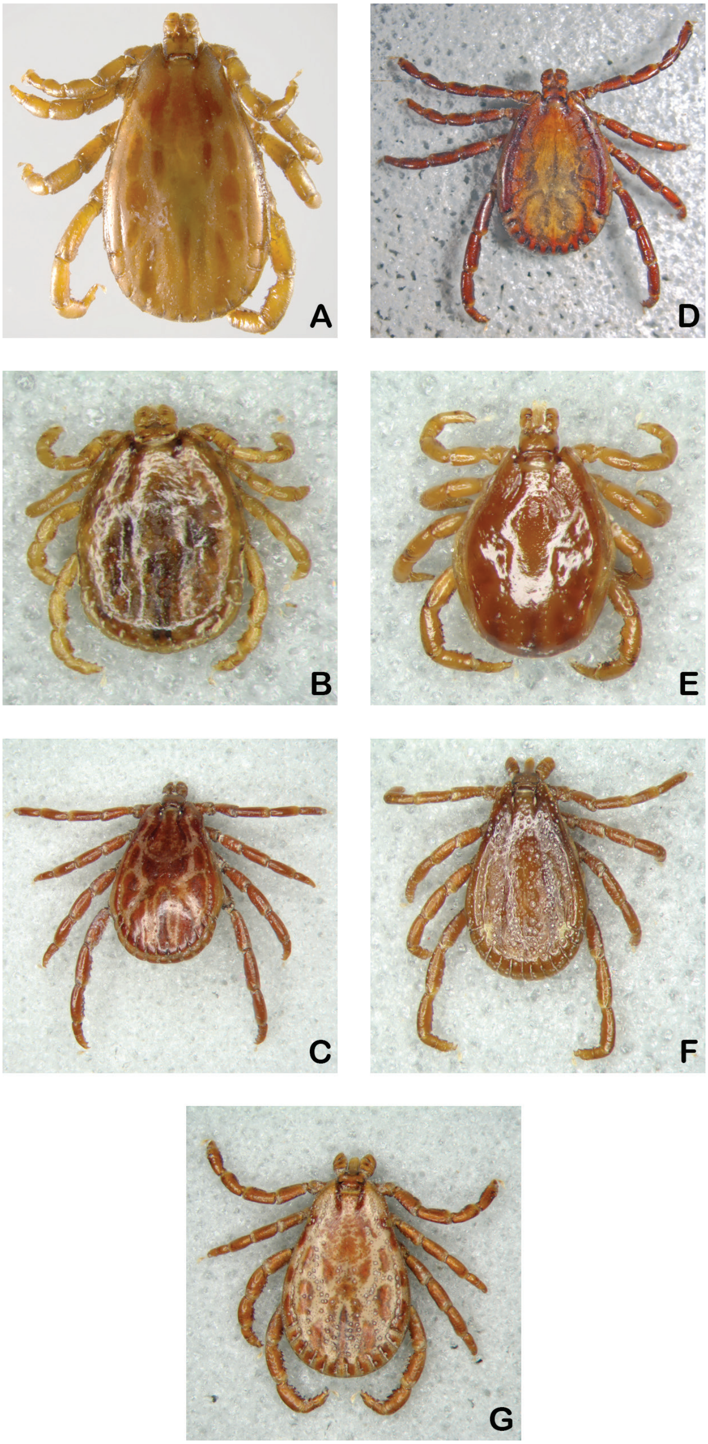

Figure 2. Males. **A** *D. albipictus* **B** *D. dissimilis* **C** *D. hunteri* **D** *D. imitans* **E** *D. nitens* **F** *D. parumapertus* **G** *D. variabilis*.

locality between Teotitlán and Cuicatlán), horses (Hoffmann 1962) (CNAC002128); ND, horses (Hoffmann and López-Campos 2000). **PUEBLA:** ND, ND (Woodham et al. 1983). **QUERÉTARO:** ND, ND (Woodham et al. 1983). **VERACRUZ:** Zongolica, horses (Kohls and Dalmat 1952); ND, horses (Hoffmann and López-Campos 2000).

New records. CHIAPAS: 9♀, 2N, Ciudad Las Casas (probably San Cristóbal de las Casas), VII-1940, horses (CNAC002147). **HIDALGO:** 2♀, Tlahuiltepa, 21-XII-1980 (CNAC002104); 6♀, 1N, San Bartolo Tutotepec, 5-IV-1969 (CNAC002272). **PUEBLA:** 1♀, Puebla, 2-IV-1995 (CNAC002126). **SINALOA:** 1♀, Ocolomé, IX-1944, *Canis familiaris* (CNAC002077). **VERACRUZ:** 4♀, Atescatitla (probably Atexcatitla, Zongolica), 31-I-1946 (CNAC002134).

Dermacentor halli McIntosh, 1931

Fig. 1C

Records. ND: ND, ND, (Vargas 1955); ND, ND (Meleney 1975); **CHIAPAS:** Ciudad Las Casas (probably San Cristóbal de las Casas), dogs (Hoffmann 1962), dogs (Hoffmann and López-Campos 2000); La Sepultura, Reserva de la Biósfera, *Tapirus bairdii* (Cruz-Aldán et al. 2006). **SAN LUIS POTOSÍ:** Taninul, human or vegetation (Fairchild et al. 1966); human, vegetation (Hoffmann and López-Campos 2000). **SINALOA:** Los Pozos, peccary (Fairchild et al. 1966); peccary (Hoffmann and López-Campos 2000).**VERACRUZ:** Atescatitla (probably Atexcatitla, Zongolica), mules (Hoffmann 1962); Zongolica, cattle (Hoffmann 1962); mules, cattle (Hoffmann and López-Campos 2000). **YUCATÁN:** Chichén Itzá (Cooley 1938); mules, cattle (Hoffmann and López-Campos 2000).

New records. JALISCO: 1♀, San Buenaventura, El Limón, 17-II- 1997, ND (CNAC005202)

Dermacentor hunteri Bishopp, 1912

Figs 1D, 2C

Records. ND: ND, ND (Vargas 1955). **BAJA CALIFORNIA:** La Rumorosa, human, vegetation, on ground (Williams 1976); ND, *Ovis canadensis* (Hoffmann 1962, Hoffmann and López-Campos 2000); Mexicali, *Ovis canadensis* (Crosbie et al. 1997); Cantil Canyon (probably Cañón Tajo-Cantil), *Ovis canadensis* (Crosbie et al. 1997). **SONORA:** Libertad (probably Puerto Libertad), ND (Cooley 1938); Libertad (probably Puerto Libertad), *Ovis canadensis* (Crosbie et al. 1997); Santa María, *Ovis canadensis* (Crosbie et al. 1997); ND, *Ovis canadensis* (Hoffmann and López-Campos 2000).

Notes. The record from Libertad cited by Crosbie et al. (1997) is probably the same record in Cooley (1938).

New records. BAJA CALIFORNIA: 1♀, 78♂, Sierra de Camulaje (probably Sierra de Calamajué), 4-III-1974, *Ovis canadensis* (CNAC002136); 3♀, 17♂, ND, "wild sheep" (probably *Ovis canadensis*) (CNAC002136).

Dermacentor imitans Warburton, 1933

Figs 1E, 2D

Records. CHIAPAS: Selvas de El Ocote, Ocozocoautla, *Pecari tajacu*, *Tayassu pecari*, *Mazama americana sartorii* (Hoffmann 1962, Fairchild et al. 1966).

Notes. *Mazama satorii* is considered a junior synonym of *Mazama temama* by Wilson and Reeder (2005), but Ramírez-Pulido et al. (2005) classify *M. satorii* as a subspecies of *Mazama americana.*

The records of Hoffmann (1962) and Fairchild et al. (1966) are identical – both reference the same RML collection numbers.

New records. CHIAPAS: 1♂, Ocosingo, Frontera Corozal, Área Natural Protegida Lacandona, 12-X-2004, vegetation (CNAC005194). **OAXACA:** 1♀, Istmo de Tehuantepec, ND, okapi (sic) (CNAC005018).

Dermacentor latus Cooley, 1937

Record. CHIAPAS: La Sepultura, Reserva de la Biósfera, *Tapirus bairdii* (Cruz-Aldán et al., 2006).

Dermacentor nitens Neumann, 1897

Figs 1F, 2E

Records. ND: ND, ND (Hooker et al. 1912); east coast of Mexico, horses, asses, mules (Bishopp and Trembley 1945); ND, horses (Becklund 1968). **CAMPECHE:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961); Rancho el Paraíso, cattle (Hoffmann 1962) (CNAC005176). **CHIAPAS:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961); Huixtla, cattle (Hoffmann 1962); El Vergel (there are two localities with this name, located in two different municipalities: Chiapa de Corzo and Pijijiapan), horses (Hoffmann 1962); Ciudad las Casas (probably San Cristóbal de las Casas), horses (Hoffmann 1962); Zoológico regional "Miguel Álvarez del Toro," *Tapirus bairdii* (Cruz-Aldán et al. 2006); Flor de Marqués, Selva Lacandona (Romero-Castañón et al. 2008). **CHIHUAHUA:** ND, ND (Woodham et al. 1983). **COLIMA:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961); Colima, cattle (Hoffmann 1962).

DISTRITO FEDERAL: near Mexico City, cattle, horse, sheep (Keirans 1985). **DURANGO:** ND, horses, sheep (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **ESTADO DE MÉXICO:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **GUERRERO:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **HIDALGO:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **MICHOACÁN:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **OAXACA:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961); Jesús Carranza (on the border of Veracruz and Oaxaca but situated in Veracruz State), ND (Keirans 1985). **PUEBLA:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **QUINTANA ROO:** southeast of Peto, horses (Bequaert 1933). **SAN LUIS POTOSÍ:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **TAMAULIPAS:** Ciudad Victoria, horse, donkey, mule (Hooker et al. 1912, Macías-Valadez 1923); Tampico, horse, donkey, mule (Hooker et al. 1912, Macías-Valadez 1923); Laguna Madre, horse (Drummond and Graham 1964). **VERACRUZ:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961). **YUCATÁN:** ND, horses, cattle (Chavarría 1941); ND, cattle, horses, donkeys, mules, dogs (Hoffmann 1961).

New records. CAMPECHE: 26♀, Candelaria, XI-1944, horse (CNAC005127); 17♀, Escárcega, XII-1944, horse (CNAC005093); 21♀, 1♂, 1N, Escárcega, XII-1944, horse (CNAC005126). **CHIAPAS:** 54♀, 44♂, Villa Flores (probably Villaflores), 6-XII-1983, horse (CNAC002087); 3♂, Rancho Agua Escondida, Villa Flores (probably Villaflores), 5-XII-1983, horse (CNAC002079). **NAYARIT:** 1♀, Acapareta (probably Acaponeta), 24-V-1981, horse (CNAC002295). **OAXACA:** 16♀, 14♂, 8N, Tlacamana (probably Tlacamama), 21-IV-1975, horse (CNAC002085); 7♀, 5♂, Cosolapa, VI-1950 (CNAC002081). **PUEBLA:** 6♀, 5♂, 2L, Huauchinango, VI-1927 (CNAC002083). **QUINTANA ROO:** 4♀, 11♂, Bacalar, VIII-1939, "tepezcuintle" (probably *Cuniculus paca*) (CNAC002088). **SINALOA:** 1♀, Ocolomé, IX-1944, *Canis familiaris* (CNAC002080). **TABASCO:** 26♀, 2 ♂, Macuspana, II- 1971, horses (CNAC002076); 3♂, Amaicote, 26-III-1971, horses (CNAC002266); 4♀, Amaicote, 26-III-1971, horses (CNAC002298). **TAMAULIPAS:** 1♀, 1♂, Rancho la Bolsa, Tampico (CNAC005158). **VERACRUZ:** 4♀, 3♂, Tuxtilla, IX-1939 (CNAC002086); 26♀, 16♂, 8N, Cosamaloapan, IX-1939, *Canis familiaris* (CNAC002082); 6♀, 2♂, Veracruz, VII-1927 (CNAC002078); 3♀, Miahuapa (probably San Pedro Miahuapan), 14-IV-1949, deer (CNAC005083); 3♀, 11♂, Miahuapa (probably San Pedro Miahuapan), 14-XI-1949, horse (CNAC005054).

Dermacentor occidentalis Marx, 1892

Records. BAJA CALIFORNIA: Tijuana, cattle (Secretaria de Agricultura y Fomento de México, 1926, 1930 in Hoffmann 1962); ND, ND (Woodham et al. 1983);

Unidad de Manejo y Conservación de Vida Silvestre (UMA) "El Tepi," Sierra San Pedro Mátir, *Odocoileus hemionus fuliginatus* (Contreras et al. 2007); ND, *Bos taurus* (Hoffmann and López-Campos 2000). **BAJA CALIFORNIA SUR:** ND, ND (Woodham et al. 1983). **COAHUILA:** ND, ND (Woodham et al. 1983). **SINALOA:** Choix (Hoffmann 1925); ND, *Bos taurus* (Hoffmann and López-Campos 2000).

Dermacentor parumapertus Neumann, 1901

Figs 1G, 2F

Records. ND: ND, ND (Vargas 1955); ND, ND (Becklund 1968). **BAJA CALIFORNIA:** Bahía de los Ángeles, *Lepus californicus* (Ryckman and Ryckman 1963); ND, rabbits, hares (Hoffmann 1961). **BAJA CALIFORNIA SUR:** La Paz, *Lepus californicus* (Hoffmann 1962); Todos Santos, rabbits (Hoffmann 1962) (CNAC002144); Puerto Chileno, hare (Hoffmann 1962) (CNAC002146). **CHIAPAS:** Ciudad Las Casas (probably San Cristóbal de las Casas), horses (Hoffmann 1962); ND, rabbits, hares (Hoffmann and López-Campos 2000). **CHIHUAHUA:** ND, cattle (Strickland and Gerrish 1965). **COAHUILA:** Región Lagunera, hares (Silva-Goytia and Elizondo 1952); ND, rabbits, hares (Hoffmann and López-Campos 2000). **DISTRITO FEDERAL:** ND, rabbits, hares (Hoffmann 1961, Hoffmann and López-Campos 2000); Camino al Desierto de los Leones, rabbits (Hoffmann 1962). **DURANGO:** Región Lagunera, hares (Silva-Goytia and Elizondo 1952); ND, rabbits, hares (Hoffmann and López-Campos 2000). **HIDALGO:** Ixmiquilpan, hares (Tovar 1944); ND, rabbits, hares (Hoffmann 1961; Hoffmann and López-Campos 2000); Actopan, rabbits (Hoffmann 1962) (CNAC002145); Taxquillo, rabbits (Hoffmann 1962) (CNAC002148). **SAN LUIS POTOSÍ:** San Luis Potosí, jack rabbits (Roberts 1934). **SONORA:** Cumuripa, hares (Hoffmann 1962) (CNAC002143); Guaymas, hares (Hoffmann 1962); ND, rabbits, hares (Hoffmann and López-Campos 2000).

New records. DURANGO: 2♀, 2♂, Ejido 18 de Marzo, Durango, 16-VIII-1976, hare (CNAC002149). **DISTRITO FEDERAL:** 1♀, 1♂, México D.F., rabbit (CNAC002141). **SONORA:** 1♀, 1♂, Guaymas, 15-VII-1924, hare (CNAC002140).

Note. In Hoffmann (1962), the record for Sonora: Guaymas is identical to our new record, except that the year is stated to be 1944, whereas the year on our collection label is 1924, and for that reason we consider our record to be different.

Dermacentor variabilis (Say, 1821)

Figs 1H, 2G

Records. ND: ND, ND (Neumann 1901); ND, ND (Hooker 1909); ND, ND (Hooker et al. 1912); ND, ND (Pinto 1930); ND, ND (Bishop and Trembley 1945); ND, ND (Vargas 1955). **BAJA CALIFORNIA:** ND, ND (Woodham et al. 1983); Unidad de Manejo y Conservación de Vida Silvestre (UMA) "El Tepi," Sierra San Pedro Mártir, *Odocoileus hemionus fuliginatus* (Contreras et al. 2007). **CHIAPAS:** ND, ND (Tovar 1945); cattle (Hoffmann 1961); Valle Central (Ortega-Gutiérrez 1979); ND, rabbits (Hoffmann and López-Campos 2000). **CHIHUAHUA:** ND, ND (Woodham et al. 1983). **COAHUILA:** ND, ND (Woodham et al. 1983). **DURANGO:** ND, ND (Woodham et al. 1983). **ESTADO DE MÉXICO:** ND, ND (Tovar 1945); cattle (Hoffmann 1961; Hoffmann and López-Campos 2000); ND, ear canal of goats (Hoffmann and López-Campos 2000). **GUANAJUATO:** ND, *Lepus callotis* (Neumann 1901). **HIDALGO:** ND, ND (Tovar 1945); cattle (Hoffmann 1961, Hoffmann 1962, Hoffmann and López-Campos 2000). **NUEVO LEÓN:** ND, ND (Woodham et al. 1983); ND, environment (Oliveira et al. 2010); Guadalupe, dog (Galaviz-Silva et al. 2013); Pesquería, dogs (Galaviz-Silva et al. 2013); Benito Juárez, dogs (Galaviz-Silva et al. 2013); Apodaca, dogs (Galaviz-Silva et al. 2013); Estanzuela, dogs (Galaviz-Silva et al. 2013); Guadalupe, dogs (Galaviz-Silva et al. 2013); Escobedo, dogs (Galaviz-Silva et al. 2013); San Nicolás de los Garza, dogs (Galaviz-Silva et al. 2013). **SAN LUIS POTOSÍ:** ND, ND (Tovar 1945). **OAXACA:** ND, ND (Tovar 1945); cattle (Hoffmann 1961, Hoffmann and López-Campos 2000). **PUEBLA:** ND, ND (Hoffmann 1962). **SAN LUIS POTOSÍ:** cattle (Hoffmann 1961); Venado, cattle (Hoffmann 1962); ND, deer (Hoffmann and López-Campos 2000). **SONORA:** cattle (Hoffmann 1961); El Maquipo, hares (Hoffmann 1962); ND, hares (Hoffmann and López-Campos 2000). **TAMAULIPAS:** Soto La Marina, Rancho La Pesca (Chavarría 1941); Soto La Marina, Hacienda Espíritu Santo (Chavarría 1941); ND, ND (Tovar 1945); cattle (Hoffmann 1961, Hoffmann and López-Campos 2000). **TLAXCALA:** ND, ND (Hoffmann 1962); ND, hares (Hoffmann and López-Campos 2000). **YUCATÁN:** Chichén Itzá, vegetation (Bequaert 1933); cattle (Hoffmann 1961, Hoffmann and López-Campos 2000). **ZACATECAS:** ND, ND (Woodham et al. 1983).

New records. COAHUILA: 1♀, 1♂, San Patricio, Villa Unión, 19-V-1975, bovine (CNAC002152). **NUEVO LEÓN:** 2♀, 3♂, Anahuac, 26-VI-1976, wildcat (CNAC002151). **TAMAULIPAS:** 2♀, 1♂, Los tres Garcia, Reynosa, 26-VIII-1976, *Canis familiaris* (CNAC002159); 1♀, 1♂ Matamoros, 19-IV-1999, *Lynx rufus* (CNAC002240).

Host-parasite List

Vegetation

Dermacentor halli
Dermacentor hunteri
Dermacentor imitans
Dermacentor variabilis

Artiodactyla

Deer

Dermacentor albipictus

Goats
Dermacentor variabilis
Sheep
Dermacentor nitens
Wild sheep
Dermacentor hunteri
Peccary
Dermacentor halli
***Bos taurus* Linnaeus (Aurochs, Cattle, Bovine)**
Dermacentor albipictus
Dermacentor halli
Dermacentor nitens
Dermacentor occidentalis
Dermacentor parumapertus
Dermacentor variabilis
***Mazama americana* (Erxleben) (South American Red Brocket)**
Dermacentor albipictus
Dermacentor nitens
***Mazama americana temama* (Kerr)**
Dermacentor imitans
***Odocoileus hemionus fuliginatus* Cowan (Southern Mule Deer)**
Dermacentor albipictus
Dermacentor nitens
Dermacentor variabilis
***Odocoileus virginianus* (Zimmermann) (White-tailed Deer)**
Dermacentor albipictus
***Ovis canadensis* Shaw (Bighorn Sheep)**
Dermacentor hunteri
***Pecari tajacu* (Linnaeus) (Collared Peccary)**
Dermacentor imitans
***Tayassu pecari* (Link) (White-lipped Peccary)**
Dermacentor imitans

Carnivora
***Canis familiaris* Linnaeus (domestic dog)**
Dermacentor dissimilis
Dermacentor halli
Dermacentor nitens
Dermacentor variabilis
***Lynx rufus* (Schreber) (Bobcat)**
Dermacentor variabilis

Lagomorpha
Hares
Dermacentor parumapertus
Dermacentor variabilis
Rabbits
Dermacentor parumapertus
Dermacentor variabilis
Lepus californicus **Gray (Black-tailed Jackrabbit)**
Dermacentor parumapertus
Lepus callotis **Wagler (White-sided Jackrabbit)**
Dermacentor variabilis

Perissodactyla
Mules
Dermacentor albipictus
Dermacentor halli
Dermacentor nitens
Equus asinus **Linnaeus (ass, donkey)**
Dermacentor albipictus
Dermacentor nitens
Equus caballus **Linnaeus (horse)**
Dermacentor albipictus
Dermacentor dissimilis
Dermacentor nitens
Dermacentor parumapertus
Tapirus bairdii **(Gill) (Baird's Tapir)**
Dermacentor halli
Dermacentor nitens

Primates
Homo sapiens **Linnaeus (human)**
Dermacentor halli
Dermacentor hunteri

Rodentia
Cuniculus paca **(Linnaeus) (Tepexcuintle, Lowland Paca)**
Dermacentor nitens
Liomys irroratus **(Gray) (Mexican Spiny Pocket Mouse)**
Dermacentor albipictus
Peromyscus boylii **(Baird) (Brush Deermouse)**
Dermacentor albipictus
Peromyscus maniculatus **(Wagner) (North American Deermouse)**
Dermacentor albipictus

List of localities

	Latitude N	**Longitude W**
East coast of Mexico	ND	ND
AGUASCALIENTES		
Asientos	22°14'18. 69"	102°5'21.92"
BAJA CALIFORNIA		
Cantil Canyon (probably Canón Tajo-Cantil)	32°15'50"	115°52'54"
Bahía de los Ángeles	28°57'5.07"	113°33'36.11"
La Rumorosa	32°31'37.93"	116°4'15.86"
Sierra de Camulaje (probably Sierra de Calamajué)	29°38'13"	114°6'39"
Tijuana	32°30'53.73"	117°2'18.37"
Unidad de Manejo y Conservación de Vida Silvestre (UMA) “El Tepi” Sierra San Pedro Mártir	31°04'36"	115°16 ’ 31”
Mexicali	32°37'26"	115°27'5"
BAJA CALIFORNIA SUR		
La Paz	24°8'33.28"	110°18'46.86"
Puerto Chileno	22°56'51"	109°48'27"
Todos Santos	23°27'23.07"	110°13'49.04"
CAMPECHE		
Candelaria	18°11'30.08"	91°2'28.68"
Campeche	19°49'49.98"	90°32'4.42"
Escárcega	18°36'32.14"	90°44'46.2"
Rancho el Paraíso	18°39'38.23"	91°46'19.84"
CHIAPAS		
Ciudad Las Casas (probably San Cristóbal de las Casas)	16°44'12"	92°38'18"
El Vergel (there are two localities with this name, located in two different municipalities: Chiapa de Corzo and Pijijiapan)		
El Vergel, Chiapa de Corzo	16°39'6"	93°00'47"
El Vergel, Pijijiapan	15°38'33"	92°58'21"
Flor de Marqués, Selva Lacandona	16°09'	90°52'
Huixtla	15°8'15.9"	92°27'57"
La Sepultura, Reserva de la Biosfera	16°00" y 16°29"	93°24" y 94°07"
Las Margaritas, abouth 45 km south Comitán	16°19'0"	91°58'57"
Loma Bonita, Selva Lacandona	16°05'	90°58'
Ocosingo Frontera Corozal, Área natural protegida Lacandona	16°49'16"	90°53'25"
Rancho Agua Escondida, Villa Flores (probably Villaflores)	16°14'4.01"	93°27'31.03"
Selva Lacandona	ND	ND
Selvas de El Ocote Ocozocoautla	16°31'56"	93°28'31"
Unión Fronteriza (probably Unión Juárez)	15°4'0"	92°5'0"
Valle Central	ND	ND
Villa Flores (probably Villaflores)	16°14'4.01"	93°27'31.03"
Zoológico regional “Miguel Álvarez del Toro”	16°43'30"	93°5'38.1"
CHIHUAHUA		
Ciudad Juárez	31°41'28.48"	106°25'28.2"
COAHUILA		
Baca de Huachi (probably Bacadéhuachi in Sonora State)	29°48'35"	109°8'28"
Ocampo	27.316261	102.405747

	Latitude N	**Longitude W**
Región Lagunera	ND	ND
San Patricio, Villa Unión	28°13'25"	100°43'47"
COLIMA		
Colima	19°14'42.7"	103°43'28"
DISTRITO FEDERAL		
Camino al Desierto de los Leones	19°19'1.6"	99°18'20.74"
Near Mexico City	ND	ND
Mexico City	19°21'11"	99°8'14"
DURANGO		
Ejido 18 de Marzo	25°43'54.4"	103°21'28.3"
Región Lagunera	ND	ND
ESTADO DE MÉXICO		
Huehuetoca	19°50'5.75"	99°12'11.09"
GUERRERO		
Arroyo, Taxco	18°32'33.03"	99°36'47.86"
GUANAJUATO		
	ND	ND
HIDALGO		
Actopan	20°16'27.58"	98°56'17.44"
Calcali (probably Calnali)	20°53'57.12"	98°35'19.1"
Hacienda del Astillero, Huichapan	20°22'16.78"	99°39'39.45"
Ixmiquilpan	20°29'03"	99°13'08"
San Bartolo Tutotepec	20°29'1.64"	98°11'41.82"
Sayula	20°12'3"	99°24'1"
Taxquillo	20°34'32.98"	99°20'31.34"
Tlahuiltepa	20°55'26.65"	98°57'2.26"
JALISCO		
San Buenaventura, El Limón	21°59'48.98"	103°34'12.97"
MICHOACÁN		
	ND	ND
NAYARIT		
Acapareta (Acaponeta)	22°27'52.2"	105 14 55.89"
NUEVO LEÓN		
Anáhuac	27°22'29.56"	100°4'47.74"
Apodaca	25°47'00"	100°11'00"
Benito Juárez	25°39'00"	100°05'00"
Escobedo	25°48'30"	100°19'36"
Estanzuela	25°32'60"	100°16'15"
Guadalupe	25°40'39"	100°15'35"
Nicolás de los Garza	25°46'00"	100°17'00"
Pesquería	25°47'00"	100°3'00"
San Antonio Peña Nevada	23°44'38.99"	101°0'36"
OAXACA		
Cosolapa	18°35'2.65"	96°39'11.3"
Teotila, Cuicatlán (probably a road between Teotitlán and Cuicatlán)	17°55'33"	97°0'21"
Istmo de Tehuantepec	ND	ND

	Latitude N	**Longitude W**
Oaxaca	17°5'00"	96°45'00"
Tlacamana (probably Tlacamama)	16°26'48.51"	98°6'42.73"
PUEBLA		
Huauchinango	20°10'30.14"	98°3'42.76"
Puebla	19°3'5"	98°13'4"
QUERÉTARO		
	ND	ND
QUINTANA ROO		
Bacalar	18°40'18.84"	88°23'53.62"
Southeast of Peto	19°59'11"	88°43'14"
SAN LUIS POTOSÍ		
San Luis Potosí	22°08'59"	100°58'30"
Taninul	21°56'09"	98°53'19"
Venado	22°56'00"	101°5'34"
SINALOA		
Choix	26°42'36"	108°19'34"
Los Pozos	23°00'40"	106°9'12"
Ocolomé	26°26'50.81"	108°36'30.67"
SONORA		
Baca de Huachi (probably Bacadéhuachi in Sonora State)	29°48'35"	109°8'28"
Cumuripa	28°9'11.41"	109°54'35.06"
El Maquipo	26°43'35"	108°43'10"
Guaymas	28°6'10.8"	111°1'47.81"
Libertad (probably Puerto Libertad)	29°54'15"	112°40'59"
Santa María	28°8'30"	110°41'35"
TABASCO		
Amaicote	17°29'5.2"	93°30'41.32"
Macuspana	17°53'13.27"	92°25'11.42"
TAMAULIPAS		
Ciudad Victoria	23°44'00"	99°8'00"
Hacienda Espíritu Santo, Soto La Marina	23°46'8"	98°12'19"
Laguna Madre	ND	ND
Los Tres García, Reynosa	25°49'36.15"	98°17'6.03"
Matamoros	25°37'7.93"	97°29'18.56"
Rancho la Bolsa, Tampico	22°15'57.34"	97°52'24.99"
Rancho La Pesca, Soto La Marina	23°47'16"	97°46'30"
Tampico	22°15'19"	97°52'7"
TLAXCALA		
	ND	ND
VERACRUZ		
Atescatitla (probably Atexcatitla, Zongolica)	18°33'25"	96°52'46"
Cosamaloapan	18°22'0.8"	95°47'40.77"
Jesús Carranza	17°26'06"	95°1'44"
Jilotepec	19°36'41"	96°56'58"
Miahuapa (probably San Pedro Miahuapan)	20°35'40.12"	97°40'18.58"
Tuxtilla	18°11'43.43"	95°51'54.75"

	Latitude N	Longitude W
Veracruz	19°11'57"	96°8'16"
Zongolica	18°40'17.54"	97°0'5.22"
YUCATÁN		
Chichen Itzá	20°40'59"	88°34'07"
Temax	21°2'55"	89°2'20"
ZACATECAS		
	ND	ND

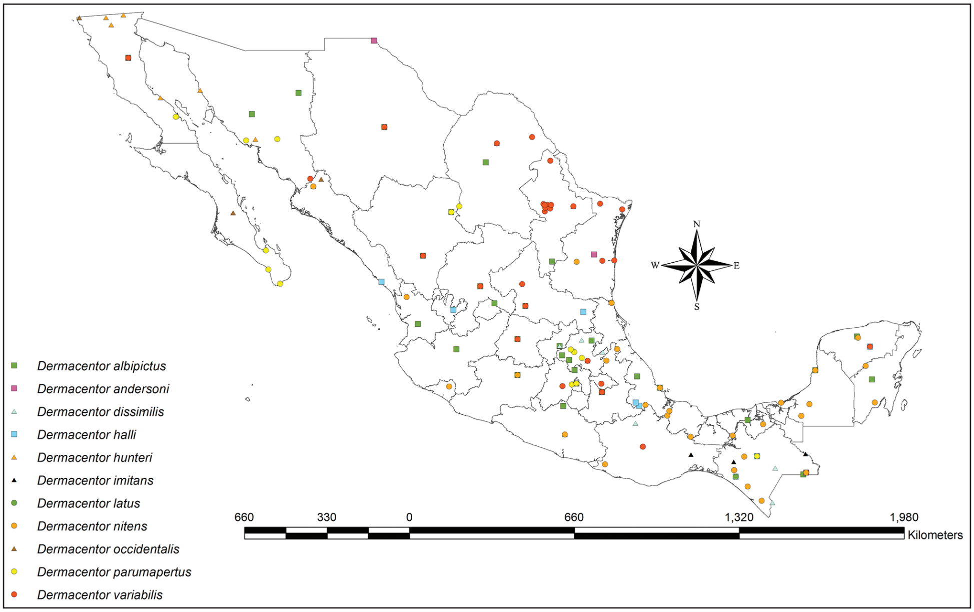

Figure 3. Distribution map of *Dermacentor* species in Mexico. Due to the lack of specific locality data for the states of Guanajuato, Michoacán, Querétaro, Tlaxcala and Zacatecas, all species symbols in those states are solely indicators of occurrence there.

Discussion

The first species records of the genus *Dermacentor* in Mexico were made by Hooker (1909) and Hooker et al. (1912), who referenced Mexican specimens of *D. variabilis* and *D. nitens*, two species of veterinary importance. The next species recorded from this country was *D. occidentalis*, cited by Hoffmann (1925) from Choix, Sinaloa. During the 1930s and 1940s, *D. albipictus*, *D. dissimilis*, *D. halli*, *D. hunteri*, and *D. parumapertus* were recorded from Mexico for the first time. Most recently, records have been published for *D. andersoni* (Vargas 1955), *D. imitans* (Hoffmann, 1962), and *D. latus* (Cruz-Aldán et al. 2006).

According to Apanaskevich and Bermúdez (2013), *D. panamensis*, which was described from specimens collected in Central America, has long been confused with *D. halli*. In Mexico, what we consider bona fide specimens of *D. halli* have been recorded in the southern part of the country, in the states of Chiapas, Yucatán and Veracruz, but there remains the possibility that *D. panamensis* may also be found in this region. Until fresh specimens of both species become available for molecular and morphological analysis, we accept the Mexican distribution of *D. halli* as described herein.

Based on literature records, 11 species of *Dermacentor* are known from Mexico, which represents 31.4% of the total number of species (35) generally recognized worldwide. However, there are two species – *D. andersoni* and *D. latus* – whose occurrence in the country needs to be confirmed. *Dermacentor andersoni* is a species of the northern Nearctic, missing from most of the North American Southwest, so the record from Chiapas seems doubtful. Moreover, the record from Tamaulipas is based on nymphs, which can be difficult to accurately determine to species, and the record from Chihuahua is suspect because Chavarría (1941) states that his tick specimens may have been collected on sheep transiting customs in Ciudad Juárez. On the other hand, *D. latus* is known only from *Tapirus bairdii* in Chiapas (Cruz-Aldán et al. 2006). This is a little-studied tick that is also thought to be endangered (Mihalca et al. 2011), and for that reason its occurrence in Mexico requires confirmation. Among the other nine *Dermacentor* species, the most widespread is *D. albipictus* (26 Mexican states), followed by *D. nitens* (20), and *D. variabilis* (18). In contrast, *D. hunteri* and *D. imitans* are both known from only two Mexican states. *Dermacentor albipictus*, *D. dissimilis*, *D. halli*, *D. nitens* and *D. variabilis* all occur in both the Neotropical and Nearctic Zoogeographic Regions. *Dermacentor hunteri* and *D. occidentalis* are chiefly regarded as Nearctic species, while *D. imitans* is considered a Neotropical species. Guglielmone et al. (2014) classify *D. parumapertus* as a Nearctic tick, but we have found records from the southern Mexican state of Chiapas; these may represent a misidentification, so we cannot conclude that this species' range extends into the Neotropics.

We have located records of *Dermacentor* species from all federal entities in Mexico except Morelos (Figure 3). The Mexican states with the largest number of localities in which *Dermacentor* ticks have been collected are Chiapas (17 localities), followed by Nuevo León (9 localities), and Hidalgo, Tamaulipas and Veracruz (8 localities each). In some cases there is a record for a state, but the collection locality is unknown (Guanajuato, Michoacán, Querétaro, Tlaxcala and Zacatecas).

Artiodactyl and perissodactyl mammals are common hosts of *Dermacentor* species. *Dermacentor albipictus* is usually associated with these large mammal hosts, although we found that this species can also be associated with rodents (*L. irroratus*, *P. boylii* and *P. maniculatus*). Unfortunately, however, no information is available concerning the stages of *D. albipictus* found on rodent hosts. *Dermacentor variabilis* is more of a generalist species, found on hosts as diverse as Carnivora and Lagomorpha. Somewhat surprisingly, in Mexico only *D. halli* and *D. hunteri* have been reported to parasitize humans. Mexican records of *D. imitans* are scarce and confined to Chiapas and Oaxaca, where this tick is associated with Artiodactyla.

Except for *D. andersoni*, *D. occidentalis* and *D. latus*, all Mexican *Dermacentor* species are represented in the CNAC. Even so, our understanding of the distribution and host relationships of this genus in Mexico is far from complete, and for that reason additional collections are urgently needed, so that we may better comprehend the biology, systematics, ecology, and zoogeography of this biomedically important genus.

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RESEARCH ARTICLE

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Three new species of the genus *Leptonetela* from Greece (Araneae, Leptonetidae)

Yi Wu[1], Chunxia Wang[2], Guo Zheng[1], Shuqiang Li[2]

1 *College of Life Science, Shenyang Normal University, Shenyang 110034, China* **2** *Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China*

Corresponding authors: *Guo Zheng* (zhengguo@synu.edu.cn); *Shuqiang Li* (lisq@ioz.ac.cn)

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Abstract

Three new species of the spider genus *Leptonetela* collected from caves in Greece are described: *L. arvanitidisi* **sp. n.** (male & female), *L. paragamiani* **sp. n.** (male & female) and *L. penevi* **sp. n.** (male & female). Detailed illustrations of the new species are provided. DNA barcodes were obtained for future use.

Keywords

Haplogynae, taxonomy, DNA-barcoding, Balkan Peninsula, spider

Introduction

The leptonetids are minute (1.0–3.0 mm) spiders that can be easily distinguished from other families by a distinctive 6-eyed pattern, with the posterior median eyes situated behind the posterior lateral eyes; however, in some cave species, the eyes are reduced to vestiges or may be completely absent (Gertsch 1974).

A total of 23 genera and 276 species of the spider family Leptonetidae are known worldwide (World Spider Catalog 2016). The genus *Leptonetela* was established by Kratochvíl (1978), using *Leptonetela kanellisi* (Deeleman-Reinhold, 1971) from a cave in Greece as the type species. *Leptonetela* can be distinguished from other genera of the family by the palpal femur without spines, the retrolateral surface of the palpal tibia with a longitudinal row of strong spines and the male palpal tarsus without appendices.

A total of 50 *Leptonetela* species are known from Europe and Asia. Two species of *Protoleptoneta* were transferred to *Leptonetela* by Brignoli in 1979: *L. strinatii* (Brignoli, 1976) from Greece and *L. deltshevi* (Brignoli, 1979) from Turkey. Deltshev described *L. andreevi* from Greece in 1985. Dunin (1990) reported *L. caucasica* from Georgia and Azerbaijan. *L. thracia* was described by Gasparo in 2005 from Greece. Subsequently, Lin and Li (2010) described 24 species occurring in the Yunnan-Guizhou Plateau, China, including *L. quinquespinata* (Chen & Zhu, 2008) which was transferred from *Qianleptoneta* Chen & Zhu, 2008. Wang and Li (2011) reported 17 *Leptonetela* species from South China, 2 species from Greece and 1 species from Vietnam.

Other than *L. deltshevi* (Brignoli, 1979) from Turkey and *L. pungitia* Wang & Li, 2011 from Vietnam which have been collected in epigean habitats, all species are found in caves. Some of them have characters typical to true troglobites, such as lacking eyes and pigmentation and elongated legs.

In this paper, three *Leptonetela* species collected from caves in Greece are described as new to science. The total number of *Leptonetela* species from Europe reaches 9 species.

Material and methods

Specimens were examined with a LEICA M205C stereomicroscope. Images were captured with an Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 dissecting microscope. Epigynes and male palps were examined after dissection from the spiders' bodies.

Terminology and abbreviations in this paper generally follow Wang and Li (2011) and Ledford (2011). The unit of measurement in this paper is millimetres (mm). Leg metric data were recorded as total length (femur, patella, tibia, metatarsus, tarsus). Leg segments were measured on their dorsal side.

DNA barcodes were obtained for future use. A partial fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified and sequenced for *Leptonetela arvanitidisi* sp. n., *L. paragamiani* sp. n. and *L. penevi* sp. n. following the protocol in Miller et al. (2010). Primers used in this study are: LCO1490 (5'-CWACAAAYCATARRGATATTGG-3') and HCO-N-2198 (5'- TAAACTTCAGGGTGACCAAAAAATCA -3') (Folmer et al. 1994). Voucher information and GenBank accession number for all samples are listed in Table 1.

The specimens studied in the current paper are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing, China.

Table 1. Voucher specimen information.

Species	Sequence length	Collecting localities	GenBank accession number
L. arvanitidisi sp. n.	620 bp	Greece Athens Attica: Leondari Cave	KU318407
L. paragamiani sp. n.	620 bp	Greece Athens: Pan Cave	KU318410
L. penevi sp. n.	620 bp	Greece Thiva: Skoteini Cave	KU318411

Taxonomy

Family Leptonetidae Simon, 1890

Genus *Leptonetela* Kratochvíl, 1978

Leptonetela: Kratochvíl 1978: 11, f. 1G. Type species *Sulcia kanellisi* Deeleman-Reinhold, 1971 from Greece.

Leptonetela arvanitidisi Wang & Li, sp. n.

http://zoobank.org/24A56F21-B537-4A53-B19B-53A86AA86C96
Figs 1–2, 7

Types. Holotype ♂ (IZCAS): GREECE, Athens, Attica, Leondari Cave, 37°59'14.61"N, 23°49'47.03"E, elevation 553 m, 28 March 2013, S. Li leg. Paratypes 2♀ (IZCAS), same data as holotype.

Etymology. The specific name is dedicated to Dr. Christos Arvanitidis of the Hellenic Centre for Marine Research in Crete, a leading taxonomist on Polychaeta; noun (name) in genitive case.

Diagnosis. *Leptonetela arvanitidisi* sp. n. is similar to *L. kanellisi* but can be separated by the basal tibial spine with bifurcated tip (Fig. 1D) (not bifurcated in *L. kanellisi*), the wave-shaped anterior margin of the atrium and the tightly twisted spermathecae (Fig. 2C); *L. kanellisi* has an arc-shaped anterior margin of the atrium and the spermathecae are loosely twisted (see Wang and Li 2011: figs 16–19).

Description. Male (holotype). Total length 1.75 (Fig. 1A). Carapace 0.83 long, 0.72 wide. Opisthosoma 1.00 long, 0.65 wide. Prosoma yellowish, with one seta in the middle of the carapace. Ocular area with a pair of setae, eyes absent. Median groove, cervical grooves and radial furrows indistinct. Clypeus 0.10 high. Sternum and legs yellowish. Opisthosoma pale brown, ovoid, lacking distinctive pattern. Leg measurements: I 8.39 (2.25, 0.38, 2.45, 2.08, 1.23); II 7.14 (1.88, 0.38, 2.03, 1.72, 1.13); III 5.93 (1.73, 0.35, 1.62, 1.55, 0.68); IV 7.83 (2.13, 0.38, 2.15, 2.00, 1.17). Male palp (Fig. 1C–D): tibia with 5 spines retrolaterally, the basal one strong, conspicuous, with bifurcated tip. Bulb with triangular embolus; prolateral lobe oval. Median apophysis (Fig. 1B) distal edge round, with six small teeth. Conductor membranous, triangular in ventral view.

Female (one of the paratypes). Similar to male in color and general features but larger and with shorter legs. Total length 2.03 (Fig. 2A–B). Carapace 0.85 long, 0.73 wide. Opisthosoma 1.23 long, 0.90 wide. Clypeus 0.10 high. Leg measurements: I 7.24 (1.90, 0.38, 2.08, 1.75, 1.13); II 5.92 (1.68, 0.33, 1.63, 1.38, 0.90); III 5.32 (1.50, 0.32, 1.42, 1.30, 0.78); IV 6.63 (1.75, 0.35, 1.80, 1.70, 1.03). Vulva (Fig. 2C): spermathecae coiled, atrium fusiform, anterior margin of the atrium wave shaped.

Distribution. Known only from the type locality.

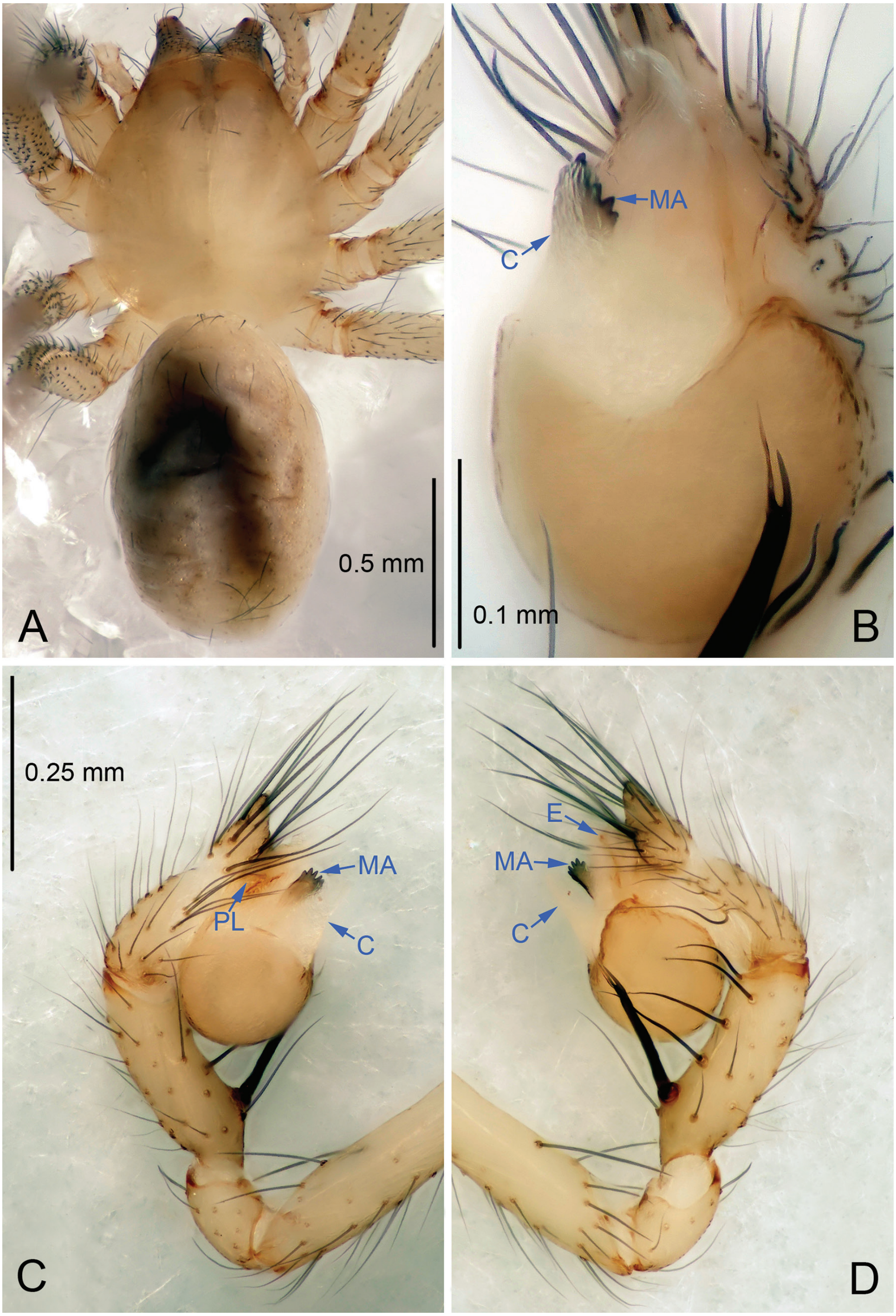

Figure 1. *Leptonetela arvanitidisi* sp. n., holotype male. **A** Habitus, dorsal view **B** Palpal bulb, ventral view **C** Palp, prolateral view **D** Palp, retrolateral view.

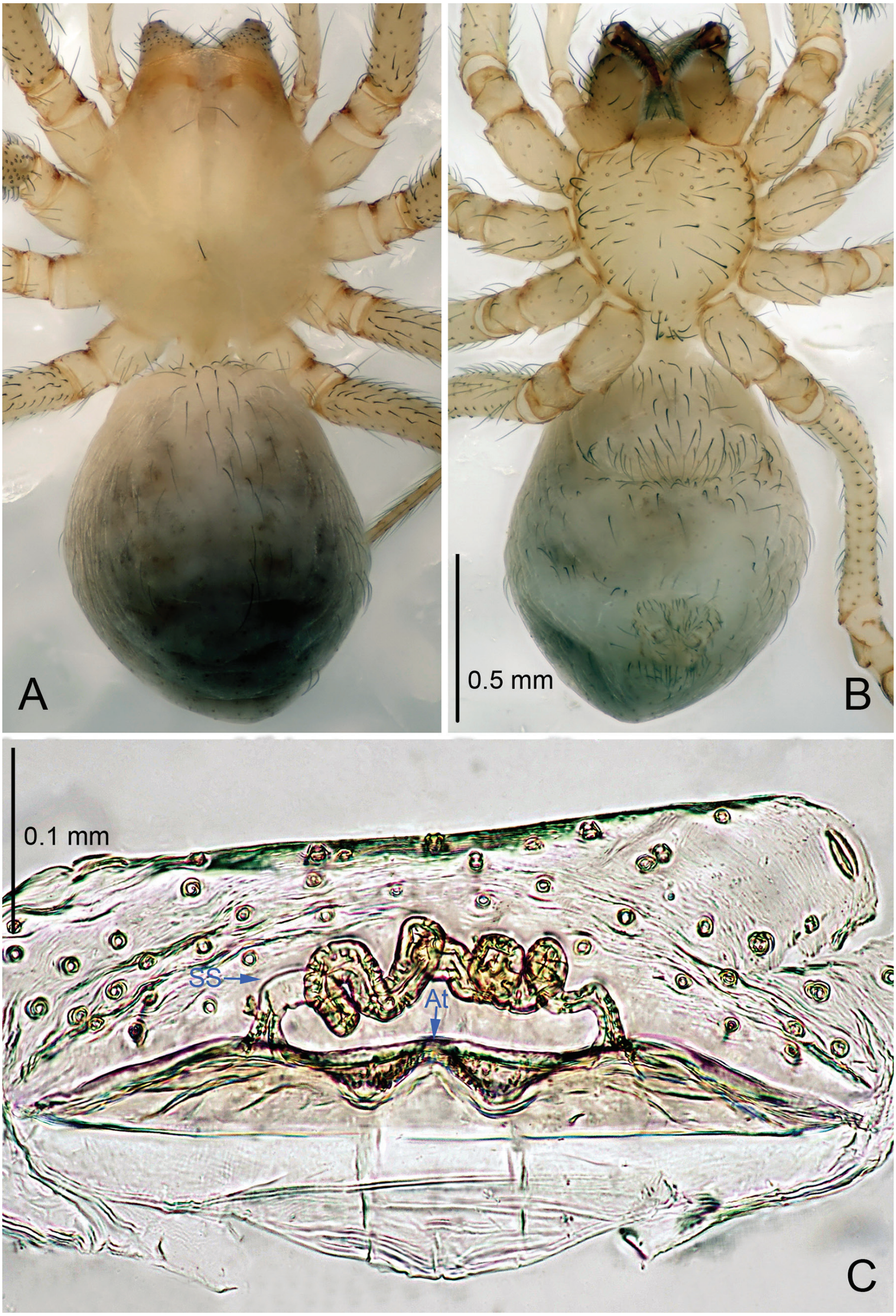

Figure 2. *Leptonetela arvanitidisi* sp. n., one of the paratype female. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Vulva, dorsal view.

Leptonetela paragamiani Wang & Li, sp. n.

http://zoobank.org/28C5914F-58AB-409B-8110-61F5B1D5004D
Figs 3–4, 7

Types. Holotype ♂ (IZCAS): GREECE, near Athens, Pan Cave, 38°08'48.54"N, 23°40'06.04"E, elevation 660 m, 7 April, 2013, S. Li leg. Paratypes 2 ♀ (IZCAS), same data as holotype.

Etymology. The specific name is dedicated to Mr. Kaloust Paragamian of the Hellenic Institute of Speleological Research in Crete, a leading speleologist in Greece; noun (name) in genitive case.

Diagnosis. *Leptonetela paragamiani* is similar to *L. kanellisi* and *L. arvanitidisi* sp. n. but can be separated by the second tibial spine, which is longest in *L. paragamiani* sp. n., whereas in *L. kanellisi* and *L. arvanitidisi* sp. n. (Fig. 3D) the basal spine is longest; the median apophysis has 3 small teeth (Fig. 3B) in *L. paragamiani* sp. n., whereas it has 6 teeth in *L. kanellisi* and *L. arvanitidisi* sp. n.; and the spermathecae are tightly twisted (Fig. 4C) compared to the spermathecae of *L. kanellisi* and *L. arvanitidisi* sp. n.

Description. Male (holotype). Total length 1.63 (Fig. 3A). Carapace 0.75 long, 0.62 wide. Opisthosoma 0.88 long, 0.62 wide. Prosoma yellowish, with one seta on the median part. Ocular area with a pair of setae, two eyes, reduced to white spots. Median groove, cervical groove and radial furrows indistinct. Clypeus 0.10 high. Sternum and legs yellowish. Opisthosoma pale brown, ovoid, lacking distinctive pattern. Leg measurements: I 5.53 (1.50, 0.28, 1.60, 1.27, 0.88); II 4.78 (1.38, 0.25, 1.27, 1.13, 0.75); III 4.01 (1.13, 0.25, 1.03, 1.00, 0.60); IV 5.25 (1.45, 0.28, 1.47, 1.25, 0.80). Male palp (Fig. 3C–D): tibia with 5 retrolateral spines, the basal one strong, conspicuous, and the second one longer than others. Bulb with spoon-shaped embolus, prolateral lobe oval. Distal edge of median apophysis round (Fig. 3B), with three small teeth, conductor membranous, shield shaped in ventral view.

Female (one of the paratypes). Similar to male in color and general features but larger and with longer legs. Total length 1.88 (Fig. 4A–B). Carapace 0.75 long, 0.68 wide. Opisthosoma 1.00 long, 0.88 wide. Clypeus 0.10 high. Leg measurements: I 6.26 (1.75, 0.28, 1.80, 1.53, 0.90); II 5.36 (1.58, 0.28, 1.50, 1.25, 0.75); III 4.69 (1.38, 0.25, 1.25, 1.13, 0.68); IV 6.19 (1.78, 0.28, 1.75, 1.50, 0.88). Vulva (Fig. 4C): spermathecae twisted, atrium oval.

Distribution. Known only from the type locality.

Leptonetela penevi Wang & Li, sp. n.

http://zoobank.org/C819DE91-5E16-4B18-B336-26AE51FFD15F
Figs 5–6, 7

Types. Holotype ♂ (IZCAS): GREECE, Thiva, Kakalitsa, Skoteini Cave, 38°29'59.81"N, 23°59'01.06"E, elevation 443 m, 29 March, 2013, S. Li leg. Paratypes 2 ♀, same data as holotype.

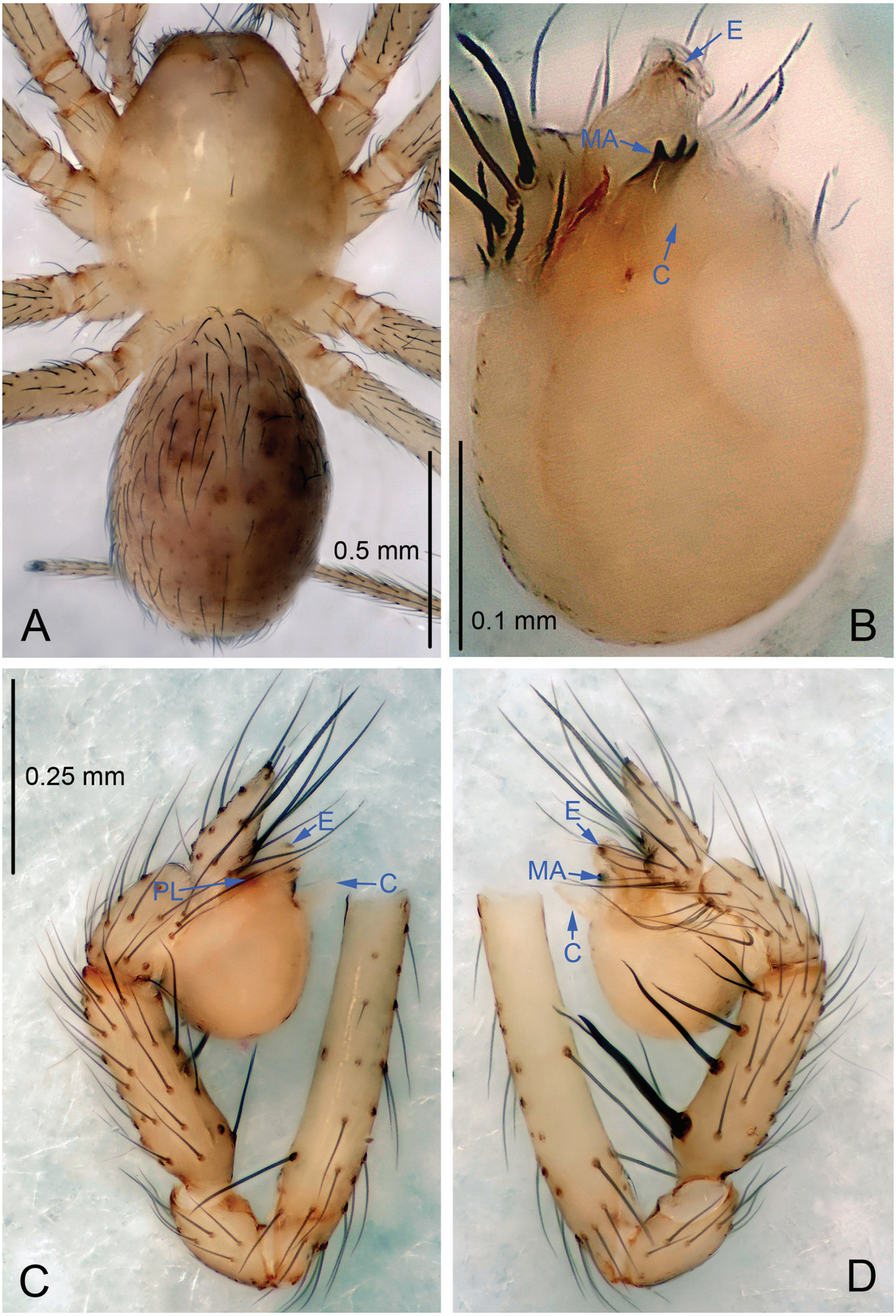

Figure 3. *Leptonetela paragamiani* sp. n., holotype male. **A** Habitus, dorsal view **B** Palpal bulb, ventral view **C** Palp, prolateral view **D** Palp, retrolateral view.

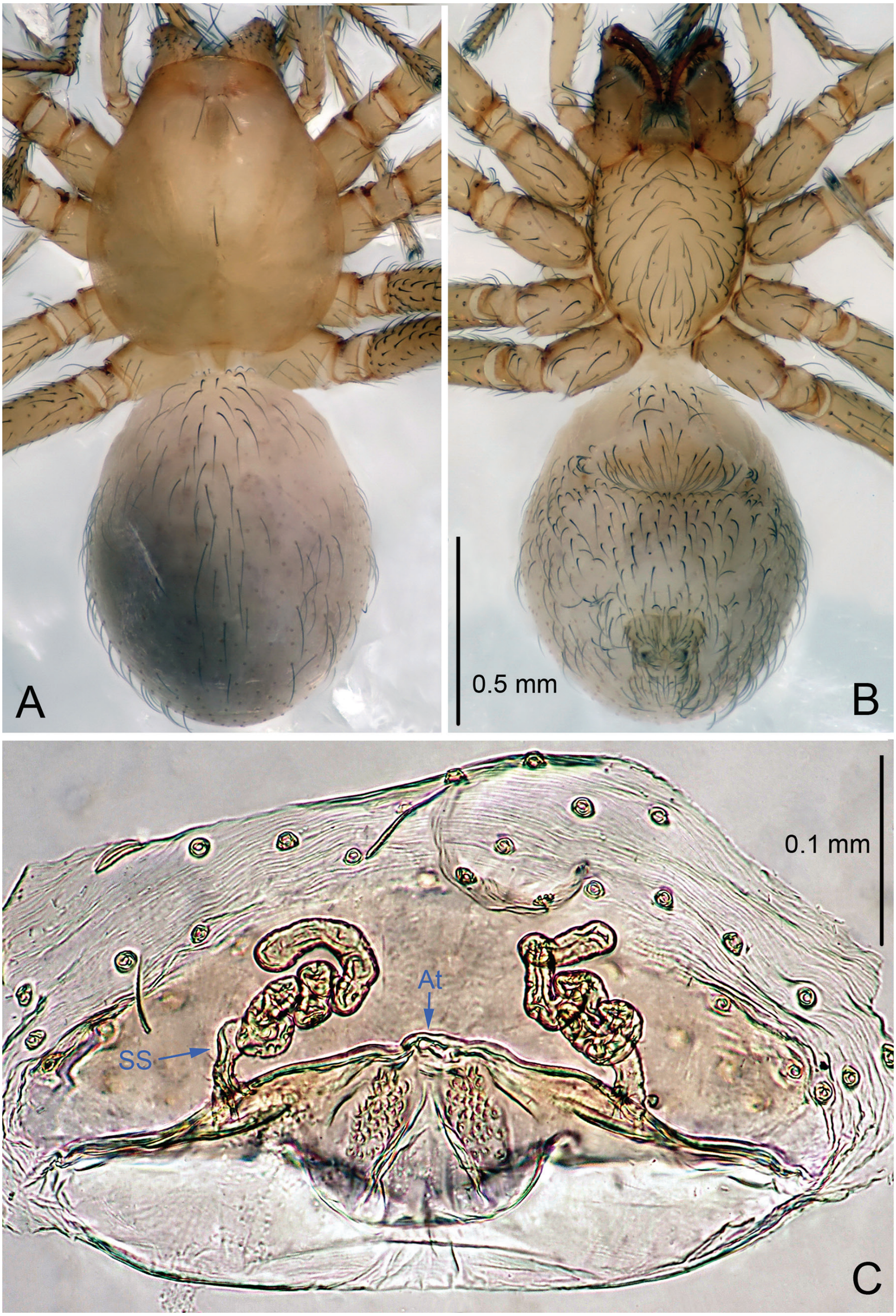

Figure 4. *Leptonetela paragamiani* sp. n., one of the paratype female. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Vulva, dorsal view.

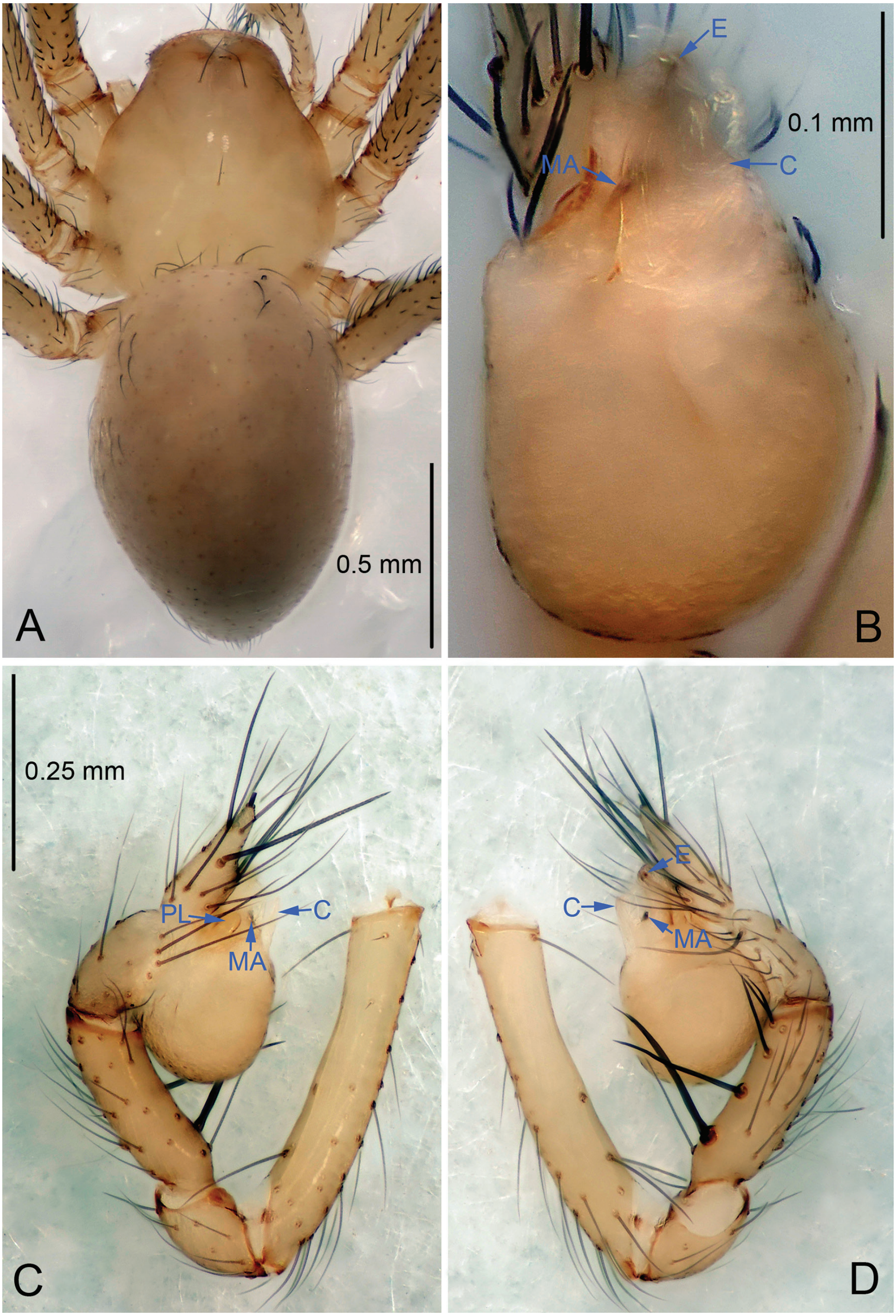

Figure 5. *Leptonetela penevi* sp. n., holotype male. **A** Habitus, dorsal view **B** Palpal bulb, ventral view **C** Palp, prolateral view **D** Palp, retrolateral view.

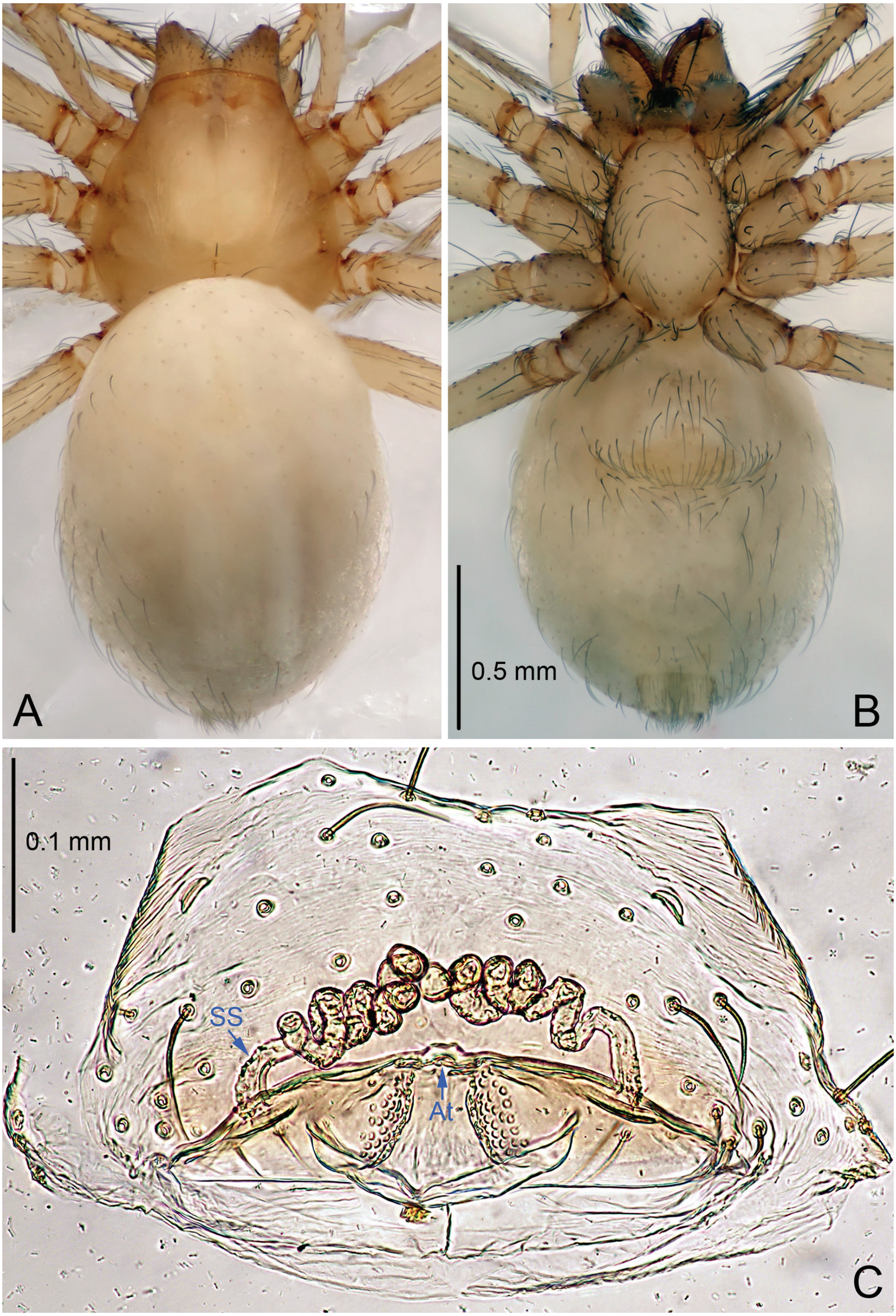

Figure 6. *Leptonetela penevi* sp. n., one of the paratype female. **A** Habitus, dorsal view **B** Habitus, ventral view **C** Vulva, dorsal view.

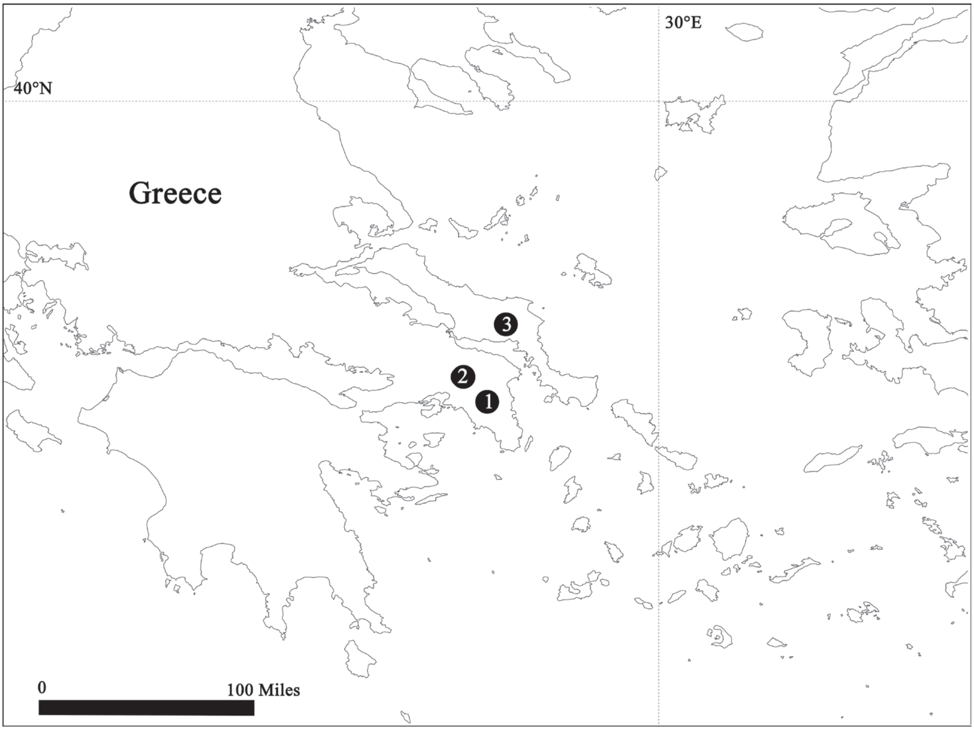

Figure 7. Locality records for three new species of *Leptonetela* in Greece: ① *L. arvanitidisi* sp. n. (Athens) ② *L. paragamiani* sp. n. (near Athens) ③ *L. penevi* sp. n. (Thiva).

Etymology. The specific name is dedicated to Prof. Dr. Lyubomir Penev, zoologist and founder of Pensoft Publishers; noun (name) in genitive case. Pensoft Publishers is a leading company in publishing taxonomic works.

Diagnosis. *Leptonetela penevi* sp. n. is similar to *L. kanellisi* and *L. paragamiani* sp. n. but can be separated by having the basal tibial spine longer than others, and slender (Fig. 5D) compared to the basal spines of *L. kanellisi* and *L. paragamiani* sp. n.; median apophysis distally without teeth (Fig. 5D) and spermathecae strongly twisted and longer than those of *L. kanellisi* and *L. paragamiani* sp. n. (Fig. 6C).

Description. Male (holotype). Total length 1.83 (Fig. 6A). Carapace 0.77 long, 0.65 wide. Opisthosoma 1.15 long, 0.80 wide. Prosoma yellowish, with one seta on the median part. Ocular area with a pair of setae, eyes absent. Median groove, cervical groove and radial furrows indistinct. Clypeus 0.10 high. Sternum and legs yellowish. Opisthosoma pale brown, ovoid, lacking distinctive pattern. Leg measurements: I 6.76 (1.88, 0.38, 1.87, 1.55, 1.08); II 5.44 (1.38, 0.33, 1.58, 1.27, 0.88); III 4.87 (1.37, 0.30, 1.25, 1.20, 0.75); IV 6.32 (1.82, 0.35, 1.73, 1.50, 0.92). Male palp (Fig. 5C–D): tibia with 5 spines retrolaterally, with the basal one strong, conspicuous, and longest. Bulb oval, with spoon-shaped embolus, prolateral lobe oval. Median apophysis (Fig. 5B) without teeth distally, conductor membranous, rugose and shield shaped in ventral view.

Female (one of the paratypes). Similar to male in color and general features but larger and with shorter legs. Total length 2.03 (Fig. 6A–B). Carapace 0.75 long, 0.72 wide. Opisthosoma 1.38 long, 0.85 wide. Clypeus 0.10 high. Leg measurements: I 6.51 (1.88, 0.38, 1.83, 1.50, 0.92); II 5.54 (1.63, 0.33, 1.55, 1.25, 0.78); III 4.91 (1.42, 0.33, 1.28, 1.13, 0.75); IV 6.31 (1.80, 0.35, 1.80, 1.48, 0.88). Vulva (Fig. 6C): spermathecae strongly twisted, atrium oval.

Distribution. Known only from the type locality.

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RESEARCH ARTICLE

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A new genus of Neelidae (Collembola) from Mexican caves

Vladimír Papáč[1], José G. Palacios-Vargas[2]

1 *State Nature Conservancy of the Slovak Republic, Slovak Caves Administration, Cave Care Department, Železničná 31, 979 01 Rimavská Sobota, Slovakia* **2** *Laboratorio de Ecologia y Sistemática de Microartrópodos, Departamento de Biologia, Facultad de Ciencias, Universidad Nacional Autónoma de México, 045 10 México, D.F.*

Corresponding author: *José Palacios Vargas* (troglolaphysa@hotmail.com)

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Abstract

The new genus *Spinaethorax*, whose proposal is based on specimens of *Megalothorax spinotricosus* Palacios-Vargas & Sánchez, 1999, is given a new name combination and a redescription. The type species comes from two caves in Campeche State, México. A new combination is also suggested for *Megalothorax tonoius* Palacios-Vargas & Sánchez, 1999. The new genus is similar to *Megalothorax* Willem, 1900 and *Neelus* Folsom, 1896, but it clearly differs from all genera within family Neelidae by a peculiar combination of characters and the presence of some new features, e.g. globular sensillum on Ant. III, sword-like macrosetae on oral fold. A comparative table and an identification key for all Neelidae genera as well as some summary tables of antennae chaetotaxy and legs setation for type species are provided.

Keywords

Neelidae, Taxonomy, Mexico

Introduction

The family Neelidae comprises five genera and 41 species in the world. However, only 9 species in 3 genera are known from Mexico (Palacios-Vargas 1997; Palacios-Vargas and Sánchez 1999). This family is mainly a euedaphic group of Collembola, whose members are usually very small (0.3–1.0 mm), they have nor eyes or scales, however, their antennae are shorter than their heads. The main differences among the genera of this family are in the structure of forehead setation, antennae, sensory fields and furcula.

Members of this family are cosmopolitan. Therefore, they have been found in various localities mainly associated with soil and litter at different altitudes from sea shore up to 3,000 m a.s.l. (García-Gómez et al. 2009). They have frequently been recorded from caves, mainly in places with rich organic material. This family has two endemic genera: *Acanthoneelidus*, with only one species from Europe, and *Zelandothorax* from New Zeland plus three cosmopolitan genera. *Megalothorax* is the most diversified with 28 species, *Neelus* with 6 species *Neelides* with 5, both widely spread, too. The new genus described herein has two species distributed in Mexican caves.

The most remarkable contribution on this family from Mexico is the work by Bonet (1947), who revised the whole family of Neelidae. The catalog by Palacios-Vargas (1997) records 7 species in this family but some of them have to be revised in the light of new characters. The most recent contribution to the taxonomy of this family from Mexico was that by Palacios-Vargas and Sánchez (1999) who described the two new species that are revised herein.

Materials and methods

The present redescription of *Megalothorax spinotricosus* Palacios-Vargas & Sánchez, 1999, is based on original slides deposited in Facultad de Ciencias, UNAM. Specimens were obtained from samples of bat guano and soil that were processed by Berlese-Tullgren funnels and preserved in 75% ethylalcohol. Slides were mounted using Hoyer´s solution.

Body length was measured on slides excluding antennae and furcula. Lengths of unguis and unguiculus were measured between the most basal (proximal) point and the tip on their inner margins. Besides the common measurements, the ratio “unguis I, II, III (inner margin): Ti. I, II, III width (middle part)” that can be used as additional character, was also included.

We followed nomenclature used in last *Neelus* revision (Kováč and Papáč 2010) for labral setae and dental spines. Nomenclature used in the most recent revision of *Megalothorax* after Schneider and D´Haese (2013) was applied for arrangement of sensory fields, wax rod crypt (wrc1–8) on head, Th. and Abd. sensilla s1, s2, s3, subsegments of dens and chaetotaxy of antennae. Forehead chaetotaxy (presence of a0 seta) is applied according to Deharveng (1978) and posterior chaetotaxy of head is used according to Palacios-Vargas and Sánchez (1999).

Abbreviations: Ant.—antennal segment; Th.—thoracic segment; Abd.—abdominal segment; Ti.—tibiotarsus; scx—subcoxae; s.f.—sensory field; wrc—free wax rod generating crypt; s1, s2, s3, s3´—swollen sensilla; dp—proximal part of dens; dd—distal part of dens; UNAM—Universidad Nacional Autónoma de México.

Taxonomy

***Spinaethorax* gen. n.**

http://zoobank.org/9E27A3C3-9464-4A6A-94B8-5BB470502D2C

Diagnosis. A genus of the Neelidae Folsom, 1896 with the following diagnostic characters:

Habitus of Neelidae. Small size, about 0.6 mm. Color white. Tegumentary grain fine and uniform. Apex of head with sword-like spines, body with several such spines, mainly around sensorial fields. Ant. III and IV fused and Ant. III with small globular sensillum in proximal position. Anterior labral setae R_1 and R_2 thick, curved and smooth. Oral fold with 1+1 sword-like macrosetae. Basomedian field of labium furnished with 6+6 setae. Presence of 3 setae around abdominal sensory fields, no E3 spine/setae on dd. Mid abdomen with swollen sensilla s3 and s3′. Base of Abd. IV sternite with 1+1 neosminthuroid setae, smooth and with pointed tip.

Type species. *Spinaethorax spinotricosus* (Palacios-Vargas & Sánchez, 1999), comb. n.

Redescription. Figs 1–15.

Type material. Holotype: female mounted on slide. Original label: 23/00/1991, Mexico, Campeche, Cueva Xtancumbilxunaan (cave), 29.xii.1996, A. Ruíz and S. Aguilar col., ext. soil. Paratypes: 1 female on slide, 23.viii. 1991, J. G. Palacios col., direct collection and 2 juveniles on slides, the same data as the holotype. Type material deposited at Facultad de Ciencias, UNAM.

Other material. Mexico, Campeche, Cueva Actún Guachapil (cave), 1 male, 1 female and 2 juveniles on slides. Original label: 14/iv/2012, 22.iii.1997, A. Ruiz and S. Aguilar col., ext. guano; 1 female on slide, 29.v. 1997, J. G. Palacios col., ext. guano. Material deposited at Facultad de Ciencias, UNAM.

Diagnosis. Unpaired seta a0 between antennal basis. Labral chaetae R_1 and R_2 thick, curved and smooth. Oral fold with 1+1 sword-like macrosetae. Basomedian field of labium with 6+6 setae. Ant. III with small globular sensillum. Manubrium with 4+4 posterior setae. Mucro with both lamellae serrated and with rounded tip.

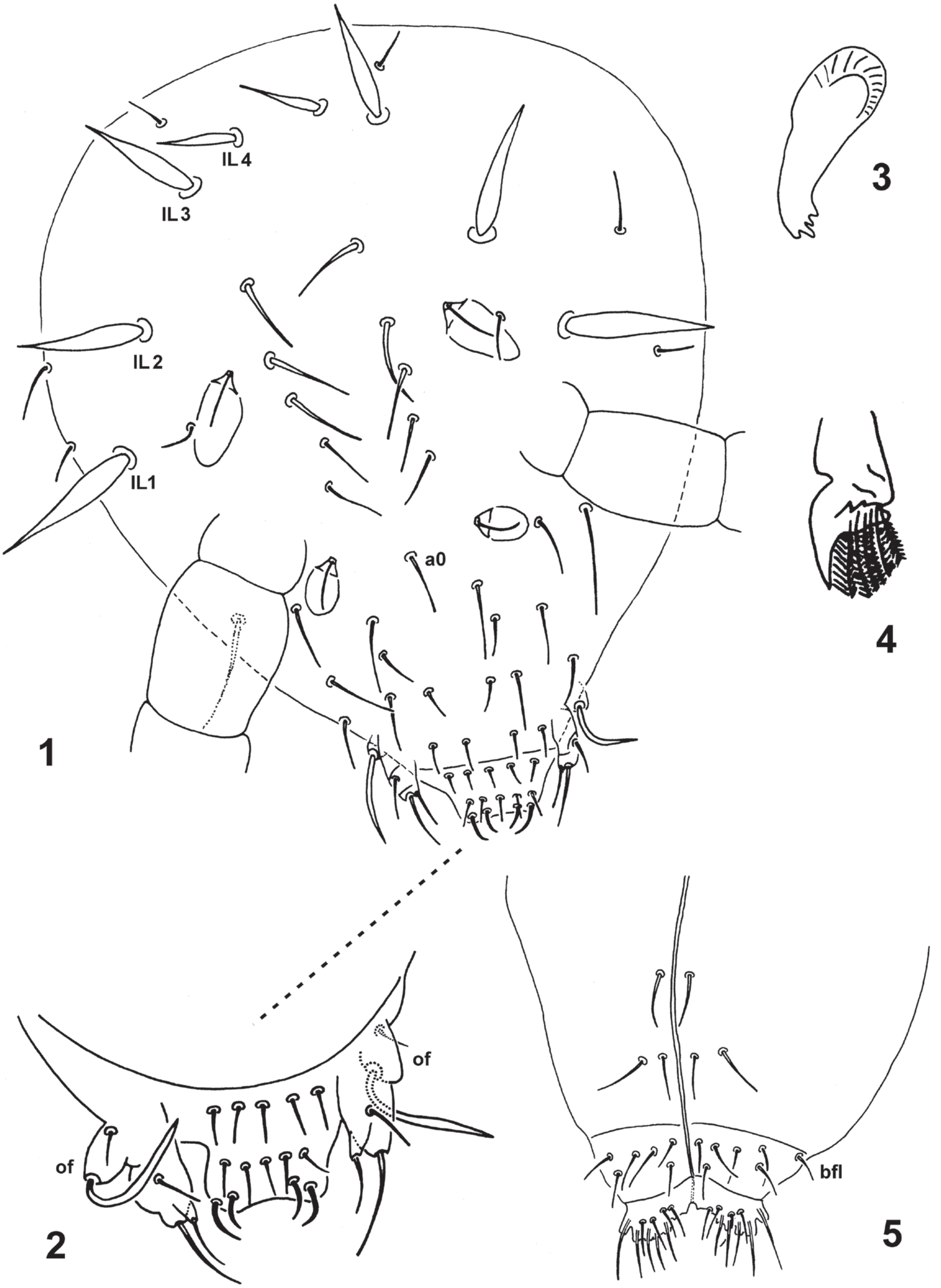
Description. For lengths of different character see Table 1. Body length 0.5–0.7 mm. Habitus globular, as other members of Neelidae. No pigmentation, cuticle finely granulated, *linea ventralis* without crossing with integumentary channels on ventral head back. Mid and hind Abd. with numerous spine-like microsetae, stouter spines around all sensory fields on body and apex of head.

Head. Head length and width 215 and 145 µm, respectively. No eyes. Head with smooth, pointed ordinary setae and spines of different width and length (Fig. 1). Frontal part with ordinary setae (lateral ones longer than axial, 26–30 µm, respectively 10–16 µm), seta a0 present; medial part between posterior s.f. ordinary or slightly spine-like setae (20 µm); posterior part with 3+3 stouter spines IL_1–IL_3 of different lengths (28–42 µm) and 1+1 smaller axial spines IL_4 (17–21 µm), others similar to smaller setae (10–14 µm). Labrum with 5,5,4 setae, 4 prelabrals. Pattern of labral setae (Fig. 2) after Massoud and Vannier (1967): a-row: $2R_1$ + $2R_2$, m-row:

Table 1. Lengths (µm, mean in parenthesis) of different morphological characters of *Spinaethorax spinotricosus* comb. n. (male, females and juvenile separately).

Body part	*Spinaethorax spinotricosus* comb. n. male	*Spinaethorax spinotricosus* comb. n., females	*Spinaethorax spinotricosus* comb. n., juvenile
Body total	520	530–720 (605)	350–500 (445)
Head width	135	133–215 (162)	100–130 (121.2)
Head length	170	183–237 (209)	130–167 (154.8)
R1 labrum	8	9–12 (10)	7–8 (7.6)
R2 labrum	10	10–14 (11.5)	8–10 (9.3)
Antenna	120	125–145 (132.6)	94–112 (106.3)
Ant. I	10	11–13 (11.4)	9
Ant. II	23	25–30 (26.4)	18–23 (21.6)
Ant. III–IV	87	90–104 (97.5)	67–81 (76)
Ant. IV sensillum Sy	16	16–18 (16.6)	10–12 (11.3)
Ant IV macrosensilla S	23–29	20–32 (23–31)	14–24 (14.8–22.6)
Tibiotarsus I width	17	17–18 (17.5)	15–17 (16.4)
Tibiotarsus II width	17	16–19 (16.8)	14–17 (16)
Tibiotarsus III width	17	16–19 (17.4)	16–18 (16.5)
Unguis I	27	26–33 (28.7)	18–23 (21.2)
Unguis II	25	24–30 (26.3)	16–21 (19)
Unguis III	23	23–30 (25)	15–21 (18.8)
Unguiculus I	14	13–16 (14.1)	10–12 (11.2)
Unguiculus II	14	14–18 (15.5)	10–12 (11)
Unguiculus III	15	14–18 (16.1)	9–13 (11.5)
Manubrium	54	56–66 (60.3)	35–45 (42.5)
Dens (proximal part, dp)	32	29–36 (32.3)	20–26 (23.3)
Dens (distal part, dd)	56	66–83 (71.2)	41–57 (55.6)
Mucro	67	65–87 (73.6)	42–58 (53.4)
Mucro width (middle part)	6	7–9 (7.7)	5–7 (6.1)
Macroseta on oral fold	25	25–30 (28)	18–23 (21)
Spines IL_1 on head	37	35–42 (37.3)	28–30 (28.8)
Spines IL_2 on head	33	28–36 (31.1)	21–26 (23.9)
Spines IL_3 on head	30	28–33 (29.8)	17–25 (22.5)
Spines IL_4 on head	20	17–21 (18.1)	12–15 (14)

m + $2r_1$ + $2r_2$ and p-row with 5 ordinary setae (11 µm). Anterior R_1 and R_2 slightly thick, smooth and curved, R_2 (11 µm) longer than R_1 (9 µm). Medial setae (m-row) equal (11 µm), smooth median setae in one line with others. Maxillary palp simple, with 1 enlarged terminal seta (18 µm), 1 basal seta (14 µm) and 1 sublobal hair (Fig. 2). Basomedian field of labium with 6+6 setae (Fig. 5), median ones slightly longer

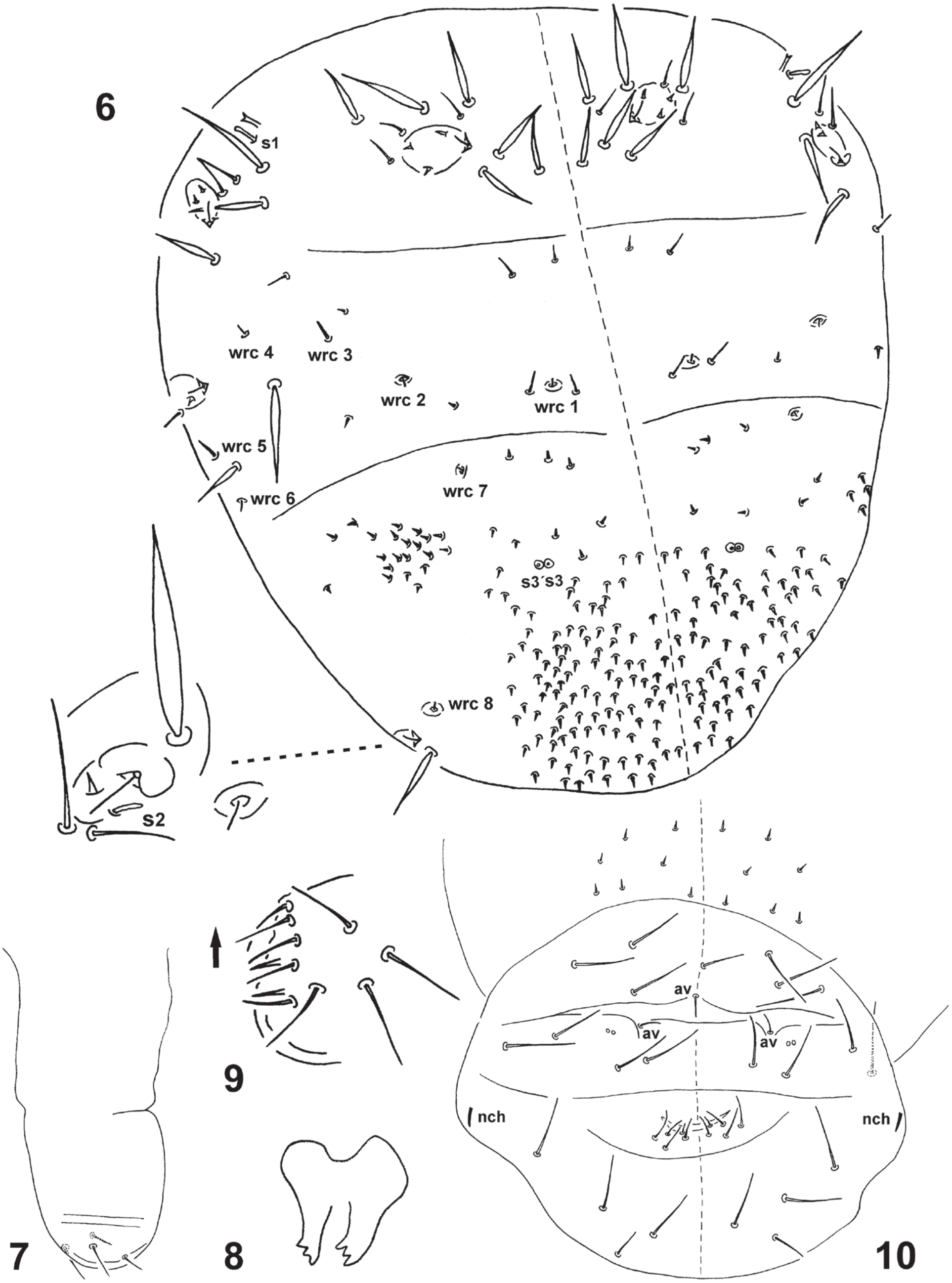


Figures 1–5. *Spinaethorax spinotricosus.* **1** dorsal chaetotaxy of head **2** anterior part of head with labrum, of—oral fold **3** mandible **4** maxilla **5** labium with ventral head back, bfl—basolateral field of labium.

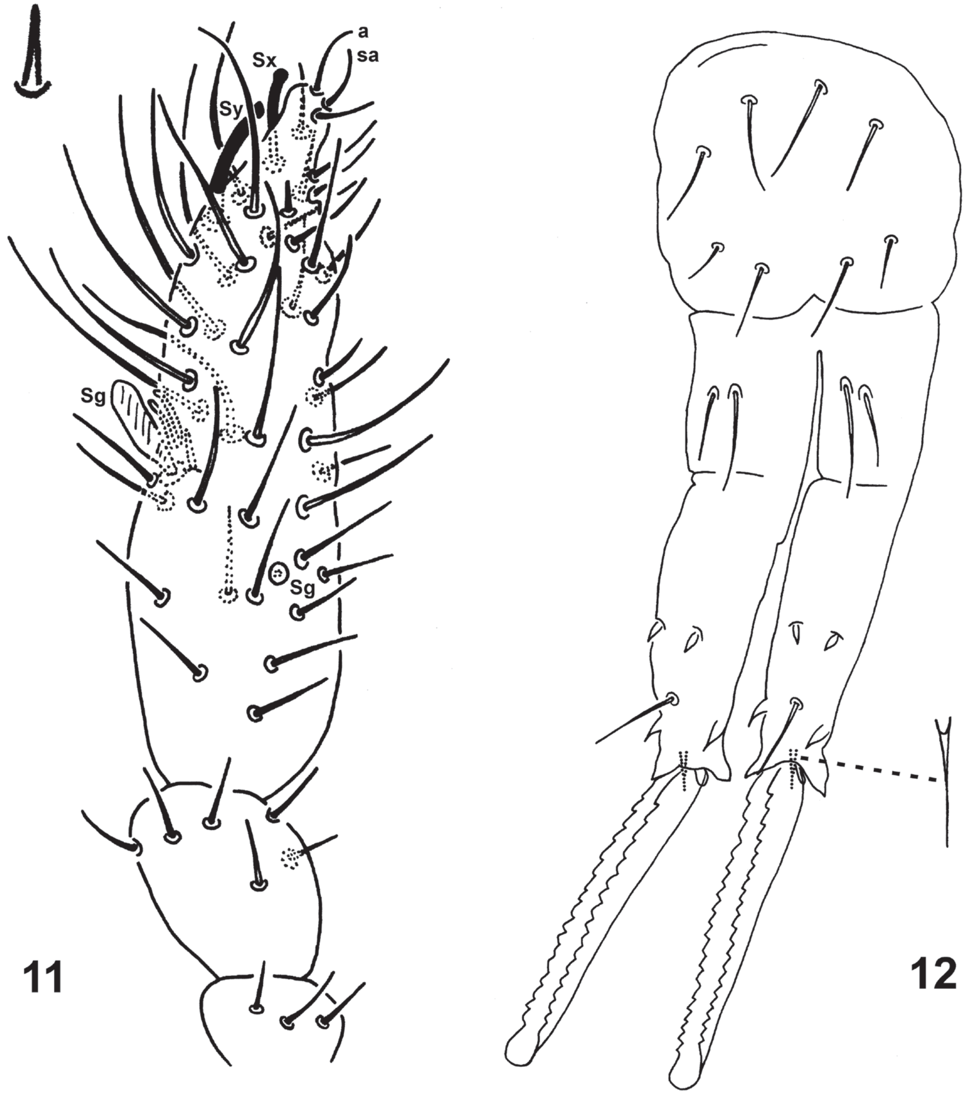
(12 µm) than others (10 µm); basolateral field with 1+1 setae (10 µm), oral fold with 1+1 basal setae (8 µm) and 1+1 terminal sword-like macrosetae (25–30 µm). Head with 3+3 smooth postmedian setae ventrally (Fig. 5); 2+2 anterior setae equal (16 µm); posterior 1+1 seta slightly curved at tip (18 µm). Mandible with 4 apical teeth, medial ones longer (Fig. 3). Maxilla as in Fig. 4.

Thorax and abdomen (Fig. 6). Dorsally with ordinary setae, swollen sensilla s1, s2, s3, s3′, spines of different size (4–6 and 25–45 µm), 6+6 wax rods (wrc1, 2, 4, 6–8) with straight setae (3–4 µm) and 2+2 (wrc 3 and 5) with thicker and longer straight setae (6–7 µm). All wrc placed in small cuticular depressions. Trichobothria or their sockets not observed. Th. II with 3+3 ordinary setae (12–14 µm) and 6+6 stouter spines (25–36 µm) around thoracic sensory fields, axial spines smaller (25 µm); sensory fields at leg II base with 2+2 ordinary setae (20 µm), 3+3 stouter spines, anterior ones longer (45 µm) than posterior (26–28 µm), 1+1 lateral sensillum s1 (8 µm) broadened at tip and 1+1 swollen transparent rod with bifid tip and no base (8 µm) above s.f. of leg II. Th. III with 5+5 ordinary setae (6–8 µm), 4+4 wrc (wrc 1–4) and several small spine-like microsetae, whose overall number is not seen clearly; at leg III base with 1+1 ordinary seta (16 µm), 3+3 stouter spines, medial ones longer (42 µm) than lateral (22 µm) and 2+2 wrc (5, 6). Anterior Abd. medially with 1+1 wrc 7 and 2+2 swollen sensilla s3 and s3′; hind Abd. with abdominal sensory fields, which are surrounded by 2+2 ordinary setae (12 and 18 µm), 1+1 stouter spines (30 µm), 1+1 swollen sensilla s2 and 1+1 wrc 8 above Abd. s.f. Dorso and dorso-lateral anterior and hind abdomen covered with numerous spine-like microsetae (4–6 µm) arranged as in Figs 6 and 10. Their overall number is not seen clearly. Abd. tergum VI with 3+3 setae (17 µm) and 1 unpaired axial seta (14 µm). Anal complex with three anal valves, each with on seta (7 µm). Abd. VI sternum with 4+4 setae (18–20 µm) and 2+2 very small globular structures (1–2 µm) next to anal valve setae. Female genital plate (Abd. V sternum, Fig. 10) with 4+4 setae (8–11 µm) and 1+1 axial microsetae (4 µm). Male genital plate with 5+5 ordinary setae (12 µm) arranged in circle and with 2+2 spine-like setae (7 µm) difficult to observe (Fig. 9), laterally surrounded with 4+4 setae (18 µm). Abd. IV sternum with 3+3 setae in one row (18–20 µm), one seta more laterally and 1+1 distal setae (8 µm). Lateral part of Abd. IV sternum with 1+1 short and pointed neosminthuroid setae (6 µm) (Fig 10).

Appendages. Ant. III and IV not separated (Fig. 11). Length of antennae 140 µm, ratio antenna/head = 0.65; length of antennal segments I, II, III–IV as 11, 26 and 103 µm. Ant. I furnished with 3 short setae (8–10 µm). Ant. II with 1 medial seta and 5 apical setae arranged in a whorl. Ant. III organ consists of 2 transparent rods (7 µm), 1 leaf-like transparent sensillum Sg (12 µm) and spine-like seta (7 µm). Proximal part of Ant. III bears 1 globular sensillum Sg (4 µm). Ant. IV with 13 curved macrosensilla S finely blunt at tip (24–32 µm); subapically with 1 long and thick subapical sensillum Sy (16–18 µm) and with 1 thick shorter apical sensillum Sx broadened at tip (12–14 µm); Ant. IV organ like a tiny, hardly visible spine (5 µm); apically with curved setae a and sa apical in position (10 µm). Complete chaetotaxy of antennae provided in Table 2. Setae numbers of legs I–III (Figs 13–15): scx I: 1, 1, 3 (one spine); scx II:

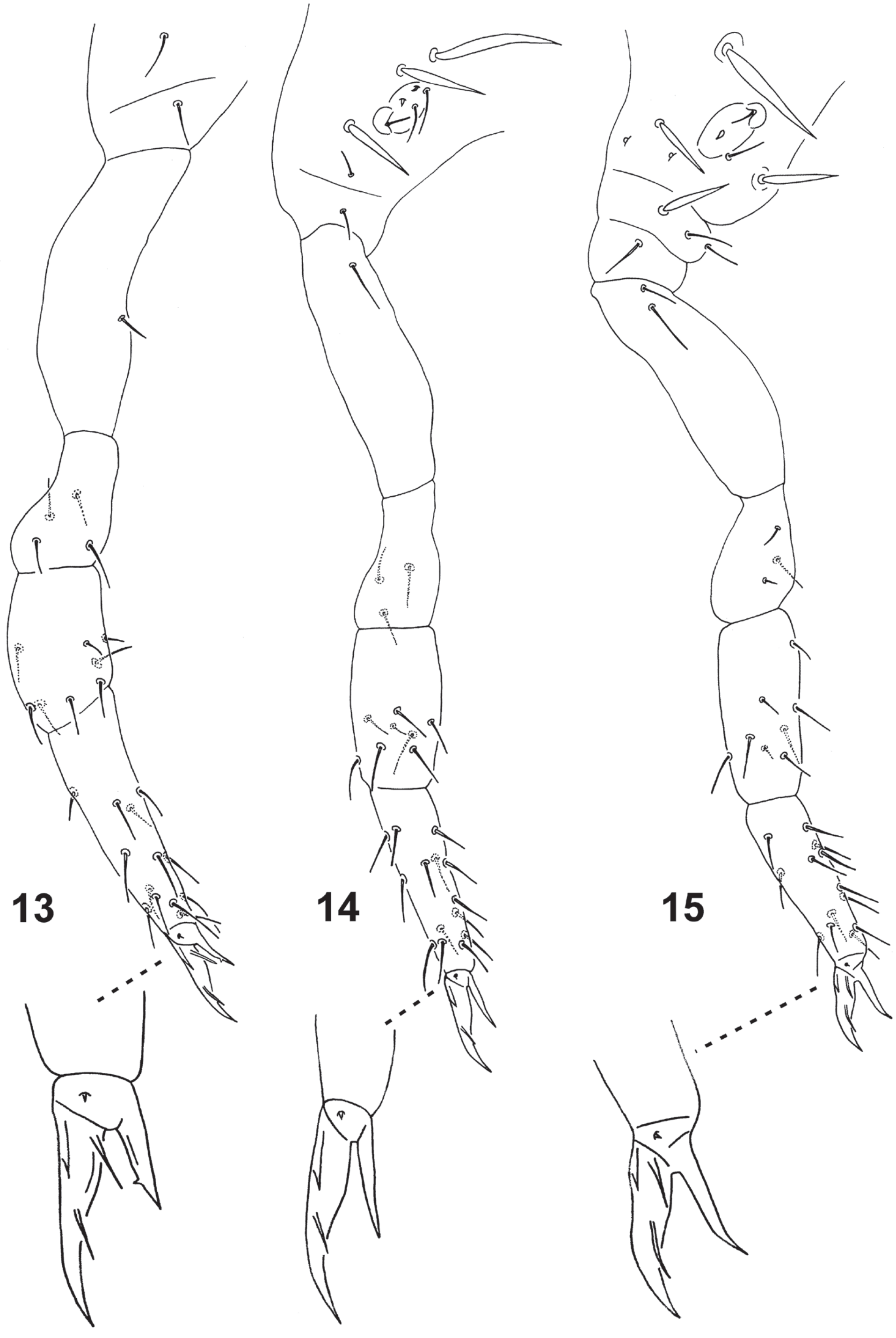


Figures 6–10. *Spinaethorax spinotricosus*. **6** thoracic and abdominal chaetotaxy with abdominal sensory field enlarged **7** ventral tube in lateral view **8** tenaculum **9** male genital plate in lateral view, arrow shows anterior direction **10** female genital plate frontal view, av—anal valve setae, nch—neosminthuroid setae.



Figures 11–12. *Spinaethorax spinotricosus.* **11** dorsal Ant. I–IV, with Ant. IV organ enlarged **12** furcula, posterior view with anterior seta enlarged.

1, 1, 1; coxae: 1, 1, 2; trochantera: 4, 3, 3; femora: 8, 8, 8 and tibiotarsi: 13, 14, 13. Thin meso- or microsetae as in following numbers on leg I: femur with 2; on leg II: femur with 1; on leg III: trochanter with 1, femur with 1. For complete setation of legs see Table 3. Tibiotarsal tenent hairs ordinary, straight and pointed (16–18 μm). Unguis narrow, in distal part bended; both unguis and unguiculus unequally long in leg I, II and III: unguis 31, 27 and 26 μm, respectively, unguiculus 14, 15 and 16 μm,



Figures 13–15. *Spinaethorax spinotricosus*. **13** leg I **14** leg II **15** leg III, with detail of each feet complex.

Table 2. Chaetotaxy of antennae in *Spinaethorax spinotricosus* comb. n.

	***Spinaethorax spinotricosus* comb. n.**			
	Chaetae	**S**	**Sg**	**Misc**
Ant. I	3	0	0	
Ant. II	6	0	0	
Ant. III	15	0	0	2 Sensory organs, 1 spine-like chaeta, 1 globular sensillum Sg, 1 leaf-like sensillum Sg
Ant. IV	13	13	0	1 Sx; 1 Sy; 1 Or; 1a; 1sa

Table 3. Setation of legs in *Spinaethorax spinotricosus* comb. n.

	***Spinaethorax spinotricosus* comb. n.**		
	Leg I	**Leg II**	**Leg III**
Subcoxae I	1	1	3
Subcoxae II	1	1	1
Coxae	1	1	2
Trochanter	4	3	3
Femur	8	8	8
Tibiotarsus	13	14	13

respectively. Length ratio unguis I, II, III / ti. I, II, III width (31, 27, 26/ 17, 16, 17 µm) = 1.8, 1.7, 1.5. Unguis I and III with three auxiliary lamellae la, lp, Bp, unguis II with only lp and Bp (Fig. 14); unguiculus I with small internal tooth on distal part, II and III untoothed and III with bended tip; unguiculi without apical filament and basal lamellae. Ventral tube with 2+2 distal setae and without posterior lobe (Fig. 7). Retinaculum with 3+3 teeth, no setae on corpus (Fig. 8). Furcula well developed (Fig. 12), length of manubrium, dens (dp and dd) and mucro: 56, 31, 71 and 74 µm, respectively. Manubrium with 4+4 setae posteriorly, lateral ones shorter (10–15 µm) than axial (16–20 µm). Dens in proximal part (dp) with 2+2 posterior setae, lateral ones (14 µm) shorter than those axial (22 µm); distal part (dd) apically with 2+2 broad, blunt lateral spines (9 µm) and 1 medial sharp spine (7 µm) on anterior side; with 2 external (E1–E2) posteriorly and 2 internal (J1–J2) spines (6–7 µm each, distal with short apical filament), and 1 medial, subapical seta (18 µm). Mucro with serrated lamellae and rounded tip. Base of mucro furnished with small scale without base. Middle mucro width 7 µm.

Sensory fields (Figs 1, 6, 14 and 15). 6+6 s.f. placed in depressions each with secretory rod (10–12 µm), i.e. blunt seta with basal part inserted on cuticle and placed in upper margin of the field. Following arrangement: (a) anterior and posterior field on head (s.f. 1, 15 × 10 and s.f. 2, 20 × 10 µm) each with secretory rod and 1 seta on margin (14–18 µm); (b) thoracic field (s.f. 3; 35 × 20 µm) with secretory rod, 3 internal spines

(4 µm) arranged in triangle, 2 external marginal setae (12–14 µm) and 6 sword-like spines of different lengths (25–36 µm), 3 spines are in anterior position and 3 spines above s.f. in axial position; (c) fields at base of legs II and III (s.f. 4, 5; 20 × 15 µm) each with secretory rod, 2 internal spines (4 µm) in s.f. 4 and 1 internal spine (4 µm) in s.f. 5. S.f 4 with 2 marginal external setae (20 µm), 3 stouter sword-like spines, medial ones longer (45 µm) than lateral (26–28 µm), 1 lateral sensillum s1 broadened at tip (8 µm) and 1 swollen rod with split tip and without base (8 µm) above s.f. 4. S.f. 5 with 1 marginal external seta (16 µm), 3 stouter sword-like spines, medial ones longer (42 µm) than lateral (22 µm); (d) abdominal field (s.f. 6; 30 × 25 µm) with secretory rod, 1 internal spine (4 µm), 2 marginal ordinary setae (12 and 18 µm), 1 sword-like spine above s.f. (30 µm) and 1 swollen sensillum s2 on the margin of s.f. (6 µm). Wrc 8 is located above Abd. s.f.

Both sexes known.

Etymology. The genus is named after the spine-like setae on thorax and abdomen.

Distribution and ecology. *Spinaethorax spinotricosus* is currently known from two caves and is putatively spread in the cave systems of Yucatán Peninsula, mainly in places with accumulation of bat guano or other type of rich organic material.

Variation. The young have only one proximal seta on dens.

Spinaethorax tonoius (Palacios-Vargas & Sánchez, 1999), comb. n.

Note. This species, described from a cave in State of Guerrero, shares with new type species of the genus similar generic characters like presence of sword-like macrosetae on oral fold, globular sensillum on Ant. III., stouter spines on Th., fusion of Ant. III and IV, 3+3 setae around Abd. s.f., 2+2 setae on proximal part of dens and absence of E3 spine on distal part of dens. The main differences consist in setation of hind Abd. (numerous thickened macrosetae in *S. tonoius* comb. n. vs. numerous spine-like microsetae in *S. spinotricosus* comb. n.), in chaetotaxy of the apex of head (only one spine IL_1 in *S. tonoius* comb. n. vs. three spines IL_1–IL_3 in *S. spinotricosus* comb. n.), in the structure of tenent hairs on Ti. (more developed in *S. tonoius* comb. n. vs. shorter in *S. spinotricosus* comb. n.) and in setation around Abd s.f. (absence of axial spine in *S. tonoius* comb. n. vs. presence of spine in *S. spinotricosus* comb. n.). Species description is based only on one adult specimen (holotype) and does not allow us to describe it in an appropriate way, along with drawings and measurements.

Discussion. The family Neelidae was for a long time an overlooked group of Collembola, mainly due to its small size and lack of diagnostic characters. In spite of the foregoing facts recent years molecular phylogenetic analysis have revealed unexpected diversity within this family (Schneider et al. 2011). Recently *Neelus* has been revised (Kováč and Papáč 2010) as well as *Megalothorax* with a redescription of the nominal species *Megalothorax minimus* (Schneider & D´Haese, 2013). Soon after additional new taxonomical characters in genus *Megalothorax* were defined (Papáč and Kováč 2013). *S. spinotrichosus* comb. n. and *S. tonoius* comb. n. share many specific characters.

Table 4. Differential characters for the genera of the order Neelipleona.

Character	***Megalothorax* Willem, 1900**	***Neelides* Caroli, 1912**	***Neelus* Folsom,1896**	***Zelandothorax* Delamare Deboutteville & Massoud, 1963**	***Acanthoneelidus* Bretfeld & Griegel 2006**	***Spinaethorax* gen. n.**
Sensory fields	yes	no	yes	yes	yes	yes
Ant. III/IV fused	yes	no	no	yes	no	yes
Retinaculum teeth	3+3 or 4+4	2+2	3+3	4+4	4+4	3+3
Neosminthuroid chaetae at the base of Abd. IV sternite	2+2	4+4 or 5+5	1+1	2+2	1+1	1+1
Dental proximal setae (dp)	1	1	2	1	1	2
Nr. of setae on basomedian field of labium	3+3 or 4+4	2+2	4+4	-	-	6+6
E3 spine/chaeta on distal part of dens (dd)	no	yes	yes	no	no	no
Nr. of setae around Abd. sf	5	absent sf	2	-	5	3

These species were included in the genus *Megalothorax* (Palacios-Vargas & Sánchez, 1999), because some features (fusion of Ant. segments III and IV, absence of E3 spine on distal dens) indicated that those specimens belonged to this genus. On the other hand, some characters link those specimens to *Neelus* (dp with 2+2 setae - except *N. fimbriatus*, 1+1 neosminthuroid setae at the base of Abd. IV sternite). Schneider and D´Haese (2013) stated that chaetotaxy of *M. spinotricosus* clearly differs from the other *Megalothorax* by the presence of great number of microsetae on Abd. and pointed out that *M. spinotricosus* deserved its own genus. On the basis of recent diagnostic features, material of these two species was re-examined arriving to the same conclusion as Schneider and D´Haese (2013) that they represent a new genus in the Neelidae. *Spinaethorax* gen. n. differs from other genera by striking morphological features and combinations, which clearly separate them, e.g. sword-like macrosetae on oral fold, six setae on basomedian field of labium, Ant. III and IV fused, presence of small globular sensillum Sg on Ant. III, three marginal setae around abdominal sensory field, absence of spine E3 on dd and dp with two seate. For comparison with other genera see Table 4.

Identifiaction key to the World genera of Neelidae

The identification key is based on that of Bretfeld (1999).

1 Head and body with well developed sensory fields; R_1 labrum setae shorter than R_2; labrum without apical fringes and split structures; retinaculum with 3+3 or 4+4 teeth **2**

– Large sensory fields absent; R_1 labrum setae longer than R_2; labrum with apical fringes and split structures; retinaculum with 2+2 teeth ....***Neelides* Caroli, 1912**, type species *Neelides folsomi* Caroli, 1912; Italy

2 Dens with short conical spines posteriorly; median labral setae present; a-row of labrum with 4 or 6 setae **3**

– Dens with broad triangular spines posteriorly; median labral setae missing; a-row of labrum with 5 setae ....***Zelandothorax* Delamare Deboutteville & Massoud, 1963**, type species *Megalothorax novozealandiae* Salmon, 1944 (New Zealand)

3 Ant. III and IV not separated........................ **4**

– Ant. III and IV separated with suture **5**

4 Sensory fields of abdomen with 5 marginal setae; 2+2 neosminthuroid setae; basomedian field of labium with 3+3 or 4+4 setae; proximal part of dens with one seta........................ ***Megalothorax* Willem, 1900**, type species *Megalothorax minimus* Willem, 1900; Belgium

– Sensory fields of abdomen with 3 marginal setae; 1+1 neosminthuroid setae; basomedian field of labium with 6+6 setae; proximal part of dens with two setae........................ ***Spinaethorax* gen. n.**, type species *Spinaethorax spinotricosus* comb. n. (Mexico)

5 Sensory fields of abdomen with 2 marginal setae; apex of head without spines; proximal part of dens with two setae (only *N. fimriatus* with one seta) .... ***Neelus* Folsom, 1896**, type species *Neelus murinus* Folsom, 1896; United States of America

– Sensory fields of abdomen with 5 marginal setae; apex of head with blunt spines; proximal part of dens with one seta .... ***Acanthoneelidus* Bretfeld & Griegel, 2006**, type species *Acanthothorax pratensis* Bretfeld & Griegel, 1999 (Poland)

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RESEARCH ARTICLE

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Dendrocerus mexicali (Hymenoptera, Ceraphronoidea, Megaspilidae): Novel antennal morphology, first description of female, and expansion of known range into the U.S.

Kyle N. Burks[1], István Mikó[1], Andrew R. Deans[1]

[1] *Pennsylvania State University, Department of Entomology, University Park, 16802, United States of America*

Corresponding author: *Kyle N. Burks* (knb151@psu.edu)

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Abstract

Dendrocerus mexicali has been described by Paul Dessart from a single male specimen collected in Mexico. Using 87 newly identified specimens we expand the known range to include the Southwestern United States and Florida, provide an expanded description of the species, and provide the first record of the female. We also use confocal laser scanning microscopy and *in vitro* hydrostatic pressure changes to investigate the functional morphology of apparently unique basally flexible antennal branches.

Keywords

Dendrocerus, morphology, taxonomy, flabellate, ramose, antennae

Introduction

Ceraphronoidea (Hymenoptera) is a widespread superfamily of parasitoid wasps comprised of two extant families: Ceraphronidae and Megaspilidae. Little is known about the biology of Ceraphronidae, but there are quite a few host records for Megaspilidae, especially for the genus *Dendrocerus* Ratzeburg, 1852 (Fergusson 1980; Dessart 1999).

Host records suggest that *Dendrocerus* parasitizes a broad range of orders, including Hemiptera, Neuroptera, Coleoptera, Diptera, Hymenoptera, (Fergusson 1980, Dessart 1995). Many of its hosts are predators or parasitoids of non-heteropteran Hemiptera, especially of aphids (Aphididae) (Fergusson 1980; Dessart 1995). Based on host records, some species are specialists, while others are generalists, and while a few may be primary parasitoids, many *Dendrocerus* are hyperparasitoids (Fergusson 1980). *D. carpenteri*, which has a very broad host range, has been recorded as being a secondary, tertiary, and even quaternary parasitoid (Fergusson 1980; Haviland 1920).

Dendrocerus mexicali was first described from a single male specimen collected on wild mustard in Mexicali, Mexico (Dessart 1999). Little is known about its natural history. The female has never been described and the host relationships of *D. mexicali* remain unknown.

Dendrocerus mexicali, like other *Dendrocerus* species of the *halidayi* species group, have antennae with long flagellar projections (flagellomeres are "branched" or "ramose"). The antennae of the male *D. mexicali* is perhaps its most distinguishing feature (Dessart 1999). While branched antennae are not uncommon across Hymenoptera, including *Dendrocerus*, the base of each flagellar process is wrinkled and is lighter than the flagellomere or the process (Figure 1A). The function of this region is unknown, and even Dessart was not sure if it was an artifact of preservation (Dessart 1999). One of the aims of this study is to investigate the function of this region.

Methods

All specimens are point-mounted and air-dried. Specimens are deposited in the University of Central Florida Arthropod Collection (UCFC) (18 males and 5 females), the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC) (9 males and 55 females) and Pennsylvania State University Collection Frost Entomological Museum (PSUC_FEM) identifier. All figures, OWL files, and supplementary files are available on Figshare (https://dx.doi.org/10.6084/m9.figshare.2063586.v1).

Dissections

Dissections were performed in glycerol or on Blue-Tack (Bostik, Inc., Wauwatosa, WI, USA) using number 2 insect pins and an Olympus SZX16 stereomicroscope, with an Olympus SDF PLAPO 1XF objective (115×) and an Olympus SDF PLAPO 2XPFC objective (230× magnification).

Confocal laser scanning microscopy (CLSM)

CLSM was used to image the male antenna and genitalia. Dissected male *D. mexicali* antennae and genitalia were placed in a droplet of glycerol between two no. 1.5 coverslips with a small amount of Blue-Tack as a spacer (Mikó and Deans 2013). Specimens were examined with an Olympus FV10i Confocal Laser Scanning Microscope. The antenna was imaged using three excitation wavelengths: 405 nm, 473 nm, and 559 nm. Autofluorescence was detected and assigned a pseudocolor using three channels with emission ranges of 420–520 nm (blue), 490–590 nm (green), and 570–670 nm (red), respectively. Volume rendered micrographs and media files were created in ImageJ (Schneider et al. 2012) using maximum intensity. The genitalia was imaged using two excitation lasers of 631 nm and 499 nm. Two channels were used to detect emissions of 647 nm (green) and 520 nm (red), respectively.

Bright field images

Bright field images were taken using an Olympus ZX41 compound microscope with an attached Olympus DP71 digital camera. Images were stacked and aligned using Zerene Stacker Version 1.04 Build T201404082055.

Antenna coiling experiment

Following the methods described by Steiner et al. (2010), we removed the antenna from one specimen stored in glycerol and one dried and pinned specimen. Both were macerated in 10% KOH for 10 minutes, and then stored in 80% ethanol overnight. We then placed the antenna in 100% ethanol for 10 minutes before transferring to distilled water.

mx autogenerated description

Specimen data, specimen images, OTU concepts and phenotypes expressed in natural language were compiled in mx (http://purl.org/NET/mx-database) and the description and material examined sections of this article were automatically generated from this software. Morphological terminology in the description and diagnosis are linked to classes in phenotype-relevant ontologies (Hymenoptera Anatomy Ontology (HAO), Phenotypic Quality Ontology (PATO), Biospatial Ontology (BSPO), OBO Relation Ontology (RO), Ontology for Biomedical Investigations (OBI), and Information Artifact Ontology (IAO); all of which are available at http://www.ontobee.org/).

Semantic statement generation

Phenotype descriptions expressed as semantic statements (Suppl. material 1, 2) were generated using Protégé Version 5.0.0 (Build beta 17) following Balhoff et al. (2013) and Mikó et al. (2014). Semantic statements for the taxonomic treatment of *Dendrocerus mexicali* are available as supplementary OWL files (Suppl. material 3) and were deposited on Figshare (https://dx.doi.org/10.6084/m9.figshare.2063586.v1).

Abbreviations used

CSB: cephalic size, HH: head height, EH: eye height, HL: head length, HW: head width, IOS: interorbital space, OOL: ocular ocellar length, LOL: lateral ocellar length, POL: posterior ocellar length, MscL: median mesoscutal line, AscW: anterior mesoscutal width, PscW: posterior mesoscutal width.

Results

Dendrocerus mexicali Dessart, 1999

Figures 1–9

Diagnosis. Male flagellomeres have projections with flexible, wrinkled regions at base (Figures 1A and 2). Both males and females have a blunt posteromedian process of the mesoscutellum, called a mucro, that is less sharp than that of *D. koyamae* (Figure 3). Both males and females have mandibular lancea (Figure 4). The sensillar plate of the male aedeagus is strongly sclerotized and greatly enlarged compared to all other described Megaspilidae (Figure 5).

Description. Body length universal: 1.4–1.7 mm (n=10). Color hue pattern: antenna, legs, mouthparts ochre; rest of body dark brown. Color intensity pattern: flagellomeres and their branches darker than scape and pedicel. Scape and pedicel same as legs. Cephalic size (csb): Mean: 400–500μm. Head height (lateral view) vs. eye height (anterior view): HH:EHf=1.4–1.8 (n=5). Head height vs. head length: HH:HL=1.4–1.8 (n=5). Head width vs. interorbital space: HW/IOS=1.8–2.0 (n=5). Head width vs. head height: HW/HH=1.2–1.4 (n=5). Male OOL:LOL: OOL/LOL=0.75–1.0 (n=2). Male OOL:POL: OOL/POL=0.24–0.43 (n=2). Female OOL:LOL: OOL 0.625–0.75× as long as LOL (n=3). Anterior ocellar fovea shape: fovea not extended ventrally to the dorsal margin of antennal scrobe. Occipital carina sculpture: smooth. Submedial flange of occipital carina count: absent. Median flange of occipital carina count: absent. Preoccipital carina and occipital carina structure: the occipital carina extends ventrally to the oral foramen with the preoccipital carina present on the vertex, but not extendinig ventrally along the gena. Preoccipital carina count: present . Preoccipital carina shape: present medially, absent laterally to lateral ocelli. Preoccipital lunula count: present. Preoccipital furrow count: present. Preoccipital furrow anterior end: preoccipital furrow ends inside ocellar triangle. Dorsal margin of occipital carina vs. dorsal margin of lateral ocellus in lateral view: occipital carina is ventral to lateral ocellus in lateral view. Transversely reticulate region on frons count: absent. Rugose region on frons count: absent. Facial pit count: facial pit present. Intertorular carina count: present. Ventral margin of antennal rim vs. dorsal margin of clypeus: not adjacent. Median region of intertorular area shape: flat. Subtorular carina count: absent. Torulo-clypeal carina count: present. Supraclypeal depression count: present. Supraclypeal depression structure: present medially, invert-

Figure 1. Bright field images of *D. mexicali* antenna. **A** male, pedicel and ramose flagellomeres **B** female, scape, pedicel, and clavate flagellomeres.

ed U-shaped. Antennal scrobe count: absent. Flagellomere shape (male): branched. Scape length relative to length of F1+F2 (male): longer or equal. 6th male flagellomere length vs. width, "sensillar" view : elongate, more than 2× as long as wide. Flagel-

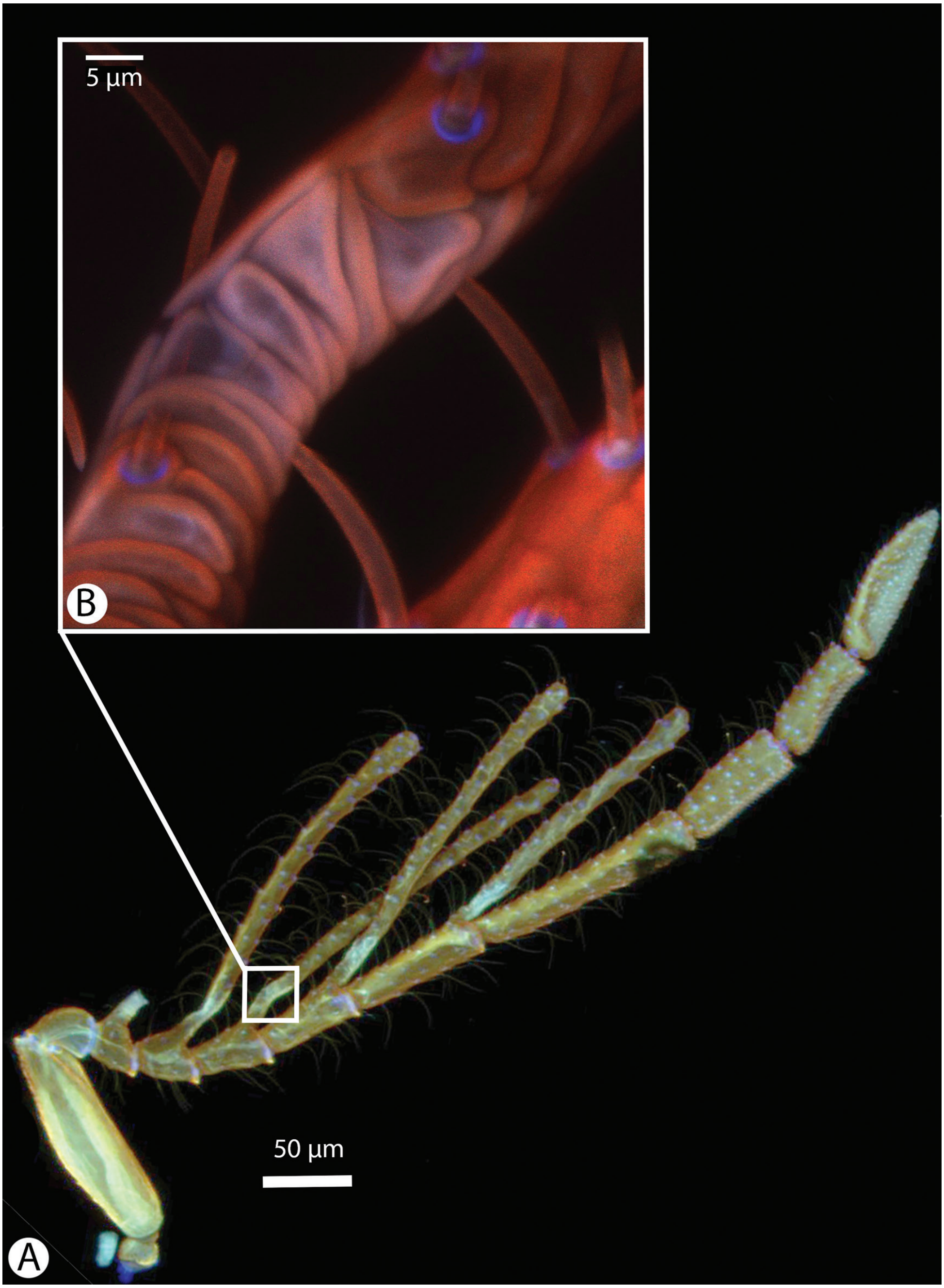

Figure 2. Confocal laser scanning microscopic images of male *D. mexicali* antenna. **A** Antenna with the most basal branch (branch of 1st flagellomere) missing. Bluish area at base of branches indicates a high concentration of resilin; orange and red indicate sclerotized regions; green indicates softer, non-sclerotized regions **B** Magnified view of branch articulation. Purple and pink areas indicate high concentrations of resilin in the cuticle; blue indicates areas of extremely high resilin concentration; red indicates strongly sclerotized regions.

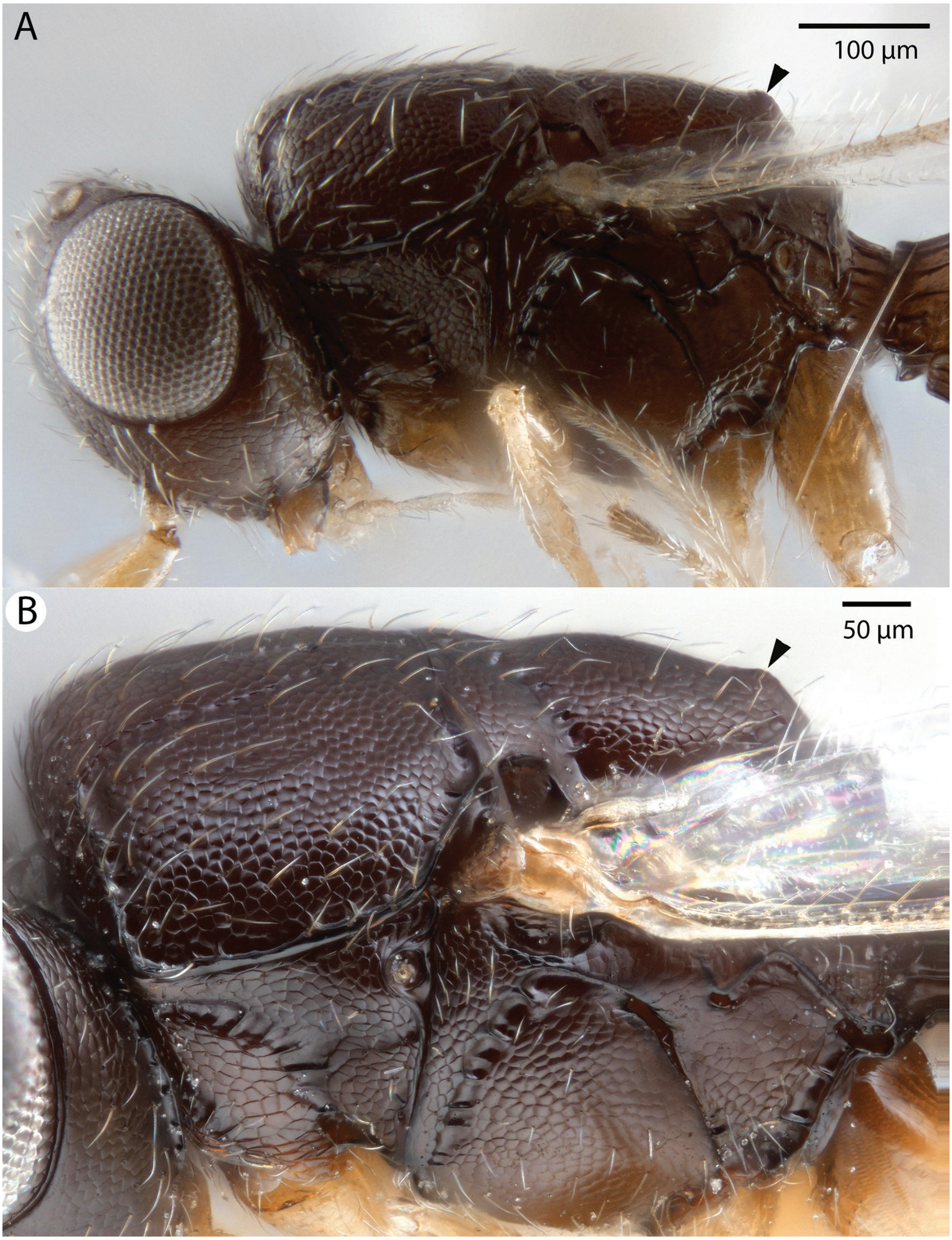

Figure 3. Bright field images of *D. mexicali* mesosoma, lateral view. Arrows indicate the location of a mucro. **A** Male **B** Female.

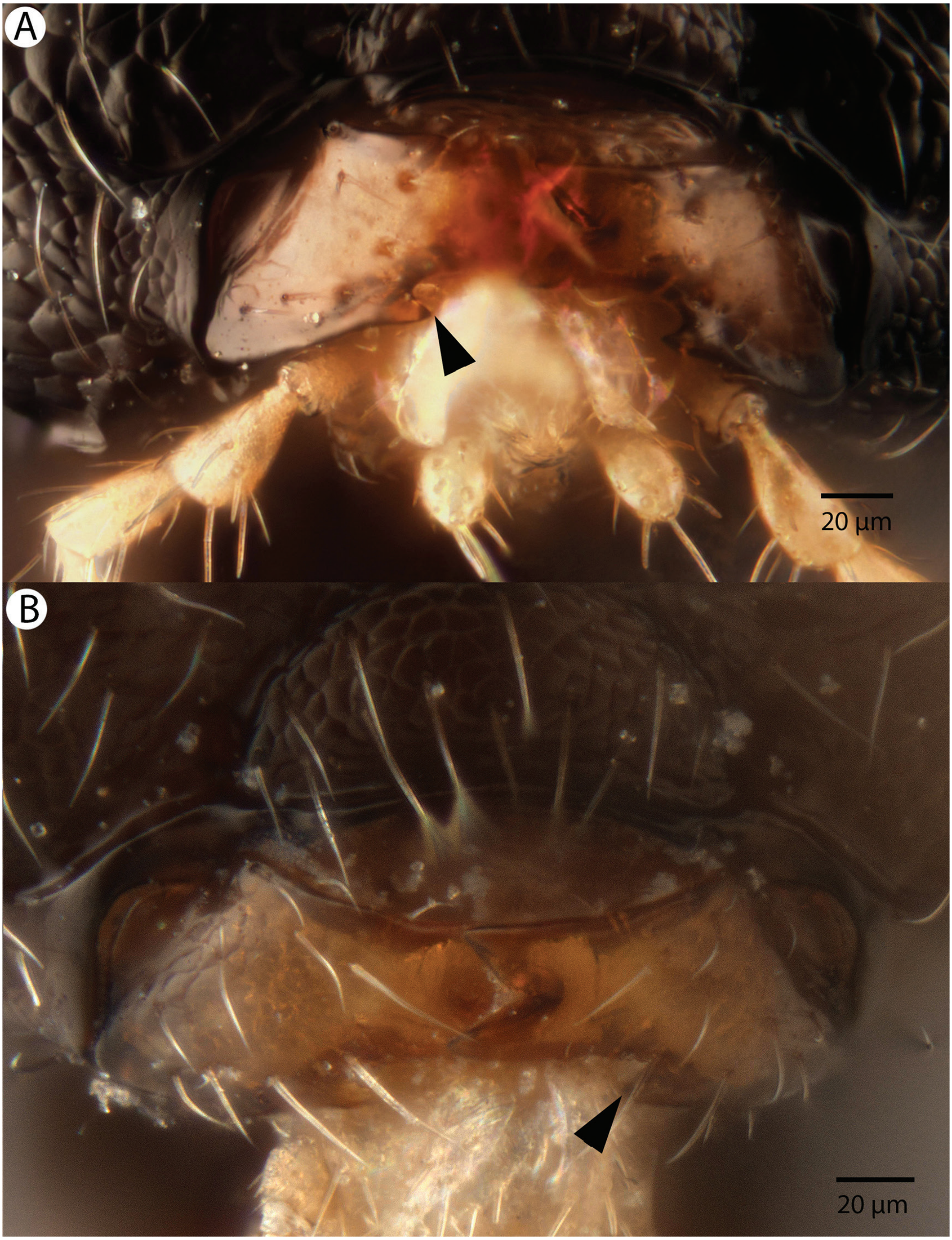

Figure 4. Bright field images of *D. mexicali* mouthparts, anterior view. Arrows indicate the location of mandibular lancea. **A** Male **B** Female.

lomere branch count: 5 branches. Branch of male flagellomere 5 length compared to flagellomere 6: Longer than length of flagellomere 6. Branch of male flagellomere 5 length compared to flagellomere 5: Longer than length of flagellomere 5. Flagel-

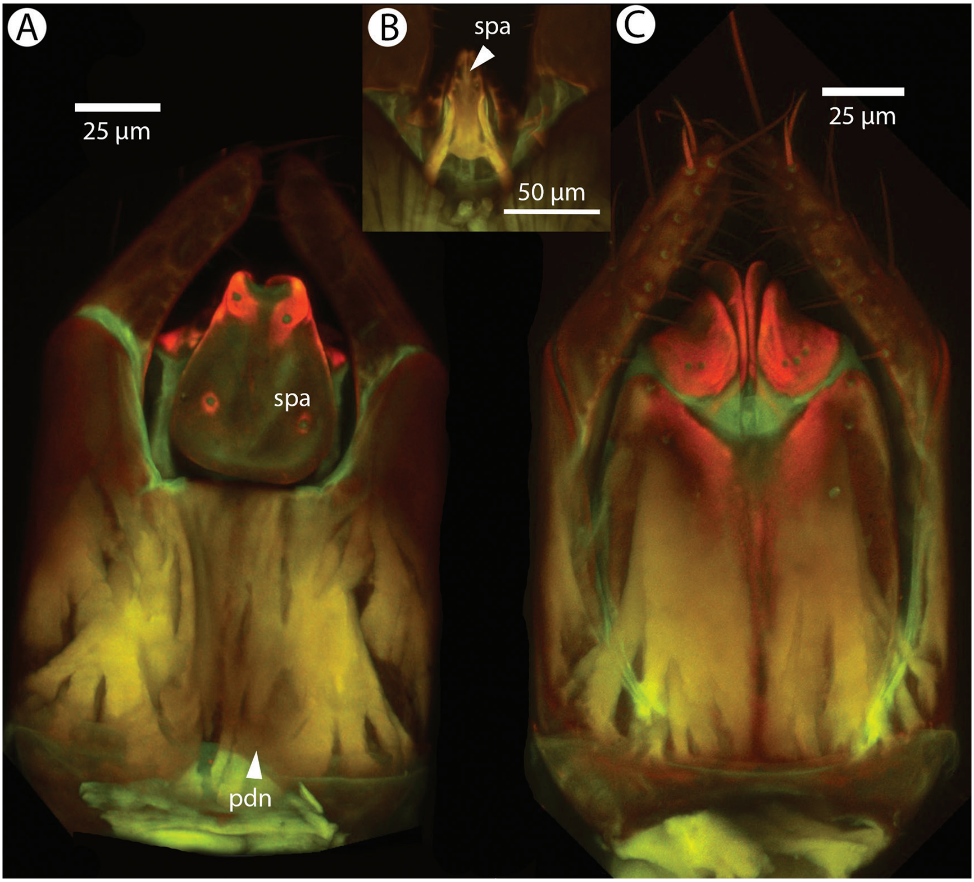

Figure 5. Confocal laser scanning microscopic images of male genitalia; spa=Sensillar plate of the aedeagus. **A** *D. mexicali* dorsal view **B** *D. ramicornis* dorsal view for spa size comparison **C** *D. mexicali* ventral view.

lomere 6 length compared to flagellomeres 7+8: Equal to the length of flagellomere 7+8. Sensillar patch of the male flagellomere pattern: F6—F9. Basal resilin-rich area of male antennal branches count: present. Female first flagellomere length vs. pedicel : F1 as long as pedicel (1.0–1.1) (n=3). Female ninth flagellomere length: F9 less than F7+F8. Mandibular tooth count: 2. Mandibular lancea count: present. Ventrolateral invagination of the pronotum count: present. Atrium of the anterior thoracic spiracle size: as wide as distal trachea. Notaulus posterior end location: adjacent to transscutal articulation. Epicnemial carina count: complete. Epicnemium posterior margin shape: anterior discrimenal pit absent; epicnemial carina straight. Speculum ventral limit: extending ventrally of pleural pit line. Sternaulus count: absent. Median mesoscutal line length vs. anterior mesoscutal width: MscL/AscW=0.6–0.9 (n=5). Anterior mesoscutal width vs. posterior mesoscutal width: AscW/PscW=0.9 (n=5). Median mesoscutal sulcus posterior end: adjacent to transscutal articulation. Axillular carina count: absent. Posteromedian process of the mesoscutellum count: present. Posteromedian process

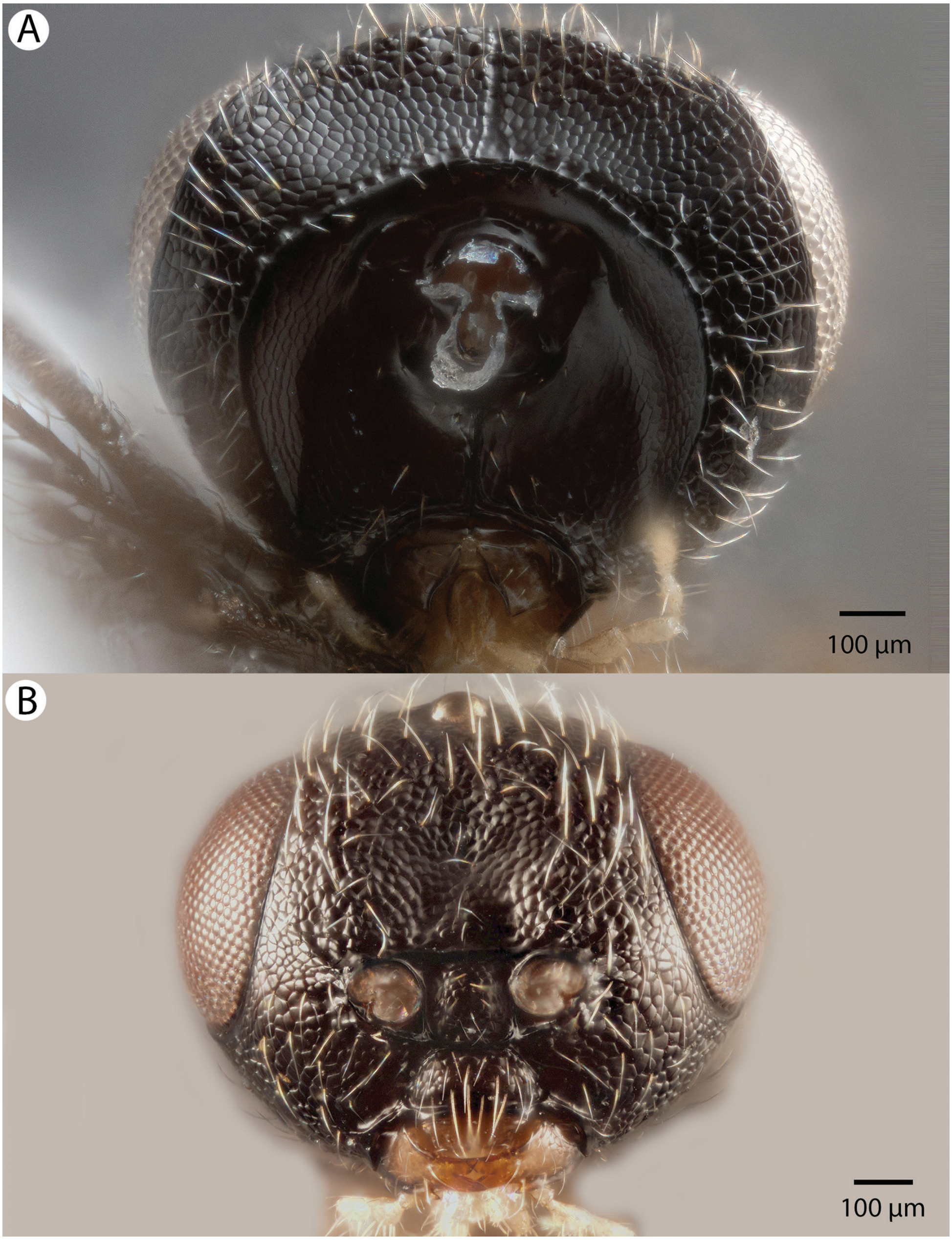

Figure 6. Bright field images of male D. mexicali head. **A** Posterior view **B** Anterior view.

of the mesoscutellum shape: blunt. Scutoscutellar sulcus vs. transscutal articulation: adjacent. Mesometapleural sulcus count: present. Posterodorsal metapleural area shape: trapezoid. Metapleural carina count: present. Anteromedian projection of the metanoto-propodeo-metapecto-mesopectal complex count: absent. Lateral propodeal

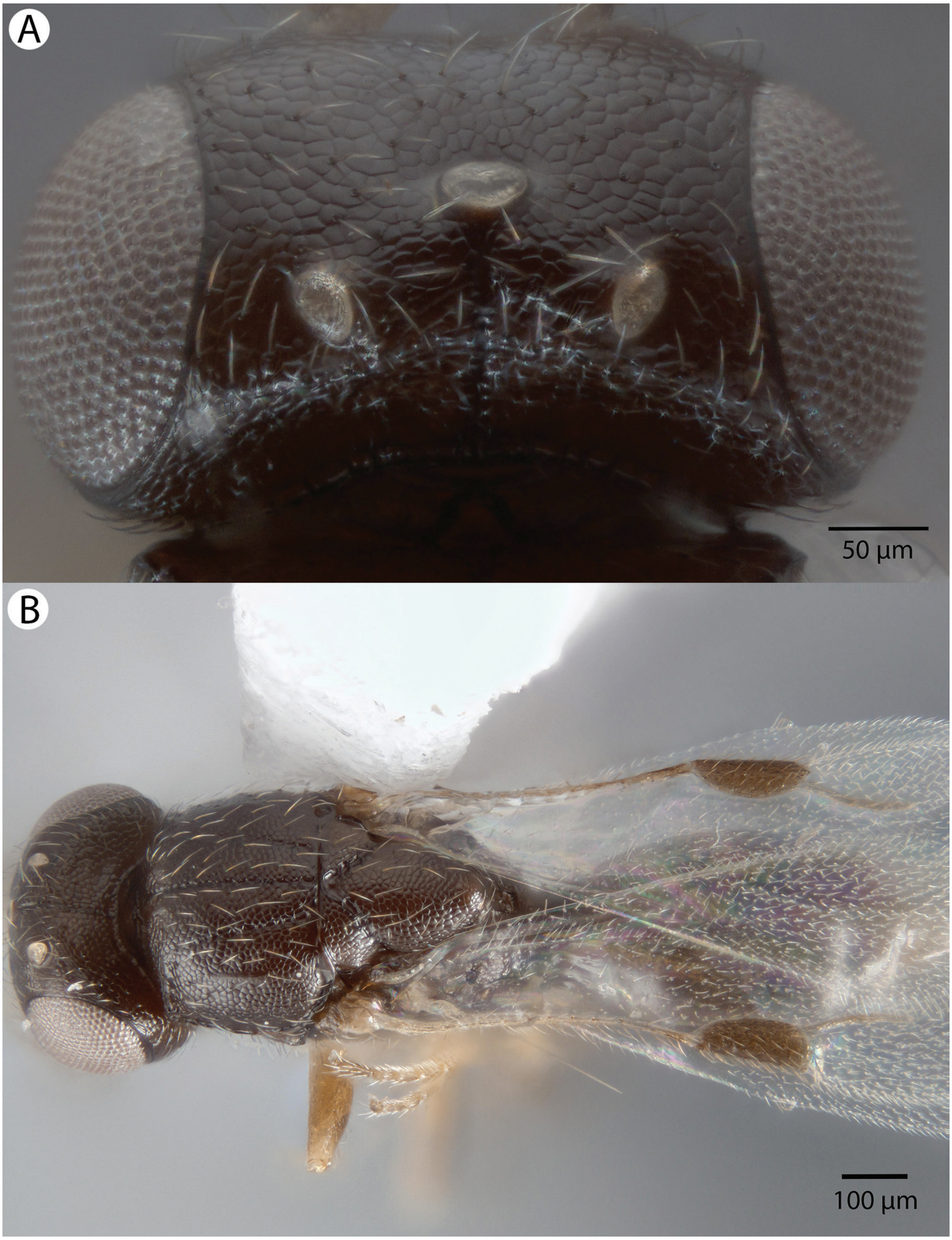

Figure 7. Bright field images of male *D. mexicali*. **A** Dorsal view of head **B** Habitus; dorsal view.

carinae shape: inverted "V" (left and right lateral propodeal carinae are adjacent medially at their intersection with antecostal sulcus of the first abdominal tergum). Lateral propodeal carina count: present. Transverse line of the metanotum-propodeum vs. antecostal sulcus of the first abdominal tergum: adjacent sublaterally. Distal margin of

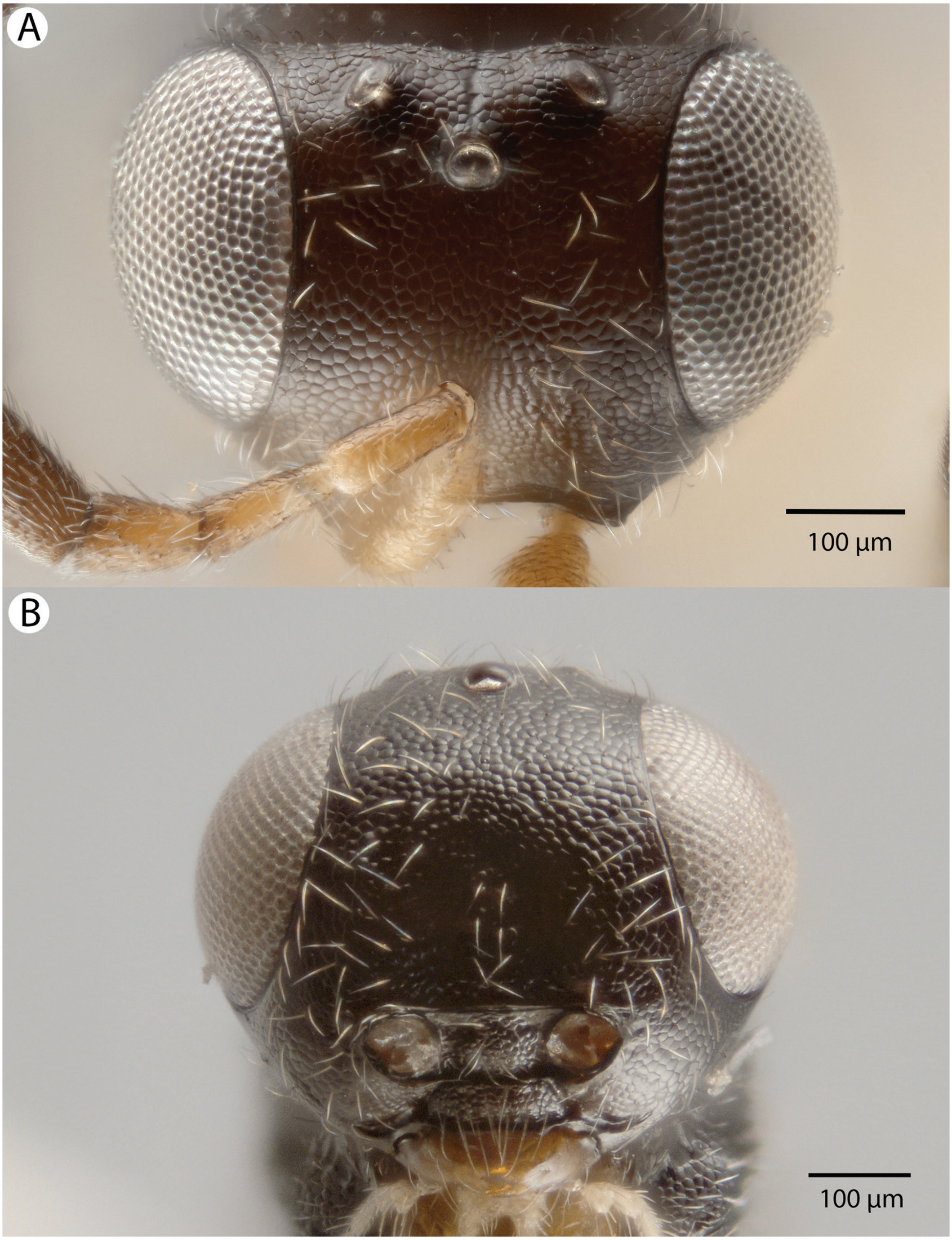

Figure 8. Bright field images of female *D. mexicali* head. **A** Dorsal view **B** Anterior view.

male abdominal sternum 9 shape: convex. Median conjunctiva of abdominal tergum 9 count: absent. Proximolateral corner of abdominal sternum 9 shape: blunt. Proximodorsal notch of cupula count: absent. Gonostyle/volsella complex proximodorsal margin shape: with deep concavity medially. Submedian conjunctiva on distoventral

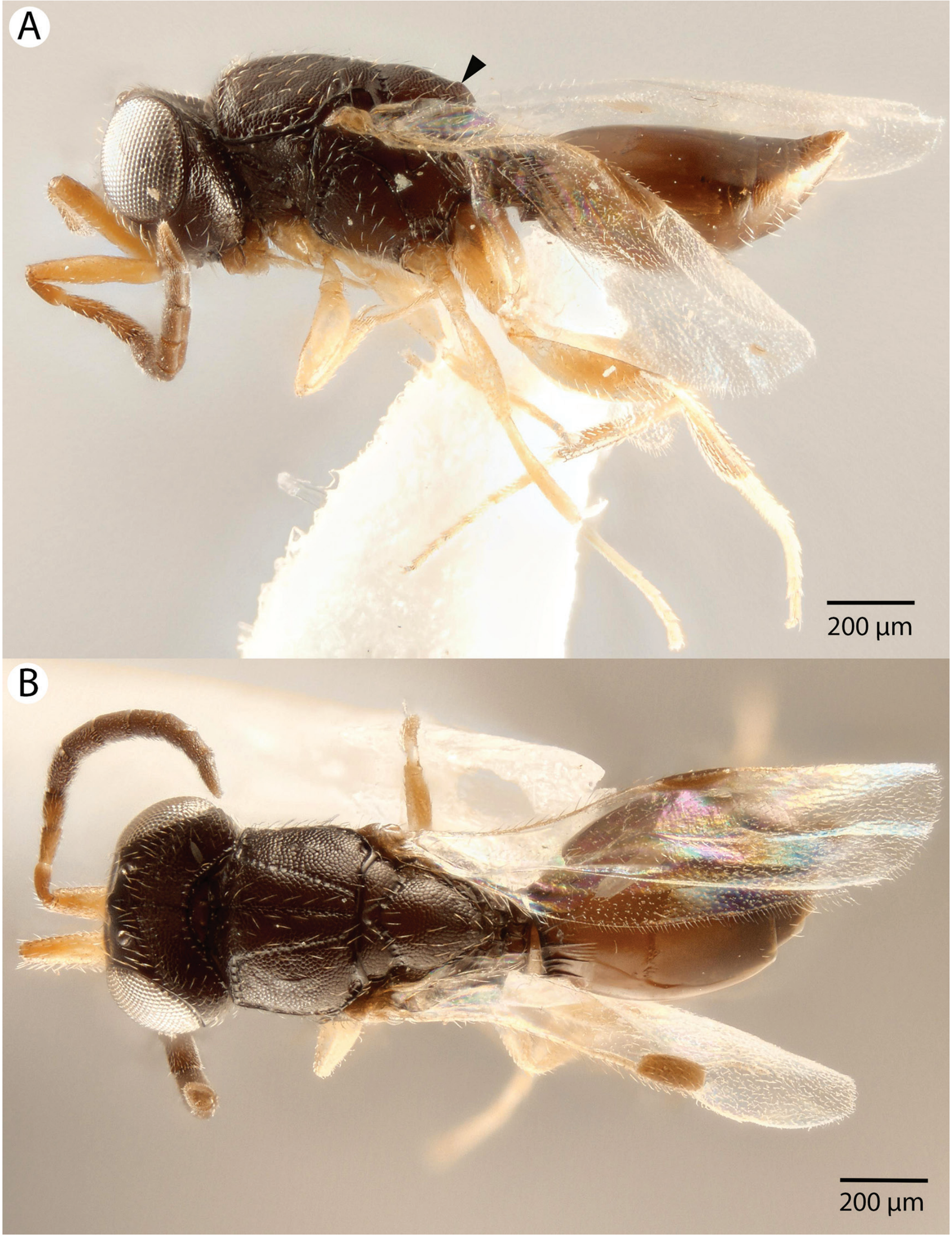

Figure 9. Bright field images of female *D. mexicali* habitus. **A** Lateral view **B** Dorsal view.

margin of gonostyle/volsella complex: length (range of fusion of parossiculus/parossiculus complex from gonostipes): more than 4/5. Apical parossiculal seta number: two. Dorsal apodeme of penisvalva count: absent. Distal projection of the penisvalva count: absent. Sensillar plate of the aedeagus shape: enlarged, about half as wide as the genitalia, and strongly sclerotized. Carina limiting posteriorly antecosta count: present. Distal projection of the parossiculus count: absent. Dorsomedian conjunctiva of the gonostyle-volsella complex count: absent. Cupula length vs. gonostyle-volsella complex length: cupula less than 1/2 the length of gonostyle-volsella complex in lateral view. Parossiculus count (parossiculus and gonostipes fusion): absent (fused with the gonostipes). Distoventral submedian corner of the cupula count: absent. Harpe length: harpe shorter than gonostipes in lateral view.

Range. Mexico (Mexicali), California, Arizona, Texas, and Florida.

Material examined. Other material (60 females, 27 males): USA:Arizona:Santa Cruz Co.: 1 male. PSUC_FEM 86285 (PSUC). USA:California:Stanislaus Co.: 1 male. IM 5156 (UCFC). USA:Florida: 8 females, 13 males. PSUC_FEM 98899, 98907 (PSUC); IM 5106, 5165, 5214; PSUC_FEM 86151, 86166, 86366, 86370, 86384, 86443 (UCFC); PSUC_FEM 56350–56352, 56397–56403 (CNC). USA:Florida:Brevard Co.: 2 males. IM 5212; PSUC_FEM 86296 (UCFC). USA:Florida:Highlands Co.: 47 females, 6 males. PSUC_FEM 56353–56359, 56361-56396, 56404–56413 (CNC). USA:Florida:Orange Co.: 3 males. IM 5210–5211; PSUC_FEM 86137 (UCFC). USA:Florida:Polk Co.: 4 females, 1 male. IM 5107; PSUC_FEM 86130, 86141, 86148, 86266 (UCFC). USA:Texas:Brazos Co.: 1 female. PSUC_FEM 56360 (CNC).

Antennal coiling experiment. After rehydration of the specimens, the rami of the flagellomeres were very flexible at their bases. After the antenna were placed in distilled water, the apical flagellomeres of both specimens curled very slightly. There was no change in the angle of the flagellomere projections or movement at their bases.

Discussion

Branched antennae are common among various groups of insects. Many Diprionidae have pectinate and bipectinate antennae, though articulated branches have not been described (Benson 1939; Benson 1945). Some Chalcidoidea, such as Eucharitidae, have ramose antennae, though none have been reported to be capable of moving the branches (pers. comm. John Heraty 2015). *Dendrocerus* of the *halidayi* species group also have ramose antennae, though none besides *D. mexicali* have articulations (Dessart 1999).

This ramose flagellomere increases the surface area of the antenna, which could aid males in detecting female pheromones. Although nothing is known of *D. mexicali* mating behavior, male *D. carpenteri* have been shown to be attracted to sex pheromones released by the females (Schwörer et al. 1999). Heavy antennation during courtship has been observed, which implies the possible presence of chemosensilla on the antenna (Liebscher 1972).

Dessart postulated that the wrinkled regions at the bases of the male antennal branches were points of movement, which is extremely likely given the high resilin content of the cuticle that we found there (Figure 2) (Dessart 1999). This evolutionary novelty might allow the branches to fold, preventing the ramose antenna from getting caught on obstacles, allowing the wasp entry into a confined space, or as a mechanical defense against breakage. Hymenoptera do not have antennal pulsatory organs, but they can change the hemolymph pressure in their antenna indirectly through movements of their pharynx (Matus and Pass 1999). This movement may be controlled by the wasp via hemolymph pressure changes and hydraulics acting antagonistically against the the resilin at the base of the branch, though it may only be a passive movement of the branches when external force is applied. We replicated the Steiner et al. (2010) antennal coiling experiment to test whether the branches might be operated hydraulically and directly by the insect. Our results offer no evidence for hydraulic movement, but this could be due to damaged specimens or a more complicated mechanism.

Author contributions

Conceived the project: IM, KNB. Character concept generation, semantic statement generation, specimen visualization and creation of plates: KNB, IM. Wrote the manuscript: KNB, IM, ARD. Commented on the final stage of the manuscript: IM, ARD.

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Supplementary material 1

Table 1S. Phenotype descriptions expressed as semantic statements

Authors: Kyle N. Burks, István Mikó, Andrew R. Deans

Data type: Excel file

Supplementary material 2

Table 2S. Phenotype descriptions expressed as semantic statements

Authors: Kyle N. Burks, István Mikó, Andrew R. Deans

Data type: Excel file

Supplementary material 3

Semantic statements for the taxonomic treatment of *Dendrocerus mexicali*

Authors: Kyle N. Burks, István Mikó, Andrew R. Deans

Data type: OWL file

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RESEARCH ARTICLE

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Two new species of *Prolyda* from the Middle Jurassic of China (Hymenoptera, Pamphilioidea)

Chen Wang[1], Chungkun Shih[1], Alexandr P. Rasnitsyn[2,3], Mei Wang[1]

1 *College of Life Sciences, Capital Normal University, 105 Xisanhuanbeilu, Haidian District, Beijing 100048, China* **2** *Palaeontological Institute, Russian Academy of Sciences, 123, Profsoyuznayaul, Moscow 117997, Russia* **3** *Department of Palaeontology, Natural History Museum, Cromwell Road, London SW7 5BD, UK*

Corresponding author: *Mei Wang* (wangmeicnu@163.com)

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Abstract

Two new species of the genus *Prolyda* Rasnitsyn, 1968, *Prolyda dimidia* **sp. n.** and *Prolyda elegantula* **sp. n.,** are described and illustrated. Both specimens were well-preserved and collected from the latest Middle Jurassic Jiulongshan Formation of Daohugou Village in Inner Mongolia, China. Based on the new morphological data, a key to the five known species of *Prolyda* is provided. In addition, *Prolyda* has an enlarged first antennal flagellomere, which means it might have revert to the elongate plesiomorphic state for the antennal configuration as previously documented.

Keywords

Daohugou, fossil insect, Jiulongshan Formation, sawfly, taxonomy

Introduction

Xyelydidae have long been regarded as the basal group of the Pamphilioidea (Rasnitsyn 1980, 1983, 2002; Grimaldi and Engel 2005). However, Wang et al. (2015a) conducted a phylogenetic study on Pamphilioidea with 33 extinct and 11 extant genera based on 45 morphological characters. The results did not support Xyelydidae as a monophyletic group – *Xyelyda* was shown to be a sister taxon to the other pamphilioids, while the relationship among the remaining xyelydids was mostly unresolved, except for the three genera of *Strophandria*, *Prolyda* and *Medilyda*, which, together with Pamphiliidae, formed a monophyletic clade defined by the synapomorphy of having 1r-m of hind wing in line with 1-M (Wang et al. 2015a, fig. 3). Because Xyelydidae is a paraphyletic group as a family and *Strophandria*, *Prolyda* and *Medilyda* form a monophyletic clade with Pamphiliidae, *Prolyda* is tentatively referred to Pamphilioidea *incertae sedis*, pending a formal reclassification of the superfamily.

A total of 12 genera and 30 species of xyelydids has been reported to date (table 1 in Wang et al. 2015a), most of which are distributed in Kyrgyzstan, P. R. China, Kazakhstan, and Russia. Most xyelydids have been reported from the Jurassic, with the oldest representatives *Sagulyda* spp. and *Ferganolyda* spp. from the Lower or Middle Jurassic Sogul Formation in Kyrgyzstan (Rasnitsyn 1983, Rasnitsyn et al. 2006, Wang et al. 2015b). Only three genera (*Novalyda*, *Fissilyda* and *Rectilyda*) of xyelydid have been described in the Early Cretaceous (Gao et al. 2013, Wang et al. 2015c); these are the latest occurrences of xyelydids in the fossil records.

The Yanliao biota at the Daohugou site has become well known because of the recent discoveries and reports from this locality a variety of excellently-preserved insects, plants, and other animals (Ren et al. 2010, 2012), among which Hymenopterans are especially well represented (Gao et al. 2009, Shih et al. 2009, Wang et al. 2012, Li et al. 2014). The age of these fossil-bearing beds is considered to belong to the latest Middle Jurassic (Bathonian-Callovian boundary), about 165 – 164 million years before present (Walker et al. 2013). We herein describe two new species of *Prolyda* Rasnitsyn, 1968, *P. dimidia* sp. n. and *P. elegantula* sp. n., based on two new specimens from the Daohugou beds, which expand the previously known geographical distribution of Prolyda and move their existence period further back in time.

Materials and methods

Both type specimens are deposited in the Key Laboratory of Insect Evolution and Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China (CNUB; Dong Ren, Curator).

The specimens were examined and photographed, either dry or moistened with 95% ethanol, with a Leica DFC500 digital camera attached to a Leica MZ165C dissecting microscope (Leica, Wetzlar, Germany). The wing venation nomenclature used in this study is modified from Rasnitsyn (1969, 1980). Venation symbols: SC, R, RS, RS+M, M, Cu, M+Cu are main (longitudinal) veins; 1-RS, 1-M *etc*. are sections of these veins; 1r-rs, 2r-rs, 2r-m, *etc*. are cross veins; 1r, 2rm, 1mcu, *etc*. are cells.

Taxonomy

Hymenoptera Linnaeus, 1758
Pamphilioidea Cameron, 1890
Family *incertae sedis*

***Prolyda* Rasnitsyn, 1968**

Type species. *Prolyda karatavica* Rasnitsyn, 1968

Other species included. *P. depressa* Rasnitsyn, 1969, *P. xyelocera* Rasnitsyn, 1968, *P. dimidia* sp. n., and *P. elegantula* sp. n.

Amended diagnosis. Head massive, circular or cube-like; mandibles curved, strong and sickle-like; pronotum short and wide; the first antennal flagellomere equal to head in length, but eight times as long as the second flagellomere; forewing pterostigma variable, completely sclerotized or partly sclerotized, or just membranous; M diverging from M+Cu at much larger angle than Cu; 1-RS proclival or somewhat vertical; angle between 1-M and RS+M almost 90°; 1cu-a distal to the middle of cell 1mcu or located at middle; 2r-rs almost in line with 2r-m; hind wing with 1r-m rather long, as long as or slightly shorter than 1-M.

***Prolyda dimidia* sp. n.**

http://zoobank.org/EF0FFA89-C470-469F-ACAF-371106977ED3
Figs 1, 2

Diagnosis. In addition to generic diagnosis, SC1 longer than SC2; SC2 relatively long, almost equal to 1-RS in length; 1-M short, about twice as long as 1-RS, and 0.6 times as long as RS+M; 2r-m slightly postfurcal; 1r-rs vertical, slightly shorter than 2r-rs and parallel to it; 3r-m located well distal to middle of cell 3r, separated from apex of cell 3r by almost its own length; 2m-cu at middle of cell 3rm.

Measurements (in mm). Body length (excluding antenna) 11.4, head length including mandible 2.03, width 2.48, forewing length up to the end of cell 3r 8.92, hind wing length up to the end of cell r 6.58.

Description. Color not reliably known because of absence of counterpart (Fig. 1A). As preserved, body and legs moderately pale except for tibiae and tarsi dark; forewing somewhat infuscated subbasally, particularly so in costal area along veins, pterostigma slightly infuscate.

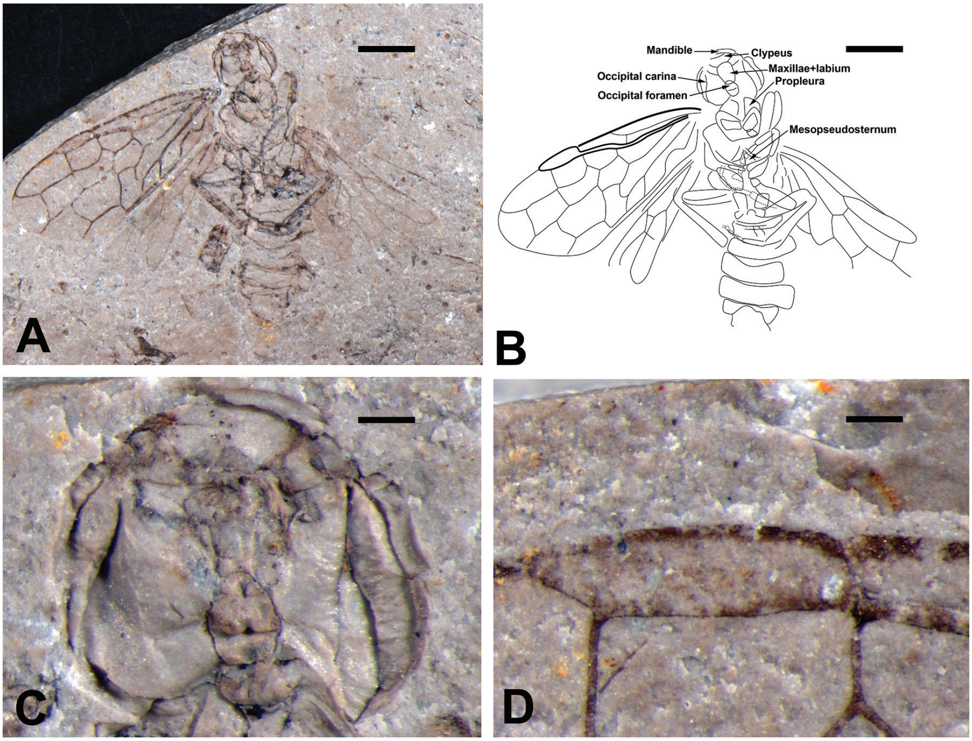

Figure 1. *Prolyda dimidia* sp. n. Holotype: **A** Photo of habitus **B** Line drawing **C** Head **D** Pterostigma. Scale bars: 2 mm (**A, B**); 0.2 mm (**C, D**).

Head circular and large (Fig. 1C), and nearly 1.38 times as wide as mesothorax; clypeus distal margin straight; mandibles incompletely preserved. Propleura large and rectangular, structure of meso- and metathorax unknown except for mesopseudosternum triangular, far from anterior margin of ventropleuron.

Three pairs of legs preserved (Fig. 1B); coxae trapezoid, and hind coxae elongate; femora short and fusiform, fore femur slightly thicker than tibia, approximately 3 times as long as wide, covered with dense bristles; tibia short and narrow, mid tibia about 1.4 times as long as mid femur; tarsi incompletely preserved, fore tarsi with 1st, 2nd and 5th segment elongate, 3rd and 4th subtriangular, with length of fore tarsal segments 1:2:3:4:5 = 3.98: 1.57: 1.18: 1: 1.46, hind tarsal segments 1: 2: 3: 4 = 4.16: 1.69: 1.35: 1.

Forewing (Fig. 2A) with pterostigma sclerotized around margins (Fig. 1D), posterior part thicker than anterior; SC with two branches, anterior branch merging with C at origin of 1-RS, posterior branch long and oblique, slightly longer than 1-RS; R curved proximal to RS base; 1-RS almost vertical, about half of 1-M; cross vein 1r-rs vertical, 0.6 times as long as 2r-rs and parallel to it; 2r-m almost interstitial, located distal to middle of cell 2mcu; 3r-m separated from the apex of cell 3r by almost its own length; M+Cu curved; RS+M vertical to 1-M, 1.8 times as long as 1-M, and nearly of

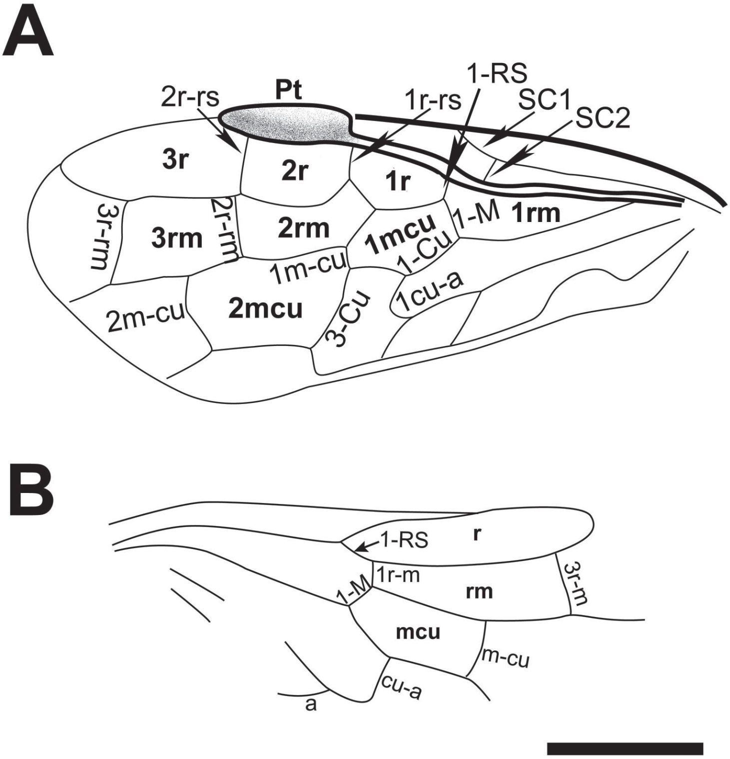

Figure 2. Interpretation of the wings of *Prolyda dimidia* sp. n. Holotype: **A** Left forewing **B** Right hind wing. Scale bar: 2 mm.

same as 1-Cu; 2-Cu curved upwards, almost as long as 2-M; 1m-cu 0.43 times and 0.25 times as long as 2-Cu and 3-Cu, respectively; 1cu-a bent distinctly towards wing apex, equal to length of 2-Cu, slightly distal to middle of cell 1mcu; 2m-cu curved nearly at its mid length, located at middle of cell 3rm; cell 1mcu 1.8 times as long as wide, 0.6 times as long as cell 2rm; cell 2rm nearly as long as cell 3rm, and cell 3r 1.7 times as long as cell 2r.

Hind wing (Fig. 2B) SC absent; cell r tapering apically; 1-RS about as long as 1-M; cross vein 1r-m far from bases of both RS and M; 3r-m oblique towards wing apex, separated from apex of cell r by 0.96 times of its own length; cross vein m-cu equal to length of 3r-m, located distal to middle of cell rm; cross vein cu-a nearly at middle of cell mcu; vein M+Cu almost straight and 1A arched upward, cross vein a distant from cu-a.

Material examined. Holotype, No. CNU-HYM-NN-2012147.

Distribution. Jiulongshan Formation; Daohugou Village, Shantou Township, Ningcheng County, Inner Mongolia, China (41°18.979'N, 119°14.318'E); latest Middle Jurassic of the Bathonian-Callovian boundary.

Etymology. The species epithet is derived from the Latin word “dimidius”, meaning half, referring to the pterostigma being more infuscate in the posterior half.

Prolyda elegantula **sp. n.**
http://zoobank.org/4CF3DA7F-BADE-4055-B3EB-86C69CD507B2
Fig. 3

Diagnosis. In addition to generic diagnosis, SC1 almost as long as SC2; 1-M long, about 0.8 times as long as RS+M; 2r-m well postfurcal; 1r-rs proclival and half as long as 2r-rs; 3r-m near apex of cell 3r, separated from apex of cell 3r by half of its length; cell 3rm widening towards apex; 2m-cu distal to middle of cell 3rm.

Measurements (in mm). Body length (excluding antenna) 12, head length including mandible 2.36, width 2.88, forewing length up to the end of cell 3r 7.02, hind wing length up to the end of cell r 4.8.

Description. Color not reliably known because of absence of counterpart (Fig. 3A). As preserved, body infuscated with part of head, mesonotum and abdomen paler than pterostigma.

Head massive and wide (Fig. 3B), about 1.5 times as wide as mesonotum, 1.73 times as wide as long (excluding mandibles); eyes oval, extending forward to mandible base, 1.6 times as long as wide, with three ocelli forming obtuse triangle; anterior clypeal margin slightly wavy; mandible (Fig. 3C) sickle-shaped, bent, almost reaching opposite side of head when closed, with long, strong apical tooth and short subapical one, almost at midlength of mandible.

Prothorax with propleura large and rectangular; as wide as mesoprescutum; mesonotum with median mesoscutellar sulcus and notauli present, mesoprescutum large, 0.4 times as long as mesonotum and 1.5 times as long as mesoscutellum; metanotum with metascutellum almost circular and metapostnotum rectangular, about 3.3 times as long as wide. Femora wide, fusiform and strong; hind femur about twice as wide as fore femur, and twice as wide as hind tibia; tibia covered with thick bristles (Fig. 3D).

Abdomen without tergum split, wider than mesonotum; abdominal segments narrow, and parallel-sided; anterior margin of the first segment incurved. Genitalia not preserved.

Forewing (Fig. 3E) pterostigma completely sclerotized; SC located at middle of cell C, with two branches, anterior and posterior branches of almost equal length, posterior one oblique, parallel to 1-RS and of same length; R distinctly angular at RS base; 1-RS proclival, about 0.45 times as long as 1-M; 1-M subvertical to RS+M, and 0.83 times as long as RS+M ; 1r-rs short and oblique, half as long as 2r-rs, with RS arching posteriorly between them; M+Cu curved; 1-Cu as long as RS+M; 2r-m separated from 2r-rs by 0.37 times its own length, located distal to middle of cell 2mcu; 3r-m separated from apex of cell 3r by half its length, 1.4 times as long as 2r-m; 1m-cu short, 0.45 times and 0.2 times as long as 2-Cu and 3-Cu, respectively; 1cu-a bent distinctly towards wing apex, 0.83 times as long as 2-Cu, distal to middle of cell 1mcu; 1-Cu 1.63 times as long as 2-Cu; 2m-cu oblique towards wing base and straight, distal to middle of cell 3rm, cell 3rm widening towards apex; cell 1mcu 1.48 times as long as wide, 0.73 times as long as cell 2rm; cell 2rm almost as long as 3rm, and 0.66 times as long as and 0.54 times as wide as, cell 2mcu, cell 3r twice as long as 2r.

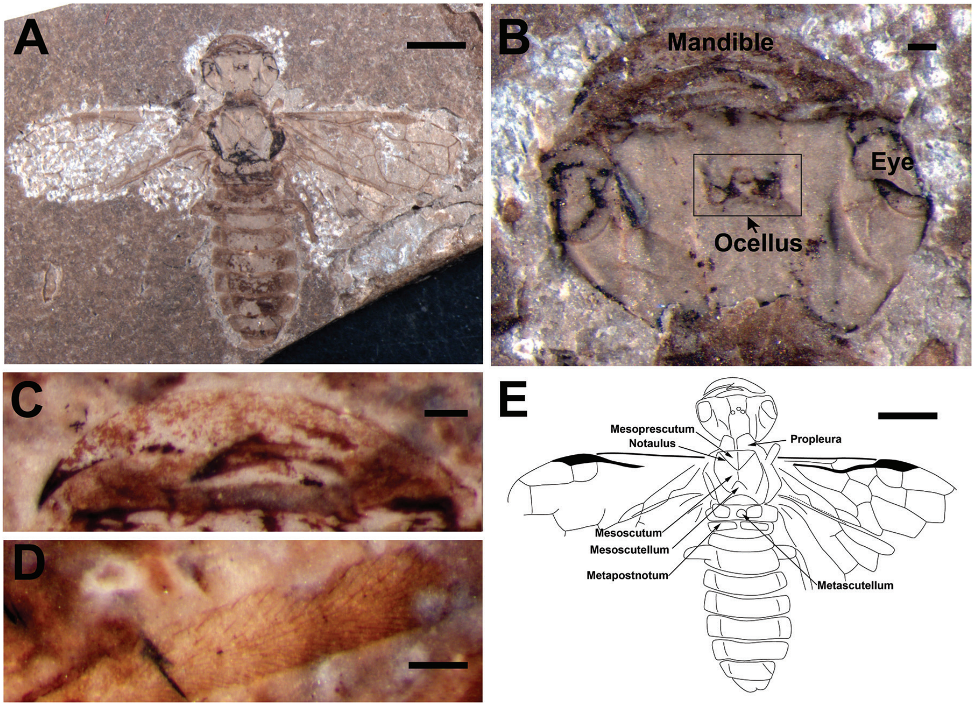

Figure 3. *Prolyda elegantula* sp. n. Holotype: **A** Photo of habitus **B** Head **C** Mandible **D** Setae on the hind tibia **E** Line drawing. Scale bars: 2 mm (**A, E**); 0.2 mm (**B–D**).

Material examined. Holotype, No. CNU-HYM-NN-2012148.

Distribution. Jiulongshan Formation; Daohugou Village, Shantou Township, Ningcheng County, Inner Mongolia, China (41°18.979'N, 119°14.318'E); latest Middle Jurassic of the Bathonian-Callovian boundary.

Etymology. The species epithet is derived from the Latin word "elegantulus", meaning graceful, referring to the habitus of this well preserved specimen.

Key to the species of *Prolyda* Rasnitsyn, 1968

1 Forewing with 2r-m and 2r-rs aligned **2**
– Forewing with 2r-m distal to 2r-rs **3**
2 Pterostigma completely sclerotized; 2m-cu located distal to cell 3rm; cell 3rm widening toward wing apex .... ***P. karatavica***
– Pterostigma membranous basally and sclerotized apically; 2m-cu located at middle of cell 3rm, and cell 3rm not widened toward apex .... ***P. dimidia* sp. n.** (Fig. 2A)
3 Forewing with 2m-cu distal to middle of cell 3rm **4**
– Forewing with 2m-cu proximal to middle of cell 3rm .... ***P. depressa***

4 1-RS short, approx. 0.4 times as long as 1-M; RS+M twice as long as 2-M; pterostigma completely sclerotized.................. ***P. elegantula* sp. n.** (Fig. 3E)

– 1-RS long, almost equal to 1-M in length; RS+M equal to 2-M in length; pterostigma partly sclerotized..***P. xyelocera***

Discussion

Prolyda were erected based on two species, *P. karatavica* and *P. xyelocera* (Rasnitsyn 1968). A third species *P. depressa* was described and illustrated by Rasnitsyn (1969). All three species were collected from the early Late Jurassic (Oxfordian or Kimmeridgian) Karatau assemblage of southern Kazakhstan (Kirichkova and Doludenko 1996, Rasnitsyn and Zhang 2004). *Prolyda dimidia* sp. n. and *P. elegantula* sp. n., from the latest Middle Jurassic, are the oldest records of the genus, and dated *Prolyda* further back in time. Furthermore, the wing venation of *Prolyda* in the two epochs is relatively stable, except for only a few characters liable to fluctuate among xyelydids and related taxa, e.g., degree of sclerotization of pterostigma, the relative positions of 2r-m and 2r-rs, and position of vein 2mcu relative to cell 3rm.

Prolyda usually possesses a relatively large mesoprescutum, which is almost half the length of the mesonotum, and the mesoscutellum nearly reaches the posterior margin of mesoprescutum. *Prolyda dimidia* sp. n. and *P. elegantula* sp. n. do not have preserved antennae. However, as shown in *P. karatavica* and *P. xyelocera*, *Prolyda* are characterized by an enlarged first flagellomere, which is as long as the head and several times as long as the second flagellomere (Rasnitsyn 1968, 1969). As suggested by Wang et al. (2015a), on basis of the ancestral-state reconstructions of first flagellomere among taxa of Pamphilioidea, that *Prolyda* might have revert to the elongate plesiomorphic state.

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RESEARCH ARTICLE

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Taxonomic revision of the Carpathian endemic *Pedicia* (*Crunobia*) *staryi* species–group (Diptera, Pediciidae) based on morphology and molecular data

Avar-Lehel Dénes[1,2], Levente-Péter Kolcsár[1], Edina Török[1,2,3], Lujza Keresztes[1]

1 *Hungarian Department of Biology and Ecology, Faculty of Biology and Geology, University Babeș–Bolyai, Clinicilor 5–7, Cluj Napoca, Romania* **2** *Interdisciplinary Research Institute on Bio–Nano–Sciences of Babeș–Bolyai University, Treboniu Laurian 42, 400271, Cluj-Napoca Romania* **3** *Romanian Academy, Institute of Biology, Splaiul Independenței 296, 060031, București, Romania*

Corresponding author: *Avar-Lehel Dénes* (avar.lehel@gmail.com)

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Abstract

Three new species of the genus *Pedicia*, subgenus *Crunobia* (Diptera: Pediciidae) belonging to the *staryi* group are described on the basis of a combination of molecular and morphology datasets, and a key to discriminate between species of the subgenus *Crunobia* is added. Geographic projection of the identified taxa suggests insular-like distribution and shows the importance of the Carpathians as a genetic center which is home to an exceptionally high aquatic diversity in Europe.

Keywords

Crunobia, cryptic lineage, new species, re-description, endemism, identification key

Introduction

The Holarctic genus *Pedicia* Latreille, 1809 is a small taxonomic unit with three subgenera, *Amalopis* Haliday, 1856 (with 12 species group taxa, including 2 subspecies of *P. tenuiloba* Alexander, 1957) and *Crunobia* Kolenati, 1859 (with 16 species).

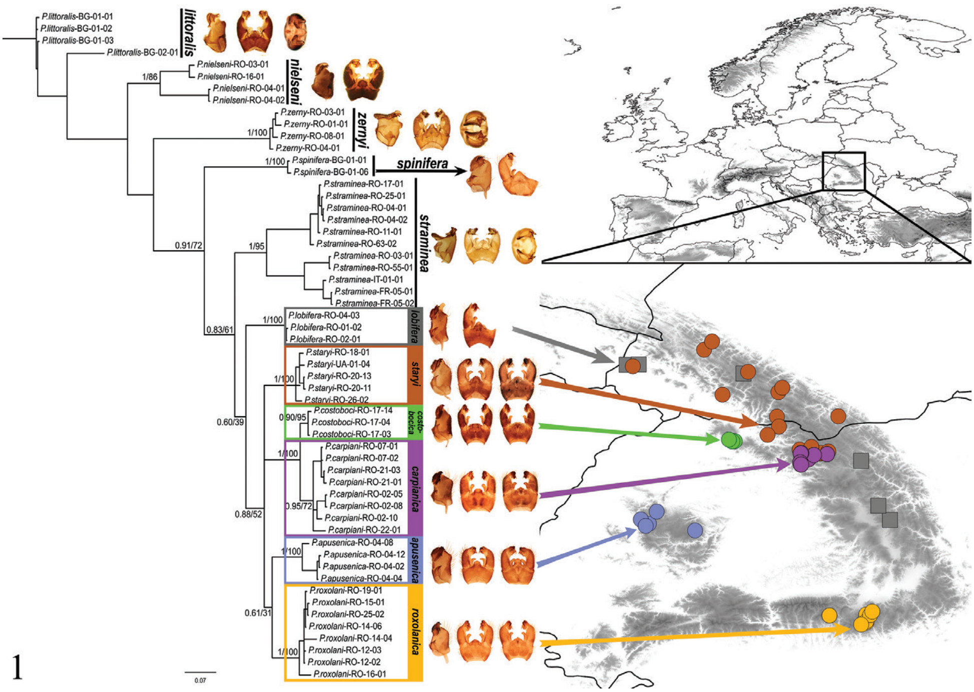
The distribution of these two subgenera is limited within the Palaearctic area, while the remaining subgenus *Pedicia* (with 33 species) extends to the whole Holarctic region (Oosterbroek 2015). The taxonomy of all three subgenera is poorly studied, and recent studies reveal incompletely described biodiversity and important cryptic diversity even from regions that have been better-studied, such as the Western Palaearctic or North America (Ujvárosi and Starý 2003, Ujvárosi and Bálint 2012, Paramonov 2009, Petersen 2006). *Crunobia* was established as a separate genus by Kolenati (1859) for the European widespread species *straminea* (Meigen, 1838) [as *C. schienerii* (Kolenati, 1859)] and later as a subgenus of *Pedicia* by Edwards (1938). The adults belonging to *Crunobia* have important features that are distinct from the features of adults of *Amalopis* or *Pedicia*. For instance, *Crunobia* adults have no conspicuous dark triangles or stripes on the wings, proctiger not sclerotized (membranous) and the gonostylus has only one complicated projection with 2–11 stout black thorns on its dorsal surface (Dienske 1987). Species belonging to this subgenus are present mostly in different mountainous ranges of Europe, with only a few species recorded from Turkey and the Northern Caucasus so far. Only a single species was discovered in Eastern Asia (Alexander 1938, Oosterbroek 2015). However, this species (*P. patens* Alexander, 1938) should be excluded from this group, due to the sharp differences of the male genital structures. Savchenko (1986) recognized two morphologically distinct species groups in *Crunobia* based on the number of chitinous thorns of the dorsal apex of the gonostylus. The most species-rich "*littoralis*" group has only two (three in the case of *P. nielseni*) black spines, while the remaining species belonging to the "*staryi*" group have more than two such projections. The systematic position of the Eastern Asian *P. patens* remains uncertain. The European *P. straminea* (Meigen, 1838) is the most widespread member of the *staryi* species group; the remaining species are endemics with limited range in the Carpathians (*P. apusenica* Ujvárosi & Starý, 2003; *P. lobifera* Savchenko, 1986; and *P. staryi* Savchenko, 1978) and the Balkan Range (*P. spinifera* Starý, 1974). *Pedicia spinifera* was first described from the Rila Mountains, and similar specimens were also later discovered in the Rhodope Mountains in Bulgaria (Kolcsár et al. 2012). These species share many common features with *P. straminea*, but differ because they have a spine in the middle of the interbasis. The remaining three species are microendemics limited to the Carpathians and found in only one or a few mountain enclaves. In the case of *P. staryi* the specimens were first described from a small area in the Ukrainian Carpathians, and were also later discovered in the Eastern and Southern Carpathians (Ujvárosi 2005). The case of *P. lobifera* is similar. First described by Savchenko in 1986, it has long been considered a species restricted to the Ukrainian Carpathians, but was later discovered in the Eastern Carpathians, Romania, as well (Kolcsár et al. 2012). The most problematic situation is with the newest member of the group, *P. apusenica* (Ujvárosi and Starý 2003), which has been found in an isolated region of the Carpathians, the Apuseni Mountains. Initially considered a sister species of *P. spinifera* (due to very limited material available at the time), the distinctive characters identified in the original description did not exemplify a clear delineation between

P. staryi and *P. apusenica*. A revision of the entire species group was suggested on the basis of an intensive sampling in the whole distribution area. Later, an exceptionally high molecular variability was detected within the *P. staryi* group, hence the emerging need for a comprehensive revision (Dénes et al. 2015).

Material and methods

Adult specimens were collected between 2003 and 2015 (Suppl. material 1) using sweep nets and were stored in 96% ethanol. The morphological characteristics of the male and female terminalia were examined in KOH–treated individuals. The genital structures were placed on a bed of fine glass and analyzed using an Olympus SZ61 stereomicroscope equipped with a Canon 650D camera and an LM Digital SLR Adapter (Micro Tech Lab, Austria). Layer photos were combined using the software Combine ZP (Hadley 2011). The drawings were created in Adobe Photoshop CS4 on the basis of the original sketches of Mendl (1974, *P. tjederi*) and Savchenko (1978, *P. semireducta*, *P. persica* and *P. dispar*). Freshly collected specimens were used as comparative materials, enabling the detection of distinct molecular and morphological features in the case of all three newly discovered taxa.

Molecular techniques. Tissue samples were collected from 152 individuals of the *P. staryi* species group (83 individuals of *P. staryi*, 17 of *P. apusenica*, 9 of *P. lobifera*, 6 of *P. spinifera* and 37 of *P. straminea*) and deposited in 96 well plates containing 30 μl of 96% ethanol. Four species (*P. littoralis* Meigen, 1804, *P. riedeli* Lackschewitz, 1940, *P. nielseni* Slipka, 1955 and *P. zernyi* Lackschewitz, 1940) representing the *littoralis* species group were used as outgroups for this study. The molecular processing—DNA extraction, PCR amplification, gel electrophoresis for PCR product checking, PCR cycle sequencing and sequencing—of 126 specimens and of the outgroup species was done at the Canadian Centre for DNA Barcoding (Ivanova et al. 2006, 2012, Ivanova and Grainger 2007, 2012). Specimen collection data, photographs, sequences, PCR, sequencing primers, and trace files are available through the Barcode of Life Data Systems (BOLD; Sujeevan and Hebert 2007) under the project name Tipuloidea of Europe [EUTIP]. An additional 26 individuals of *P. staryi* were processed at the Interdisciplinary Research Institute on Bio–Nano–Sciences of Babeș–Bolyai University. Genomic DNA was extracted using a commercial kit (Qiagen, DNeasy Blood and Tissue Kit) and in accordance with the protocols provided by the manufacturer. The mitochondrial cytochrome c oxidase subunit I (COI) sequences were amplified using the standard LCO1490 and HCO2198 primer pair (Folmer et al. 1994). PCR was performed in a volume of 50 μl reaction mixture at 47 °C. PCR products were visualized on a 1% agarose gel and purified with a commercial kit (Promega, Wizard SV Gel and PCR Clean–Up System, USA). Sequencing was performed by Macrogen Inc. (Korea). Sequences were verified at the NCBI website using a Basic Local Alignment Search Tool (BLAST) (Johnson et al. 2008) and deposited in GenBank (accession numbers KT983903 to KT983910).



1

Figure 1. Bayesian inference (BI) tree with hypopygium profiles of species. Posterior probabilities (PP) and bootstrap values (BP, %) for the nodes are shown under the branches. Carpathian endemic species of *staryi* group are mapped. Color codes: *Pedicia lobifera* (gray square), *P. staryi* (brown point), *P. apusenica* (blue point), *P. carpianica* (purple point), *P. costobocica* (green point), *P. roxolanica* (orange point).

The sequences were downloaded and aligned using Clustal W in MEGA6 (Tamura et al. 2013). Phylogenetic reconstructions were performed on the haplotype dataset using a Maximum Likelihood (ML) and a Bayesian inference (BI) algorithm assuming a General Time Reversible model with a gamma-distributed variation rate across sites (G). The ML tree was estimated with Seaview, version 4 (Gouy et al. 2010) and the BI was implemented in MrBayes version 3.2.2 (Ronquist et al. 2012). Intra– and interspecific Kimura–two parameter (K2P) distance was calculated in MEGA6 (Tamura et al. 2013).

Results

The COI alignment of the five species belonging to the *P. staryi* species group was represented by 44 haplotypes. The ML and BI analysis resulted in congruent tree topologies with high posterior probability (PP) and bootstrap (BP) values, showing a clade that includes four well-differentiated lineages of *P. staryi* (Fig. 1). The K2P distance between *P. lobifera*, *P. apusenica*, *P. staryi* and the three discovered cryptic groups ranged between 4.90% (between the cryptic groups from the Gutâi Mountains and from

Table 1. Pairwise K2P distances between species within *Pedicia staryi* species group.

	P. straminea	*P. spinifera*	*P. lobifera*	*P. apusenica*	*P. roxolanica*	*P. staryi*	*P. costobocica*
P. spinifera	16,2						
P. lobifera	12,8	12,4					
P. apusenica	12	11,8	9,61				
P. roxolanica	11,5	12,5	9,82	6,16			
P. staryi	13,2	13,1	11,4	6,1	6,85		
P. costobocica	13,3	13,4	11,2	9,25	8,58	9,05	
P. carpianica	14	13,4	11,6	8,94	8,77	9,34	4,91

the southern slopes of the Rodnei Mountains) and 11.59% (between *P. lobifera* and the group from the southern slopes of the Rodnei Mountains), with a mean value of 8.94%, which corresponds to the interspecific divergences generally used for molecular taxonomy of Diptera (Renaud et al. 2012, Ashfaq et al. 2014) (Table 1).

Pedicia (*Crunobia*) *apusenica* Ujvárosi & Starý 2003, redescription

BOLD accession number: EUTIP718 to 720 and EUTIP725

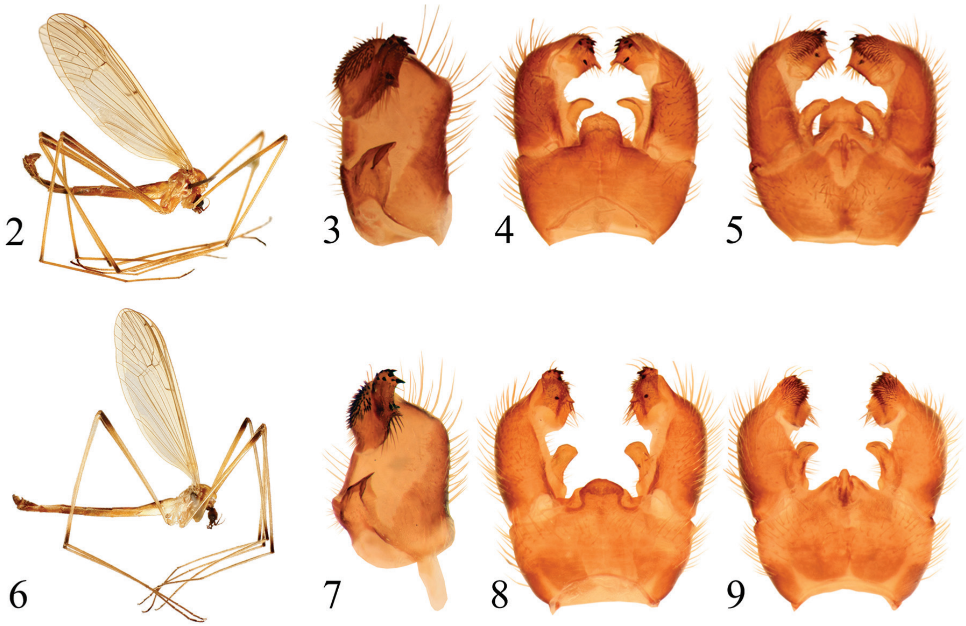
Figs 2, 3, 4, 5

Type material. Holotype male and three paratype males collected in Romania, Apuseni Mountains, Padiș Protected Area, 1 km west of Poiana Vărășoaia, near the Cetatea Rădesei Cave, 1320 m, 46°37.806'N, 22°42.486'E, 21.July 1999, leg. L. Ujvárosi (L. Keresztes). The holotype (CN: TI96) and paratypes (CN: TI97, TI98, TI99) are deposited in the Museum of Zoology of the Babeș–Bolyai University (MZBBU), Cluj Napoca, Romania.

Other material. Romania: Apuseni Mountains, Padiș Protected Area, 1 km west of Poiana Vărășoaia, near the Cetatea Rădesei Cave, 1320 m, 46°37.800'N, 22°42.480'E, 03.Aug.2003, 9 ♂♂, leg. L. Keresztes, 20.Aug.2013, 12 ♂♂, leg. A.L. Dénes; Apuseni Mountains, Stâna de Vale, 1140 m, 46°41.250'N, 22°36.546'E, 19.Aug.2013, 1♂ 1♀ leg. A.L. Dénes; Apuseni Mountains, 2 km west of Rogojel, 1290 m, 22°48.528'E, 22°48.528'E, 14.July.2014, 3 ♂♂ leg. L.P. Kolcsár; Apuseni Mountains, Boga, Boga Valley, 700 m, 46°36.576'N, 22°40.674'E, 15.Aug.2015, 1 ♂, leg. E. Török. Materials are stored in 96% ethanol or pinned dry and are deposited in the Diptera Collection of the Faculty of Biology and Geology, Cluj-Napoca, Romania

Diagnosis. The species is distinguished from all other species of the *Pedicia staryi* group by the following combination of characteristics: all flagellomeres are almost uniformly colored, and there are no dark lines between antennae; the abdominal stripe starts from the second segment; the tip of the last palpus segment is darker than other segments; 9th tergite has a rounded median lobe, with a small apical emersion.

Redescription. Large species of a yellowish orange color (Fig. 2). Male body length is 14–15 mm, (mean 14 mm, n=20), wing length 13–15 mm (mean =14.1 mm, n=20),



Figures 2–9. 2–5 *Pedicia apusenica* male: **2** lateral habitus **3** inner lateral view of the gonocoxite **4** male hypopygium dorsal view **5** male hypopygium ventral view **6–9** *Pedicia roxolanica* sp. n. holotype male: **6** lateral habitus **7** inner lateral view of the gonocoxite **8** male hypopygium dorsal view **9** male hypopygium ventral view.

antenna 1.9–2.1 mm (mean 1.95, n=9). Head with light brown vertex. The antenna is 16–segmented; scape and pedicel are light brown, flagellomeres are almost uniformly yellowish. Scape is cylindrical, approximately 1.8 times longer than it is wide, pedicel oval. Flagellomere 1 (f1) and f2 are fused in some cases, first segment 2–2.5 times longer than it is wide (or 1.2 times), f2–f6 are approximately oval, f7–f14 are fusiform or cylindrical. All flagellomeres are equipped with 4–6 black bristles, about half as long as the flagellomeres. Palpus is 5–segmented; the first segment extremely short, second three palpomeres are dark brown, the ventral part is lighter and more membranous than dorsal parts; the last palpomere is dark brown only at the tip, the remaining parts are yellowish, membranous. Dorsal and lateral parts of thorax are yellowish orange. Scutum is yellow to orange, with two longitudinal lines of setae. Wing venation is yellowish brown. Pterostigma is light brown, more or less distinct. Small spots are present at Sc2, at base of Rs, at fork of Rs (mostly around r–m) and around R2 (Fig. 2). Halters have yellowish stem and dark orange to light brown knob (uniform yellowish orange in the case of specimens stored in alcohol). Legs have femora and tibiae are yellowish brown, black at the tip. Tarsi are light brown to dark brown. First abdominal segment is yellowish orange. A dark brown longitudinal stripe starts dorsally from the second abdominal segment, which widens through the 7th and 8th tergite and covers it. Sternites are yellowish orange to light brown, anterior sternites are lighter than caudal. Male terminalia is considerably broad (Figs 4, 5). The 9th tergite is in some case darker than the remaining parts of the hypopygium. Posterior margin of 9th tergite has a rounded median lobe, with a small apical top. Gonocoxite is stout, cylindrical and truncated at distal end. A flat spoon-like extension is present at the distal end of gonocoxite ventrally, directed inwards, densely covered with short black spinules (Fig. 3). Gonostylus is subterminal, inserted laterally at distal inner side of the gonocoxite and forming a nearly right angle with the long axis of the latter. Gonostylus is generally quadrangular in dorsal or ventral views, with 7–10 strong black spines mostly situated at the outer distal margin and with short slender projection at the lower (caudal) margin distally. Interbase is simple, broadened and rounded distally. **Female** has a body length of 15 mm; wing length 9 mm. Head is uniformly yellowish orange. Pedicel and scape are yellowish orange. Flagellomeres absent. Palpus is uniformly yellowish orange. Dorsal and lateral parts of the thorax are uniformly yellowish. Legs have coxae and trochanters yellowish orange. Wings are partly reduced, and the females are flightless. Venation is yellowish orange, having no spots on the wing. Abdomen is uniformly light orange. Female terminalia has a wide cercus, dagger-like, and raised upward at the tip. Both Hypovalvae are wide, darker than the tenth sternite. At the dorsal margin, there are seven pairs of curved, strong, needle–like setae. One seta is separated distally from the rest. A strong sensory seta is visible on the distal part of the hypovalvae, which extends beyond the end of hypovalvae.

***Pedicia* (*Crunobia*) *roxolanica* Kolcsár, Keresztes & Dénes, sp. n.**

http://zoobank.org/69D3AFD5-E970-4429-8C00-98B08789E01A
GenBank accession number: KT983903; BOLD accession number: EUTIP669, EUTIP670, EUTIP691 to 694 and EUTIP710
Figs 6, 7, 8, 9, 25, 26

Type material. Holotype male and two paratype males collected in Romania, Baiu Mountains, Azuga, Limbășel Valley, 1200 m, 45°29.574'N, 25°35.910'E, 26.Aug.2014, L.P. Kolcsár. The pinned dry holotype (Catalog Number–CN: TI101) and paratypes (CN: TI102, TI103) are deposited in the Museum of Zoology of the Babeș–Bolyai University (MZBBU), Cluj Napoca, Romania.

Other material. Romania: Bucegi Mountains, Sinaia, Cota 1400, 1400 m, 45°21.258'N, 25°31.278'E, 21.July.2004, 4 ♂♂, leg. L. Keresztes; Iezer–Păpușa Mountains, Lerești, Voina Hut, 970 m, 45°26.526'N, 25°2.670'E, 03.Aug.2006, 4 ♂♂, leg. L. Keresztes; Bucegi Mountains, Sinaia, Peleș Valley, 1300 m, 45°22.092'N, 25°30.978'E, 04.Aug.2006, 1 ♂, leg. L. Keresztes; same site, 26.July. 2013, 6 ♂♂, leg. L. Keresztes and Á. Péter; Baiu Mountains, Azuga, Casariei Valley, 1025 m, 45°26.868'N, 25°34.260'E, 20.June.2013, 1 ♂, leg. E. Török & L. Keresztes; Bucegi Mountains, Moroeni, Dichan Hut, 1575 m, 45°19.506'N, 25°27.294'E, 26.July.2013, 1 ♂, leg. L. Keresztes & Á. Péter; Baiu Mountains, Azuga, Limbășel Valley, 1200 m, 45°29.574'N, 25°35.910'E, 26.Aug.2014, 3 ♂♂, leg. L.P. Kolcsár; Bucegi Mountains, Moroeni, Cheile Orzei, 1366 m, 45°17.682'N, 25°25.434'E, 18.Aug.2015, 2 ♂♂, leg. L. Keresztes. All materials are stored in 96% ethanol and deposited in the Diptera Collection of the Faculty of Biology and Geology, Cluj-Napoca, Romania.

Etymology. The species is named after an ancient population from the southern border of the Carpathians, suggesting its ancient origin, which is revealed by deep genetic and morphological structuring.

Diagnosis. The new species is distinguished from all other species of the *Pedicia staryi* group by the following combination of characteristics: all flagellomeres are almost uniformly colored; the abdominal stripe starts from the first segment; the tip of last palpus segment is the same color as the other segments; 9th tergite has a rounded or rarely five angled median lobe, with a notch on the tip or rarely with a small apical emergence.

Description. Large species of a yellowishorange color (Fig. 6). Males body length is 13–15 mm, (mean 14.2 mm, n=7), wing length 13–14.5 mm (mean =13.9 mm, n=7), antenna 1.6–1.9 mm (mean 1.7, n=5). The head has vertex yellowish orange to dark brown (Fig. 25). Antenna is 13–15 segmented; scape and pedicel are yellowish brown to light brown, flagellomeres are almost uniformly yellowish brown. Scape is cylindrical approximately 2 times longer than width, pedicel wider apically than basally, 1.2–1.5 times longer than width in middle. F1 and f2 fused in some cases, then first segment 2 times longer than width (else 1.2 times), f2–f6 approximately oval, f7–f12 fusiform, last one or two segments elongated or orb-like. All flagellomeres with 4–6 black bristles, about half as long as the flagellomeres. Palpus 5–segmented; 2–4 palpomeres dark brown, the ventral parts lighter and more membranous than dorsal parts; the basal part of the last palpomere is light brown, dark brown at tip. Rostrum is slightly darker than vertex, margin of the labellum brown. Lateral parts of the thorax are yellowish orange, only the front margin of the katepisternum is light brown in some cases. Scutum is dark orange, with two longitudinal hair lines. Center of postnotum is yellowish orange, lateral margin is dark orange to light brown. Wing venation is yellowish brown (Fig. 6). Pterostigma is light orange, less visible. Small spots are present at Sc2 and around R2, spots are not visible around r–m and base of Rs, only the venation is slightly darkener. Halters have yellowish stem and orange knob. The legs femora is light brown and tibiae yellowish brown, and both are black at tips. Tarsi are light brown to dark brown. Dorsally on the abdomen is a pale to dark brown longitudinal stripe starting from the first abdominal segment. The 7th and 8th sternites and tergites are light brown. Male terminalia is considerably broad (Figs 8, 9). The 9th tergite is generally darker than the remaining parts of the hypopygium. Posterior margin of 9th tergite has a rounded or rarely five angled median lobe, with a notch on the tip or rarely with a small apical emergence. Gonocoxite is stout, cylindrical, and truncated at distal end (Fig. 7). Flat spoon-like extension is present at the distal end of gonocoxite ventrally, directed inwardly, covered densely in short black spinules and partly hides the gonostylus in ventral view. Gonostylus is subterminal, inserted laterally at distal inner side of the gonocoxite, forming a nearly right angle with the latter. Gonostylus is generally quadrangular in dorsal or ventral views, with 7–11 strong black spines mostly situated at outer distal margin and with short slender projection at lower (caudal) margin distally, which is less conspicuous in some cases. Interbase simple, broadened and rounded distally.

Female is unknown.

***Pedicia* (*Crunobia*) *costobocica* Kolcsár, Keresztes & Dénes, sp. n.**

http://zoobank.org/98237E45-3EF8-4F65-8462-1087DA5DFEB1
BOLD accession number: EUTIP695, EUTIP698 and EUTIP708
Figs 10, 11, 12, 13

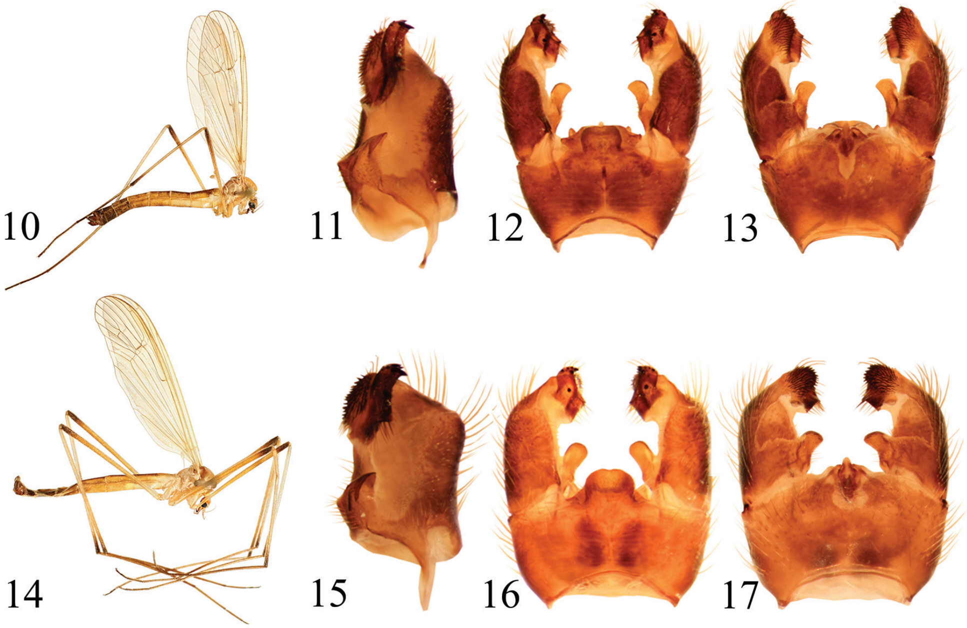
Type material. Holotype male and paratype male collected in Romania, Gutâi Mountains, Baia Sprie, Arinieşi brook, 1015 m, 47°43.068'N, 23°44.628'E, 15.May.2014, leg. L.P. Kolcsár. The pinned dry holotype (CN: TI104) and paratype (CN: TI105) are deposited in the Museum of Zoology of the Babeş–Bolyai University (MZBBU), Cluj Napoca, Romania.

Other material. Romania: Gutâi Mountains, Baia Sprie, Gutâi Pass, 1070 m, 47°41.898'N, 23°49.128'E, 26.May.2012, 2 ♂♂, leg. E. Török, L.P. Kolcsár & L. Keresztes; Gutâi Mountains, Baia Sprie, Gutâi Pass, 47°41.634'N, 23°47.226’, 15.May.2013, 1 ♂ 1 ♀, E. Török & L. Keresztes; Gutâi Mountains, Baia Sprie, Arinieşi brook, 1015 m, 47°43.068'N, 23°44.628'E, 15.May.2014, 7 ♂♂, leg. L.P. Kolcsár. All material is stored in 96% ethanol and deposited in the Diptera Collection of the Faculty of Biology and Geology, Cluj-Napoca, Romania.

Etymology. The species is named after an ancient population from the northern part of the Eastern Carpathians suggesting its ancient origin revealed by deep genetic and morphological structuring.

Diagnosis. The new species is distinguished from all other species of the *Pedicia staryi* group by the following combination of characteristics: the last 1–2 antennal segments are darker than others; black line between antennae; the abdominal stripe starts from the second segment; mean body size reach 13 mm; pedicel and scape darker than first flagellomere, face dark brown; 9th tergite has a rounded median lobe, usually with a notch on the tip.

Description. Medium sized species of a yellowish orange color (Fig. 10). **Male** body length is 10–14 mm, (mean 12.9 mm, n=8), wing length 11–14.5 mm (mean =13 mm, n=8), antenna 1.7 mm (mean 1.7, n=5). The head has vertex dark orange to light brown, the frontal part is brown mostly around the antennas and a narrow dark line is present between antennae. Antenna is 15–16 segmented; scape and pedicel is light brown, flagellum is almost uniformly yellowish, only the last 1–2 segments are darker. Scape is cylindrical approximately 2 times longer than width; pedicel is wider apically than basally, 1.2–1.5 times longer than width in the middle. First flagellomere is 1.3–1.4 times longer than width, flagellomeres 2 to 11 are approximately oval, apical flagellomeres are more elongated. Border between flagellomeres 12 and 13 is less distinct, frequently merge together; flagellomere 13 is elongated. All flagellomeres have 4–6 black bristles, about half as long as flagellomeres. Palpus is 5–segmented; 2–4



Figures 10–17. 10–13 *Pedicia costobocica* sp. n. holotype male: **10** lateral habitus **11** inner lateral view of the gonocoxite **12** male hypopygium dorsal view **13** male hypopygium ventral view **14–17** *Pedicia carpianica* sp. n. holotype male: **14** lateral habitus **15** inner lateral view of the gonocoxite **16** male hypopygium dorsal view **17** male hypopygium ventral view.

palpomeres are dark brown, the ventral parts are lighter and more membranous than dorsal parts; the last palpomere is dark brown only in the basal part and at tip. Rostrum is slightly darker than vertex, margin of the labellum is brown. Dorsal and lateral parts of thorax are yellowish orange. Scutum is orange, with two yellowish longitudinal lines of setae. Wing has yellowish brown venation (Fig. 10). Pterostigma is light orange, less visible. Small spots are present at Sc2, at base of Rs, around r–m and around R2. Halters with yellowish stem and dark orange knob. Legs have femora light brown and tibiae yellowish brown, and both are black at the tip. Tarsi are light brown to dark brown. First abdominal segment yellowish orange, sometimes the posterior margin brown. Dark brown longitudinal stripe in the abdominal tergum starts from the second segment. The 7th and 8th sternites and tergites are dark brown. The male terminalia is considerably broad (Fig. 12, 13). The 9th tergite is generally darker than the remaining parts of hypopygium. Posterior margin of 9th tergite has a rounded median lobe, usually with a notch on the tip. Gonocoxite is stout, cylindrical and narrowing at distal end (Fig. 11). A flat spoon-like extension is present at the distal end of gonocoxite ventrally, directed inwards, densely covered with short black spinules. Gonostylus is subterminal, inserted laterally at the distal inner side of gonocoxite, forming a nearly right angle with the latter. Gonostylus is generally quadrangular in dorsal or ventral views, with 6–9 strong black spines mostly situated at outer distal margin and distally with short slender projection at lower (caudal) margin, which is less conspicuous in some cases. Interbase is simple, broadened and rounded distally.

Female has a body length of 12 mm, wing length 11 mm, antenna 1.6 mm. General color is yellowish. Head is dark orange, frontal part is light brown mostly around the antennas, a narrow dark line is present between antennas. Pedicel and scape are dark orange. The antenna has 14 yellowish orange flagellomeres. Palpus is uniformly yellowish orange. The dorsal and lateral parts of the thorax are yellowish. Legs have coxae and trochanters yellowish orange. Wings are well-developed, having the ability to fly. Wing venation is yellowish orange, small spots are present at Sc2, and at around r–m. Abdomen is uniformly light orange. Female terminalia has wide cercus, dagger-like, with tip raised upward. Hypovalvae are each wide, darker than the tenth sternite with nine pairs of curved knitting needle-like strong setae at the dorsal margin. The distal seta is isolated from the rest. Two pairs of sensory setae are visible at the end of the hypovalvae, and they extend beyond the end of hypovalvae.

***Pedicia* (*Crunobia*) *carpianica* Kolcsár, Keresztes & Dénes, sp. n.**

http://zoobank.org/3180ABB8-A7CC-4A56-9ABA-BEF6E119C821
Gen Bank accession number: KT983904 to KT983906; BOLD accession number: EUTIP095, EUTIP096, EUTIP475, EUTIP478 and EUTIP480
Figs 14, 15, 16, 17, 27

Type material. Holotype male and two paratypes males collected in Romania, Rodnei Mountains, Anieș, Tomnaticul Valley, 700 m, 47°27.768'N, 24°45.696'E, 19.Aug.2014, leg. L.P. Kolcsár. The pinned dry holotype (CN: TI106) and paratypes (CN: TI107, TI108) are deposited in the Museum of Zoology of the Babeș–Bolyai University (MZBBU), Cluj Napoca, Romania.

Other material. Romania: Rodnei Mountains, Rodna, Vinului Valley, 1000 m, 47°30.918'N, 24°50.094'E, 15.Aug.2008, 2 ♂♂, leg. L. Keresztes; Rodnei Mountains, Cormaia, Cormaia Valley, 750 m, 47°26.328'N, 24°39.702'E, 26.Aug.2010, 11 ♂♂, leg. R. Vaida; Rodnei Mountains, Anieș, Izvorul Mare Valley, 1220 m, 47°32.274'N, 24°40.362'E, 28.July.2011, 1 ♂, leg. R. Vaida; Rodnei Mountains, Anieș, Cepelor Spring, 1165 m, 47°31.404'N, 24°45.024'E, 19.Aug.2014, 3 ♂♂ leg. L.P. Kolcsár; Rodnei Mountains, Anieș, Tomnaticul Valley, 700 m, 47°27.768'N, 24°45.696'E, 19.Aug.2014, 11 ♂♂ leg. L.P. Kolcsár; Rodnei Mountains, Valea Mare, Rotunda Pass, 1165 m, 47°31.812'N, 25°0.810'E, 19.Aug.2014, 1 ♂, leg. L.P. Kolcsár. Material is stored in 96% ethanol or pinned dry and deposited in the Diptera Collection of the Faculty of Biology and Geology, Cluj-Napoca, Romania.

Etymology. The species is named after an ancient population from the Eastern Carpathians suggesting its ancient origin revealed by deep genetic and moprhological structuring.

Diagnosis. The new species is distinguished from all other species of the *P. staryi* group by the following combination of characteristics: the last 1–2 antennal segments are darker than the others, black line between antennae; the abdominal stripe starts from the second segment; mean body size reaches 15.4 mm; pedicel and scape have the same color as the first flagellomeres; only the lump is darker than other parts of the head; 9th tergite has a rounded or five angled median lobe, usually with a notch on the tip.

Description. Large species with yellowish orange color (Fig. 14). **Male** body length is 13–17 mm, (mean 15.4 mm, n=13), wing length 13.5–17 mm (mean =15.4 mm, n=13), antenna 1.9–2.1 mm (mean 1.98, n=7). Head has vertex light brown with light yellow setae. Rostrum is slightly darker than vertex, margin of the labellum is dark brown, a narrow dark (grayish brown in the case of dry specimens) line present between antennas. Antenna is 14–16 segmented, almost uniformly yellowish, only the last 2–3 segments are darker. Scape is cylindrical, approximately 2 times longer than width, pedicel slightly wider apically than basally, 1.2–1.5 times longer than its width in the middle. First flagellomere (f1) fusiform, f2–f11(13) oval, only the last (f14) is cylindrical. All flagellomeres with 4–6 black bristles, about half as long as the flagellomeres. Palpus is 5–segmented, 2–4 palpomeres are dark brown, the ventral parts lighter and more membranous than the dorsal parts; the last palpomere dark brown at the base and tip. The dorsal and lateral parts of the thorax are yellowish orange. Scutum is orange, with two yellowish longitudinal lines of setae. In few cases the lateral margin of the postnotum is dark orange or light brown. Wing venation is yellowish brown (Fig. 14). Pterostigma is light orange, less conspicuous. Small spots present around Sc2, at the base of Rs, r–m and around R2 and R1, but in some cases less visible at the end of R1, only the venation is darker. Halters have yellowish stem and dark orange knob. Legs have light brown femora and yellowish brown tibiae, and are both black at the tip. Tarsi are light brown to dark brown. First abdominal segment yellowish orange, sometimes the posterior margin brown (Fig. 27). The dark brown dorsal longitudinal stripe on the abdomen starts from the second segment (Fig. 27). The 7th and 8th sternites and tergites are dark brown. Male terminalia is considerably broader (Figs 16, 17). The 9th tergite generally darker than the remaining parts of hypopygium. Posterior margin of 9th tergite has a rounded or five angled median lobe, usually with a notch on the tip. Gonocoxite is stout, cylindrical, and truncated at the distal end (Fig. 15). A flat spoon-like ventral extension is present at the distal end of gonocoxite, directed inwardly and densely covered with short black spinules. Gonostylus has subterminal position, inserted laterally at the distal inner side of gonocoxite and forming a nearly right angle with the long axis of the latter. Gonostylus is generally quadrangular in dorsal or ventral view, with 6–9 strong black spines mostly situated at the outer distal margin and with a short slender projection at the lower (caudal) margin distally. Interbase is simple, broadened and rounded distally.

Female is unknown.

***Pedicia* (*Crunobia*) *staryi* Savchenko, 1978, redescription**

Gen Bank accession number: KT983907 to KT983910; BOLD accession number: EUTIP709

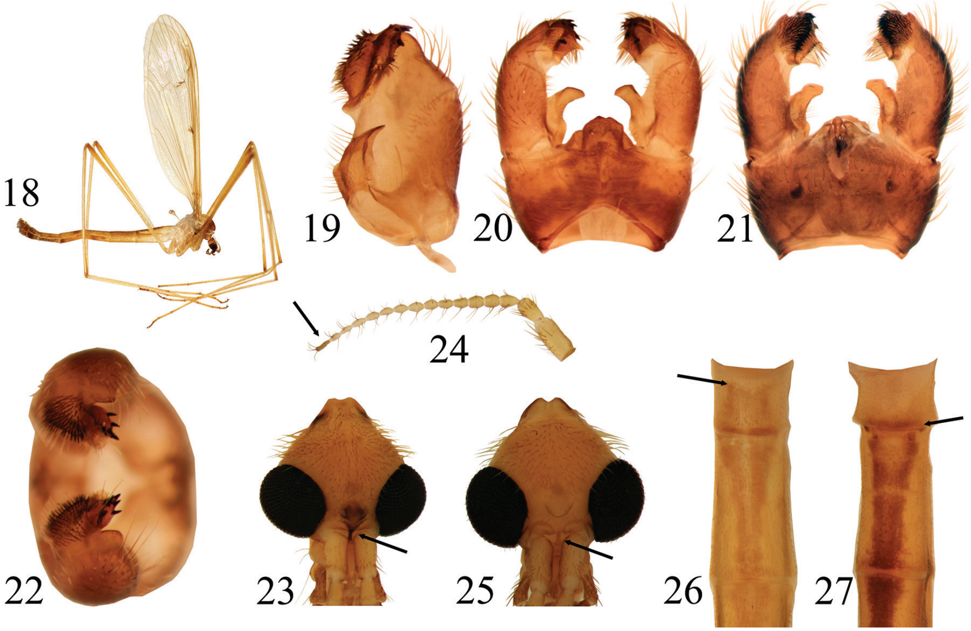
Figs 18, 19, 20, 21, 22, 23, 24

Type material. Holotype male Ukraine: Ivano–Frankivsk Oblast, environs settlements Vorokhta, Verkhovinsk region, 18.VI 1962 leg. E.N Savtshenko (Savchenko); deposited in the National Museum of Natural History, Kiev, Ukraine. Based of photos taken by Valery A. Korneyev.

Other material. Ukraine: Gorgan Mt., Bukovel, 1120 m, 48°23.340'N, 24°26.010'E, 29.July.2014, 21 ♂♂ 1 ♀, leg. E. Török & L.P. Kolcsár. Romania: Rodnei Mountains, Borșa, Cailor Waterfall, 1700 m, 47°35.292'N, 24°39.510'E, 16.June.2010, 1 ♂, leg. R. Vaida; Maramureș Mountains, Borșa, Vișeu River, 1000 m, 47°37.374'N, 24°48.582'E, 17.May.2013, 1 ♂ leg. E. Török; Rodnei Mountains, Gura Lalei, Lalei Valley, 1200–1800 m, 47°32.070'N, 24°54.930'E, 20.July.2013, 13 ♂♂, leg. L. Keresztes & L.P. Kolcsár; Maramureș Hills, Leordina, 490 m, 47°46.752'N, 24°14.574'E, 17.May.2014, 3 ♂♂, leg. L.P. Kolcsár; Maramureș Mountains, Repedea, Repedea Valley, 790 m, 47°53.202'N, 24°23.418'E, 17.May.2014, 20 ♂♂ 1 ♀, leg. L.P. Kolcsár; Maramureș Mountains, Gura Lalei, Bistrița River, 1025 m, 47°33.858'N, 25°1.800'E, 20.Aug.2014, 5 ♂♂ leg. L.P. Kolcsár. Material stored in 96% ethanol or pinned dry and is deposited in the Diptera Collection of the Faculty of Biology and Geology, Cluj-Napoca, Romania.

Diagnosis. The species is distinguished from all other species of the *Pedicia staryi* group by the following characters combination: the last 1–3 antennal segments are darker than others; black line between antennae; two brown spots on the scutum; abdominal dorsal stripe starts from first abdominal segment; 9th tergite has a rounded or five angled median lobe, sometimes with a notch on the tip.

Redescription. Large species having general color yellowish orange (Fig. 18). **Male** body length is 13–16 mm, (mean 14.2 mm, n=20), wing length 13–15 mm (mean =14.1 mm, n=20), antenna 1.9–2.1 mm (mean 1.95, n=9). Head has vertex light brown (specimens from Maramureș Mountains and Rodnei Mt.) or yellowish orange (specimens from Gorgan Mt.), with rostrum light brown to brown mostly around the antennas, a narrow dark (greyish brown in the case of dry specimens) line present between antennas (Fig. 23). Antenna is 16–segmented; almost uniformly yellowish, only the last 1–3 segments are darker (Fig. 24). Scape is cylindrical approximately 2.5 times longer than wide; pedicel is little wider apically than basally, 1.2–1.5 times longer than wide in the middle. First flagellomere (f1) is narrower than f2, f2–f6 are approximately ovals, f7–f12 are fusiforms, f13–14 cylindricals. All flagellomeres have 4–6 black bristles, about half as long as the flagellomeres. Palpus is 5–segmented; 2–4 palpomeres are dark brown, the ventral parts are lighter and more membranous than dorsal parts; the last palpomere is dark brown in the basal and at the tip. Lateral parts of the thorax are yellowish orange, only the frontal margin of the katepisternum is light brown in some cases. Scutum is dark orange, with two longitudinal lines of setae. Savtshenko



Figures 18–27. 18–24 *Pedicia staryi* male: **18** lateral habitus **19** inner lateral view of the gonocoxite **20** male hypopygium dorsal view **21** male hypopygium ventral view **22** male hypopygium caudal view **23** head dorsal view **24** antenna **25**–**26** *Pedicia roxolanica* sp. n.: **25** head dorsal view **26** dorsal view of the cranial part of abdomen **27** *Pedicia carpianica* sp. n.: dorsal view of the cranial part of abdomen.

(1978) mentions in the original description of the species the presence of an ochre yellow longitudinal stripe on the prescutum. However, this feature is visible only in some dry preserved specimens. Two diffuse dark orange to brown spots are presents near the scutellum. Postnotum is yellowish orange with an oval brown spot, which is lighter in the middle and sometimes a light line separates it in two parts. Wing has yellowish brown venation (Fig. 18). Pterostigma is light orange, less conspicuous. Small spots are present at Sc2, at base of Rs, at fork of Rs (mostly around r–m) and around R2. The spots at the base of Rs and around r–m are less visible, only the venation is darker. Halters stems are yellowish and knobs are dark orange (uniform yellowish orange in case of specimens stored in alcohol). Legs have femora dark orange to light brown and tibiae are yellowish brown, both black at the tip. Tarsi are light brown to dark brown. A light brown to dark brown longitudinal stripe is present on the abdomen, positioned dorsally to first segment. The 7th and 8th sternites and tergites are dark brown. Male terminalia is considerably broader than abdominal segments (Figs 20, 21). The 9th tergite is generally darker than remaining parts of hypopygium. Posterior margin of 9th tergite has a rounded or five angled median lobe, sometimes with a notch on the tip. Gonocoxite is stout, cylindrical, and truncated at distal end (Fig. 19). A flat spoon-like extension is present ventrally at the distal end of gonocoxite, directed inwardly, and densely covered with short black spinules. Gonostylus is subterminal, inserted laterally at distal inner side of gonocoxite and forming a nearly right angle with the long axis of the latter. Gonostylus is generally quadrangular in dorsal or ventral views, with 5–8 strong black spines, mostly situated at the outer distal margin and with short slender projection at lower (caudal) margin distally. Interbase simple, broadened and rounded distally. **Female** body length is 16.5–17 mm, wing length 12–13 mm, antenna 1.7 mm. Head is uniformly dark orange. Pedicel, scape and flagellomeres are yellowish orange, only the last flagellomere is darker. Palpus is light brown. The dorsal and lateral parts of the thorax are yellowish. Legs have coxae and trochanters yellowish orange. Femora, tibia and tarsomeres (I–IV) are orange. Last tarsomere (V) is black. Wings are developed, and females are able to fly. Wing venation is yellowish orange, having no spots on the wing. Abdomen is dark orange, only the first and second tergite are light brown. Female terminalia has wide cerci, widest in middle and raised upward towards the tip. Hypovalvae are each wide, darker than the tenth sternite. Dorsal margin has 7–8 pairs curved knitting needle-like strong setae. One seta is distally isolated from the rest. Sensory setae are visible in hypovalvae, and they extend beyond the end of hypovalvae.

Key to species of *Crunobia* subgenus

1 Gonostylus has dorsally only two or three big thorn-like dark spines (Figs 29, 33, 40, 41) **2**

– Gonostylus has dorsally 5–11 claw-like dark spines (Figs 2, 7, 22, 46, 47) .... (*staryi* species group) **11**

2 Gonostylus extends ventrally and forms a big obtuse projection, densely covered by black spinule (Figs 29, 31, 41, 43) **3**

– Gonostylus without such projection (Figs 33, 35) **8**

3 Wings strongly reduced, much shorter than the abdomen .... ***P.* (*C.*) *semireducta* Savchenko, 1978**

– Wings well-developed and as long or sometimes longer than the abdomen (Fig. 28) **4**

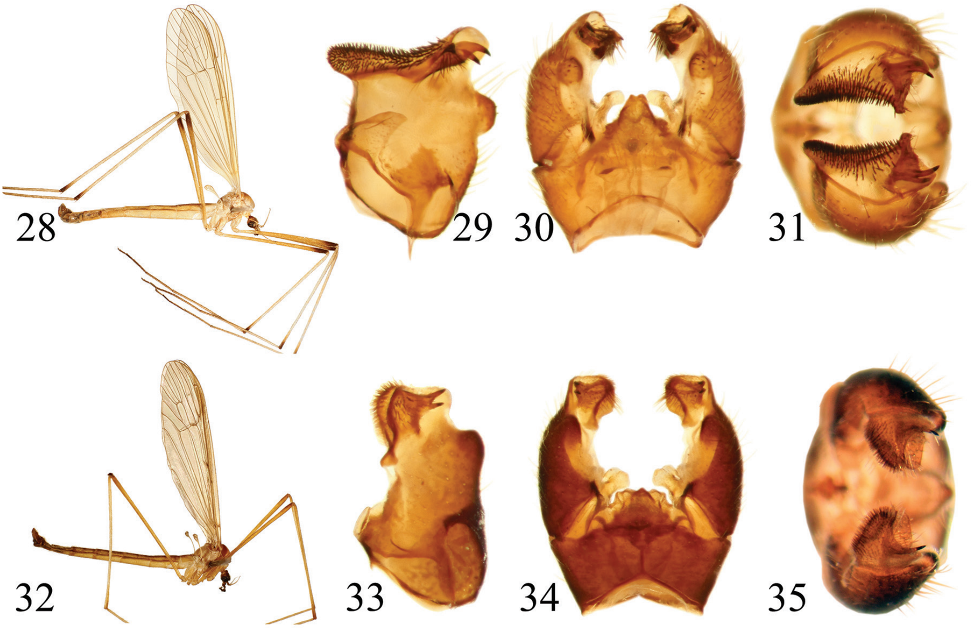
4 Gonostylus ventral projection short, not wider than gonocoxite, 9th abdominal tergite with a small triangular lobe with a big notch in the middle of the distal margin (Fig. 52) .... ***P.* (*C.*) *tjederi* Mendl, 1974**

– Gonostylus ventral projection long, wider than the gonocoxite, 9th abdominal tergite is differently shaped (Figs 29, 30, 31, 41, 42, 43) **5**

5 Gonostylus ventral projection (in caudal view) is densely covered by black spinule only in the ventral parts (Fig. 43); 9th abdominal lobe without notch (Figs 41, 42) .... ***P.* (*C.*) *zangheriana* Nielsen, 1950**

– Gonostylus ventral projection (in caudal view) uniformly covered by black spinule (Fig. 31); 9th abdominal lobe has a notch (Fig. 30) **6**

6 Lateral side of scutum, prescutum and wings' base is lighter (Fig. 28) .... ***P.* (*C.*) *pallens* Savchenko, 1978**



Figures 28–35. 28–31 *Pedicia pallens* male: **28** lateral habitus **29** inner lateral view of the gonocoxite **30** male hypopygium dorsal view **31** male hypopygium caudal view **32–35** *Pedicia littoralis* male: **32** lateral habitus **33** inner lateral view of the gonocoxite **34** male hypopygium dorsal view **35** male hypopygium caudal view.

– Lateral side of scutum, prescutum and wings' base is darker 7

7 Wings are transparent, with more or less conspicuous pattern, 9th abdominal tergite is narrow, narrower at its base (Fig. 54).. **P. (C.) *persica* Alexander, 1975**

– Wings are yellowish, only with an obscure dark pattern; 9th tergite wider at its base.. **P. (C.) *zernyi* (Lackschewitz, 1940)**

8 Ochre–yellow species. Femora uniformly colored, the tip of femora without dark rings (Fig. 32) ....**P. (C.) *littoralis* (Meigen, 1804)**

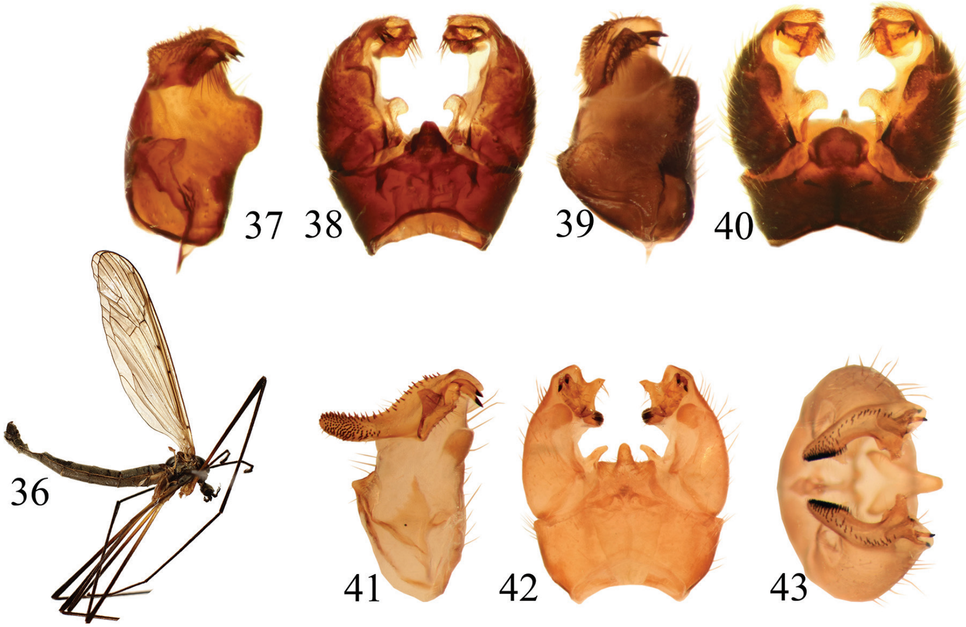
– Dark colored species. Femora brown, dark–brown, basally lighter (Fig. 36) **9**

9 Thorax mainly brownish yellow, with brown or yellowish brown prescutal stripes, 9th abdominal tergite lobe with a big notch (Fig. 55)..**P. (C). *dispar* Savchenko, 1978**

– Thorax mainly gray, with grayish–brown prescutal stripes; 9th abdominal tergite lobe without a notch (Figs 38, 40) **10**

10 Medial lobe of the 9th abdominal tergite triangular, narrowed towards the tip (Figs 39, 40). Wings usually grayish.... **P. (C.) *riedeli* (Lackschewitz, 1940)**

– Medial lobe of the 9th abdominal tergite hexagonal, slightly narrow at the base. Wings usually yellowish (Figs 36, 37, 38) ......**P. (C.) *nielseni* (Slipka, 1955)**



Figures 36–43. 36–38 *Pedicia nielseni* male: **36** lateral habitus **37** inner lateral view of the gonocoxite **38** male hypopygium dorsal view **39**–**40** *Pedicia riedeli*: **39** inner lateral view of the gonocoxite **40** male hypopygium dorsal view **41–43** *Pedicia zangheriana* male: **41** inner lateral view of the gonocoxite **42** male hypopygium dorsal view **43** male hypopygium caudal view.

11 Gonostylus extended ventrally and forms a big obtuse projection (Figs 44, 45, 46, 47) .... ***P.* (*C.*) *straminea* (Meigen, 1838)**

– Gonostylus without this projection (Figs 3, 7, 11, 15, 19, 22, 48, 50) **12**

12 Interbases with a spine-like outgrowth (Figs 48, 49) .... ***P.* (*C.*) *spinifera* Starý, 1974**

– Interbases without spine-like outgrowth (Fig. 4, 20, 51) 13

13 Gonocoxite has on the top a conspicuous, isolated rounded lobe (figs 50, 51) .... ***P.* (*C.*) *lobifera* Savchenko, 1986**

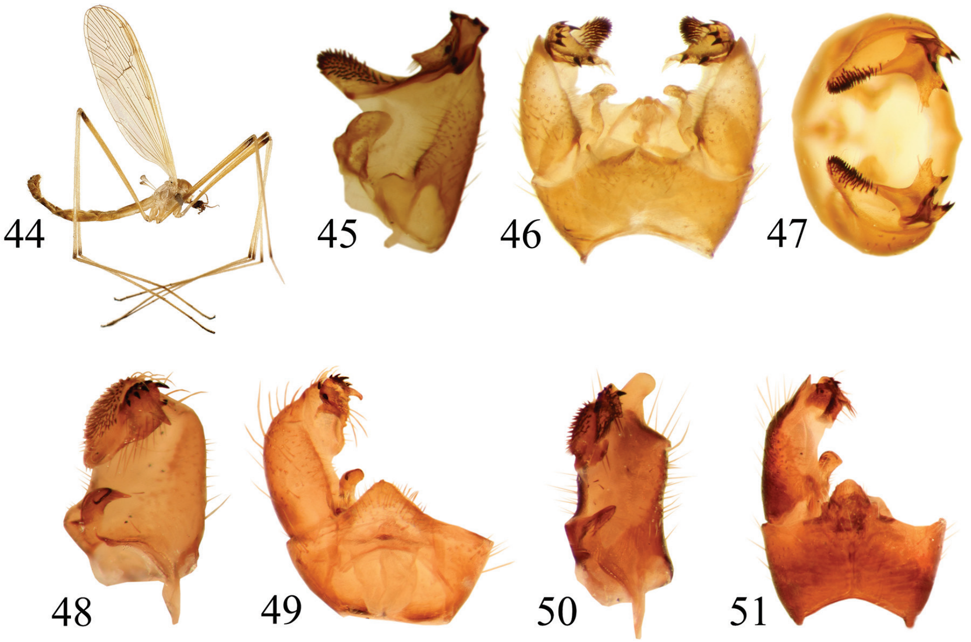
– Gonocoxite on the top without such rounded lobe (Figs 3, 7, 11, 15 19) **14**

14 All flagella almost uniformly colored, none darker, no black or dark brown line between antennae (Fig. 25) **15**

– The last 1–3 antennal segments are darker than others (Fig. 23), black or dark brown line between antennae (Fig. 24) **16**

15 Abdominal dorsal stripe starts from the second abdominal segment (Fig. 27); tip of the 5th palpus segment darker than other segments; 9th tergite has a rounded median lobe, with a small apical emersion (Figs 4, 5)...................... .... ***P.* (*C.*) *apusenica* Ujvárosi & Starý, 2003**

– The abdominal stripe starts from the first segment (Fig. 26); tip of 5th palpus segment is the same color as the other segments; medial lobe of 9th abdominal

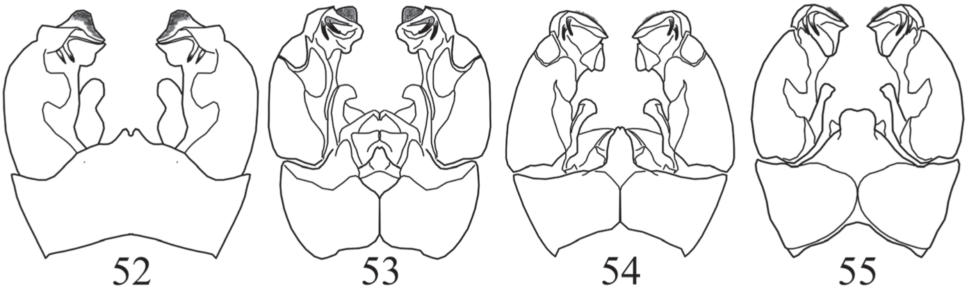


Figures 44–51. 44–47 *Pedicia straminea*: **44** male lateral habitus **45** inner lateral view of the gonocoxite **46** male hypopygium dorsal view **47** male hypopygium caudal view **48–49** *Pedicia spinifera*: **48** inner lateral view of the gonocoxite **49** male hypopygium dorsal view **50–51** *Pedicia lobifera*: **50** inner lateral view of the gonocoxite **51** male hypopygium dorsal view.

tergite rounded or with a notch on the tip, or rarely with a small apical emergence (Figs 8, 9)...... ***P.* (*C.*) *roxolanica* Kolcsár, Keresztes & Dénes, sp. n.**
16 Two brown spots on the scutum, abdominal dorsal stripe starts from first abdominal segment (Fig. 26) .... ***P.* (*C.*) *staryi* Savchenko, 1978**
– No brown spots on scutum, stripe starts from second abdominal segment (Fig. 27)..**17**
17 Smaller species, mean body size reach 13 mm; pedicel and scape darker than first flagellomere, face dark brown.. .... ***P.* (*C.*) *costobocica* Kolcsár, Keresztes & Dénes, sp. n.**
– Larger species, mean body size is 15.4 mm; pedicel and scape is the same color as the first flagellomere, only the lump is darker than other parts of the head........................ ***P.* (*C.*) *carpianica* Kolcsár, Keresztes & Dénes, sp. n.**

Discussion

The three newly described species, *P. costobocica*, *P. carpianica* and *P. roxolanica* can clearly be attributed to the "*staryi*" species group *sensu* Savchenko (1986) because they have more than two black spines on the top of the gonostylus. In concordance with



Figures 52–55. *Pedicia tjederi*: **52** male hypopygium dorsal view *Pedicia semireducta*: **53** male hypopygium dorsal view *Pedicia persica*: **54** male hypopygium dorsal view *Pedicia dispar*: **55** male hypopygium dorsal view.

the previous taxonomic hypotheses, the Maximum Likelihood and Bayesian Inference phylogenetic analyses also support the *P. staryi* group as a monophyletic unit. However, the six Carpathian endemics, *P. apusenica*, *P. lobifera*, *P. staryi*, and the newly described *P. costobocica* sp. n., *P. carpianica* sp. n. and *P. roxolanica* sp. n. form a well-defined and highly distant clade from the other two members of the group, with an average genetic distance of 12.81% in the case of *P. straminea* and 12.67% in the case of *P. spinifera.* Such deep genetic structures suggest autochthonous evolutionary histories with ancient divergences in the Carpathian Area (see also Dénes et al. 2015). Our genetic results are highly congruent with the morphological analyses. The newly described species are closely related to *P. staryi* and *P. apusenica*, by a combination of important morphological features. Morphology and genetic comparison show similar patterns. *Pedicia apusenica* and *P. roxolanica* are unified in a separate group, differing from the remaining species by the uniformly colored antennae and the lack of a dark line between them. Genetic results also suggest more similarity between *P. apusenica* and *P. roxolanica*. *Pedicia staryi*, *P. costobocica* and *P. carpianica* share highly similar features, but *P. staryi* is distinguished from *P. costobocica* and *P. carpianica* by the presence of two brown spots on the scutum and by the abdominal dorsal stripe that starts from the first segment.

The rhitral biome in Central Europe is home to an exceptionally high aquatic diversity with an important number of endemics, in contrast with the arboreal elements, which are related mostly with some classical Mediterranean core areas (Mey and Botoşăneanu 1985, Pauls et al. 2009, Bálint et al. 2011). The rithral biome of the Alps and the Pyrenees has been intensively explored over the course of the past few years; however, the Carpathians are constantly neglected, despite their basic role as important hot spots of aquatic diversity in Europe (Bálint et al. 2008, Stewart et al. 2010, Schmitt and Varga 2012, Graf et al. 2014, Török et al. 2015). The geographic projection of the newly discovered species suggests insular-like distribution in the Carpathians. The pattern is similar to the distribution of the already recognized *P. apusenica*, *P. staryi* or *P. lobifera*, and it represents new evidence concerning the importance of the Carpathians as a significant genetic center of aquatic diversity. The northern part of the Eastern Carpathians is exceptionally rich in endemics, as suggested by a high number of other aquatic insects, such as caddisflies (Bálint et al. 2011). In our case, 4 species (*P. staryi*, *P. apusenica*, *P. costobocica*, *P. carpianica* and *P. lobifera*) belonging to the *P. staryi* group are exclusive inhabitants of this important northern refuge-like area, confined between the western limit of the northern Carpathians in Ukraine and the Haghimaș Mountains in Romania, where they are isolated in one or a few limited enclaves (the refugia within refugia pattern, Varga 2010). The Apuseni Mountains harbor an exceptionally high terrestrial diversity (ex. earth worms) (Csuzdi and Pop 2007), due to their isolated position from the rest of the Carpathians. The aquatic diversity however, is lower (ex. caddis flies) (Ujvárosi et al. 2008)hence they are home to only one endemic species from the group under examination: *P. apusenica*. In the Southern Carpathians, the eastern part shelters a single endemic species of the group: *Pedicia roxolanica*. Despite its role as an important genetic center for a series of terrestrial (Varga 2010) and aquatic species (Bálint et al. 2009, Ujvárosi et al. 2008), the western part of the Southern Carpathians has no endemic members belonging to the *staryi* species group. Most of the newly discovered microendemic species of the Carpathians are highly specialized rithral elements concentrated near the sources of cold stenotherm springs, with an important degree of hitherto undiscovered diversity.

Acknowledgements

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Supplementary material 1

Sampling information

Authors: Avar-Lehel Dénes, Levente-Péter Kolcsár, Edina Török, Lujza Keresztes

Data type: Collection data

Explanation note: Information for sampling of the five microendemic Carpathian species (*Pedicia lobifera*, *P. staryi*, *P. apusenica*, *P. carpianica*, *P. costobocica*, *P. roxolanica*), containing the name of the species, the date of collection, with localities, mountains, locations and geographic coordinates

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RESEARCH ARTICLE

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A new species of *Peckoltia* from the Upper Orinoco (Siluriformes, Loricariidae)

Jonathan W. Armbruster[1], Nathan K. Lujan[2]

1 *Department of Biological Sciences, 101 Life Sciences Building, Auburn University, AL 36849, USA*
2 *Department of Biological Science, University of Toronto Scarborough, Toronto, ON M1C1A4, Canada*

Corresponding author: *Jonathan W. Armbruster* (armbrjw@auburn.edu)

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Abstract

A new species of the suckermouth armored catfish genus *Peckoltia* is described from the lower Ventuari River, a tributary of the upper Orinoco River in Amazonas State, Venezuela. Specimens of this species were formerly included in the wide-ranging Amazonian species *P. vittata*, but a recent molecular phylogeny found Orinoco individuals to be distantly related to Amazon Basin individuals spanning the range of *P. vittata* syntypes. Detailed morphological examination confirmed distinctiveness of Orinoco specimens, and found them to be diagnosable from true *P. vittata* by having generally greater than 25 teeth (vs. less), spots on the nape (vs. nape lacking spots), the upper lip with two to three black bar-shaped markings in a line like a moustache (vs. lips generally with a hyaline wash), and by the snout having a medial black line disconnected from the moustache markings (vs. medial snout stripe connected to a bar just above the lip). *Peckoltia wernekei* displays remarkable genetic similarity to its sister species, *P. lujani*, but differs morphologically by having dentary tooth rows meet at an angle less than 90° (vs. >90°), by having large faint blotches on the abdomen (vs. abdomen with no blotches), by a smaller internares width (21.2–26.6% vs. 28.5–46.5% of interorbital width), and a larger dorsal spine (148.1–178.6% vs. 80.1–134.5% of abdominal length).

Keywords

Ancistrini, Hypostominae, Molecular Phylogeny, Morphology, *Peckoltia*, Systematics, Taxonomy

Introduction

Peckoltia Miranda Ribeiro, 1912 is a genus of suckermouth armored catfishes (Loricariidae) with 18 currently described species (Armbruster et al. 2015). Armbruster (2004; 2008) had restricted *Peckoltia* to those species of Ancistrini that had dentary tooth rows meeting at an angle of 90° or less and that lacked the synapomorphies of similar genera like *Hypancistrus* and *Panaqolus* (the latter then the *Panaque dentex* group); however, this arbitrary definition was not supported by the molecular analysis of Lujan et al. (2015), so Armbruster et al. (2015) recognized an expanded *Peckoltia* that currently lacks a morphological diagnosis, but is strongly monophyletic based on molecular evidence (Lujan et al. 2015).

Armbruster (2008) had recognized a wide-ranging *Peckoltia vittata* (Steindachner, 1881) that included specimens from the Maranhão to the Madeira and upper Orinoco, but suggested that this putative range of *P. vittata* included multiple species. The syntype series of *Peckoltia vittata* itself contains specimens spanning more than 600 km of the main channel of the Amazon River, from the Xingu River to the Madeira River, making it difficult to know where one might reliably find the true *P. vittata*. Specimens from the Xingu, Madeira and Orinoco that were morphologically consistent with *Peckoltia vittata* were found to be polyphyletic in Lujan et al. (2015) and Lujan et al. (in review, Fig. 1). In addition, *P. greedoi* was recognized as distinct from *P. vittata* by Armbruster et al. (2015; specimens of *P. greedoi* were originally included among the specimens of *P. vittata* examined by Armbruster 2008).

Lujan et al. (2015) found the putative specimens of *Peckoltia vittata* from the Orinoco to be strongly supported as sister to another Orinoco species, *P. lujani* Armbruster, Werneke and Tan, and part of a clade with another undescribed species known as *Peckoltia* sp. n. Meta L147. In this study, we re-examine specimens identified as *P. vittata* from the upper Orinoco and describe them as a new species clearly distinguishable from Amazon Basin *P. vittata*. In addition, we provide a key to all of the current species of *Peckoltia*.

Methods

Methods follow Armbruster (2003) with the addition of counts of mid-dorsal and mid-ventral plates (the number of plates in these series from the head to caudal fin and excluding the last, triangular plate, which is beyond the hypural). Institutional abbreviations are as in Sabaj Pérez (2014). Names of skeletal characteristics are as in Schaefer (1987) and Geerinckx et al. (2007) and of plate rows as in Schaefer (1997). Full morphometric dataset is presented in Suppl. material 1, locality information for species described below is presented in Suppl. material 2.

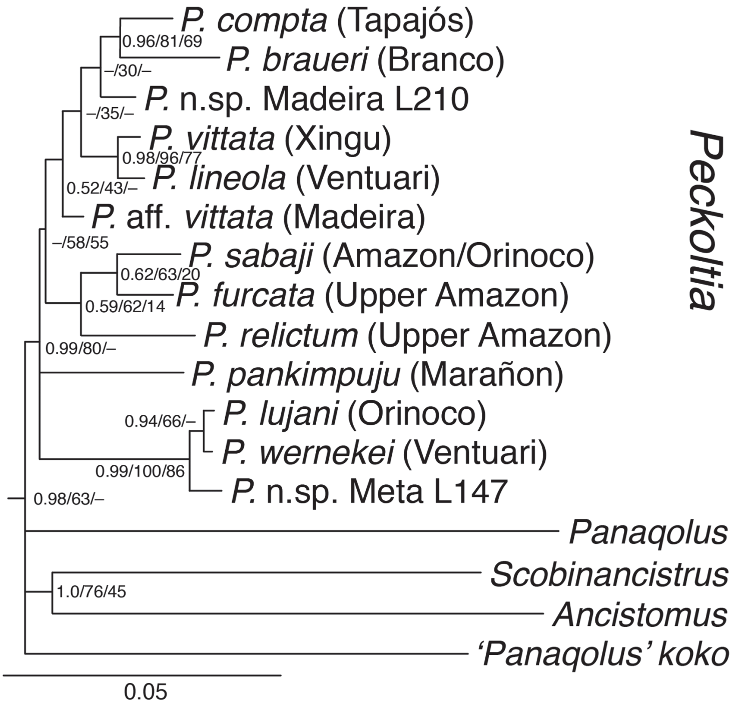

Figure 1. Phylogenetic relationships within the *Peckoltia* Clade (sensu Lujan et al. 2015), from Lujan et al. (in review). Results based on analysis of a 4293 base pair alignment consisting of two mitochondrial (16S, Cyt b) and three nuclear loci (RAG1, RAG2, MyH6). Node support values given in order as Bayesian posterior probability, maximum likelihood bootstrap and maximum parsimony bootstrap.

Taxonomy

***Peckoltia wernekei* Armbruster & Lujan, sp. n.**
http://zoobank.org/3488FBE3-34F0-4F5B-94D2-60BFA849D945
Figs 3–4 and 5b, Table 1

Peckoltia aff. *vittata* (Orinoco) Lujan et al., 2015 [molecular phylogeny]

Type locality. Ventuari River drainage, Amazonas State, Venezuela, South America

Holotype. AUM 54314, 104.6 mm SL, VENEZUELA, Amazonas State, Ventuari River drainage, Marujeta Creek, 159 km E of San Fernando de Atabapo, 04.2948°, -066.2889°, N.K. Lujan, M. Sabaj Pérez, D.C. Werneke, T. Carvalho, V. Meza-Vargas, 02 April 2010.

Paratypes. All specimens Venezuela, Amazonas State, Ventuari River drainage: AUM 39248, 1, 63.2 mm SL, Ventuari River at beach at village of Moriche, 116 km NE of Macuruco, 169 km NE of San Fernando de Atabapo, 04.7503°, -066.3549°, D.C. Werneke, N.K. Lujan, M.H. Sabaj, L.S. de Souza, 7 April 2004. AUM 39313, 13, 2 cs, 56.8–87.3 mm SL, Manapiare River, 14.5 km NW of San Juan de Manapi-

Table 1. Selected morphometrics of *Peckoltia wernekei*. Numbers in parentheses refer to landmark numbers in Armbruster (2003).

	Holotype	N	Mean	SD	Min	Max
SL, mm (1–20)	104.6	23	70.2		52.6	104.6
%SL						
Predorsal Length (1–10)	38.8	23	42.2	1.5	38.8	45.2
Head Length (1–7)	36.5	23	35.5	1.2	32.5	37.5
Head–dorsal Length (7–10)	6.7	23	6.5	0.7	5.0	7.9
Cleithral Width (8–9)	26.4	23	28.5	1.2	25.6	30.7
Head-pectoral Length (1–12)	25.8	23	27.2	0.7	25.8	28.5
Thorax Length (12–13)	22.2	23	22.7	1.1	20.2	24.8
Pectoral-spine Length (12–29)	31.2	23	32.0	1.2	29.6	34.7
Abdominal Length (13–14)	22.0	23	20.3	0.6	19.2	22.0
Pelvic-spine Length (13–30)	27.9	23	27.2	1.2	25.3	29.0
Postanal Length (14–15)	39.0	23	35.2	1.5	32.5	39.0
Anal-fin spine Length (14–31)	18.1	23	16.3	1.1	14.8	19.1
Dorsal–pectoral Distance (10–12)	24.5	23	26.8	1.6	23.8	29.7
Dorsal spine Length (10–11)	32.6	22	32.7	1.7	28.8	35.7
Dorsal-pelvic Distance (10–13)	24.9	23	22.3	1.4	18.8	24.9
Dorsal-fin base Length (10–16)	29.3	23	28.9	0.9	27.0	30.9
Dorsal-adipose Distance (16–17)	15.3	23	15.2	1.1	12.0	17.4
Adipose-spine Length (17–18)	9.4	23	10.7	0.7	9.4	12.0
Adipose-upper caudal Distance (17–19)	16.2	23	17.1	1.0	15.3	19.3
Caudal-peduncle Depth (15–19)	10.8	23	11.7	0.5	10.8	13.2
Adipose-lower caudal Distance (15–17)	22.9	23	23.0	0.9	21.6	24.8
Adipose-anal Distance (14–17)	20.8	23	20.3	0.5	19.3	21.0
Dorsal-anal Distance (14–16)	14.9	23	15.0	0.6	14.2	16.3
Pelvic-dorsal Distance (13–16)	27.5	23	22.5	1.3	20.8	27.5
% Head Length						
Head-eye Length (5–7)	31.4	23	36.3	2.0	31.4	41.3
Orbit Diameter (4–5)	18.9	23	21.9	1.0	18.9	23.7
Snout Length (1–4)	53.7	23	56.3	1.5	53.7	59.5
Internares Width (2–3)	11.2	23	11.3	0.5	10.3	12.3
Interorbital Width (5–6)	43.0	23	46.8	1.8	43.0	49.6
Head Depth (7–12)	59.6	23	67.0	2.6	59.6	73.5
Mouth Length (1–24)	41.9	23	41.9	1.8	38.0	46.1
Mouth Width (21–22)	42.4	23	43.1	2.2	36.3	46.0
Barbel Length (22–23)	16.9	23	17.5	2.5	12.6	22.3
Dentary Tooth Cup Length (25–26)	11.3	23	11.1	1.5	7.9	13.8
Premaxillary Tooth Cup Length (27–28)	10.6	23	12.0	1.5	9.2	15.4

are, 05.4286°, -066.1362°, N.K. Lujan, M.H. Sabaj, L.S. de Souza, D.C. Werneke, 12 April 2004. AUM 39839, 1, 31.5 mm SL, Manapiare River, 10 km NW of San Juan de Manapiare, 05.3868°, -066.1159°, N.K. Lujan, L.S. de Souza, D.C. Werneke,

Figure 2. Mouth of *Peckoltia multispinis*, holotype, NMW 8952. Photo by M.H. Sabaj and K. Luckenbill.

M.H. Sabaj, 14 April 2004. MCNG 56680, 13, 52.6–80.2 mm SL, same data as AUM 39313.

Diagnosis. *Peckoltia wernekei* can be separated from all other *Peckoltia* by having a broken black line of pigment on the upper jaw (vs. solid line of pigment along snout edge or snout uniformly colored or mottled). *Peckoltia wernekei* can be further separated from *P. vittata* by generally having 25 or more teeth in at least one dentary or one premaxilla (vs. generally 24 or fewer; one specimen of *P. wernekei* had both upper and lower jaws with <25 teeth/ramus), by having a largely naked abdomen (abdomen with a few plates below pectoral girdle, between pelvic fins and along sides of abdomen; vs. most of ventral surface from the throat to the anus with small plates), and by having large, faint blotches on the abdomen (vs. abdomen uniform). *Peckoltia wernekei* can be further separated from upper Orinoco congeners as follows: from *P. brevis* and *P. lineola* by lacking short lines and spots on the head (vs. lines and spots present), from *P. brevis*, *P. caenosa* and *P. lineola* by having a largely naked abdomen (vs. abdomen fully plated), and by generally having 25 or more teeth per jaw ramus (vs. 22 or fewer in *P. brevis*, 21 or fewer in *P. caenosa*, and 19 or fewer in *P. lineola*); from *P. lujani* by having the dentaries meet at an angle less than 90° (vs. >90°), by having large, faint

Figure 3. Holotype of *Peckoltia wernekei* sp. n., AUM 54314, 104.6 mm SL, dorsal, lateral, and ventral views. Scale = 1 cm. Photos by J.W. Armbruster.

blotches on the abdomen (vs. abdomen with no blotches), by a smaller internares width to interorbital width ratio (21.2–26.6% vs. 28.5–46.5%), and a larger dorsal spine to abdominal length ratio (148.1–178.6% vs. 80.1–134.5%); and from *P. sabaji* by having bands in the dorsal and caudal fins (vs. spots) and prominent dorsal saddles on the body (vs. large spots).

Description. Morphometrics in Table 1. Counts and measurements based on 23 specimens. Small to medium-sized loricariids, largest specimen examined 104.6 mm SL. Body stout, but slightly narrower than *P. vittata*. Head gently sloped to supraoccipital. Supraoccipital with tall, rounded crest. Supraoccipital crest raised slightly above nuchal region. Nuchal region rises slightly to nuchal plate. Dorsal slope decreasing in straight line to insertion of dorsal procurrent caudal-fin rays then ascending to

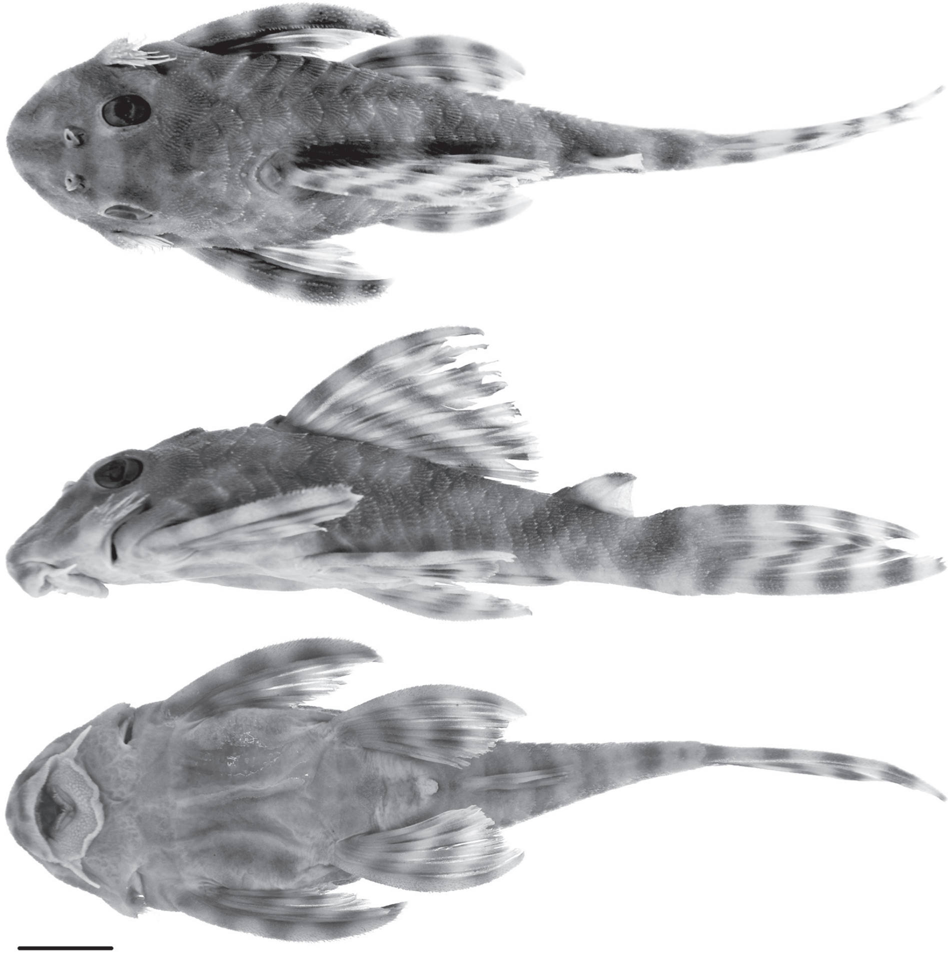

Figure 4. Paratype of *Peckoltia wernekei* sp. n., AUM 39313, 73.5 mm SL dorsal, lateral, and ventral views. Scale = 1 cm. Photos by J.W. Armbruster.

caudal fin. Body depth greatest at anteriormost insertion of dorsal fin. Ventral profile flat to caudal fin. Caudal peduncle trapezoidal in cross section with dorsal surface flattened. Body widest at insertion of pectoral fins, narrowest at insertion of caudal fin. Snout rounded.

Eye moderately sized (orbit diameter 18.9 ± 1.0% of head length), dorsal rim of orbit forming tall crest that continues forward to area just anterior of nares as low, rounded ridge. Iris operculum present. Interorbital space with slight, rounded, median hump that is contiguous with ridge of parieto-supraoccipital. Parieto-supraoccipital pointed posteriorly with posterior point raised above nuchal region in small crest. Infraorbitals, frontal, nasal, compound pterotic and parieto-supraoccipital supporting odontodes. Preopercle generally supporting a single column of odontodes that gener-

Figure 5. Live photos of **A** *Peckoltia lujani* (uncataloged), photograph by N.K. Lujan, and **B** *P. wernekei* sp. n. AUM 39313 (was used as a live photo of *P. vittata* in Armbruster 2008). Photograph by M.H. Sabaj Pérez.

ally decrease in number with increasing body size; largest specimen with some odontodes located posterodorsally on preopercle and two individuals without preopercular odontodes. Opercle with one to three rows of odontodes with numbers of rows and numbers of odontodes generally decreasing with increasing body size; largest individual without odontodes.

Lips covered with short, wide papillae. Lower lip wide, reaching just to or slightly short of pectoral girdle; upper lip narrow. Edge of lower lip smooth. Maxillary barbel only barbel present, reaching about two-thirds of distance to gill opening from base of barbel.

Median plates 24–26 (mode 24). Plates unkeeled, but first four or five plates of mid-ventral series bent to form slight ridge. Five caudal peduncle plate rows. Plates on all dorsolateral surfaces of body. Throat naked. Abdomen mostly naked except for a line one to three platelets wide along and slightly posterior to anterior margin of pectoral girdle, few uneven rows of platelets ventral to ventral plate series, patch of platelets below posterior section of pelvic girdle; number of platelets on abdomen increases with body size. Evertible cheek plates supporting hypertrophied odontodes evertible perpendicular to head. Cheek odontodes 17–40 (mode 33). Longest evertible cheek odontode almost reaching vertical through posterior edge of pectoral-fin spine. Hypertrophied cheek odontodes relatively weak. Odontodes slightly longer than average body odontodes present along dorsal-, adipose-, pelvic-, caudal-, and pectoral-fin spines; larger individuals with hypertrophied odontodes at tip of pectoral spine.

Dorsal fin ii,7; dorsal spinelet *V*-shaped, dorsal-fin locking mechanism present, last ray of dorsal fin not reaching or just reaching preadipose plate when adpressed. Adipose fin with single preadipose plate and moderately long spine. Caudal fin i,14,i; caudal fin forked, ventral lobe longer than dorsal lobe; dorsal and ventral procurrent caudal-fin rays five. Pectoral fin i,6; pectoral-fin spine reaching just posterior to pelvic fin when adpressed ventral to pelvic fin. Pelvic fin i,5; pelvic-fin spine extending one to two plates posterior to anal fin when adpressed. Anal fin i,4; unbranched anal-fin ray slightly shorter than first branched ray.

Teeth bicuspid with lateral lobe one-half to three-quarters length of medial lobe and lateral cusp half width of medial cusp. Eighteen to 32 left dentary teeth (mode 31; 1 of 23 with less than 25); 22–35 left premaxillary teeth (mode 32; 1 of 23 with less than 25); all specimens with at least one jaw ramus having 25 or more teeth.

Color. Base color reddish brown. Head and nape mottled dark brown with distinct, medial dark line along mesethmoid and slightly less distinct lines from lateral portion of naris to lip mark. Parieto-supraoccipital crest darker than surrounding areas. Lip with dark mark consisting of two or three ovoid dashes of brown, lateral portions continue as line to lateral portions of nares (Fig. 6). Dark portion of lips separated from mesethmoid line by distinctly lighter *C*-shaped region (Fig. 6), which may continue less distinctly between mesethmoid line and lines lateral to nares. Lips may also have other spots. Head colors less distinct in larger specimens. Body with four distinct, oblique bars, first below anterior of dorsal fin, second below posterior end of dorsal fin and anterior part of interdorsal space, third beginning at preadipose plate to about posterior edge of adipose spine, and fourth at end of caudal peduncle; first and second bars fade into a dark wash ventrally connecting the two bars; third and fourth bars continuing around caudal peduncle; bars connected at median plate series. Secondary bars sometimes present between any two primary bars, but generally not as dark, darker ventrally than dorsally; posterior secondary bars darker than anterior; secondary bars may connect across caudal peduncle. Pectoral-fin spine dark brown with alternating, similarly-sized dark and light spots, spots continuing as bands on fin; two to five dark bands on fin with number increasing with body size. Pelvic fin as pectoral but with two to four dark bands. Dorsal fin as pectoral but with dark bands distinctly wider than light bands, three or four dark bands. Anal and caudal fins as pectoral, but with light bands wider than dark bands (one to three dark bands in anal and three to five bands in caudal). Adipose spine with dorsal section of third dark bar covering base in all specimens, with some also having dark spot at tip of spine; in largest individual (holotype) basal and distal spots combine along posterior margin of spine, with ante-

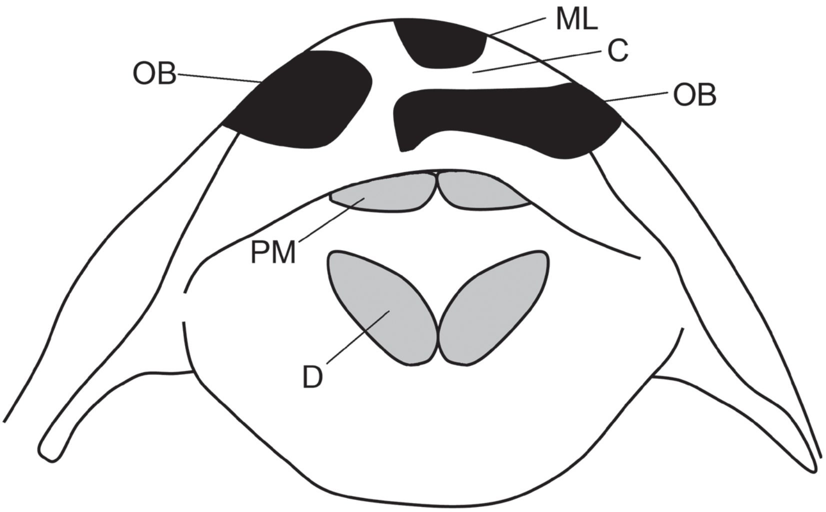

Figure 6. Schematic of the mouth of *Peckoltia wernekei* based on the specimen in Figure 3. Dentaries (D) form less than a 90° angle with one another whereas premaxillae (PM) are relatively straight. A broad, broken band is formed from ovate blotches (OB) on the upper lip, separated from a line that runs along the mesethmoid (ML) by a *C*-shaped light space (C).

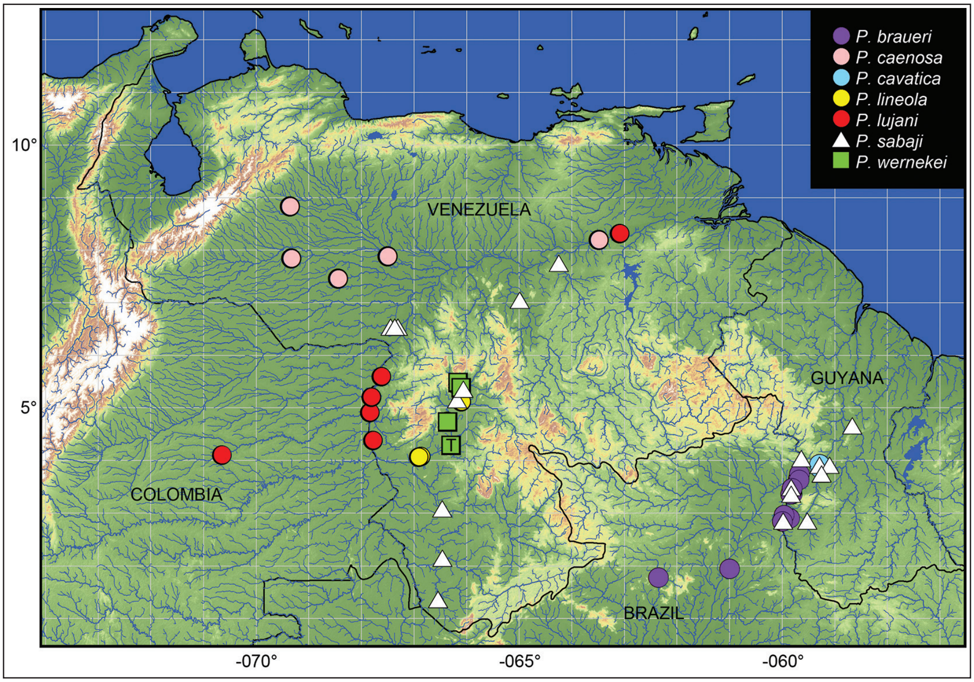

Figure 7. Distribution of *Peckoltia* around the western Guiana Shield. 'T' indicates type locality of *P. wernekei* sp. n.

rior edge having light space between spots. Abdomen mostly light, but with four to six large, faint blotches laterally and occasionally with one or two median faint blotches.

Sexual dimorphism. None observed.

Distribution. Known only from the Ventuari River, a right-bank tributary of the upper Orinoco River in Amazonas State, Venezuela (Fig. 7).

Etymology. Patronym honoring David C. Werneke, Collection Manager of Fishes at the Auburn University Museum, for his diligence, camaraderie and humor during three expeditions to the upper Orinoco Basin and for his long service as a Collection Manager at Auburn University.

Key to the species of *Peckoltia* (after Armbruster (2008) and the present paper)

1 Eyes reduced (orbit diameter <10% of HL); long caudal-fin filaments; lacking pigment or color reduced, light tan with very slightly darker saddles, mottled black and white or completely black .... ***Peckoltia pankimpuju***
– Eyes normal (orbit diameter >13% of HL); caudal fin lacking filaments; color normal, with dark brown saddles, spots, or lines on a tan background) **2**
2 Dentaries meeting at an angle less than 90° to just slightly greater **3**
– Dentaries meeting at an angle greater than 130° **17**
3 Posterior margin of lower lip with finely branched fimbriate papillae (Fig. 2)..... .... ***Peckoltia multispinis***
– Posterior margin of lower lip with simple fimbriae or smooth..................... **4**
4 Distinct round spots present on head and/or sides of body **5**
– Color pattern on head consisting of large blotches, saddles or lines, lacking distinct round spots on head or sides **12**
5 Some spots on the parieto-supraoccipital and/or compound pterotic combining to form lines **6**
– All spots distinct, none combining to form lines **7**
6 Lines on head most prominent on compound pterotic, not radiating from a central point on the parieto-supraoccipital; lines on head approximately same width or wider than pupil .... ***Peckoltia lineola***
– Lines on the head most prominent on the parieto-supraoccipital where they radiate from a central point; lines on head narrower than pupil .... ***Peckoltia vermiculata***
7 Bands present on caudal fin (bands may be composed of spots arranged linearly) **8**
– Spots present on caudal fin separate, not forming bands **10**
8 Lateral body surfaces with spots .... ***Peckoltia otali***
– Lateral body surfaces with dorsal saddles.. **9**
9 Spots on the dorsal fin; no spots on the abdomen; caudal fin lunate with upper caudal-fin spine longer than lower spine (usually the tail is broken and this character is not observable) .... ***Peckoltia furcata***

– Bands on the dorsal fin; spots on abdomen of large juveniles and adults; caudal fin truncate or emarginated with lower caudal-fin spine longer than upper .... ***Peckoltia brevis***

10 Spots absent on head .... ***Peckoltia capitulata***

– Spots present on head **11**

11 Unworn teeth with lateral lobe about 2/3 or less the length of the medial lobe, longest cheek odontode not extending beyond cleithrum .... ***Peckoltia oligospila***

– Unworn teeth with lobes approximately equal (lateral lobe just slightly shorter than medial lobe), longest cheek odontode extending beyond cleithrum .... ***Peckoltia simulata***

12 Caudal fin with dark bands much wider (approximately four or more times) than light bands; dorsal fin with white spots; abdomen with large dark spots with at least some spots merging to form vermiculations .... ***Peckoltia caenosa***

– Caudal fin with dark and light bands of approximately equal width; dorsal fin with bands or uniformly colored; abdomen uniformly light or with faint dark spots that do not merge into vermiculations **13**

13 Bold pattern of contrasting light and dark bars on body, usually five or more bars behind head, bars extending to or near ventrum without fading below the median plate series .... ***Peckoltia compta***

– Dark saddles and lighter background colors not boldly contrasting, usually only four dorsal saddles behind head that fade below the median plate series and generally do not reach ventrum **14**

14 Dark blotch between eyes and on snout, head mottled or with a bold patch of pigment in the form of an *E* on the snout with the central branch of the *E* located mid-dorsally and the top and bottom branches located just lateral to the nares, all three branches extending anteriorly and the main stem of the *E* running transversely centered on the nares; none of the plates of the head or nape outlined in black; dorsal and caudal fins without orange edge in life **15**

– Head and snout uniformly brown or with the plates outlined in black; *E* mark absent; posterior plates of the head and nape outlined in black; dorsal and caudal fins with an orange band at the edges in life **16**

15 Upper lip mottled; teeth usually 24 or fewer per jaw ramus (only one specimen examined greater than 25); abdomen plated from throat to anus .... ***Peckoltia vittata***

– Upper lip with a broken dark line of pigment, teeth usually 26 or more per jaw ramus (only one specimen examined with less than than 25); abdomen mostly unplated (a few plates below pectoral girdle, between pelvic fins, and along sides of abdomen) .... ***Peckoltia wernekei***

16 Vermiculations on compound pterotic; plates of head and nape not completely outlined in heavy black lines; caudal fin with at least one broken dark band; marginal orange bands of dorsal and caudal fins narrow .... ***Peckoltia braueri***

– No markings on compound pterotic; all bones of head and nape outlined in faint black lines; caudal fin without dark bands; marginal orange bands of dorsal and caudal fins wide .... ***Peckoltia cavatica***

17 Cheek odontodes evertible to less than 45° from head, 0–10 hypertrophied cheek odontodes, cheek odontodes very short, not extending to cleithrum (no longer than 15× length of those on lateral plates)...... ***Peckoltia relictum***

– Cheek odontodes evertible to greater than 80° from head, 20 or more evertible cheek odontodes, cheek odontodes very long, extending to at least middle of cleithrum (much greater than 15× length of those on lateral plates **18**

18 No spots or bands in dorsal fin .... ***Peckoltia ephippiata***

– Spots or bands present on dorsal fin.......... **19**

19 Abdomen fully plated, caudal fin with upper lobe longer than lower lobe in adults.......... ***Peckoltia furcata***

– Abdomen with large naked areas, particularly between pectoral girdle and anus. Caudal fin with lower lobe longer than upper lobe **20**

20 Head, sides and fins with large, bold spots.......... ***Peckoltia sabaji***

– Head mottled or with faint spots, sides with dorsal saddles, fins with bands..... **21**

21a No spots on the posterolateral surface of head and nape; pectoral spine in relaxed position angled dorsally (pointing at insertion of dorsal fin); and pectoral-fin spine reaching two or more plates of the ventral series beyond the pelvic base when adpressed ventral to pelvic fin .... ***Peckoltia greedoi***

– Distinct spots on the posterolateral surface of head and nape; pectoral spine in relaxed position angled only slightly dorsally (pointing maximally to dorsal insertion of caudal fin); and pectoral-fin spine reaching less than one plate of the ventral series beyond the pelvic base when adpressed ventral to pelvic fin........ **22**

22 Spots larger than twice naris diameter, often indistinct, irregularly spaced and merging into irregular shapes, especially posterior of dorsal-fin origin, spots generally dark gray on a light gray base .... ***Peckoltia lujani***

– Spots on snout naris sized or smaller, distinct and evenly spaced, growing to larger than orbit size and/or merging into oblique bars posterior of dorsal-fin origin, spots generally dark brown on a light brown base ....
.... ***Peckoltia* sp. n.** Meta L147

Discussion

The disparity between the morphological and molecular phylogenies of Armbruster (2004; 2008) and Lujan et al. (2015) and Lujan et al. (in review) are likely due to homoplasy and convergence in the morphological dataset, and nowhere is this more obvious than in the upper Orinoco clade containing *Peckoltia lujani*, *P. wernekei* and *Peckoltia* sp. n. Meta L147 (Fig. 1). *Peckoltia wernekei* was included in *Peckoltia sensu* Armbruster (2008) because of its stocky body and dentaries forming an angle of less than 90° (Fig. 6). The molecular phylogeny found *P. wernekei* to be sister to *P. lujani*, a species with dentary tooth rows forming almost a straight line and a more elongate body (Fig. 5a). Despite the differences in jaw angle, which likely has ecological and functional repercussions (Lujan and Armbruster 2012), *P. wernekei* and *P. lujani* differ from one another by only one of approximately 600 base pairs (bp; <0.2%) sequenced from the mitochondrial 16s gene and three of approximately 1150 bp (<0.3%) sequenced from the mitochondrial cytochrome *b* gene, whereas *P. wernekei* has 9.6% cyt *b* sequence divergence from *P. vittata* in the Xingu and 7.5% divergence from *P. vittata* in the Madeira (Lujan et al. in review).

Given the nested phylogenetic position of *Peckoltia wernekei* among two other species with relatively straight tooth rows (*P. lujani* and *P.* n.sp. Meta L147; Fig. 1), its highly-angled dentary tooth row angle is likely derived and an example of convergence upon the condition observed in many other *Peckoltia*. Given the limited sequence divergence between *P. wernekei* and *P. lujani*, this jaw evolution has either occurred very quickly or there has been very recent mitochondrial introgression. Mitochondrial introgression was observed between the sympatric *Panaqolus koko* and *Peckoltia otali* (Fisch Muller et al., 2012) where the two species had similar sequences for the mitochondrial COI gene suggesting close relationships, but sequences in the nuclear F-Reticulon4 gene showed no such close relationship. In this case, *P. wernekei* and *P. lujani* are not known to be sympatric and nuclear genes are also very similar (≤0.3% divergence in RAG1, RAG2 and MYH6; Lujan et al., in review), so we believe that the genetic similarity between these species is indicative of close evolutionary relationships and not hybridization. If such a pattern can be seen among closely related species, then many elements of jaw morphology are likely to be convergent across the phylogeny. Indeed, highly-angled jaws were seen in several clades even within the morphological phylogeny (Armbruster 2004; 2008). We are now testing this hypothesis with a phylogenetically explicit examination of jaw morphological diversity across the Hypostominae.

Peckoltia wernekei is illustrative of an increasingly clear biogeographical pattern in which the fish fauna of the Orinoco River upstream of its confluence with the Ventuari undergoes replacement or turnover between this confluence and the large Autures Rapids downstream. We hypothesize that this is due to an environmental filter similar to that documented by Winemiller et al. (2008) in the nearby Casiquiare Canal. The limnology of the Casiquiare Canal displays a gradient from relatively neutral or slightly acidic pH with few tannins at its origin in the upper Orinoco, to highly acidic pH with high tannin load at its confluence with the Negro River. Likewise, the physicochemical parameters of the clearwater upper Orinoco River shift a short distance downstream from the Ventuari River in response to its confluence with one major blackwater tributary (the Atabapo River) and one major whitewater river (the Guaviare/Inirida River) along its left-bank.

Many loricariid species are known only from the Ventuari River and nearby reaches of the Orinoco main channel. These include: *Baryancistrus demantoides*, *B. beggini*, *Hemiancistrus subviridis*, *Hypancistrus contradens*, *H. furunculus*, *H. lunaorum*, *Leporacanthicus* cf. *galaxias*, *L. triactis*, *Hypancistrus vandragti*, *Peckoltia lineola*, *Pseudolithoxus tigris*, and *Pseudancistrus pectegenitor* (Armbruster 2008, Armbruster et al. 2007, Lujan and Armbruster 2011, Lujan and Birindelli 2011, Lujan et al. 2007, 2009, Werneke et al. 2005). At least two other species have a disjunct distribution inclusive of the Ventuari River and the Caura River but are absent from intervening reaches of the Orinoco River main channel (*Limatulichthys nasarcus*, and *Pseudolithoxus anthrax*; Lujan and Birindelli 2011, Londoño-Burbano et al. 2014), and in four of these instances there is strong morphological or molecular evidence that sister species are allopatrically distributed upstream vs. downstream of the confluence of the Orinoco and Atabapo/Guaviare/Inirida rivers. In addition to the *Peckoltia lujani*/*P. wernekei* pair, there is *Hypancistrus debilittera* and *H. furunculus*, *Pseudolithoxus kelsorum* and *P. tigris*, and *Hemiancistrus* sp. n. L128 (Dignall 2014) and *Hemiancistrus subviridis*.

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Supplementary material 1

Morphometrics and meristics for species of *Peckoltia* and *Etsaputu*

Authors: Jonathan W. Armbruster, Nathan K. Lujan

Data type: (measurement/occurence/multimedia/etc.)

Explanation note: Morphometirc and meristic data based on Armbruster (2003) for all specimens of *Peckoltia* examined.

Supplementary material 2

Locality information for all collections of the three species described in this paper

Authors: Jonathan W. Armbruster, Nathan K. Lujan

Data type: Occurences

Explanation note: Database containing catalog numbers, numbers of specimens, and localities for *Peckoltia wernekei*.

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http://zookeys.pensoft.net

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Biodiversity data mining from Argus-eyed citizens: the first illegal introduction record of *Lepomis macrochirus macrochirus* Rafinesque, 1819 in Japan based on Twitter information

Yusuke Miyazaki[1], Akinori Teramura[2], Hiroshi Senou[1]

1 *Kanagawa Prefectural Museum of Natural History, 499 Iryuda, Odawara-shi, Kanagawa 250-0031, Japan*
2 *Faculty of Marine Science, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan*

Corresponding author: *Yusuke Miyazaki* (miyazaki@nh.kanagawa-museum.jp)

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Abstract

An apparent illegal introduction of *Lepomis macrochirus macrochirus* from Yokohama City, Kanagawa Prefecture, Japan, is reported based on a juvenile specimen and a photograph of two adults collected on 14 June 2015 and deposited in the Kangawa Prefectural Museum of Natural History. The specimens and photographs were initially reported on the internet-based social networking site, Twitter. Two specimens of *Carassius auratus*, including an aquarium form, were also reported at the same locality and date, suggesting that the illegal introductions originated from an aquarium release. Our report demonstrates an example of web data mining in the discipline of Citizen Science.

Keywords

Biotope, Centrarchidae, environmental education, Invasive Alien Species Act, recreational fishing, tweet, voucher

Introduction

Rapid biodiversity decline is a serious problem requiring a global response. The spread and resultant establishment of invasive non-native species is one of the most critical contributing factors to biodiversity decline (Puth and Post 2005; Blackburn et al. 2011), and the detection of invasive species is required globally to safeguard biodiversity.

Japan's "Invasive Alien Species Act (IASA)" was established in 2005 under the Basic Biodiversity Act, within the Environmental Act (Oikawa 2010). The IASA prohibits the introduction and spread of Invasive Alien Species (IAS) as defined by the law in Japan. That is, the breeding, cultivation, storage (in either natural or artificial conditions), transportation, transfer, delivery, importing and releasing from an already established place to anywhere else including artificial habitats, planting and dispersing to outdoors of IAS are strictly prohibited. Any person who violates the IASA faces a fine of <3 million yen or <3 year of penal servitude, while a corporation that transgress faces a fine of <100 million yen (Study Group on Impacts and Managements of Alien Species 2008).

The first suggested illegal introduction under the IASA was a report of the centrachid fish *Micropterus salmoides* (Lacepède, 1802), which remains, unfortunately, one of the most popular recreational fishing targets in Japan. The species was apparently illegally released into an irrigation pond in Ichinoseki City, Iwate Prefecture, during October 2007–May 2008 (Miyazaki 2010). The second report also recorded the introduction of the same species in the same prefecture. This IAS was illegally released into three irrigation ponds of Oshu City, Iwate Prefecture, between 2008 and 2009 (Tsunoda et al. 2011). Following this, on 19 August 2009, the first arrestee under the IASA, a black bass fishing fan who had transferred two live specimens of *M. salmoides*, was reported by several mass media outlets such as Jiji Press, The Asahi Shimbun, and Nara Newspaper (e.g., Miyazaki 2010).

Another IAS, *Lepomis macrochirus macrochirus* Rafinesque, 1819, also belongs the family Centrarchidae. The first recorded introduction of the species in the natural waters of Japan was in 1963 (Matsuzawa and Senou 2008; Senou and Hayashi 2013). The origin of all Japanese populations of the species is the 18 individuals donated from the Shedd Aquarium (Mississippi River population) by the Chicago mayor of October 1960 (based on the analyses of mtDNA of the Japanese populations: Kawamura et al. 2004, 2010); these were bred at the Freshwater Fisheries Research Laboratory of Fisheries Agency, Japan. After the donation, they were introduced to four man-made lakes in Miyazaki, Kochi, and Tokushima Prefectures during 1963–1964 ahead of the ceremonial introductions by the Japanese prince in April 1966 to Lake Ippeki, Shizuoka Prefecture. The species was then introduced widely across Japan for the purposes of commercial fisheries and ceremonies (Nakai 2002). Since the 1970s, this species has been often released together with *M. salmoides* because several columns in Japanese recreational fishing magazines encouraged the widespread and combined introductions of the two species (Nakai 2002; Matsuzawa and Senou 2008). In addition, *L. macrochirus macrochirus* was unintentionally spread during the widespread release of *Plecoglossus altivelis altivelis* (Temminck & Schlegel, 1846) from Lake Biwa; the latter was commercially stocked in

Lake Biwa and then widely released during the 1970s–2000s (see also Miyazaki et al. 2015a). Both *M. salmoides* and *L. macrochirus macrochirus* had spread to all Japanese prefectures by 2001 (Kiriu 1992; Hayashi 2002; Maruyama 2002; Nakai 2002). However, the former two examples of illegal introductions were of *M. salmoides* and, despite the circumstantial evidence of the illegal introduction of *L. macrochirus macrochirus*, this illegal activity has not been previously reported from Japanese waters.

Here, we detail the first apparent introduction of *L. macrochirus macrochirus* in Japan since the IASA was adopted.

Integrating information from a student's tweet to museum collections

On 14 June 2015, the second author (AT), an undergraduate student of the Tokyo University of Marine Science and Technology, tweeted a comment along with two images on a social networking service, Twitter, via the internet (Fig. 1). It stated that he had identified *L. macrochirus macrochirus* from a public outdoor swimming pool and was obviously surprised that people could be so irresponsible as to release an invasive species. The first author (YM) saw the tweet, and contacted AT with the idea of publishing the information as a scientific report.

As a result, we deposited a juvenile specimen of *L. macrochirus macrochirus* collected from the artificial outdoor pool in Chigasaki Park, Yokohama City (35°32'20.8"N, 139°34'54.0"E; 40–50 m at middle latitudes), on 14 June 2015 in the Kanagawa Prefectural Museum of Natural History, Japan (KPM-NI 39654; Fig. 2A, B). The

てらむら @orca_lf · 6月14日
ある市民プールで掃除をした結果笑
こんなもんプールに侵入して逃がすバカが
おるんは信じられんわ

13
お気に入り

Figure 1. A tweet including two fish images posted by AT on 14 June 2015. The image on the left shows two adults of *Lepomis macrochirus macrochirus*, while the one on the right shows juveniles of *L. macrochirus macrochirus* and two young of *Carassius auratus* (however, the specimens in the latter image are not able to be identified even to family level from the image alone). The comments are translated into English as follows: "The results of a pool cleaning. I cannot believe the foolishness of the person who introduced these fish into the pool." https://twitter.com/orca_lf/status/610088107476516864 [Accessed on 25 November 2015]

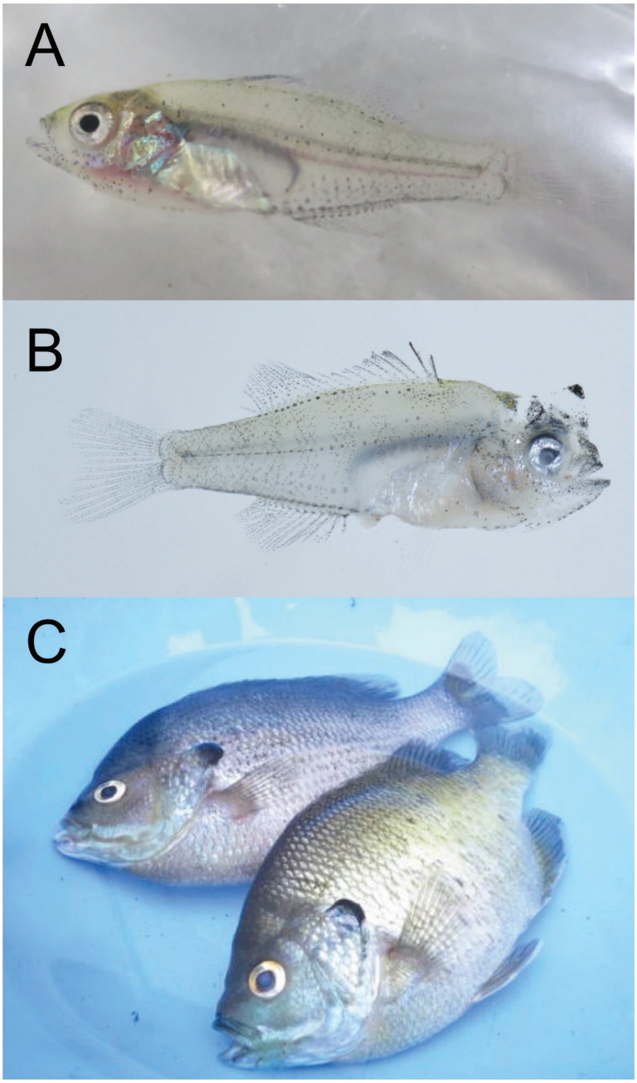

Figure 2. Photographs of *Lepomis macrochirus macrochirus* collected from the outdoor pool of Chigasaki Park, Yokohama City, Kanagawa Prefecture, Japan on 14 June 2015. **A** voucher specimen, juvenile, KPM-NI 38654 (photo: KPM-NR 164120 by AT), 22.6 mm SL **B** voucher specimen, juvenile (right side), KPM-NR 108928 by YM (the same individual as KPM-NI 38654) **C** adult specimens, photograph, KPM-NR 164118 by AT.

pool has been isolated from natural waters since its construction (Fig. 3). Additionally, the public museum catalogs and stores fish images as well as specimens (Miyazaki et al. 2014), so we also registered the above specimen's photographs (KPM-NR 108928, 164120; Fig. 2A, B), and the original image of two *L. macrochirus macrochirus* adults that was posted on the internet via Twitter on the image database (KPM-NR 164118; Figs 1, 2C). Based on the above voucher specimen and photographs, we identified

Figure 3. Photograph of the outdoor pool of Chigasaki Park, Yokohama City, Kanagawa Prefecture, Japan.

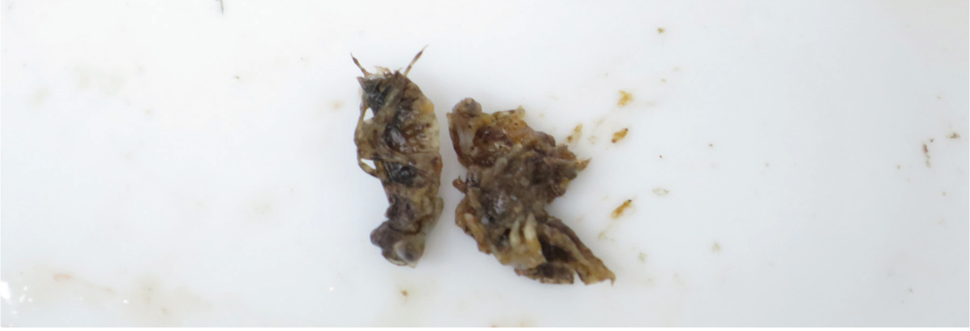

Figure 4. A portion of gut components of *Lepomis macrochirus macrochirus* collected from the outdoor pool of Chigasaki Park, Yokohama City, Kanagawa Prefecture, Japan, on 14 June 2015. Dragonfly nymphs, *Sympetrum* sp., were included.

the specimens as the invasive fish species, *Lepomis macrochirus macrochirus* (Scott and Crossman 1973; Ross et al. 2001; Yamamoto and Yodo 2014), noting the following external morphological characters. In adults (photograph): mouth small; posterior end of maxilla not reaching anterior margin of eye; posterior part of opercle with flap; deep body; dorsal, pelvic and anal fins with spines. In juvenile (specimen): D X, 10; A III, 10; P_1 13; P_2 I, 5; dark transverse bands on body.

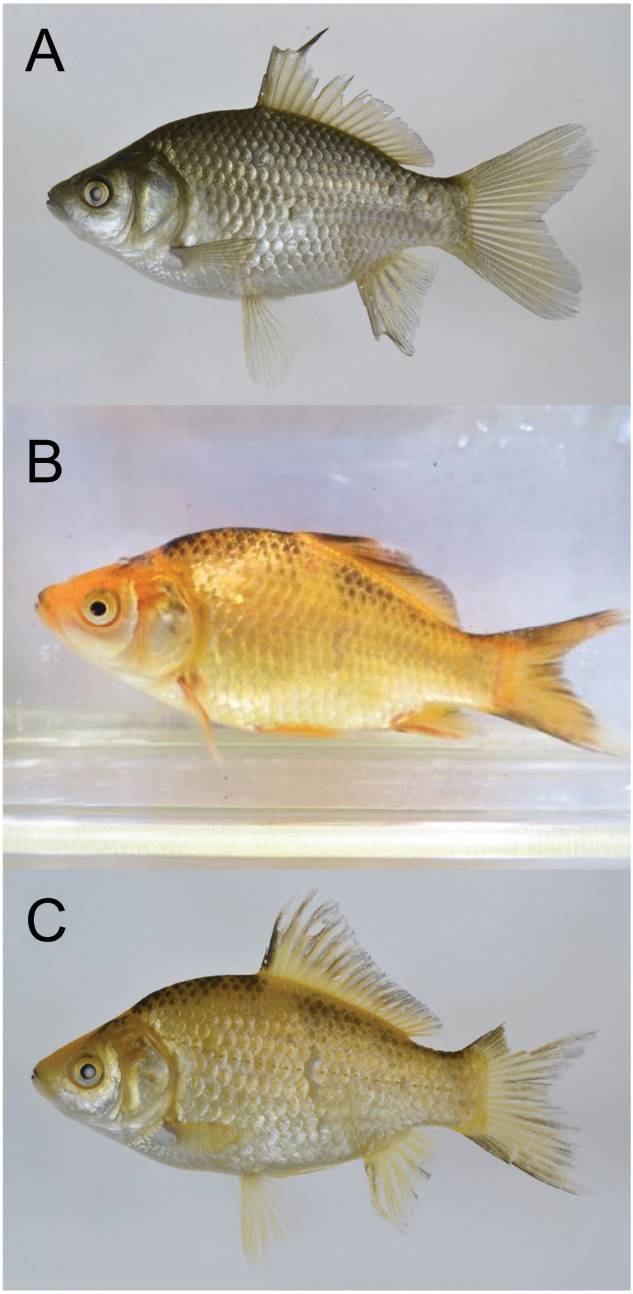

Figure 5. The voucher specimens and photographs of *Carassius auratus* collected from the outdoor pool of Chigasaki Park, Yokohama City, Kanagawa Prefecture, Japan, on 14 June 2015. **A** voucher specimen, KPM-NI 38655 (photo: KPM-NR 108929 by YM), 64.9 mm SL **B** voucher specimen (live), KPM-NI 38656 (photo: KPM-NR 164119 by AT), 61.3 mm SL **C** voucher specimen (fresh), KPM-NR 108930 by YM (the same individual as KPM-NI 38656).

We also identified some of the gut contents of an adult *L. macrochirus macrochirus* as dragonfly nymphs of the genus *Sympetrum* (Fig. 4). This identification follows Ishida (1996) and is based on the following characters: laterally long and inverted trapezoid head, large and prominent compound eyes, and presence of lateral spines in abdominal segments 8–9.

In addition, we also deposited two specimens and photographs of the goldfish, *Carassius auratus* (Linnaeus, 1758), which were collected and recorded along with the *Lepomis macrochirus macrochirus* specimens (Fig. 5). The goldfish specimens had the following characters (compared with Japanese *Carassius* spp.): higher body depth (45.1–46.8% of standard length), fewer gill rakers (37–40), fewer numbers of pores in lateral line (each 28), longer fins, and silvery–golden and/or yellowish body colors.

We note, in passing, that *C. auratus* is native to China and the Korean Peninsula but not the Japanese archipelago (Kalous et al. 2012). Specimens from Japan have been comfirmed by mtDNA analyses to have originated from Chinese populations (Komiyama et al. 2009). That is, it is an exotic non-native species in Japan. Although molecular analyses (Takada et al. 2010; Sakai et al. 2011; Kalous et al. 2012) strongly suggest that some Japanese *Carassius* spp. are endemic, the species' morphological identifications have been confused, except for *C. cuvieri* Temminck & Schlegel, 1846 (see also Taniguchi 1982; Hosoya 2002, 2013; Iguchi et al. 2003; Suzuki et al. 2005; Yamamoto et al. 2010; Saitoh and Uchiyama 2015). Generally, Japanese taxonomists have labelled the Japanese endemic taxa as *C. langsdorfii* Temminck & Schlegel, 1846 or *C. buergeri* Temminck & Schlegel, 1846 and their subspecies, but their classification schemes have not been published in the English literature.

Discussion

In 2014, the public outdoor pool where the IAS and goldfish specimens were found operated between 12 July and 7 September; this suggests that *L. macrochirus macrochirus* and *C. auratus* were introduced sometime between 8 September 2014 and 14 June 2015. This report is the first circumstantial evidence of the illegal introduction of *L. macrochirus macrochirus* in Japan based on the IASA after it was enacted on 1 June 2005.

Generally, outdoor swimming pools that are not in operation during autumn and spring usually function as spawning and nursery habitats for native aquatic insects such as dragonflies and diving beetles (e.g., Lee et al. 1998; Taguchi et al. 2000; Ido and Goto 2002; Takeyama et al. 2002). Therefore, they are sometimes used for environmental education activities, utilizing them as a biotope. The present report is a case in point, where the pool was being cleaned and its organisms observed. The invasion of *L. macrochirus macrochirus* frequently causes population decline of aquatic insects (sometimes including threatened species). In fact, AT found dragonfly larvae or juveniles in the gut components of individuals of the invasive fish species collected from the pool (see Fig. 4).

The discovery of *C. auratus* at the same locality and date as the IAS, suggests that the fishes might have originated from an aquarium fish release from a local shop or an aquarist who no longer wanted them. However, based on the IASA, keeping *L. macrochirus macrochirus* in a home aquarium is illegal, and of course its release is also strictly prohibited. Any specimens collected from Japanese waters should be destroyed. From a bioethical viewpoint, several groups (particularly bass-fishing fans) do not support such killings (e.g., Mizuguchi 2005). However, they are required only because of the irresponsible actions of people who release invasive species from controlled environments such as aquaria to nature. Without such releases, the killings would be unnecessary. Community awareness of this issue needs to be improved, and widespread reporting of cases such as this one will help.

More than 10 years have passed since the IASA was enacted, and the illegal introductions of *M. salmoides* and *L. macrochirus macrochirus* have possibly occurred mostly via the younger generation who are not aware of the law. It has been pointed out that they probably contribute to the wider distribution of invasive species such as those discussed here (e.g., Yodo and Iguchi 2004; Miyazaki 2010). Ideally, the IASA should be advertised again to all Japanese generations.

Web data mining has been rapidly developing over recent years, and its potential continues to expand (Liu 2011). This report shows an example of web data mining in the discipline of Citizen Science, as similarly shown by Miyazaki et al. (2015b). It demonstrates the “accidental” crowdsourcing approach but not the “systematic” crowdsourcing approach such as iSpot (Silvertown et al. 2015).

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RESEARCH ARTICLE

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A revision of the distribution of sea kraits (Reptilia, *Laticauda*) with an updated occurrence dataset for ecological and conservation research

Iulian Gherghel[1,2], Monica Papeş[1], François Brischoux[3],
Tiberiu Sahlean[4,5], Alexandru Strugariu[6]

1 Department of Integrative Biology, Oklahoma State University, 501 Life Sciences West, Stillwater 74078, Oklahoma, USA **2** *Current address: Department of Biology, Case Western Reserve University, 2080 Adelbert Road, Cleveland 44106, Ohio USA* **3** *Centre d'Etudes Biologiques de Chizé, CEBC-CNRS UMR 7372, 79360 Villiers en Bois, France* **4** *Faculty of Biology, University of Bucharest, Bvd. Mihail Kogalniceanu no. 36-46 Bucharest, Romania* **5** *Department of Terrestrial Fauna, "Grigore Antipa" National Museum of Natural History, Kiseleff no 1, 011341, Bucharest, Romania* **6** *Faculty of Biology, "Alexandru Ioan Cuza" University of Iași, 20th Carol 1st Boulevard, 700505, Iasi, Romania*

Corresponding author: *Iulian Gherghel* (iuliangherghel@case.edu)

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Abstract

The genus *Laticauda* (Reptilia: Elapidae), commonly known as sea kraits, comprises eight species of marine amphibious snakes distributed along the shores of the Western Pacific Ocean and the Eastern Indian Ocean. We review the information available on the geographic range of sea kraits and analyze their distribution patterns. Generally, we found that south and south-west of Japan, Philippines Archipelago, parts of Indonesia, and Vanuatu have the highest diversity of sea krait species. Further, we compiled the information available on sea kraits' occurrences from a variety of sources, including museum records, field surveys, and the scientific literature. The final database comprises 694 occurrence records, with *L. colubrina* having the highest number of records and *L. schistorhyncha* the lowest. The occurrence records were georeferenced and compiled as a database for each sea krait species. This database can be freely used for future studies.

Keywords

Amphibious snakes, Elapidae, geodatabase, distribution, marine, open access, sea snakes

Introduction

Sea kraits (genus *Laticauda*) are a group of amphibious, marine snakes from the family Elapidae (Heatwole et al. 2005; Pyron et al. 2011; Shine et al. 2002, 2003), distributed in tropical and subtropical coastal waters of the eastern Indian Ocean, south-east Asia, and archipelagoes of the western Pacific Ocean (Heatwole et al. 2005).

The taxonomic status of the group has been and continues to be subject to much debate. For instance, Vitt and Caldwell (2009) recently elevated Laticaudinae as a separate subfamily within elapids. In contrast, Kharin and Czeblukov (2013) elevated the subfamily Laticaudinae to familial level (Laticaudidae) and divided the genus *Laticauda* into two genera (*Laticauda* and *Pseudolaticauda*), based on morphological characters. However, this split is not widely accepted (e.g., Elfes et al. 2013) and the sea kraits are considered to belong to a single genus, *Laticauda*. Furthermore, in a phylogenetic analysis of the group, Pyron et al. (2011) did not recognize any subfamilies within elapids, as none of the previously described ones (Elapineae, Hydrophiinae and Laticaudinae) formed well-supported monophyletic groups.

In contrast with the taxonomic disputes, from an ecological perspective, this clade is divided into three major complexes (Heatwole et al. 2005), which broadly overlap in geographic range, but differ in their relative use of terrestrial versus marine environments. Species from the “*Laticauda colubrina* complex” (Yellow-banded sea kraits, composed of *L. colubrina*, *L. frontalis*, *L. guineai*, and *L. saintgironsi*) are more terrestrial; species from the “*L. semifasciata* complex” (Black-banded sea kraits, composed of *L. semifasciata* and *L. schistorhyncha*) are more aquatic; and species from the “*L. laticaudata* complex” (Blue-banded sea kraits, composed of *L. laticaudata* and *L. crockeri*) are considered intermediate (Greer 1997; Heatwole 1999, Brischoux et al. 2013).

As amphibious animals, sea kraits have unique characteristics that allow them to perform well in both marine and terrestrial environments. For instance, as sea snakes, they display a paddle-shaped tail that allows them to move efficiently in the water (Brischoux et al. 2010; Brischoux and Shine 2011), but have retained terrestrial characteristics such as large ventral scales that allow them to crawl efficiently on land (Bonnet et al. 2005; Shine and Shetty 2001). Although they prey mostly on eels in coral reefs, sea kraits need to return on land (to digest, rest, slough their skin, mate, and lay eggs; Heatwole 1999) where they manifest a high degree of philopatry (Brischoux et al. 2009; Brischoux et al. 2007; Shetty and Shine 2002).

Interestingly, it has been recently shown that acquisition of fresh water is crucial for sea kraits (Kidera et al. 2013; Lillywhite et al. 2008) and that a combination of availability of fresh water on land and low oceanic salinity at sea may determine environmental tolerances and geographic distributions of sea kraits (Brischoux et al. 2012, 2013). Also, studies indicate that sea snakes may act as indicators of the effects of climate change (Lillywhite et al. 2008, 2014; Lillywhite and Tu 2011), and there is growing interest in their conservation (Bonnet 2012; Bonnet et al. 2009; Brischoux et al. 2009; Elfes et al. 2013).

As such, detailed knowledge regarding the distribution of sea kraits is key for applying conservation measures, planning conservation reserves, and evaluating the impact of human activities (Elith et al. 2006; Ferrier and Watson 1997; Funk and Richardson 2002; Rushton et al. 2004). In the present study, we review the information available on the geographic range of the three sea krait groups and analyze their distribution patterns. In addition, we provide an occurrence database for each sea krait species for use in future studies.

Materials and methods

Occurrence records

A database of sea krait occurrences was created using a combination of data extracted from online repositories (GBIF, HerpNet, iOBIS), from published scientific literature, and from field surveys. Most of the occurrences came from the marine environment, as data on terrestrial localities are scarce for this group. Because of philopatry, sea kraits generally avoid venturing very far from the shore line (Lane and Shine 2011a). Occurrences without spatial data were manually georeferenced to the finest scale possible using the information provided by the source and Google Earth 7. Country taxa lists or locations that could not be georeferenced due to lack of detailed locality descriptions (e.g., name of islands or provinces within countries; McCarty 1986; David and Ineich 1999) had to be excluded from our dataset, as the descriptions were too general. The resolution of the final dataset is 9 km and was projected to WGS84. This resolution is standardized in accordance with existing environmental data (e.g., Bio-ORACLE, www.oracle.ugent.be/) that can be used to answer various biogeographic, conservation or evolutionary questions.

Distribution patterns of sea kraits

Distribution maps for all species of sea kraits were created in ArcGIS 10.2 (ESRI 2011) by intersecting the occurrence points with the 100 km Military Grid System (MGRS) available on-line from the National Geospatial-Intelligence Agency (NGA 2014).

The Extent of Occurrence (EOO) and the Area of Occupancy (AOO) were calculated according to the methodology proposed by IUCN (2012) using ArcGIS 10.2 (ESRI 2011). The calculations for EOO were based on the occurrence records gathered, while the AOO also relied on the 100 km MGRS. In our study, the AOO is overestimated because of the 100 km MGRS used for measurements.

An optimized hot spot analysis was performed using the occurrence points for all species of sea kraits and the whole grid in order to identify statistically significant clusters of high values (hot spots) or low values (cold spots) (ESRI 2011). Subsequently, the analysis was re-run using all occurrence points for the whole *Laticauda* group, but the area was limited to those cells that contained at least one occurrence. The aim was to detect areas in the distribution range of sea kraits that can be viewed as hot spots for the group (i.e., areas with high clustering of species).

Finally, the Shannon-Wiener diversity index was calculated for the group based on the number of occurrences in each cell of the grid, using the Marine Geospatial Ecology Tools (MGET) toolbox (Roberts et al. 2010). The classification scheme used for the index was based on the Natural Breaks (Jenks) algorithm (ESRI 2014).

Results

The final database was comprised of 694 unique records of occurrence at a spatial resolution of 9 km (Suppl. material 1). The bulk of these records belonged to the yellow-banded sea krait (*L. colubrina*, 64.55% of all records compiled), while the lowest number of occurrences was for *L. schistorhynchus* (0.86% of all records) (Table 1).

The EOO registered very high values for *L. colubrina* as a result of its wide distribution range, while *L. schistorhynchus* had the smallest EOO, with only 180.99km^2 (Table 2). The AOO was also the smallest for *L. schistorhynchus*, while the largest AOOs were for *L. colubrina* and *L. laticaudata* (Table 3).

The optimized hot spot analysis based on the whole MGRS grid identified the bulk of the range (western shores of Myanmar and Thailand, Indonesia, Malaysia, Philippines, Papua New Guinea, Solomon Islands, Vanuatu, and Fiji) as an area of high spatial clustering, with 99% confidence ($\bar{z}$ = 9.015; $\bar{p}$ = <0.001), while areas based on 95% confidence ($\bar{z}$ = 3.21; $\bar{\mathrm{p}}$ = 0.001) generally omitted them (Figure 1). Important regions of high spatial clustering, but with a reduced degree of confidence (90%) ($\bar{z}$ = 2.84; p = 0.004), were located around the island of Palau and to the north of Papua New Guinea (Figure 1).

The Shannon–Wiener diversity index registered values between 0 and 1.089 and the diversity map created (Figure 2) showed high diversity values (>0.82) for sea kraits on the islands south-west of Japan and south-east of Taiwan, in the Visayan Sea from the Philippines Archipelago, in the northern part of Celebes Sea, on the northern shores of Halmahera, Indonesia, in New Caledonia around the atoll Ouvéa, and in the Coral Sea around the island of Efate, Republic of Vanuatu (Figure 2).

Discussion

Through the current study we provide the first set of comprehensive distribution maps for all exant species of sea kraits *Laticauda* spp. (but see also The IUCN Red List online maps). Following the pertinent suggestions of Heatwole and Cogger (2013), cases of vagrancy were excluded from the current analysis. Complementary analyses regarding

Table 1. Number of occurrence records available for each species in the *Laticauda* group.

Species	Number of occurrence records	% of total no. of occurrences
L. colubrina	448	64.55
L. frontalis	18	2.6
L. guineai	10	1.44
L. laticaudata	108	15.56
L. saintgironsi	75	10.81
L. schistorhynchus	6	0.86
L. semifasciata	29	4.18

Table 2. Extent of Occurrence (EOO) for the species of *Laticauda* group.

Species	Extent of occurrence (sq. km.)	Of which	
		% Land	% Ocean
L. colubrina	31,651,270.32	18.43	81.57
L. frontalis	93,874.79	7.99	92.01
L. guineai	6,461.75	83.27	16.73
L. laticaudata	27,350,493.24	15.74	84.26
L. saintgironsi	87,825.41	24.19	75.81
L. schistorhynchus	180.99	83.68	16.32
L. semifasciata	6,006,752.15	15.50	84.50

Table 3. Area of Occupancy (AOO) for the species of *Laticauda* group, calculated based on a 100 km Military Grid (NGA 2014).

Species	Area of occupancy (sq. km.)	Of which	
		% Land	% Ocean
L. colubrina	1,988,055.71	22.64	77.36
L. frontalis	63,909.17	9.03	90.97
L. guineai	13,228.66	69.00	31.00
L. laticauda	603,380.15	19.75	80.25
L. saintgironsi	152,370.06	13.67	86.33
L. schistorhynchus	11,239.95	2.30	97.70
L. semifasciata	204,637.92	14.29	85.71

the conservation and distribution of marine elapid snakes (including sea kraits) have been published by Elfes et al. (2013).

Laticauda colubrina has the largest range of any sea krait species (Table 1; Figure 3; Suppl. material 1), spanning from Tonga, in the south-east, through Fiji, Vanuatu, the Solomon Islands, New Guinea, Palau, most of the Indonesian coast, the Philipines, Taiwan, and reaching its northernmost limits in southern Japan and its westernmost limits in the Bay of Bengal, in the Andaman Islands and on the Myanmar coast (Figure 3; Suppl. material 1). Although the species exhibits a great degree of morphologi-

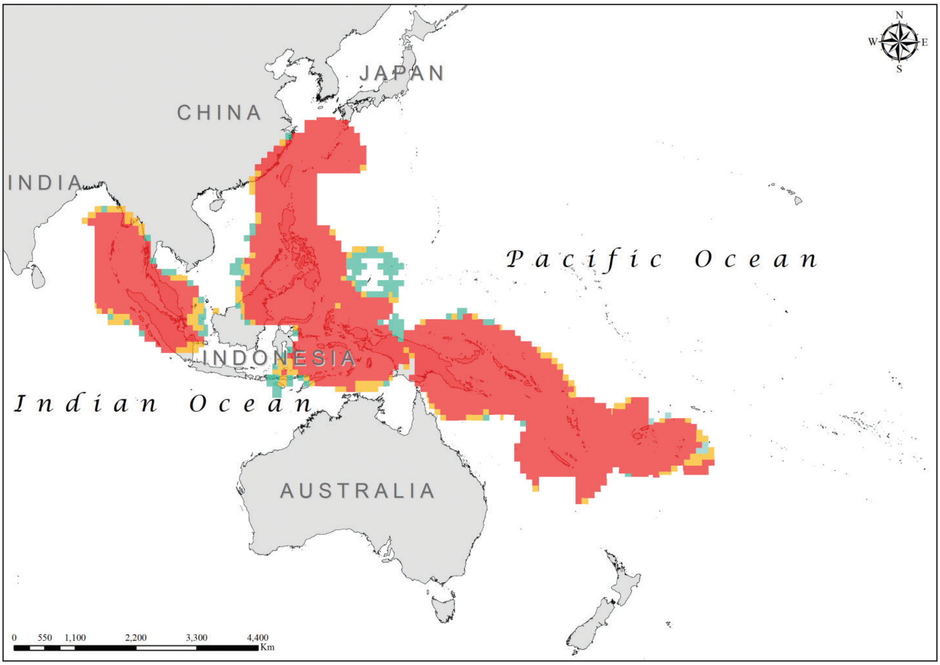

Figure 1. Distribution of hot spots for the *Laticauda* group (color codes reflect statistical confidence; red for 99% confidence level, orange for 95% confidence level, and green for 90% confidence level).

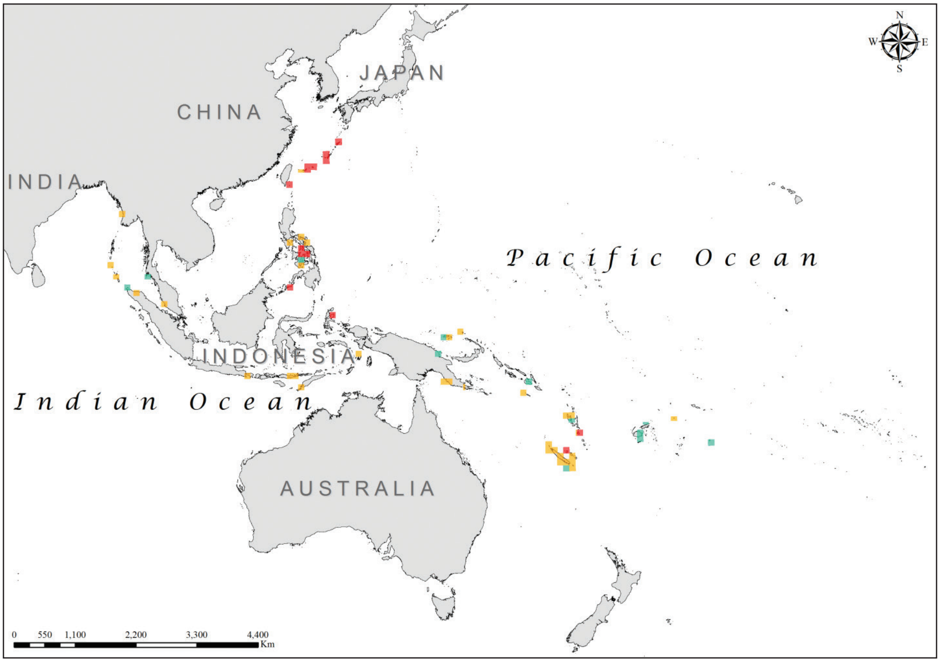

Figure 2. Shannon-Wiener diversity index for the *Laticauda* group (green: H' = 0.000001–0.56; orange: H' = 0.57–0.82; red: H' = 0.82–1.08).

cal variability across its distribution range, all populations are currently regarded as a single species (Heatwole 2010; Heatwole et al. 2005; Heatwole and Cogger 2013; Lane and Shine 2011b). In contrast, all other species of the *L. colubrina* group have very narrow distribution ranges. *Laticauda frontalis* is considered endemic to Loyalty Islands and the islands of Vanuatu (Cogger and Heatwole 2006) (Figure 4; Suppl. material 1). Except for this latter location, the available records indicate that the species is sympatric with *L. colubrina* throughout Vanuatu (Figures 3 and 4; Suppl. material 1). *Laticauda guineai* has a very small distribution range, known from only two areas in southern Papua New Guinea (Heatwole et al. 2005) (Figure 5; Suppl. material 1). *Laticauda saintgironsi* is endemic to New Caledonia, including the Loyalty Islands (Figure 6; Suppl. material 1), as reported by other authors (Cogger and Heatwole 2006; Heatwole and Cogger 2013). In the Loyalty Islands, the species occurs in sympatry with *L. frontalis* (Figures 4 and 6; Suppl. material 1).

Laticauda laticaudata has a very wide range, similar to that of *L. colubrina* (Figures 3 and 7; Suppl. material 1), but the range of *L. laticaudata* is much more fragmented and the species' EOO and AOO are considerably smaller (Tables 2–3) than those of *L. colubrina.* The easternmost location of *L. laticaudata* is the Island of Niue while the northernmost and westernmost limits for the species are the same as for *L. colubrina* (Figures 3 and 7; Suppl. material 1). However, *L. laticaudata* also occurs in New Caledonia, where it is sympatric with *L. saintgironsi*, and in Vanuatu, where it is sympatric with *L. frontalis* (Figures 4, 6, and 7; Suppl. material 1). Contrasting with this very broad range, the only other sea krait species from the *L. laticaudata* group, *L. crockeri*, is known from a single location, the Lake Te'Nggano from Rennell Island, Solomon Islands (e.g., Elfes et al. 2013; Heatwole and Cogger 2013). The ecology of this species is virtually unknown (but see Cogger et al. 1987) and the species is considered highly vulnerable (Elfes et al. 2013), thus ecological and conservation research should be prioritized for this species.

The *L. semifasciata* group comprises another two species with contrasting and allopatric distribution ranges: *L. schistorhyncha*, endemic to the Island of Niue (Figure 8; Suppl. material 1) (Heatwole and Cogger 2013), and *L. semifasciata*, with a relatively wide but fragmented distribution range, southern Japan being its northernmost limit. Although the southern limit for the species was previously considered to be the Maluku Islands (Heatwole and Cogger 2013), our database indicates an extension of the known range, with new records from southern Indonesia (Figure 9; Suppl. material 1).

With the exception of one species, all sea kraits presented a terrestrial EOO < 25%. The notable exception is *L. guineai* for which more than 80% of the EOO comprises terrestrial surfaces. In a recent attempt to assess the conservation status of the world's marine elapids, Elfes et al. (2013) have also calculated values for the extent of occurrence of several *Laticauda* species. The EOO calculated by us for *L. frontalis* (93,874 km^2) considerably exceeds the value reported by Elfes et al. (2013), which was less than 15,000 km^2. This discrepancy is due to a coarser grid (NGA 2014) that was used in our study.

For the entire Hydrophiinae, the greatest diversity of hot spots comprise the Gulf of Thailand, the Java Sea, the Timor Sea, Arafura Sea, and the Gulf of Carpentaria

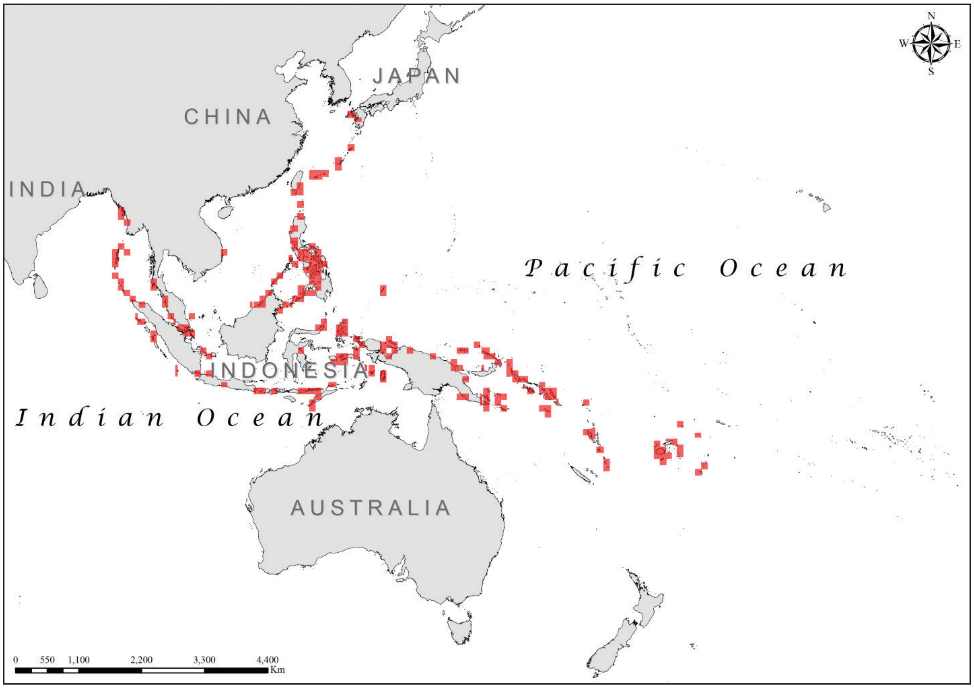

Figure 3. Distribution of *L. colubrina*.

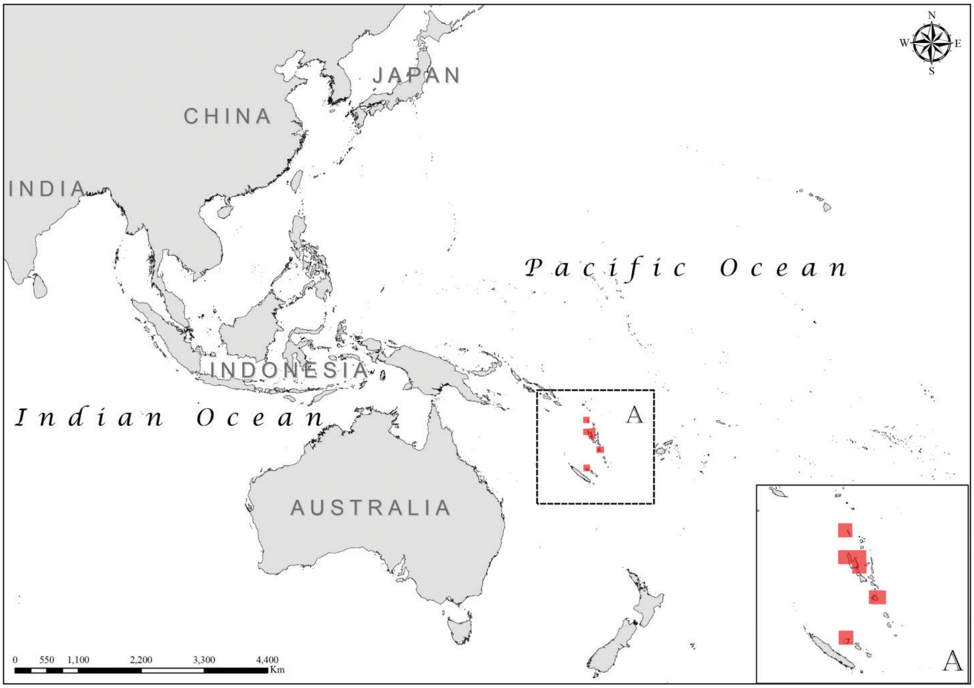

Figure 4. Distribution of *L. frontalis*, regional view and zoomed in (**A**).

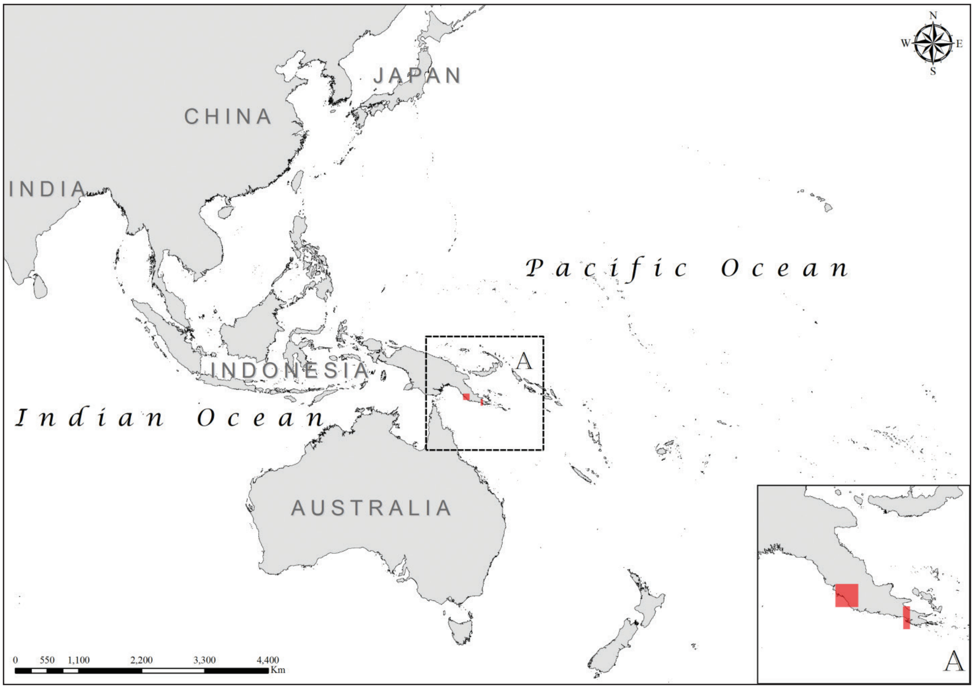

Figure 5. Distribution of *L. guineai*, regional view and zoomed in (**A**).

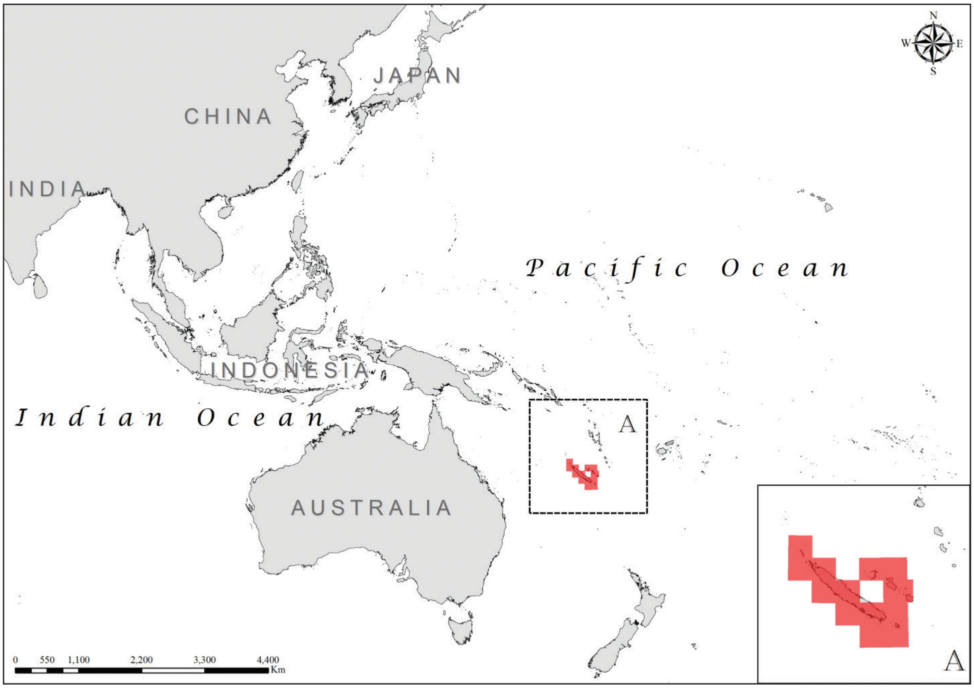

Figure 6. Distribution of *L. saintgironsi*, regional view and zoomed in (**A**).

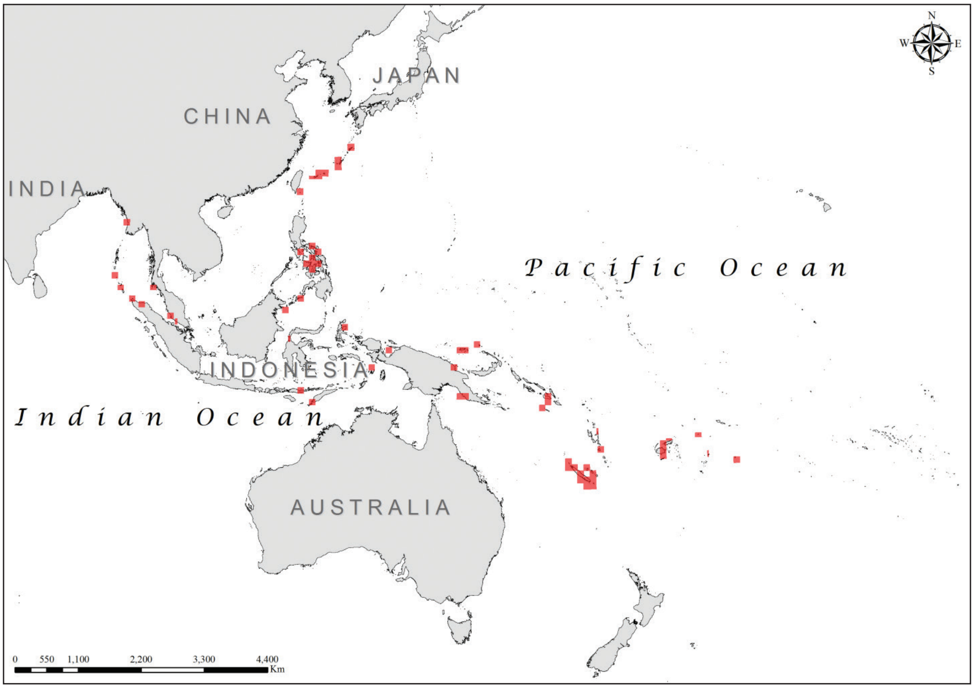

Figure 7. Distribution of *L. laticaudata*.

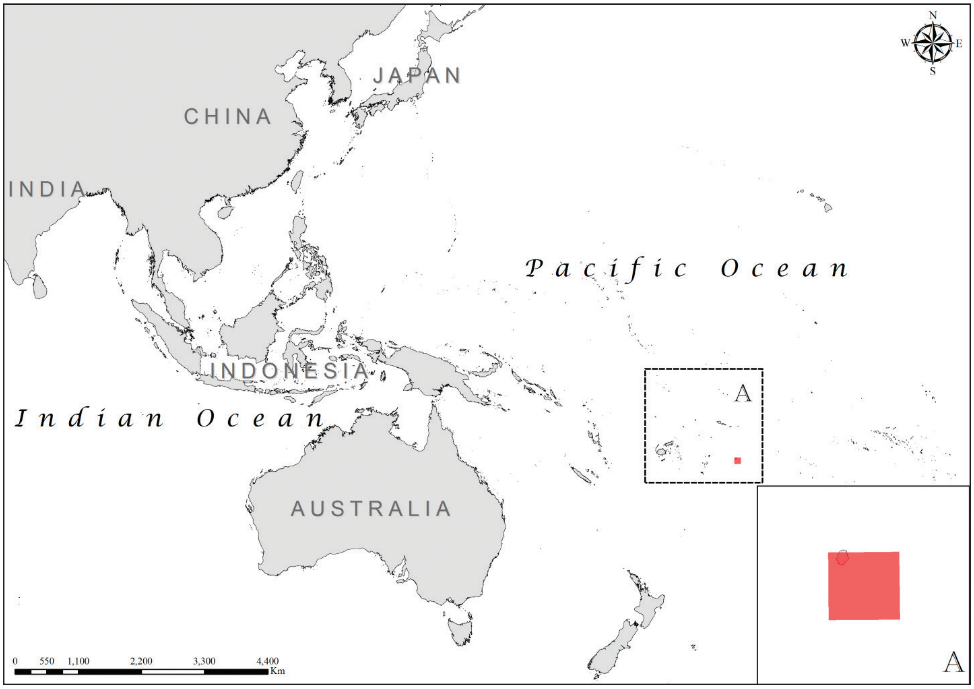

Figure 8. Distribution of *L. schistorhynchus*, regional view and zoomed in (**A**).

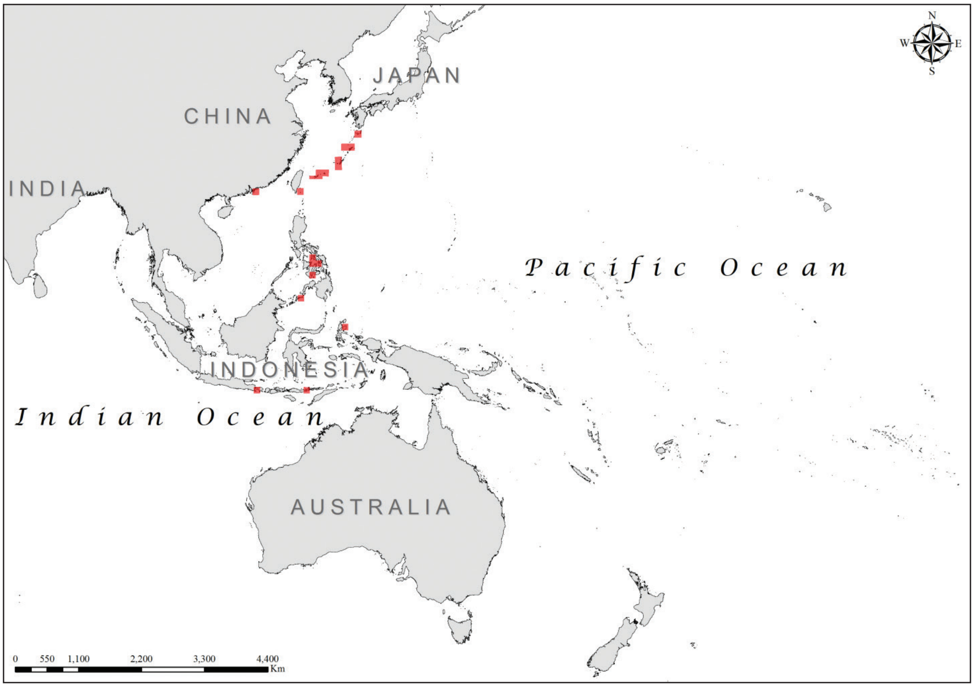

Figure 9. Distribution of *L. semifasciata*.

(Elfes et al. 2013). Remarkably, the areas of greatest diversity of sea kraits specifically, as identified by the current study, are outside the major hot spots for Hydrophiinae (Elfes et al. 2013), despite broad preferences of most marine Elapids for benthic habitats and coral reefs (Heatwole 1999). This difference suggest that the center of origin for the transition to marine life in Hydrophiinae and in sea kraits may be different, albeit geographically relatively close (Brischoux et al. 2012).

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Supplementary material 1

Occurrence dataset of all sea krait species included in this study

Authors: Iulian Gherghel, Monica Papeş, François Brischoux, Tiberiu Sahlean, Alexandru Strugariu

Data type: Occurrences

Explanation note: Geodatabase of occurrence records of all sea krait species compiled in the current study.