

Checklist of ascidians (Chordata, Tunicata) from the southern Gulf of Mexico

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Abstract

This study is the first inventory of ascidians from shallow waters (0–25 m) of coastal and reef habitats in the southern Gulf of Mexico where ascidian diversity is poorly known. Sampled environments in 14 locations (38 sites) with 134 samples collected from 2015 to 2017 included coral reefs, coastal lagoons, mangroves, seagrass, ports, and artificial platforms. The 31 identified species comprise 19 genera and 13 families. Ten species are newly reported in the Gulf of Mexico: *Ascidia panamensis* Bonnet & Rocha, 2011; *Ecteinascidia styeioides* (Traustedt, 1882); *Cystodytes roseolus* Hartmeyer, 1912; *Eudistoma* aff. *amanitum* Paiva & Rocha, 2018; *Eudistoma recifense* Millar, 1977; *Eubherdmania fasciculata* Monniot, 1983; *Eubherdmania* aff. *vitrea* Millar, 1961; *Polycarpa cartilaginea* (Sluiter, 1885); *Botrylloides magnicoecum* (Hartmeyer, 1912) and *Didemnum granulatum* Tokioka, 1954. Two new species will be described separately (*Clavelina* sp. and *Pyura* sp.). This study provides the first records for 26 species ascidians for the region as well as describes increased distributions of ten Atlantic species. Thus, our data provide a starting point for future ecological, experimental and taxonomic studies of ascidians of the Gulf of Mexico.

Keywords

Ascidacea, biodiversity, Gulf of Mexico, Yucatán

Introduction

The Ascidacea is the most diverse class of tunicates with ca 3000 recognized species, with representatives found in all marine habitats (Shenkar and Swalla 2011). Local ascidian species diversity depends primarily on availability and diversity of hard substrates, as well as temperature and salinity (Lambert 2005), while population density depends on food availability (organic particles suspended in water; Monniot et al. 1991). Ascidians are active suspension filter-feeders and are key organisms at times when they contribute to the control of phytoplankton (Petersen and Riisgard 1992) and may reduce eutrophication or contaminant concentration (Naranjo et al. 1996, Draughon et al. 2010). Many species colonize most artificial substrates and thereby become among the dominant members of “fouling” communities (Carballo 2000). Among foulers there are species known for their invasion potential worldwide (Lambert 2005). Ascidians are also known because of the presence of bioactive metabolites with potential biomedical interest (Erba et al. 2001).

Ascidian diversity in the Gulf of Mexico includes records of 79 species in 15 families in the northern Gulf of Mexico (Carballo 2000, Cole and Lambert 2009, CONABIO 2016, Fortaleza and Lotufo 2018). The southern Gulf of Mexico, however, is much less known and, despite the ecological and biotechnological importance of ascidians, only includes nine reported species: *Aplidium exile* (Van Name, 1902); *Polyclinum constellatum* Savigny, 1816; *Ecteinascidia turbinata* Herdman, 1880; *Eudistoma capsulatum* (Van Name, 1902); *Eudistoma hepaticum* (Van Name, 1921); *Stomozoa roseola* (Millar, 1955); *Botrylloides niger* Herdman, 1886; *Symplegma brakenhielmi* (Michaelsen, 1904) and *Symplegma viride* Herdman, 1886 (Van Name 1945; Carballo 2000). Essentially there are no studies from Mexico and the seven reported species is far below the expected number considering the great diversity of suitable habitats. To fill this gap, here we provide an inventory of the coastal species of ascidians in reefs and other shallow habitats in the southern Gulf of Mexico, along the Yucatán Peninsula.

Material and methods

Samples were collected in 14 locations and 38 sites from 2015 to 2017 in coral reefs, coastal lagoons, mangroves, seagrass, ports and artificial platforms by free diving and SCUBA, in the states of Veracruz, Tabasco, Campeche, Yucatan and Quintana Roo (Fig. 1, Table 2). Specimens were anesthetized in menthol and fixed with 4% formaldehyde in seawater. External characters of ascidians provide little information for determining their taxonomy and therefore dissection is required, for which a list of multiple characters is available (Monniot and Monniot 1972; Monniot et al. 1991; Rocha et al. 2012). Dissection was carried out following Monniot and Monniot (1972) and internal structures were stained with Harris hematoxylin dye (see: <https://bocasarts.weebly.com/tunicate-tools.html>). Families and genera were identified following Rocha et al. (2012) for species of the Atlantic Ocean.

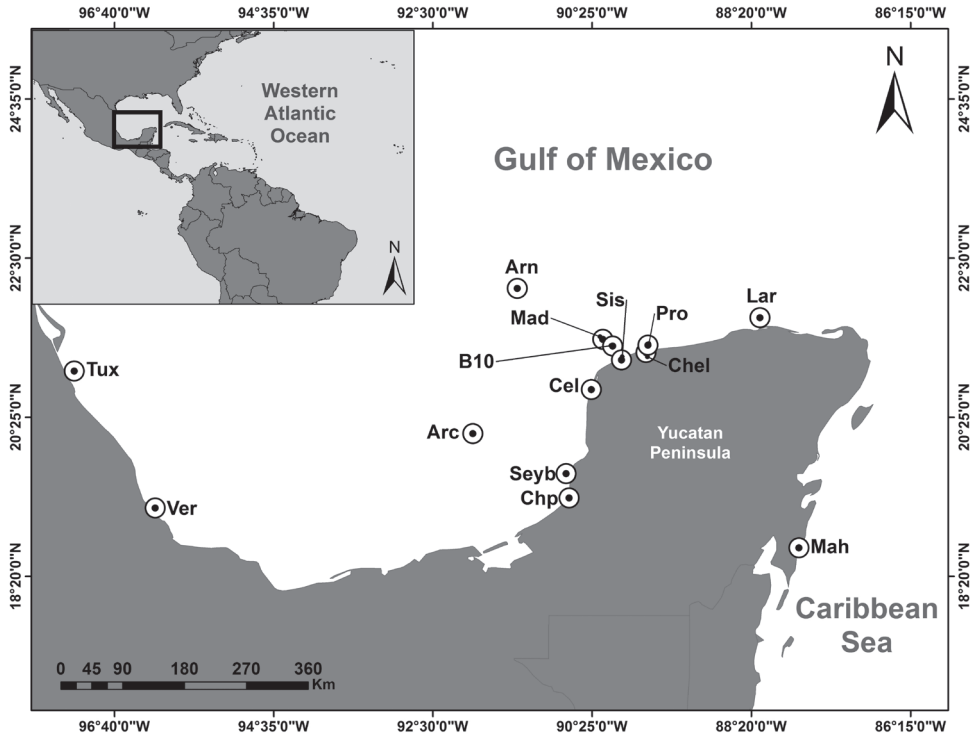


Figure 1. Study area in the southern Gulf of México. Abbreviations: Tuxpan Reef – Tux, Veracruz Reef – Ver, Arcas Cay Reef – Arc, Seybaplaya – Sey, Champotón – Chp, Celestún – Cel, Arenas Cays – Arn, Madagascar Reef – Mad, Bajo 10 Reef – B10, Chelém Coastal Lagoon – Chel, Progreso Harbor – Pro, Ría Lagartos – Lar, Mahahual Harbor – Mah, and Sisal Harbor – Sis.

Specimens were deposited in the Colección de Ascidias del Golfo de México (CAGoM), which is part of the collection of the Marine Invertebrates of Gulf of Mexico, National Autonomous University of Mexico (UNAM), Mérida, Yucatán. The resulting dataset has been uploaded to the Zenodo data repository (Alvarez et al. 2018).

Results

In 134 samples we identified 31 species in 19 genera and 13 families in 14 locations at 38 sites (see Table 2). We report ten species for the first time in the Gulf of Mexico: *Ascidia panamensis* Bonnet & Rocha, 2011; *Cystodytes roseolus* Hartmeyer, 1912; *Ecteinascidia styloloides* (Traustedt, 1882); *Eudistoma* aff. *amanitum* Paiva & Rocha, 2018; *Eudistoma recifense* Millar, 1977; *Euherdmania fasciculata* Monniot, 1983; *Euherdmania* aff. *vitrea* Millar, 1961; *Polycarpa cartilaginea* (Sluiter, 1885); *Botrylloides magnicoecus* (Hartmeyer, 1912) and *Didemnum granulatum* Tokioka, 1954 (Table 1) and two new species that will be reported somewhere else. Most specimens were found on natural substrates (rocks, corals and algae) followed by artificial substrates (oil platforms, docks and sunken ships).

Table 1. Species checklist of ascidians in south Gulf of Mexico. Abbreviations: (Tux) Reef Tuxpan, (VeR) Veracruz Reef, (Arc) Reef Arcas Cay, (Sey) Seybaplaya, (Chp) Champotón, (Cel) Celestún, (Arn) Arenas Cays, (Mad) Reef Madagascar, (B10) Reef Bajo 10, (Chel) Coastal Lagoon Chelém, (Pro) Progreso Harbor, (Lar) Ría Lagartos (Sis) Sisal Harbor, and (Mah) Mahahual Harbor. (*) New records for Gulf of Mexico.

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Table 2. Study localities in south of Gulf of Mexico.

Localities	Latitude	Longitude
Progreso Harbor		
Pro 1	21°19'56.4"N	89°41'17.8"W
Pro 2	21°20'58.1"N	89°40'49.1"W
Pro 3	21°21'41.12"N	89°41'7.02"W
Reef Arcas Cay		
Arc 1	20°12'11"N	91°58'56"W
Arc 2	20°12'13"N	91°58'34"W
Arc 3	20°12'16.62"N	91°57'48.13"W
Arc 4	20°12'16.9"N	91°58'39.8"W
Arc 5	20°12'17.17"N	91°57'48.06"W
Arc 6	20°12'19.95"N	91°57'39.19"W
Arc 7	20°12'31.1"N	91°57'51.37"W
Arc 8	20°12'32.14"N	91°57'41.04"W
Arc 9	20°12'35.6"N	91°58'0.7"W
Arc 10	20°12'36.36"N	91°57'51.08"W
Arc 11	20°12'41.6"N	91°57'49.1"W
Arc 12	20°12'56.6"N	91°58'31.3"W
Reef Arenas Cays		
Arn 1	22°6'12.73"N	91°23'41.64"W
Arn 2	22°6'54.11"N	91°23'42.17"W
Reef Madagascar		
Mad 1	21°26'16.1"N	90°16'36.6"W
Mad 2	21°26'16.4"N	90°16'39.3"W
Mad 3	21°26'17.5"N	90°16'34.9"W
Mad 4	21°26'17.7"N	90°16'39.7"W
Reef Bajo 10		
B10	21°20'58"N	90°8'52.3"W
Celestún		
Cel 1	20°46'43.4"N	90°25'36.1"W
Cel 2	20°49'0.4"N	90°25'59.3"W
Champotón		
Chp 1	19°21'18.98"N	90°43'35.77"W
Chp 2	19°21'41.8"N	90°43'3.4"W
Coastal Lagoon Chelém		
Chel 1	21°15'47"N	89°44'28.82"W
Chel 2	21°15'55.26"N	89°42'39.08"W
Mahahual Harbor		
Mha	18°42'30"N	87°42'40"W
Sisal Harbor		
Sis	21°10'4.29"N	90°1'55.3"W
Ría Lagartos		
Lar 1	21°43'19.9"N	88°13'11.8"W
Lar 2	21°43'23.6"N	88°13'6.5"W
Lar 3	21°43'8.4"N	88°12'27.1"W
Seybaplaya		
Sey 1	19°39'3.3"N	90°42'31.4"W
Sey 2	19°40'44.3"N	90°45'20.6"W
Sey 3	19°44'11.7"N	90°48'22.8"W
Reef Tuxpan		
Tux	21°1'21.5"N	97°11'27.4"W
Veracruz Reef		
VeR	19°12'25.5"N	97°4'7"W

Systematics

Subphylum Tunicata Lamarck, 1816

Class Ascidiacea Blainville, 1824

Order Phlebobranchia Lahille, 1886

Family Ascidiidae Herdman, 1882

Genus *Ascidia* Linnaeus, 1767

***Ascidia panamensis* Bonnet & Rocha, 2011**

Fig. 2A

Material examined. CAGoM-0023, Mad 1, 9 m, 20-04-2015, leg. L. Palomino-Alvarez; CAGoM-0182, Arc 3, 3 m, 30-10-2015, leg. L. Palomino-Alvarez; CAGoM-0187, CAGoM-00189, Arc 6, 2 m, 30-10-2015, leg. L. Palomino-Alvarez; CAGoM-0190, CAGoM-0191, Arc 8, 2 m, 31-10-2015, leg. L. Palomino-Alvarez.

Remarks. These specimens are of uniformly dark coloration inside the siphons, in contrast to specimens from Panama which have white lines between the siphon lobes (Bonnet and Rocha 2011a). Mexican specimens are also smaller with conical papillae on the tunic in the area of the oral siphon, the shape of the dorsal tubercle is variable, and the anus is smooth. All specimens were found under rocks, two to three specimens per rock, in association with *Polycarpa cartilaginea* (Sluiter, 1898) and *Corella minuta* Traustedt, 1882. Symbiosis was also noted with palaemonid crustaceans (*Ascidonia miserabilis* (Holthuis, 1951)) living in the pharynx of some of the larger specimens.

Global distribution. Mexico (as described here) and Panamá (Bonnet and Rocha 2011a).

Genus *Phallusia* Savigny, 1816

***Phallusia nigra* Savigny, 1816**

Material examined. CAGoM-0062, Pro 1, 4 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0085, CAGoM-0089, Sey 1, 11 m, 12-06-2015, leg. L. Palomino-Alvarez; CAGoM-0733, Sis, 1 m, 21-03-2018, leg. Bryan Flores;

Photographed record (no specimens in the collection): Tux, 5 m, 21-09-2015.

Remarks. This species was only recorded on artificial substrates and shallow rocks near the shore.

Global distribution. United States (Van Name 1921, 1945; Plough 1978), Bermudas (Herdman 1882; Verrill 1900; Van Name 1902, 1945; Monniot 1973), Panama (Collin et al. 2005; Rocha et al. 2005; Bonnet and Rocha 2011a), Curaçao (Millar 1962; Goodbody 1984), Venezuela (Bermudez and Grimaldi 1975), Guadeloupe (Monniot 1983a), Martinique (Monniot 2018a), French Guiana (Monniot 2016),

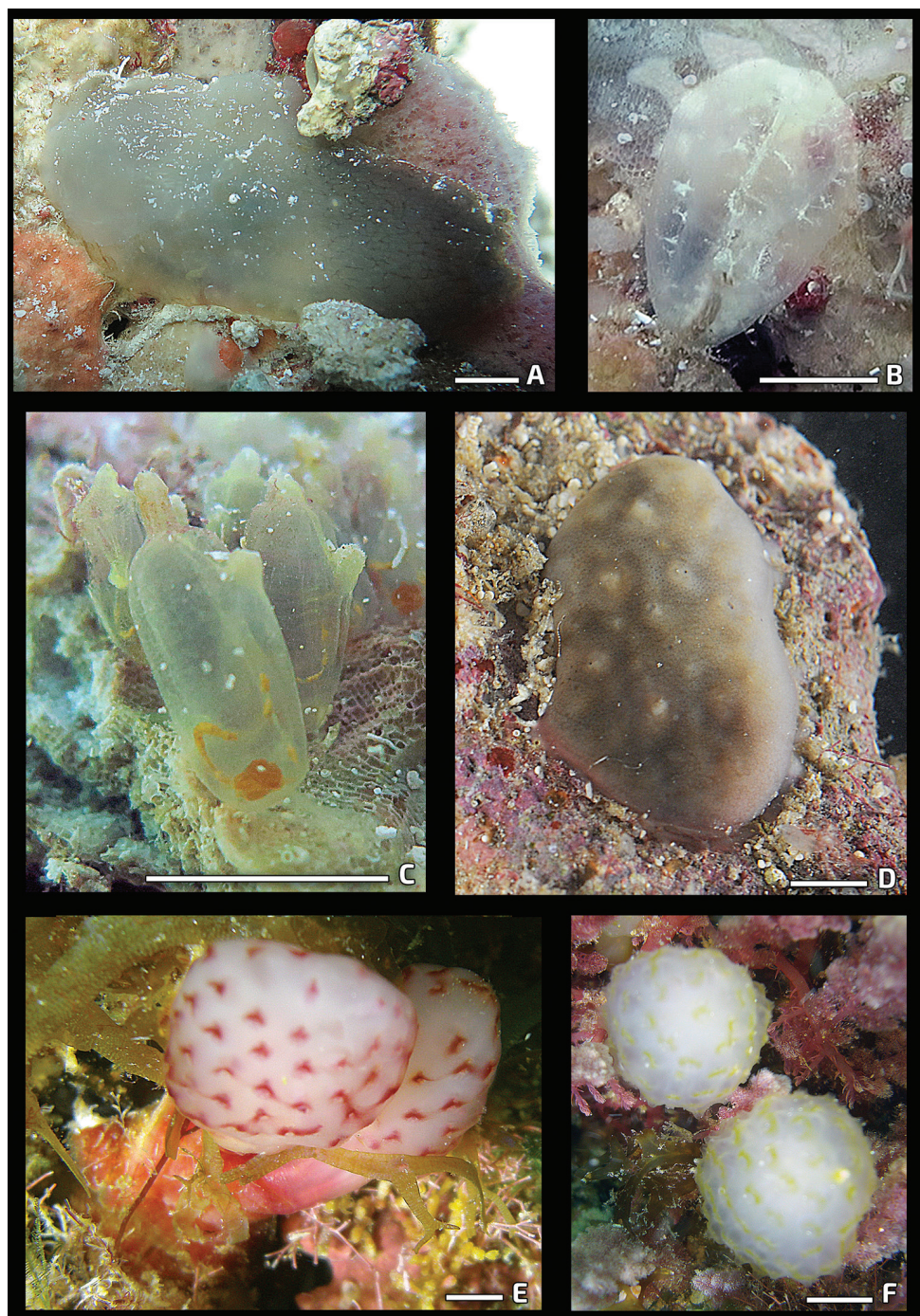


Figure 2. Photos of live specimens in situ in the field. **A** *Ascidia panamensis* **B** *Corella minuta* **C** *Ecteinascidia styloloides* **D** *Cystodytes roseolus* **E, F** *Eudistoma* aff. *amanitum*. Scale bar: 1 cm.

Brazil (Van Name 1921, 1945; Millar 1958; Monniot 1970; Rodrigues 1962; Rocha and Costa 2005; Bonnet and Rocha 2011a; Dias et al. 2012), South Africa (Herdman 1880), Angola (Millar 1965), Greece (Kondilatos et al. 2010), Suez Canal (Harant 1927; Ghobashy and Abdel Messeih 1991), Israel (Pérès 1958; Shenkar 2012), Red Sea (Michaelsen 1918; Savigny 1816), Micronesia (unconfirmed – Nishikawa 1984; Lambert 2003).

Family Corellidae Lahille, 1888

Genus *Corella* Alder & Hancock, 1870

***Corella minuta* Traustedt, 1882**

Fig. 2B

Material examined. CAGoM-0369, Arc 5, 2 m, 21-08-2016, leg. L. Palomino-Alvarez; CAGoM-0384, Arcas Cay Reef, Yucatán, Arc 4, 2 m, 22-08-2016, leg. L. Palomino-Alvarez; CAGoM-0447, Arcas Cay Reef, Yucatán, Arc 7, 4 m, 25-08-2016, leg. L. Palomino-Alvarez.

Remarks. Specimens were found in a single location under rocks, together with *A. panamensis* and *Polycarpa cartilaginea*.

Global distribution. United States (Van Name 1921, 1930, 1945), Curaçao (Van Name 1924), Guadeloupe (Monniot 1983), Martinique (Monniot 2018), Mozambique (Monniot 1997), Japan (Tokioaka and Nishikawa 1975), New Caledonia (Monniot 1987, 1991), Micronesia (Nishikawa 1984; Lambert 2003), and French Polynesia (Monniot and Monniot 1987a).

Family Perophoridae Giard, 1872

Genus *Ecteinascidia* Herdman, 1880

***Ecteinascidia styeloides* (Traustedt, 1882)**

Fig. 2C

Material examined. CAGoM-0441, Arc 9, 9 m, 27-08-2016, leg. L. Palomino-Alvarez; CAGoM-0442, CAGoM-0444, Bajo 10 Reef, Yucatán, B10, 7 m, 19-10-2016, leg. L. Palomino-Alvarez.

Remarks. Colonies of many individuals and many sizes were found on seaweed and under rocks.

Global distribution. Jamaica (Goodbody 1984, Goodbody and Cole 2006), Guadeloupe (Monniot 1983a), Belize (Goodbody 2004; Goodbody and Cole 2006), Panama (Collin et al. 2005; Rocha et al. 2005), Venezuela (Goodbody 2004; Rocha et al. 2010), Mozambique (Monniot 1997).

***Ecteinascidia turbinata* Herdman, 1880**

Material examined. CAGoM-0020, Arn 2, 7 m, 03-19-2015, leg. L. Palomino-Alvarez; CAGoM-0026, CAGoM-0027, CAGoM-0028, CAGoM-0031, CAGoM-0033, CAGoM-0034, CAGoM-0035, Chel 2, 1 m, 11-05-2015, leg. L. Palomino-Alvarez; CAGoM-0054, Pro 1, 3 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0063, Chp 1, 4 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0171, Arc 2, 9.4 m, 30-10-2015, leg. L. Palomino-Alvarez.

Remarks. Colonies with the largest number of zooids were found in coastal lagoons on wooden piers or mangrove prop roots as well as coral reefs far from the shore. This was the most common species in Chelém (an enclosed, very salty, lagoon), comprising two morphotypes. Some had orange zooids and others had transparent, uncolored, zooids with a ring of orange along the siphon rim. This species was never found in disturbed port areas.

Global distribution. United States (Van Name 1921, 1945; Plough 1978), Bermudas (Herdman 1882; Verrill 1900; Berrill 1932, 1935; Monniot 1972; Van Name 1902, 1945), Cuba (Hernández-Zanuy and Carballo 2001), Jamaica (Goodbody 2003; Goodbody and Cole 2006), Turks and Caicos Islands (Millar 1962), Mexico, Yucatán Peninsula (Carballo 2000), Belize (Goodbody 2000), Panama (Collin et al. 2005), Curacao (Goodbody 1984), Venezuela (Goodbody 1984a; Rocha et al. 2010; Carballo-Pérez and Díaz 2011), Guadeloupe (Monniot 1983a), Martinique (Monniot 2018a), Guyana (Millar 1978), French Guiana (Monniot 2016), Senegal (Pérès 1949, 1951; Lafargue and Wahl 1990; Monniot and Monniot 1994), Sierra Leone (Millar 1956), Gibraltar (Naranjo and García-Gómez 1994), Balears Islands (Ramos et al. 1993; Spain (Casso et al. 2018), France (Harant 1927, Harant and Vernières 1933, Thessalou-Legaki et al. 2012), Tunisia (Pérès 1954), and Egypt (Harant 1927).

Order Aplousobranchia Lahille, 1886**Family Clavelinidae Forbes & Hanley, 1848****Genus *Clavelina* Savigny, 1816*****Clavelina oblonga* Herdman, 1880**

Material examined. CAGoM-0081, CAGoM-0082, Sey 3, 11 m, 19-03-2015, leg. L. Palomino-Alvarez; CAGoM-0093, Mad 2, 7 m, 27-05-2015, leg. L. Palomino-Alvarez.

Remarks. Specimens from Seybaplaya, Campeche were associated with the hydroid *Macrorhynchia philippina* Kirchenpauer, 1872 (Cnidaria: Hydrozoa), from which they may gain protection from predators. The details of this association should be investigated.

Global distribution. United States (Van Name 1945; Plough 1978; Lambert et al. 2005), Bermudas (Herdman 1880, 1882; Monniot 1972; Van Name 1902, 1945; Verrill

1900; Berrill 1932), Jamaica (Goodbody 1993, 2003), Southwestern Gulf of Mexico (Van Name 1921), Curaçao (Goodbody 1984; Millar 1962), Venezuela (Rocha et al. 2010), Guadeloupe (Monniot 1983), Tobago (Cole 2012), Brazil (Millar 1958; Rocha et al. 2005a; Rocha and Costa 2005; Rocha and Kremer 2005; Rodrigues et al. 1998), Azores (Monniot and Monniot 1994), Madeira (Harant 1929); Senegal (Pérès 1951; Monniot 1969; Lafargue and Wahl 1987), Spain and Italy (Ordóñez et al. 2016; Casso et al. 2018).

Clavelina sp.

Material examined. CAGoM-0006, CAGoM-0007, Arn 1, 2 m, 19-03-2015, leg. L. Palomino-Alvarez; CAGoM-0021, Arn 2, 6 m, 19-03-2015, leg. L. Palomino-Alvarez.

Remarks. This species is dark blue with characteristics that do not match any known species and will be described elsewhere. The single colony found was small with few zooids (Table 1).

Family Polycitoridae Michaelsen, 1904

Genus *Cystodytes* Drasche, 1884

Cystodytes dellechiaiei (Della Valle, 1877)

Material examined. CAGoM-0135, CAGoM-0449, B10, 11 m, 17-06-2015, leg. L. Palomino-Alvarez.

Remarks. This is the only known west Atlantic species of the genus which has been reported from nine countries, both in tropical and subtropical regions (Rocha et al. 2005, 2012). The species is very rare in the southern Gulf of Mexico (Table 1); it has been found in shallow waters of the northern Gulf of Mexico in Florida (Van Name 1945).

Global distribution. United States (Van Name 1945; Plough 1978); Bermudas (Monniot 1972; Van Name 1902, 1945), Panamá (Collin et al. 2005), Les Saints, Martinique (Gravier 1955), Guyana (Millar 1977), Brazil (Millar 1977; Rocha et al. 2005), Azores (Michaelsen 1923; Monniot 1971; Monniot and Monniot 1994; Monniot 1975), Canary Islands (Ríos 1985), Senegal (Michaelsen 1915; Monniot 1969; Pérès 1949, 1951; Lafargue and Wahl 1987), Iberic Mediterranean (López-Legentil and Turon 2005), France (Harant 1927; Harant and Vernières 1933; Lafargue 1970), Italy (Drasche 1883; Brunetti 1994), Philippines (Van Name 1918), and Australia (Michaelsen 1930; Millar 1953; Kott 1990).

Cystodytes roseolus Hartmeyer, 1912

Fig. 2D

Material examined. CAGoM-0114, B10, 11 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0043, Pro 2, 7 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0064, Chp 1,

2 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0105, Mad 4, 5 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0465, B10, 11 m, 17-06-2015, leg. L. Palomino-Alvarez.

Remarks. *Cystodytes roseolus* might have been found in Atlantic Panama in 2003 but identification needs to be confirmed due to the disjunct distribution (Rocha et al. 2005). This second report of the species in the Gulf of Mexico indicates that this species occurs on both sides of the Atlantic. Finding this species in Progreso Harbor and nearby locations suggests that this species was introduced to the Yucatán peninsula from Africa by ship transport.

Global distribution. Senegal (Pérès 1949; Monniot 1969; Lafargue and Wahl 1987; Monniot and Monniot 1994), South Africa (Hartmeyer 1912; Michaelsen 1919, 1934; Millar 1962), Seychelles (Michaelsen 1919).

Genus *Eudistoma* Caullery, 1909

Eudistoma aff. *amanitum* Paiva & Rocha, 2018

Fig. 2E, F

Material examined. CAGoM-0074, Mad 2, 9 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-0070, Mad 2, 10 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-0100, Mad 4, 12 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0112, B10, 7 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0115, B10, 9 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0139, CAGoM-0140, CAGoM-0142, Lar 1, 10 m, 07-10-2015, leg. L. Palomino-Alvarez; CAGoM-0149, CAGoM-0150, Lar 2, 10 m, 07-10-2015, leg. L. Palomino-Alvarez; CAGoM-0152, Lar 2, 12 m, 07-10-2015, leg. L. Palomino-Alvarez; CAGoM-0163, Lar 3, 12 m, 07-10-2015, leg. L. Palomino-Alvarez.

Remarks. Colonies from Mexico and Panama vary by location in the number of heads per peduncle and shape, zooid size, zooid position within the tunic, and color (Paiva and Rocha 2018). Gonads were undeveloped and no larvae were found, thus this species identification remains to be confirmed.

Global distribution. Southern Gulf of Mexico (described herein) and Panama (Paiva and Rocha 2018).

Eudistoma clarum (Van Name, 1902)

Material examined. CAGoM-0041, Pro 3, 8 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0051, Pro 1, 14 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0077, Mad 3, 12 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-0103, Mad 4, 9 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-00166, Lar 3, 12 m, 07-10-2015, leg. L. Palomino-Alvarez.

Remarks. Records of *E. clarum* have been found in mangroves and to a depth of 20 m in coral reefs (Goodbody, 2000). We found specimens mainly in coral reefs and near shore in places with strong anthropogenic impact.

Global distribution. United States (Van Name 1921), Bermudas (Van Name 1902, 1945; Berrill 1932; Monniot 1972), Belize (Goodbody 2000), Panama (Collin et al. 2005; Rocha et al. 2005), Bonaire (Millar 1962), Venezuela and Caribbean Islands (Millar 1962; Goodbody 1984), Tobago (Cole 2012), Guadeloupe (Monniot 1983), Senegal (Pérès 1949), and French Polynesia (Monniot and Monniot 1987a).

***Eudistoma hepaticum* (Van Name, 1921)**

Fig. 3G

Material examined. CAGoM-0052, Pro 1, 6 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0068, Chp1, 26-05-2015, 2 m, leg. L. Palomino-Alvarez; CAGoM-0091, Sey 2, 12-06-2015, 11 m, leg. Palomino-Palomino Alvarez; CAGoM-0088, Sey 1, 12-06-2015, 11 m, leg. Palomino-Palomino Alvarez; CAGoM-0107, Mad 4, 17-06-2015, 9 m, leg. L. Palomino-Alvarez; CAGoM-0072, Mad 2, 13 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-0039, Cel 1, 11-05-2015, 1 m, leg. L. Palomino-Alvarez; CAGoM-0116, B10, 9 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0087, Sey 2, 11 m, 12-06-2015, leg. L. Palomino-Alvarez.

Remarks. We found large (about 20 cm in diameter) purple or blue colonies on cement columns in Progreso Harbor and smaller colonies were found on coral reefs. This is the most common species of *Eudistoma* in the region.

Global distribution. United States (Van Name 1945; Plough 1978), Bermudas (Van Name 1902), Jamaica (Goodbody 2003, Van Name 1921), St. Thomas (Van Name 1921), Mexico (Van Name 1945), Curaçao (Goodbody 1984b), Venezuela (Goodbody 1984a), and Guadeloupe (Monniot 1983c).

***Eudistoma obscuratum* (Van Name, 1902)**

Material examined. CAGoM-0073, Mad 2, 8 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-0101, CAGoM-0109, Mad 4, 12 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0159, Lar 2, 14 m, 07-10-2015, leg. L. Palomino-Alvarez.

Remarks. Colonies are small and found beneath rocks and on bivalve shells.

Global distribution. United States (Van Name 1921, 1945), Bermuda (Monniot 1972; Van Name 1902, 1945), Virgin Islands (Van Name 1921, 1945), and Belize (Goodbody 2000).

***Eudistoma olivaceum* (Van Name, 1902)**

Fig. 3H

Material examined. CAGoM-0016, Mha, 1 m, 12-03-2015, leg. L. Palomino-Alvarez; CAGoM-0078, Chp 2, 0.5 m, 11-06-2015, leg. L. Palomino-Alvarez; CAGoM-0025,

Mad 4, 9 m, 04-04-2015, leg. L. Palomino-Alvarez; CAGoM-0060, Pro 1, 8 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0036, Chel 2, 11-05-2015, 1 m, leg. L. Palomino-Alvarez.

Remarks. Zooids of some samples have a third opening at the base of the thorax through which fecal pellets are expelled. While colonies appeared healthy, this third opening may have been due to body wall rupture caused by obstruction of the atrial canal by incubating larvae in the atrial cavity or excess sediments in the water. The appearance of third siphons may be induced by experimental injuries in a few solitary ascidians (Jeffery et al. 2015).

Global distribution. United States (Van Name 1921, 1945; Plough 1978), Bermudas (Berrill 1932; Monniot 1972; Van Name 1902, 1945), Jamaica (Goodbody 2003), Cuba (Van Name 1921), Puerto Rico (Van Name 1921), Guadeloupe (Monniot 1983c), Belize (Goodbody 2004), Curaçao (Van Name 1924; Millar 1962; Goodbody 1984), Venezuela (Millar 1962; Goodbody 1984a), Tobago (Cole 2012), Senegal (Lafargue and Wahl 1987), Micronesia (Nishikawa 1984).

Eudistoma recifense Millar, 1977

Fig. 3I

Material examined. CAGoM-0071, Mad 2, 11 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-0047, Pro 1, 8 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0137, B10, 11 m, 17-06-2015, leg. L. Palomino-Alvarez.

Remarks. Commonly found on grass beds, coral reefs and cement columns of harbors near the shore.

Global distribution. Southern Gulf of Mexico (present study) and Brazil (Millar 1977; Oliveira et al. 2014).

Family Stomozoidae Kott, 1990

Genus *Stomozoa* Kott, 1957

Stomozoa roseola (Millar, 1955)

Material examined. CAGoM-0076, Mad 3, 12 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-69, Chp 1, 5 m, 26-05-2015, leg. L. Palomino-Alvarez.

Remarks. Colonies were found on dead coral and between large rocks. The tunic is very firm and dark purple, similar to colonies from the Red Sea and Madagascar.

Global distribution. United States (Van Name 1945; Plough 1978); Mexico (Van Name 1945), French Guiana (Monniot 2016), Brazil (Millar 1977), South Africa (Millar 1955), Madagascar (Monniot 2012), Red Sea (Kott 1957), Indonesia (Monniot and Monniot 1996), and New Caledonia (Monniot 1988).

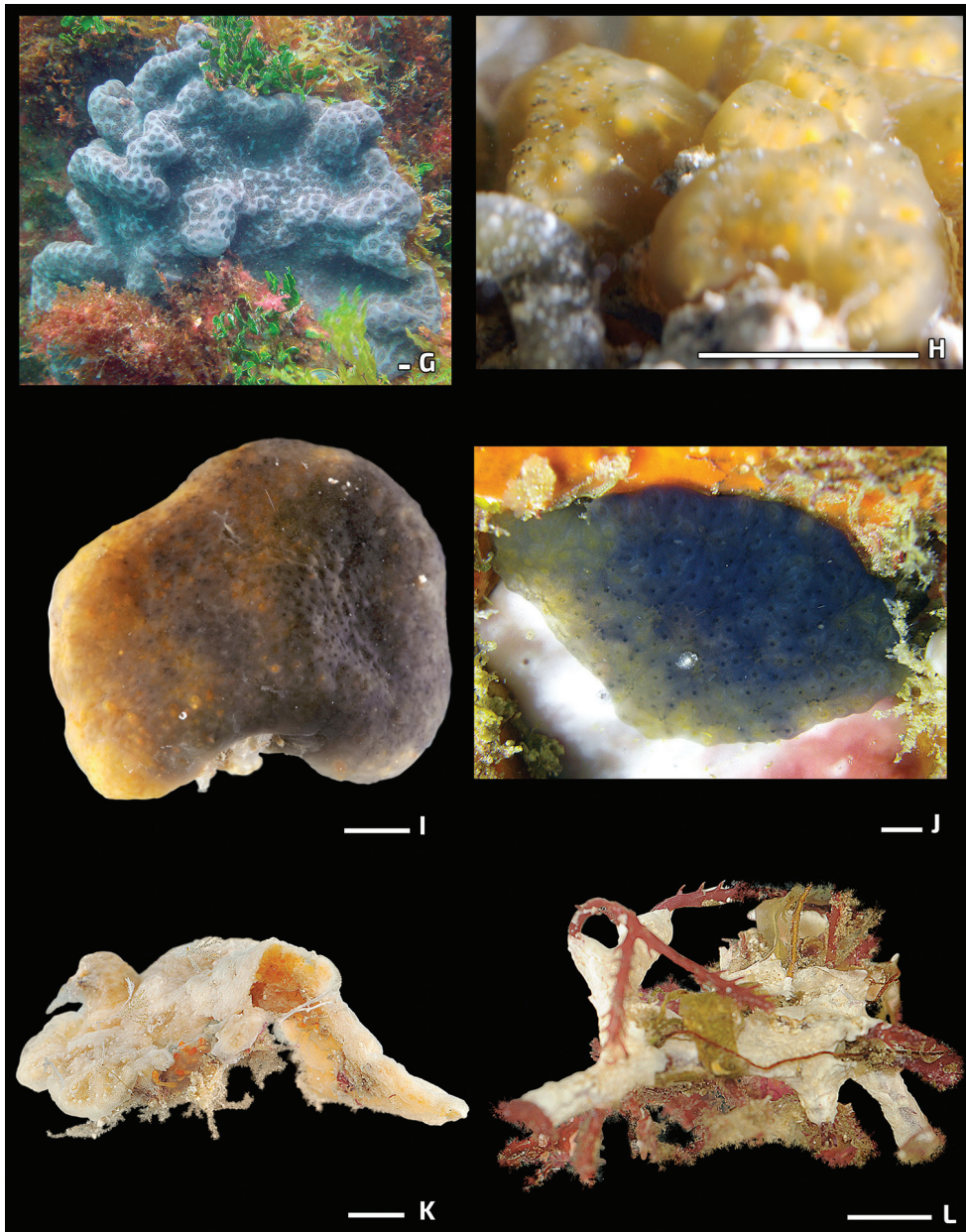


Figure 3. Photos of live specimens in the field (continued). **G** *Eudistoma hepaticum* **H** *Eudistoma olivaceum* **I** *Eudistoma recifense* **J** *Distaplia bermudensis* **K** *Polysyncraton amethysteum* (preserved specimen) **L** *Lissoclinum fragile*. Scale bar: 1 cm.

Family Holozoidae Berrill, 1950**Genus *Distaplia* Della Valle, 1881*****Distaplia bermudensis* Van Name, 1902**

Fig. 3J

Material examined. CAGoM-00102, Mad 4, 14 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0095 Mad 4, 17-06-2015, 9 m, leg. L. Palomino-Alvarez.

Remarks. Although this species is common in many Caribbean countries, we found it in only one location.

Global distribution. United States (Van Name 1921, 1945; Plough 1978), Bermudas (Van Name 1902, 1945; Berrill 1932; Gravier 1955; Monniot 1972), Cuba (Hernandez 1990), Jamaica (Goodbody 2003), Puerto Rico (Van Name 1921), Virgin Islands, St Thomas (Van Name 1921), Guadeloupe (Monniot 1983); Belize (Goodbody 2004), Panamá (Collin et al. 2005, Rocha et al. 2005), Curaçao (Millar 1962; Goodbody 1984), Venezuela (Millar 1962; Goodbody 1984; Rocha et al. 2010), Guyana (Millar 1978), French Guiana (Monniot 2016), Brazil (Millar 1958, 1977; Rodrigues and Rocha 1993; Rodrigues et al. 1998; Rocha et al. 2005; Rocha and Costa 2005; Rocha and Kremer 2005), Senegal (Pérès 1949), and Italy (Mastrototaro and Brunetti 2006).

Family Didemnidae Giard, 1872**Genus *Polysyncraton* Nott, 1892*****Polysyncraton amethysteum* Van Name, 1902**

Fig. 3K

Material examined. CAGoM-0158, Lar 2, 9 m, 07-10-2015, leg. L. Palomino-Alvarez; CAGoM-0118, B10, 9 m, 17-06-2015, leg. L. Palomino-Alvarez.

Remarks. Colony found on corals and rocks. Orange zooids when alive and in preservation.

Global distribution. United States (Van Name 1921), Bermuda (Van Name 1902, 1921, 1945), Puerto Rico (Van Name 1945), Guadeloupe (Gravier 1955), Martinique (Gravier 1955); Colombia (Van Name 1945), French Guiana (Monniot 2016), Brazil (Millar 1958, 1977; Rodrigues and Rocha 1993; Rocha et al. 2005; Rocha and Kremer 2005), Cape Verde (Monniot and Monniot 1967), Senegal (Pérès 1948, 1949), Ghana (Millar 1953), Tunisia (Pérès 1954).

Genus *Lissoclinum* Verrill, 1871***Lissoclinum fragile* (Van Name, 1902)**

Fig. 3L

Material examined. CAGoM-0143, Lar 1, 12 m, 07-10-2015, leg. L. Palomino-Alvarez.

Remarks. We found colonies in a single location growing on algae. *Lissoclinum fragile* is reported from tropical and subtropical regions where it is very common in marinas on artificial substrates, which suggests that it has been extensively introduced while the original geographical distribution remains unknown.

Global distribution. United States (Van Name 1921; Lambert et al. 2005), Bermuda (Van Name 1902; Berrill 1932), St Thomas (Van Name 1921), Jamaica (Goodbody 1993), Guadeloupe (Monniot 1983a), Belize (Goodbody 2004), Costa Rica (Tokioka 1972), Curaçao (Millar 1962; Van Name 1924), Venezuela (Millar 1962; Rocha et al. 2010), Tobago (Cole 2012), Brazil (Rodrigues et al. 1998; Rocha and Kremer 2005; Rocha and Faria 2005), Azores (Monniot 1974), Sierra Leone (Monniot and Monniot 1994), Madagascar (Vasseur 1970), Persian Gulf (Monniot 1997), India (Renganathan 1982), Philippines (Tokioka 1967), Indonesia (Lafargue and Vasseur 1989), Japan (Tokioka 1954), New Caledonia (Monniot 1992), Guam (Monniot and Monniot 2001; Lambert 2003), and French Polynesia (Monniot and Monniot 1987a; Monniot et al. 1985).

Genus *Didemnum* Savigny, 1816***Didemnum duplicatum* Monniot, 1983**

Material examined. CAGoM-050, CAGoM-0475, Pro 1, 13 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0080, Sey 3, 8 m, 12-06-2015, leg. L. Palomino-Alvarez; CAGoM-0108, Mad 4, 11 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0126, CAGoM-0133, B10, 7 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0186, Arc 6, 8 m, 30-10-2015, leg. L. Palomino-Alvarez.

Remarks. Colonies were found only near shore (harbors) and on artificial reefs. Recent molecular unpublished data (RMR) suggests that this might be a complex of three species.

Global distribution. United States (Lambert et al. 2005), Jamaica (Goodbody 2003), Guadeloupe (Monniot 1983a), Belize (Goodbody 2000), Curaçao (Goodbody 1984), Venezuela (Goodbody 1984; Rocha et al. 2010), Tobago (Cole 2012), French Guiana (Monniot 2016).

***Didemnum granulatum* Tokioka, 1954**

Material examined. CAGoM-0075, Mad 3, 7 m, 27-05-2015, leg. L. Palomino-Alvarez; CAGoM-0153, Lar 2, 10 m, 07-10-2015, leg. L. Palomino-Alvarez.

Remarks. With a global distribution, *D. granulatum* is known to rapidly colonize artificial substrates (Oren and Benayahu 1998), so it may be widely introduced, but its origin is unknown. In the southern Gulf of Mexico colonies were found on corals, rocks and algae near shore.

Global distribution. Panama (Rocha et al. 2005), French Guiana (Monniot 2016), Brazil (Dias et al. 2012; Paiva et al. 2015), Senegal (Monniot and Monniot 1994), South Africa (Monniot et al. 2001), Red Sea (Shenkar 2012), Hong Kong (Kott and Goodbody 1982), Philippines (Monniot and Monniot 2001), Papua New Guinea (Monniot and Monniot 2001), Japan (Tokioka 1954), Australia (Kott 2001), New Caledonia (Monniot 1995), French Polynesia (Monniot and Monniot 1987a), and Fiji (Kott 1981).

Family Polyclinidae Milne Edwards, 1841

Genus *Polyclinum* Savigny, 1816

Polyclinum constellatum Savigny, 1816

Fig. 4M

Material examined. CAGoM-0731, CAGoM-0732, CAGoM-0736, Chel 1, 0 m, 21-03-2018, leg. R.M. Rocha.

Remarks. This is another widespread species that was probably introduced in the southern Gulf of Mexico, yet we only found it in one harbor. The high salinity tolerance has been observed in Margarita Island, Venezuela where the species has also been found in an estuary with salinity > 50 ppt (Rocha et al. 2010).

Global distribution. United States (Van Name 1945), Gulf of Mexico (Van Name 1945; Lambert et al. 2005), Bahamas (Van Name 1945), Bermuda (Monniot 1972), Cuba (Van Name 1945), Jamaica (Van Name 1945, Goodbody 1993), Puerto Rico (Van Name 1921), Guadeloupe (Monniot 1983b), Martinique (Gravier 1955), Belize (Goodbody 2000), Panama (Carman et al. 2010), Colombia (Van Name 1945), Curaçao (Millar 1962; Goodbody 1984), Venezuela (Rocha et al. 2010; Carballo-Pérez and Díaz 2011), French Guiana (Monniot 2016), Brazil (Millar 1958; Rodrigues and Rocha 1993; Rocha et al. 2005, 2011), South Africa (Millar 1955), Mozambique (Michaelsen 1919, Monniot and Monniot 1976), Madagascar (Vasseur 1970), Mauritius Island (Savigny 1816), Persian Gulf (Monniot and Monniot 1997), Japan (Tokioka 1963, 1967), China (Michaelsen 1923), New Caledonia (Monniot 2007).

Family Euherdmaniidae Ritter, 1904

Genus *Euherdmania* Ritter, 1904

Euherdmania fasciculata Monniot, 1983

Material examined. CAGoM-00471, Pro 1, 13 m, 26-05-2015, leg. L. Palomino-Alvarez.

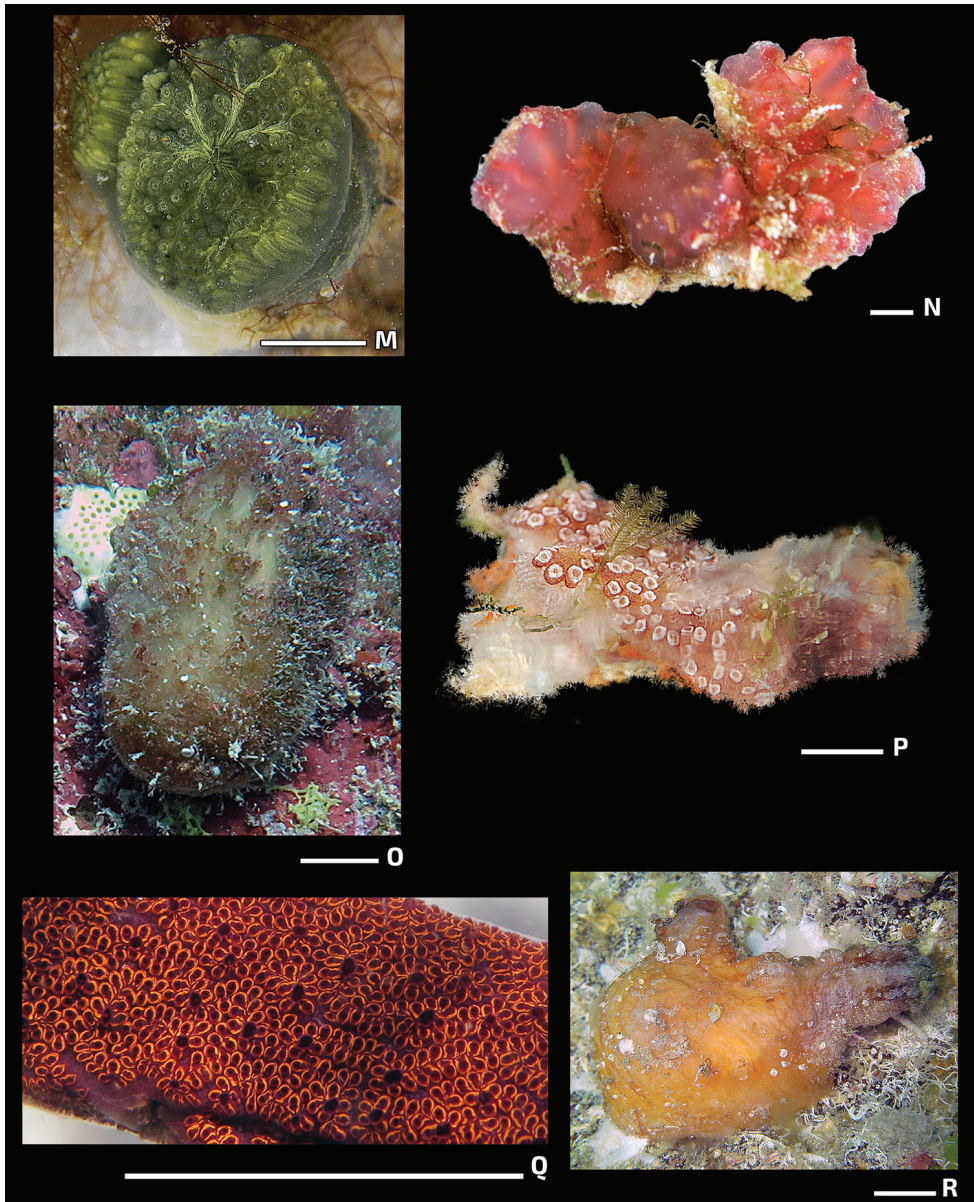


Figure 4. Photos of live specimens in the field (continued). **M** *Polyclinum constellatum* **N** *Euherdmania* aff. *vitrea* **O** *Polycarpa cartilaginea* **P** *Botrylloides magnicoecus* **Q** *Botrylloides niger* **R** *Microcosmus exasperatus*. Scale bar: 1 cm.

Remarks. The specimen was found in a disturbed environment (near-shore artificial reef).

Global distribution. Southern Gulf of Mexico (present study), Guadeloupe (Monniot 1983b), French Guiana (Monniot 2016)

***Eubherdmania* aff. *vitrea* Millar, 1961**

Fig. 4N

Material examined. CAGoM-00104, Mad 4, 11 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-00120, B10, 10 m, 17-06-2015, leg. L. Palomino-Alvarez.

Remarks. Morphological patterns of colony shape, zooids completely embedded in the tunic, conspicuous musculature throughout the body, number of siphon lobes, number of stigmal rows, and the testis position correspond with *E. vitrea* by Millar (1961), but the absence of the developed gonads and larvae prevent confirmation of this species. Also, the tunic is dark red colored while all colonies collected in Brazil are uncolored.

Global distribution. Southern Gulf of Mexico (present study), Brazil (Millar 1961; Rocha et al. 2005).

Order Stolidobranchia Lahille, 1886**Family Styelidae Sluiter, 1895****Genus *Polycarpa* Heller, 1877*****Polycarpa cartilaginea* (Sluiter, 1898)**

Fig. 4O

Material examined. CAGoM-0010, Arn 1, 12 m, 19-03- 2015, leg. L. Palomino-Alvarez; CAGoM-0176, Arc 1, 4 m, 19-03- 2015, leg. L. Palomino-Alvarez; CAGoM-0364, CAGoM-0365, Arc 11, 7 m, 20-08- 2016, leg. L. Palomino-Alvarez; CAGoM-0386, CAGoM-00468, Arc 4, 6 m, 22-08-2016, leg. L. Palomino-Alvarez; CAGoM-0408, CAGoM-412, CAGoM-0420, CAGoM-0421, Arc 12, 6 m, 24-08-2016, leg. L. Palomino-Alvarez; CAGoM-0426, CAGoM-0479, Arc 7, 9 m, 25-08-2016, leg. L. Palomino-Alvarez; CAGoM-0437, Acr 10, 12 m, 26-08- 2016, leg. L. Palomino-Alvarez.

Remarks. All the specimens from Arcas Cay Reef were found under large rocks with other species of ascidians (*Ascidia panamensis*, *Corella minuta* and *Ecteinascidia styeloides*).

Global distribution. Belize (Goodbody 2000), Panama (Collin et al. 2005; Rocha et al. 2005), Curaçao (Millar 1962; Van der Sloot 1969; Goodbody 1984), Colombia (Sluiter 1898), Guadeloupe (Monniot 1983), Martinique (Monniot 2018b).

***Polycarpa spongiabilis* Traustedt, 1883**

Material examined. CAGoM-0022, Arn 2, 6 m, 19-03- 2015, leg. L. Palomino-Alvarez; CAGoM-0448, Arc 10, 4 m, 26-08-2016, leg. L. Palomino-Alvarez.

Remarks. Amphipods were found between folds of the pharynx of *P. spongiabilis* (two males of *Leucothoe wuriti* Thomas & Klebba, 2007).

Global distribution. United States (Van Name 1921, 1945; Plough 1978), Bermuda (Verrill 1900; Van Name 1902, 1945; Gravier 1955; Monniot 1972), Cuba (Hernandez 1990), Jamaica (Sluiter 1898; Goodbody 1993), Puerto Rico (Van Name 1921, 1930), St. Thomas (Traustedt 1883), Guadeloupe (Gravier 1955; Monniot 1983b), Martinique (Monniot 2018b), Belize (Goodbody 2000), Panamá (Collin et al. 2005; Rocha et al. 2005), Curaçao (Sluiter 1898; Goodbody 1984), Venezuela (Sluiter 1898; Goodbody 1984; Rocha et al. 2010), Tobago (Cole 2012), Guyana (Millar 1978), Brazil (Rodrigues 1962; Millar 1977; Rocha and Kremer 2005).

Genus *Botrylloides* Milne Edwards, 1841

Botrylloides magnicoecus (Hartmeyer, 1912)

Fig. 4P

Material examined. CAGoM-0125, B10, 11 m, 17-06-2015, leg. L. Palomino-Alvarez.

Remarks. This is the first record of *B. magnicoecus* in the Gulf of Mexico. General characteristics agree with descriptions by Brunetti (2010), but caecum size in the Mexico specimens is larger.

Global distribution. According to Brunetti (2010) the records from South Africa have characteristics in agreement with the type and are trustful (Michaelsen 1934; Millar 1955). Recently a new record from French Guiana (Monniot 2016) has been published. A more detailed study of the species in the type locality should be performed to permit the revision of other records in the Atlantic (Guadeloupe – Monniot 1983b, Belize – Goodbody 2004).

Botrylloides niger Herdman, 1886

Fig. 4Q

Material examined. CAGoM-0029, CAGoM-0030, CAGoM-0032, CAGoM-0037, CAGoM-0038, Chel 2, 1 m, 11-05-2015, leg. L. Palomino-Alvarez; CAGoM-0040, Cel 2, 1 m, 11-05-2015, leg. L. Palomino-Alvarez; CAGoM-0185, Arc 3, 3 m, 30-10-2015, leg. L. Palomino-Alvarez.

Photographed record (no specimens in collection): VeR, 8 m, 20-09-2015, L. Palomino-Alvarez

Remarks. *Botrylloides niger* is among the species considered common and abundant in tropical waters (Rocha et al. 2010) with a widespread geographical distribution, where it attaches to natural and artificial substrates (Sheets et al. 2016 – as *B. nigrum*). We found it beneath rocks, on coral reefs, in ports and lagoons.

Global distribution. United States (Van Name 1945), Mexico (Van Name 1945), Bermuda (Herdman 1886; Van Name 1902, 1945; Monniot 1972), Belize (Goodbody 2000), Panama (Carman et al. 2011), Curaçao (Goodbody 1984), Bonaire (Millar 1962), Venezuela (Rocha et al. 2010; Carballo-Pérez and Díaz 2011), Cuba (Van Name 1945),

Jamaica (Goodbody 2003), Puerto Rico (Van Name), Guadeloupe (Monniot 1983b), Martinique (Monniot 2018b as *B. nigrum*), Antilles (Gravier 1955; Van Name 1930), Tobago (Cole 2012), Brazil (Rodrigues 1962; Rocha and Costa 2005; Rocha and Kremer 2005; Rocha and Bonnet 2009; Dias et al. 2012), Morocco (Millar 1698), Senegal (Monniot 1969), Israel Mediterranean (Sheets et al. 2016), Singapore (Sheets et al. 2016), Somalia (Millar 1988), Madagascar (Vasseur 1970), French Polynesia (Monniot et al. 1985).

Family Pyuridae Hartmeyer, 1908

Genus *Pyura* Molina, 1782

Pyura sp.

Material examined. CAGoM-0129, CAGoM-0128, CAGoM-0134, B10, 11 m, 17-06-2017, leg. L. Palomino-Alvarez; CAGoM-0160, CAGoM-0162, CAGoM-0146, Pro 1, 7 m, 26-05-2015, leg. L. Palomino-Alvarez.

Remarks. Specimens were compared with Panamanian specimens and are similar to a new species being described (Skinner et al. in press). In Yucatan peninsula they are 9–10 cm long, and were found mainly on coral reefs. Oral tentacles are more numerous (43–58) and one of the specimens had the following vessel formula:

E 11 (28) 9 (28) 6 (34) 6 (36) 5 (33) 5 (31) 6 LD 3 (30) 5 (28) 5 (27) 6 (32) 6 (30) 6 (22) 10. All other characters were within the variation found in Panama.

Genus *Microcosmus* Heller, 1877

Microcosmus exasperatus Heller, 1878

Fig. 4R

Material examined. CAGoM-0131, B10, 11 m, 17-06-2015, leg. L. Palomino-Alvarez; CAGoM-0048, Pro 1, 8 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-00476, Chp 1, 5 m, 26-05-2015, leg. L. Palomino-Alvarez; CAGoM-0731, Sis, 1 m, 21-03-2018, leg. L. Palomino-Alvarez.

Remarks. Can be very common on mangrove prop roots in the Caribbean Sea (Panama – Rocha et al. 2005, Venezuela – Rocha et al. 2010). Here we found it mainly in harbors, with > 20 specimens found in Sisal harbour, suggesting that it was introduced.

Global distribution. United States (Van Name 1921, 1945), Bermudas (Berrill 1932; Monniot 1972), Jamaica (Heller 1878; Goodbody 2003), Belize (Goodbody 2000), Panamá (Collin et al. 2005; Carman et al. 2011), Curaçao (Van Name 1924; Millar 1962; Goodbody 1984), Venezuela (Rocha et al. 2010; Carballo-Pérez and Díaz 2011), Tobago (Cole 2012), Martinique (Gravier 1955; Monniot 2018c), Antilles (Sluiter 1898; Van Name 1921, 1931), Guadeloupe (Monniot 1983), Brazil (Rodrigues 1962; Rodrigues et al. 1998; Monniot and Monniot 2001; Rocha et al. 2012; Rocha

and Costa 2005; Rocha and Kremer 2005; Rocha et al. 2005), Azores (Harant 1929), Cape Verde (Harant 1929), Mediterranean sea (Streftaris et al. 2005; Turon et al. 2007), Red Sea (Shenkar 2012), Philippine Sea (Van Name 1918), Mindoro (Tokiooka 1970), Truuk Islands (Nishikawa 1984), Guam (Lambert 2003), Australia (Kott 1985; Monniot 1992).

Family Molgulidae Lacaze-Duthiers, 1877

Genus *Molgula* Forbes, 1848

***Molgula occidentalis* Traustedt, 1883**

Material examined. CAGoM-0734, Sis, 1 m, 21-03-2018, leg. L. Palomino-Alvarez.

Remarks. The single specimen was found with *M. exasperatus* on a cement column.

Global distribution. Unites States (Van Name 1945), Panama (Collin et al., 2005; Rocha et al. 2005), Curaçao (Van Name 1924; Millar 1962; Goodbody 1984), Venezuela (Goodbody 1984; Rocha et al. 2010), Virgin Islands (Traustedt 1883), Puerto Rico (Van Name 1921, 1930), Brazil (Monniot 1970), Senegal (Pérès 1949; Monniot 1969; Lafargue and Wahl 1987; Monniot and Monniot 1994), Italy (Monniot 1970).

Discussion

With this first checklist from the southern Gulf of Mexico, we list 31 species, five in the order Phlebobranchia, 19 Aplousobranchia and seven Stolidobranchia. Ascidians found in the southern Gulf of Mexico comprise 24% of the species, 24% of the genera and 60% of the families of ascidians that have been found throughout the Gulf of Mexico (Van Name 1945; Abbott 1951; Carballo 2000; Lambert et al. 2005; Cole and Lambert 2009). Also, they comprise 7% of the species, 22% of the genera and 53% of the families reported from the Atlantic Ocean (Rocha et al. 2012). Styelidae and Polycitoridae are the most species-rich families.

The number of species we found is surprisingly less than expected and that have been reported from other regions of the Caribbean, including Belize, Bocas del Toro (Panama), Jamaica and Guadeloupe, while similar to the number of species found in Cuba, Curaçao and Puerto Rico (Rocha et al., 2005). A possible explanation for fewer species is simply variation in sampling effort among studies, or that greater diversity has been found in association with mangrove roots in some of those studies. Mangroves are less common in the southern Gulf of Mexico surveyed here and therefore they were sampled less often in this study. In the present study we visited eight coral reefs, where ascidians are mainly found beneath pieces of dead coral and in crevices and pits where they are not easily found. Sampling effort was somewhat greater in the two sites with more species (Madagascar and Bajo 10). Four sites were in harbors or associated with marinas and urban construction, one of which had many species (Progreso). Harbors are known as entrances for exotic species, among which only *C. roseolus*, *P. constellatum*

Table 3. World distribution of the ascidian species found in the present survey in southern Gulf of Mexico.

	Gulf N	Gulf S	Caribbean	West Atl	East Atl	Medit	Indian	Pacif
Total #	19	31	25	21	16	9	10	10
<i>Ascidia panamensis</i>		x	x					
<i>Phallusia nigra</i>	x	x	x	x	x	x	x	
<i>Corella minuta</i>	x	x	x				x	x
<i>Ecteinascidia styeloides</i>		x	x				x	
<i>Ecteinascidia turbinata</i>	x	x	x	x	x	x		
<i>Clavelina oblonga</i>	x	x	x	x	x	x		
<i>Clavelina</i> sp.		x						
<i>Cystodytes dellechiaiei</i>	x	x	x	x	x	x		x
<i>Cystodytes roseolus</i>		x			x		x	
<i>Eudistoma</i> aff. <i>amanitum</i>		x	x					
<i>Eudistoma clarum</i>	x	x	x		x			x
<i>Eudistoma hepaticum</i>	x	x	x					
<i>Eudistoma obscuratum</i>	x	x	x	x				
<i>Eudistoma olivaceum</i>	x	x	x	x	x			x
<i>Eudistoma recifense</i>		x		x				
<i>Stromozoa roseola</i>	x	x		x	x		x	x
<i>Distaplia bermudensis</i>	x	x	x	x	x	x		
<i>Polysyncraton amethysteum</i>	x	x	x	x	x	x		
<i>Lissoclinum fragile</i>	x	x	x	x	x		x	x
<i>Didemnum duplicatum</i>	x	x	x	x				
<i>Didemnum granulatum</i>		x	x	x	x		x	x
<i>Polyclinum constellatum</i>	x	x	x	x	x		x	x
<i>Euherdmania fasciculata</i>		x	x	x				
<i>Euherdmania</i> aff. <i>vitrea</i>		x		x				
<i>Polycarpa cartilaginea</i>		x	x					
<i>Polycarpa spongiabilis</i>	x	x	x	x				
<i>Botrylloides magnicoecus</i>		x		x	x			
<i>Botrylloides niger</i>	x	x	x	x	x	x	x	x
<i>Pyura</i> sp.		x	x					
<i>Microcosmus exasperatus</i>	x	x	x	x	x	x	x	x
<i>Molgula occidentalis</i>	x	x	x	x		x		

and *M. exasperatus* are likely to be introduced. Some species found in anthropogenic sites are widely distributed and have been introduced elsewhere (e.g., *D. perlucidum*, *L. fragile*, *E. turbinata*, *P. nigra* – Renganathan 1982; Monniot et al. 1985; Sheehy and Vik 2010; Thessalou et al. 2012; Vandepas et al. 2015).

Major affinities of the ascidian fauna in southern Gulf of Mexico are with the Caribbean Sea (25 shared species) and West Atlantic countries with tropical or warm waters (21 species), and only then with the northern region of the Gulf (19 species). Half of the species are also found in the east Atlantic region, and 13 species have wide geographical distribution including either or both Indian and Pacific oceans waters (Table 3). In contrast, we found a few species that are very common elsewhere in the Caribbean Sea, including *Rhopalaea abdominalis*, *Ascidia curvata*, *A. interrupta*, *Symplegma rubra*, *S. brakenhielmi*, *Pyura vittata*, and *Herdmania pallida*. Whether there are oceanographic or biological barriers preventing species from entering the southern

Gulf of Mexico remains to be tested. Also, increasing sampling effort will certainly uncover more species.

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References

- Abbott DP (1951) *Bostrichobranchus digonas*, a new molgulid ascidian from Florida. Journal of the Washington Academy of Sciences 41(9): 302–308.
- Alvarez L, Simoes N, Rocha R (2018) Checklist of ascidians (Chordata: Tunicata) from south of the Gulf of Mexico. Zenodo. [Data set] <http://doi.org/10.5281/zenodo.1478185>
- Bermúdez IR, Grimaldi JR (1975) Estudio comparativo de cuatro especies de ascidias de la Bahía de Mochima, Edo, Sucre. Lagena 35–36: 31–49.
- Berrill NJ (1932) Ascidians of the Bermudas. The Biological Bulletin 62: 77–88. <https://doi.org/10.2307/1537145>
- Berrill NJ (1935) Studies in tunicate development. Part III. Differential retardation and acceleration. Philosophical Transactions of the Royal Society of London (B) 225(525): 255–326.
- Berrill NJ (1950) The Tunicata. With an account of the British species. Ray Society, London, 354 pp.
- Bonnet NYK, Rocha RM (2011a) The family Ascidiidae Herdman (Tunicata: Ascidiacea) in Bocas del Toro, Panama. Description of six new species. Zootaxa 2864: 1–33.
- Bonnet NYK, Rocha RM (2011b) The Ascidiidae (Ascidiacea: Tunicata) of coastal Brazil. Zoological Studies 50(6): 809–825.
- Brunetti R (1994) Ascidians of the northern Adriatic Sea. Aplousobranchia 1. Bollettino di Zoologia Italiana 61: 89–96. <https://doi.org/10.1080/11250009409355864>

- Brunetti R (2010) Redescription of *Botrylloides magnicoecum* (Hartmeyer, 1912) based on the analysis of the type (Tunicata, Ascidiacea, Styelidae, Botryllinae). *Bollettino del Museo civico di storia naturale di Venezia* 61: 45–48.
- Carballo JL (2000) Distribution of *Ecteinascidia turbinata* (Ascidiacea: Perophoridae) in mangroves of the Yucatan Peninsula, Mexico. *Revista de Biología Tropical* 48: 365–369.
- Carballo-Pérez V, Díaz O (2011) Ascidians (Tunicata: Ascidiacea) of Cariaco Gulf, Venezuela. *Boletín del Instituto Oceanográfico de Venezuela* 50(2): 233–244.
- Carman MR, Bullard SG, Rocha RM, Lambert G, Dijkstra JA, Roper JJ, Goodwin A, Carman MM, Vail EM (2011) Ascidians at the Pacific and Atlantic entrances to the Panama Canal. *Aquatic Invasions* 6: 371–380. <https://doi.org/10.3391/ai.2011.6.4.02>
- Casso M, Navarro M, Ordóñez V, Fernández-Tejedor M, Pascual M, Turon X (2018) Seasonal patterns of settlement and growth of introduced and native ascidians in bivalve cultures in the Ebro Delta (NE Iberian Peninsula). *Regional Studies in Marine Science* 23: 12–22. <https://doi.org/10.1016/j.rsma.2017.11.002>
- Cole LL, Lambert G (2009) Tunicata (Urochordata) of the Gulf of Mexico. In: Felder D, Camp DK (Eds) *Gulf of Mexico – Origins, Waters, and Biota Biodiversity*. Texas A and M Press, College Station, 1209–1216.
- Cole LL (2012) Diversity and distribution of Tunicata (Urochordata) in Tobago. *Journal of Life Sciences* 6: 221–232.
- Collin R, Díaz MC, Norenbur J, Rocha RM, Sanchez J, Schulze A, Schwartz M (2005) Photographic identification guide to some common marine invertebrates of Bocas del Toro. *Caribbean Journal of Science* 41(3): 638–707.
- CONABIO (2016) Comisión Nacional para el Uso y conocimiento de la Biodiversidad. Sistema Nacional de Información sobre Biodiversidad de México (SNIB). <http://www.conabio.gob.mx/remib/doctos/snib.html>
- Della Valle A (1877) Contribuzioni alla storia naturale delle ascidie composte del Golfo di Napoli. Napoli, tip. Comuni, 48 pp.
- Dias G, Rocha RM, Lotufo TMC, Kremer LP (2012) Fifty years of ascidian biodiversity research in São Sebastião, Brazil. *Journal of the Marine Biological Association of the United Kingdom* 93(1): 273–282.
- Drasche R (1883) Die Synascidien der Bucht von Rovigno (Istrien). In: *Ein Beitrag zur Fauna der Adria*. Carl Gerolds Sohn, Wien, 41.
- Draughon D, Scarpa J, Hartmann JX (2010) Are filtration rates for the rough tunicate *Styela plicata* independent of weight or size? *Journal of Environmental Science and Health* 45: 168–176.
- Erba E, Bergamaschi D, Bassano L, Damia G, Ronzoni S, Faircloth GT, D’Incalci M (2001) Ecteinascidin-743 (ET-743), a natural marine compound, with a unique mechanism of action. *European Journal of Cancer* 37: 97–105. [https://doi.org/10.1016/S0959-8049\(00\)00357-9](https://doi.org/10.1016/S0959-8049(00)00357-9)
- Fortaleza MTV, Lotufo TMC (2018) Polyclinidae (Ascidiacea: Aplousobranchia) from the Gulf of Mexico, collected during the “Hourglass” cruises (1965–1967). *Zootaxa* 4422: 519–536. <https://doi.org/10.11646/zootaxa.4422.4.4>
- Ghobashy AFA, Abdel Messeih MK (1991) Ascidians in Egyptian waters. *Journal of the Egyptian-German Society of Zoology* 4: 313–326.

- Goodbody I (1984a) Ascidiens from Caribbean shallow water localities. Studies on the Fauna of Curaçao and other Caribbean Islands 47(203): 62–76.
- Goodbody I (1984b) The ascidian fauna of two contrasting lagoons in the Netherland Antilles: Pescadera baai, Curaçao, and the lac of Bonaire. Studies on the Fauna of Curaçao and other Caribbean Islands 48(202): 21–61.
- Goodbody I (1993) The ascidian fauna of a Jamaican lagoon: thirty years of change. Revista de Biología Tropical (Suppl.) 41(1): 35–38.
- Goodbody I (2000) Diversity and distribution of ascidians (Tunicata) in the Pelican Cays, Belize. Atoll Research Bulletin 480: 1–33. <https://doi.org/10.5479/si.00775630.480>
- Goodbody I (2003) The ascidian fauna of Port Royal, Jamaica 1. Harbor and mangrove dwelling species. Bulletin of Marine Science 73: 457–476.
- Goodbody I (2004) Diversity and distribution of ascidians (Tunicata) at Twin Cays, Belize. Atoll Research Bulletin 524: 1–22. <https://doi.org/10.5479/si.00775630.524.1>
- Goodbody I, Cole L (2006) The tropical western atlantic Perophoridae (Ascidia) 11. The genus Ecteinascidia. Bulletin of Marine Science 79(1): 49–70.
- Gravier R (1955) Ascidiens récoltées par le ‘President Theodore Tissier’ (Campagne de printemps 1951). Révue des Travaux de l’Institut des Pêches Maritimes 19(4): 611–631.
- Harant H (1927) Introduction synoptique a une faune de France des tuniciers. 1. Ascidiens Stolidobranchiata. Bulletin de l’Institut océanographique de Monaco 508: 1–10.
- Harant H (1929) Ascidiens provenant des croisières du Prince Albert Ier de Monaco. Résultats des Campagnes Scientifiques Monaco 75: 1–112.
- Harant H, Vernières P (1933) Tuniciers. Fasc. 1, Ascidiens. Faune de France 27: 1–101.
- Hartmeyer R (1912) Die ascidien der Deutschen Tiefsee-Expedition. Deutschen Tiefsee-Exp 17: 223–392.
- Heller C (1878) Beiträge zur näheren Kenntniss der Tunicaten. Sitzber. Denkschriften der Kaiserlichen Akademie der Wissenschaften 77(1): 2–28.
- Herdman WA (1880) Preliminary report on the Tunicata of the Challenger expedition. Part 2. Proceedings of the Royal Society of Edinburgh 10(2): 714–726. <https://doi.org/10.1017/S0370164600044606>
- Herdman WA (1882) Report on the Tunicata collected during the voyage of H.M.S. Challenger during the years 1873–1876, part 1, Ascidia simplices. Rep. sci. Res. Voy. H.M.S. “Challenger”. Zoology 6(17): 1–296.
- Herdman WA (1886) Report on the Tunicata collected during the years 1873–1876. Part 2, Ascidia compositae. Journal of Experimental Zoology 14(38): 1–425.
- Hernández-Zanuy A (1990) Lista de ascidias cubanas. Poeyana 388: 1–7.
- Hernández-Zanuy A, Carballo J (2001) Distribution and abundance of ascidian assemblages in Caribbean reef zones of the Golfo de Batabanó (Cuba). Coral Reefs 20: 159–162. <https://doi.org/10.1007/s003380100154>
- Jeffery WR (2015) Closing the wounds: One hundred and twenty-five years of regenerative biology in the ascidians *Ciona intestinalis*. Genesis 53: 48–65. <https://doi.org/10.1002/dvg.22799>

- Kondilatos G, Corsini-Foka M, Pancucci-Papadopoulou MA (2010) Occurrence of the first non-indigenous ascidian *Phallusia nigra* Savigny, 1816 (Tunicata: Ascidacea) in Greek waters. *Aquatic Invasions* 5(2): 181–184. <https://doi.org/10.3391/ai.2010.5.2.08>
- Kott P (1957) The sessile tunicata. The John Murray Expedition, 1933–1934, Scientific Reports 10(4): 129–149.
- Kott P (1981) The ascidians of the Reef Flats of Fiji. *Proceedings of the Linnean Society of New South Wales* 105(3): 147–212.
- Kott P (1985) The Australian Ascidacea Part 1, Phlebobranchia and Stolidobranchia. *Memoirs of the Queensland Museum* 23: 1–440.
- Kott P (1990) The Australian Ascidacea, part 2, Aplousobranchia (1). *Memoirs of the Queensland Museum* 29(1): 1–226.
- Kott P (2001) The Australian Ascidacea Part 4, Aplousobranchia (3), Didemnidae. *Memoirs of the Queensland Museum* 47(1): 1–407.
- Kott P, Goodbody I (1982) The ascidians of Hong Kong. In: Morton BS, Tseng CK (Eds) *The marine flora and fauna of Hong Kong and Southern China*. University Press, Hong Kong, 503–554.
- Lafargue F (1970) Ascidies de Port-Cros (Parc national). *Annales de la Société des Sciences Naturelles de Toulon* 22: 128–132.
- Lafargue F, Vasseur P (1989) Ascidies des récifs coralliens du N. E. du canal de Mozambique (Campagne “Bentedi” du N. “Suroit”, 17 mars – 14 avril 1977). *Mésogée* 49: 59–66.
- Lafargue F, Wahl M (1987) Contribution to the knowledge of littoral ascidians (Ascidacea, Tunicata) of the Senegalese coast. *Bulletin de l’Institut Fondamental d’Afrique Noire* 46(3–4): 385–402.
- Lambert G (2003) Marine biodiversity of Guam: the Ascidacea. *Micronesica* 35–36: 588–597.
- Lambert G (2005) Ecology and natural history of the protochordates. *Canadian Journal of Zoology* 83: 34–50. <https://doi.org/10.1139/z04-156>
- Lambert G, Faulkes Z, Lambert CC, Scofield VL (2005) Ascidians of South Padre Island, Texas, with a key to species. *Texas Journal of Science* 57: 251–262.
- López-Legentil S, Turon X (2005) How do morphotypes and chemotypes relate to genotypes? The colonial ascidian *Cystodytes* (Ascidacea: Polycitoridae). *Zoologica Scripta* 34(1): 3–14. <https://doi.org/10.1111/j.1463-6409.2005.00167.x>
- Mastrototaro F, Brunetti R (2006) The non-indigenous ascidian *Distaplia bermudensis* in the Mediterranean: comparison with the native species *Distaplia magnilarva* and *Distaplia lucillae* sp. nov. *Journal of the Marine Biological Association of the United Kingdom* 86: 181–185. <https://doi.org/10.1017/S0025315406013014>
- Michaelsen W (1915) Tunicata. In: *Beiträge zur Kenntnis der Meeresfauna westafrikas*. Beiträge zur Kenntnis der Meeresfauna Westafrikas 1(3): 322–518.
- Michaelsen W (1918) Die Ptychobranchen und Diktyobranchen Ascidien des westlichen Indischen Ozeans. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten* 35(2): 1–73.
- Michaelsen W (1919) Die Krikobranchien Ascidien des Westlichen Indischen Ozeans: Claveliniden und Synoiciden. *Mitteilungen aus dem Naturhistorischen Museum in Hamburg* 34: 74–98.

- Michaelsen W (1923) Südafrikanische Ascidien. Meddelanden fran Göteborgs Musei Zoologiska avdelning 24: 1–23.
- Michaelsen W (1930) Ascidae Krikobranchiae. Fauna Sudvest-Australiensis 5: 461–558.
- Michaelsen W (1934) The ascidians of the Cape Province of South Africa. Transactions of the Royal Society of South Africa 22: 129–163. <https://doi.org/10.1080/00359193409519335>
- Millar RH (1953) On the collection of ascidians from the Gold Coast. Proceedings of the Zoological Society of London 123(2): 277–325. <https://doi.org/10.1111/j.1096-3642.1953.tb00176.x>
- Millar RH (1955) On a collection of ascidians from South Africa. Proceedings of the Zoological Society of London 125(1): 169–221. <https://doi.org/10.1111/j.1096-3642.1955.tb00597.x>
- Millar RH (1956) Notes on some ascidians from Sierra Leone and Gambia. The Annals and magazine of natural history 9(12): 409–417. <https://doi.org/10.1080/00222935608655834>
- Millar RH (1958) Some ascidians from Brazil. The Annals and magazine of natural history 13(1): 497–514. <https://doi.org/10.1080/00222935808650975>
- Millar RH (1961) *Euherdmania vitrea*, a new species of ascidian from Brazil. The Annals and magazine of natural history ser13, 4: 143–147. <https://doi.org/10.1080/00222936108655793>
- Millar RH (1962a) Some ascidians from the Caribbean. Studies on the Fauna of Curaçao and other Caribbean Islands 13: 61–7.
- Millar RH (1962b) Further descriptions of South African ascidians. Annals of the South African Museum 46(7): 113–221.
- Millar RH (1965) Ascidians from the tropical coast of west Africa. Atlantide Reports 8: 247–255.
- Millar RH (1968) Ascidian of Rabat Morocco. Bulletin de la Société des sciences naturelles du Maroc 47(1–2): 201–206.
- Millar RH (1977) Ascidians (Tunicata: Ascidiacea) from the northern and north-eastern Brazilian shelf. The Annals and magazine of natural history 11(2): 169–223. <https://doi.org/10.1080/00222937700770131>
- Millar RH (1988) Ascidians collected during the International Indian Ocean Expedition. The Annals and magazine of natural history 22: 823–848. <https://doi.org/10.1080/00222938800770541>
- Monniot C (1969) Sur une collection d'ascidies de Dakar (Phlébobranches et Stolidobranches). Bulletin du Muséum National d'Histoire Naturelle 41(3): 622–654.
- Monniot C (1970) Ascidies Phlébobranches et Stolidobranches des Iles Kerguelen recoltées par P. Grua. Bulletin du Muséum National d'Histoire Naturelle ser 2, 42(2): 340–354.
- Monniot C (1971) Quelques ascidies infralittorales de Sao Miguel (Açores). Bulletin du Muséum National d'Histoire Naturelle ser 2, 42(6): 1200–1207.
- Monniot C (1972a) Ascidies stolidobranches des Bermudes. Bulletin du Muséum National d'Histoire Naturelle ser 3, 43(57): 617–643.
- Monniot C (1972b) Ascidies phlébobranches des Bermudes. Bulletin du Muséum National d'Histoire Naturelle ser 3, 6(82): 939–948.
- Monniot C (1983a) Ascidies littorales de Guadeloupe. II. Phlébobranches. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 5(1): 51–71.
- Monniot C (1983b) Ascidies littorales de Guadeloupe. IV. Styelidae. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 5(2): 423–456.

- Monniot C (1987) Ascidies de Nouvelle-Calédonie. II. Les genres *Polycarpa* et *Polyandrocarpa*. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 9(2): 275–310.
- Monniot C (1992) Ascidies de Nouvelle-Calédonie. XI. Phlébobranches et Stolidobranches du plateau des Chesterfield. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 14(1): 3–22.
- Monniot C (1997) Ascidies phlébobranches du canal du Mozambique. Zoosystema 19(4): 557–571.
- Monniot C, Monniot F (1967) Tuniciers benthiques. Campagne de la Calypso aux îles du Cap Vert (1959). Annales de l'Institut océanographique 45(2): 1–18.
- Monniot C, Monniot F (1972) Clé mondiale des genres d'ascidies. Archives de zoologie expérimentale et générale 113(3): 311–367.
- Monniot C, Monniot F (1987a) Les ascidies de Polynésie française. Mémoires du Muséum d'histoire naturelle, séc A, 136: 1–155.
- Monniot C, Monniot F (1994) Additions to the inventory of eastern tropical Atlantic ascidians: arrival of cosmopolitan species. Bulletin of Marine Science 54(1): 71–93.
- Monniot C, Monniot F (1997) Records of ascidians from Bahrain, Arabian Gulf with three new species. Journal of Natural History 31: 1623–1643. <https://doi.org/10.1080/00222939700770871>
- Monniot C, Monniot F, Laboute P (1985) Ascidies du port de Papeete (Polynésie française): relations avec le milieu naturel et d'autres ports. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 7(3): 481–495.
- Monniot C, Monniot F, Laboute P (1991) Coral reef ascidians of New Caledonia. Collection Faune tropicale, Editions de l'ORSTOM, Paris 30, 247 pp.
- Monniot F (1969) Sur une collection d'ascidies composées de Dakar. Bulletin du Museum national d'histoire naturelles ser 2, 41(2): 426–447.
- Monniot F (1972) Ascidies aplousobranches des Bermudes. Polyclinidae et Polycitoridae Bulletin du Muséum National d'Histoire Naturelle ser 3, 82(1): 949–962.
- Monniot F (1983a) Ascidies littorales de Guadeloupe I. Didemnidae. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 5(1): 5–49.
- Monniot F (1983b) Ascidies littorales de Guadeloupe III. Polyclinidae. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 5(2): 413–422.
- Monniot F (1983c) Ascidies littorales de Guadeloupe V. Polycitoridae. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 5(4): 999–1019.
- Monniot F (1988) Ascidies de Nouvelle Calédonie V. Polycitoridae du lagon. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, (2): 197–235.
- Monniot F (1992) Ascidies de Nouvelle Calédonie XII. Le genre *Lissoclinum* (Didemnidae) dans le lagon sud. Bulletin du Muséum National d'Histoire Naturelle ser 4, sec A, 14(3–4): 565–589.
- Monniot F (1995) Ascidies de Nouvelle-Calédonie. XV. Le genre *Didemnum*. Bulletin du Muséum National d'Histoire Naturelle 4 ser, sec A, 16(2–4): 299–344.
- Monniot F (2007) Some comments on the Ascidians of New Caledonia. In: Pyari CE, Rieher de Forges B (Eds) Compendium of marine species from New Caledonia. Documents Scientifiques et Techniques, EDITOR (117): 349–356.
- Monniot F (2012) Some ascidians from the southern coast of Madagascar collected during the “AtimoVatae” survey. Zootaxa 3197: 1–42.

- Monniot F (2016) Ascidiens (Tunicata) of the French Guiana Expedition. *Zootaxa* 4114(3): 201–245. <https://doi.org/10.11646/zootaxa.4114.3.1>
- Monniot F (2018a) Ascidiens collected during the Madibenthos expedition in Martinique: 1. Phlebobranchia. *Zootaxa* 4387(3): 451–472. <https://doi.org/10.11646/zootaxa.4387.3.3>
- Monniot F (2018b) Ascidiens collected during the Madibenthos expedition in Martinique: 2. Stolidobranchia, Styelidae. *Zootaxa* 4410: 291–318. <https://doi.org/10.11646/zootaxa.4410.2.3>
- Monniot F (2018c) Ascidiens collected during the Madibenthos expedition in Martinique 3. Stolidobranchia, Pyuridae and Molgulidae. *Zootaxa* 4459: 401–430. <https://doi.org/10.11646/zootaxa.4459.3.1>
- Monniot F, Monniot C (1996) New collections of ascidians from the Western Pacific and Southeastern Asia. *Micronesica* 29(2): 133–279.
- Monniot F, Monniot C (2001) Ascidiens from the tropical western Pacific. *Zoosystema* 23(2): 201–383.
- Naranjo SA, García-Gómez JC (1994) Ascidia litorales del estrecho de Gibraltar: nuevas aportaciones faunísticas. *Graellsia* 50: 57–69.
- Naranjo SA, Carballo JL, García-Gómez JC (1996) Effects of environmental stress on ascidian populations in Algeciras Bay (southern Spain). Possible marine bioindicators? *Marine Ecology Progress Series* 144: 119–131. <https://doi.org/10.3354/meps144119>
- Nishikawa T (1984) Ascidiens from the Trunk Island, Ponape Island and Majuro atoll (Tunicata: Ascidiacea). *Proceedings of the Japanese Society of Systematic Zoology* 27: 107–140.
- Nishikawa T (1991) The ascidiens of the Japan Sea 2. *Publications of the Seto Marine Biological Laboratory* 35 (1/3): 25–170. <https://doi.org/10.5134/176172>
- Oliveira LM, Gamba GA, Rocha RM (2014) *Eudistoma* (Ascidiacea: Polycitoridae) from tropical Brazil. *Zoologia* 31(2): 195–208. <https://doi.org/10.1590/S1984-46702014000200011>
- Ordóñez V, Pascual M, Fernández-Tejedor M, Turon X (2016) When invasion biology meets taxonomy: *Clavelina oblonga* (Ascidiacea) is an old invader in the Mediterranean Sea. *Biological Invasions* 18(4): 1203–1215. <https://doi.org/10.1007/s10530-016-1062-0>
- Oren U, Benayahu Y (1998) Didemnid ascidiens: rapid colonizers of artificial reefs in Eilat (Red Sea). *Bulletin of Marine Science* 63(1): 199–206.
- Paiva SV, Rocha MR (2018) A large marine Caribbean mushroom field: description of *Eudistoma amanitum* sp.nov. (Ascidiacea: Polycitoridae). *Zootaxa* 4399(3): 443–450. <https://doi.org/10.11646/zootaxa.4399.3.13>
- Paiva SV, Oliveira-Filho RR, Lotufo TMC (2015) Ascidiens from Rocas Atoll, northeast Brazil. *Frontiers in Marine Science* 2: 39. <https://doi.org/10.3389/fmars.2015.00039>
- Pérès JM (1949) Contribution à l'étude des Ascidiens de la côte occidentale d'Afrique. *Bulletin de l'Institut fondamental d'Afrique noire* 11(1–2): 159–207.
- Pérès JM (1951) Nouvelle contribution à l'étude des ascidiens de la côte occidentale d'Afrique. *Bull Institut fondamental d'Afrique noire* 13: 1051–1071.
- Pérès JM (1958) Ascidiens récoltées sur les côtes Méditerranéennes d'Israel. *Bulletin of the Research Council of Israel (B Zool)* 7(3–4): 143–150.
- Petersen JK, Riisgard HU (1992) Filtration capacity of the ascidian *Ciona intestinalis* and its grazing impact in a shallow fjord. *Marine Ecology Progress Series* 88(1): 9–17. <https://doi.org/10.3354/meps088009>

- Plough HH (1978) Sea Squirts of the Atlantic Continental Shelf from Maine to Texas. Johns Hopkins Univ. Press, Baltimore, 118 pp.
- Renganathan TK (1982) On the occurrence of a colonial ascidian *Lissoclinum fragile* (Van Name, 1902) from India. *Current Science* 51(3): 149.
- Ramos A, Buencuerpo V, Vazquez E, Lafarque F (1993) Distribución bionómica de las ascidias litorales del Estrecho de Gibraltar. *Publicación Especial del Instituto Español de Oceanografía* 11: 2–36.
- Ríos M (1985) Ascidias (Ascidacea, Tunicata) de la isla de Fuerteventura. *Vieraea* 15(1–2): 123–138.
- Rocha RM, Bonnet NYK (2009) Ascídias (Tunicata, Ascidiacea) introduzidas no Arquipélago de Alcatrazes, São Paulo. *Iheringia, Série Zoologia* 99(1): 27–35. <https://doi.org/10.1590/S0073-47212009000100004>
- Rocha RM, Costa LVG (2005) Ascidians (Urochordata: Ascidiacea) from Arraial do Cabo, Rio de Janeiro, Brazil. *Iheringia, Série Zoologia* 95(1): 57–64. <https://doi.org/10.1590/S0073-47212005000100009>
- Rocha RM, Faria SB (2005) Ascidians at Currais Islands, Paraná, Brazil: taxonomy and distribution. *Biota Neotropica* 5(2): 1–20. <https://doi.org/10.1590/S1676-06032005000300013>
- Rocha RM, Kremer LP (2005) Introduced Ascidians in Paranaguá Bay, Paraná, Southern Brazil. *Revista Brasileira de Zoologia* 22(4): 1170–1184. <https://doi.org/10.1590/S0101-81752005000400052>
- Rocha RM, Bonnet NYK, Baptista MS, Beltramin FS (2012) Introduced and native Phlebobranch and Stolidobranch solitary ascidians (Tunicata: Ascidiacea) around Salvador, Bahia, Brazil. *Zoologia* 29(1): 39–53. <https://doi.org/10.1590/S1984-46702012000100005>
- Rocha RM, Farias SB, Moreno TR (2005) Ascidians from Bocas del Toro, Panamá. I Biodiversity. *Caribbean Journal of Science* 41: 600–612.
- Rocha RM, Guerra-Castro E, Lira C, Pauls SM, Hernandez I, Pérez A, Sardi A, Pérez J, Herrera C, Carbonini AK, Caraballo V, Salazar D, Diaz MC, Cruz-Motta JJ (2010) Inventory of ascidians (Tunicata, Ascidiacea) from the National Park La Restinga, Isla Margarita, Venezuela. *Biota Neotropica* 10(1): 1–10. <https://doi.org/10.1590/S1676-06032010000100021>
- Rocha RM, Moreno TR, Metri R (2005) Ascídias (Tunicata, Ascidiacea) da Reserva Biológica Marinha do Arvoredo, Santa Catarina, Brasil. *Revista Brasileira de Zoologia* 22(2): 461–476. <https://doi.org/10.1590/S0101-81752005000200024>
- Rocha RM, Zanata TB, Moreno TR (2012) Keys for identification of families and genera of Atlantic shallow water ascidians. *Biota Neotropica* 12(1): 1–35. <https://doi.org/10.1590/S1676-06032012000100022>
- Rodrigues SA (1962) Algumas ascídias do litoral sul do Brasil. *Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, ser Zool* 24:193–215. <https://doi.org/10.11606/issn.2526-3382.bfclzoologia.1962.120585>
- Rodrigues SA, Rocha RM (1993) Littoral compound ascidians (Tunicata) from São Sebastião, Estado de São Paulo, Brazil. *The Proceedings of the Biological Society of Washington* 106(4): 728–739.
- Savigny JC (1816) *Mémoires sur les animaux sans vertèbres*. Paris 2: 1–239.

- Sheehy DJ, Vik SF (2010) The role of constructed reefs in non-indigenous species introductions and range expansions. *Ecological Engineering* 36: 1–11. <https://doi.org/10.1016/j.ecoleng.2009.09.012>
- Sheets EA, Cohen CS, Ruis, GM, Rocha RM (2016) Investigating the widespread introduction of a tropical marine fouling species. *Ecology and Evolution* 6(8): 2453–2471. <https://doi.org/10.1002/ece3.2065>
- Shenkar N (2012) Ascidian (Chordata, Ascidiacea) diversity in the Red Sea. *Marine Biodiversity Records* 42(4): 459–469. <https://doi.org/10.1007/s12526-012-0124-5>
- Shenkar N, Swalla BJ (2011) Global Diversity of Ascidiacea. *PLoS ONE* 6(6): e20657. <https://doi.org/10.1371/journal.pone.0020657>
- Skinner LF, Rocha RM, Counts BK (2019) *Pyura gangelion* and *Pyura beta* sp. nov. (Ascidiacea: Pyuridae): an exotic and a new tunicate from the West Atlantic. *Zootaxa* (in press). <https://doi.org/10.11646/zootaxa.4545.2.6>
- Sluiter CP (1898) Tuniciers recueilli en 1896, par la Chazalie, dans la mer des Antilles. *Mémoires de la Société zoologique de France* 11: 5–34.
- Streftaris N, Zenetos A, Papathanassiou E (2005) Globalisation in marine ecosystems: the story of non-indigenous marine species across European seas. *Oceanography and Marine Biology Annual Review* 43: 419–453.
- Thessalou-Legaki M, Aydogan O, Bekas P, Bilge G, Boyaci Y, Brunelli E, Circosta V, Crocetta F, Durucan F, Erdem M, Ergolavou A, Filiz H, Fois F, Gouva E, Kapiris K, Katsanevakis S, Kljajic Z, Konstantinidis E, Konstantinou G, Koutsogiannopoulos D, Lamon S, Macic V, Mazzette R, Meloni D, Mureddu A, Paschos I, Perdikaris C, Piras F, Poursanidis D, Ramos-Espla A, Rosso A, Sordino P, Sperone E, Sterioti A, Taskin E, Toscano F, Tripepi S, Tsiakkios L, Zenetos A (2012) New Mediterranean Biodiversity Records. *Mediterranean Marine Science* 13(2): 312–327. <https://doi.org/10.12681/mms.313>
- Tokioka T (1954) Contribution to Japanese ascidian fauna. 7. Invertebrate fauna of the intertidal zone of the Tokara Islands. *Ascidians. Publications of the Seto Marine Biological Laboratory* 3(3): 239–264. <https://doi.org/10.5134/174491>
- Tokioka T (1963) Contributions to Japanese ascidian fauna. XX. The outline of Japanese ascidian fauna as compared with that of the Pacific coast of North America. *Publications of the Seto Marine Biological Laboratory* 11(1): 131–156. <https://doi.org/10.5134/175319>
- Tokioka T (1967) Pacific Tunicata of the United States National Museum. *Bulletin (United States National Museum)* 251: 1–247. <https://doi.org/10.5479/si.03629236.251.1>
- Tokioka T (1970) Ascidiaceans from Mindoro Island, the Philippines. *Publications of the Seto Marine Biological Laboratory* 18(2): 75–107. <https://doi.org/10.5134/175626>
- Tokioka T (1972) On a small collection of ascidians from the Pacific coast of Costa Rica. *Publications of the Seto Marine Biological Laboratory* 19(6): 383–408. <https://doi.org/10.5134/175738>
- Tokioka T, Nishikawa T (1975) Contributions to the Japanese ascidian fauna XXVII. Some ascidians from Okinawa, with notes on a small collection from Hong Kong. *Publications of the Seto Marine Biological Laboratory* 22(5): 323–341.
- Traustedt MPA (1882) Vestindiske Ascidiæ Simpliciter, Forsie Afdeling. Phallusiadae. *Videnskabelige meddelelser fra Dansk Naturhistorisk Forening*, ann 1881: 257–288.

- Traustedt MPA (1883) Vestindiske Ascidiae Simples, Anden Afdeling. Molgulidae og Cynthiadae. Videnskabelige meddelser fra Dansk Naturhistorisk Forening, ann 1882: 108–136. <https://doi.org/10.1016/j.jembe.2006.10.040>
- Turon X, Nishikawa T, Rius M (2007) Spread of *Microcosmus squamiger* (Ascidacea: Pyuridae) in the Mediterranean Sea and adjacent waters. Journal of experimental Marine Biology and Ecology 342(1): 185–188. <https://doi.org/10.1016/J.JEMBE.2006.10.040>
- Vandepas LE, Oliveira LM, Lee SSC, Hirose E, Rocha RM, Swalla BJ (2015) Biogeography of *Phallusia nigra*: Is it really black and white? The Biological Bulletin 228: 52–64. <https://doi.org/10.1086/BBLv228n1p52>
- Van Name WG (1902) The ascidians of the Bermuda Islands. Transactions of the Connecticut Academy of Arts and Sciences 11: 325–412.
- Van Name WG (1918) Ascidians from the Philippines and adjacent waters. Bulletin of the American Museum of Natural History 100(1): 49–174.
- Van Name WG (1921) Ascidians of the West Indian region and southeastern United States. Bulletin of the American Museum of Natural History 44: 283–494.
- Van Name WG (1924) Ascidians from Curaçao. Bijdragen Tot de Dierkunde 23: 23–32.
- Van Name WG (1930) The ascidians of Porto Rico and the Virgin Islands. Transactions of the New York Academy of Sciences 10: 401–512.
- Van Name WG (1945) The North and South American ascidians. Bulletin of the American Museum of Natural History 84: 1–476.
- Van der Sloot CJ (1969) Ascidians of the family Styelidae from the Caribbean. Studies on the Fauna of Curaçao and other Caribbean Islands 30: 1–57.
- Vasseur P (1970) Contribution à l'étude des Ascidies de Madagascar (région de Tuléar). III. La faune ascidiologique des herbiers à phanérogames marines. Recueil des Travaux de la Station marine d'Endoume, Fasc hors serie suppl 10: 209–221.
- Verrill AE (1900) Addition to the Tunicata and Molluscoidea of the Bermudas. Transactions of the Connecticut Academy of Arts and Sciences 10(2): 588–594. <https://doi.org/10.5962/bhl.part.7034>

A new interstitial species of the genus *Caecianiropsis* Menzies & Pettit, 1956 (Isopoda, Asellota) from Korea

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Abstract

A new interstitial species, *Caecianiropsis goseongensis* **sp. n.** is described from littoral off the east coast of Korea (Sea of Japan). The species can be distinguished from its congeners by the number of antennular articles, shape of the male appendix masculina, setation of pereopods, and length ratio of the uropodal rami. To aid species identification a taxonomic key to all species of *Caecianiropsis* Menzies & Pettit, 1956 is also provided as well as a partial 16S mitochondrial ribosome RNA of the new species, which is the first genetic information for the genus.

Keywords

East Asia, Janiridae, marine benthos, taxonomic key

Introduction

Menzies and Pettit (1956) established *Caecianiropsis* for the new species *Caecianiropsis psammophila* Menzies & Pettit, 1956 collected from a coarse sand beach in California. This species was distinct from all other members of the family Janiridae by the following morphological characters: an elongated body (6.5 times longer than wide), distolateral extension forming an angular shape, and coiled appendix masculina in the relaxed position. So far only three species were described: *Caecianiropsis psammophila* from northern California (Menzies and Pettit 1956; Kussakin 1988), *C. birsteini* Kus-

sakin, 1979 from Okhotsk Sea (Kussakin 1979, 1988), and *C. ectiformis* (Vanhöffen, 1914) from Kerguelen (Vanhöffen 1914), St. Paul (Kensley 1976), and Marion and Prince Edward Islands (Branch et al. 1991). Wilson and Wägele (1994) provided a brief review of *Caecianiropsis* and divided it into two species groups based on the region from where they were collected. The northern group included *C. psammophila* and *C. birsteini*, and was characterized by a relatively longer antennal flagellum, a similar width of the rostrum and the antennular peduncle, and an angular lateral extension of the male pleopod I. The southern group included only *C. ectiformis*, a species with a compact antennal flagellum, a much broader rostrum, and lacking the lateral extension on the male pleopod. *Caecianiropsis ectiformis* was originally described in the genus *Austroniscus* Vanhöffen, 1914 and transferred to its present systematic position by Menzies and Pettit (1956). Nevertheless, Wilson and Wägele (1994) questioned this because of its distinct morphology and suggested a thorough reexamination of the species with the type material.

A new species of *Caecianiropsis* was collected from shallow water of the East Sea (Sea of Japan) near Goseong (Gangwondo, Korea). It has a typical body plan of *Caecianiropsis* but also a unique combination of characters which clearly distinguish the new species from all other congeners. This paper provides an illustrated description of a new species, a revised generic diagnosis, and an identification key to the four species of *Caecianiropsis*. In addition, a partial mitochondrial sequence of 16S ribosome RNA gene was obtained and this may be useful for the future phylogenetic study of *Caecianiropsis*.

Materials and methods

Specimen collection and examination

Samples were collected from littoral off the East coast of Korea (depth 15 m), by scuba diving with plastic corer and initially kept in a plastic bag. Sediment was transferred to 250 ml bottles and immediately preserved in 99% ethanol. Sorting from sediment sample and dissection of specimens were done under an Olympus SZX 12 stereo-binocular microscope. Dissected appendages were mounted onto glass slides in lactophenol. The line drawings were prepared using Olympus BX 51 compound microscope equipped with a *camera lucida*. All studied material was deposited at the invertebrate collection of the National Institute of Biological Resources (NIBR), Korea. One male and one female were transferred to isoamyl acetate for 20 minutes and dried in a critical-point dryer Hitachi E-1010. Dried specimens were mounted onto a SEM stub and coated with gold using a sputter coater to a thickness of 15–30nm. Coated specimens were examined and photographed with a Hitachi S-3400 scanning electron microscope at Eulji University (Seongnam, Korea). Measurements were made following the method of Riehl and Brandt (2010). All measurements were taken from the dorsal view of line drawings using the distance measurement tools of Adobe Acrobat Professional. The ratios of appendages were given in distal to proximal order, excluding

setae. The body ratios were given in anteromedial point to posteromedial point order excluding appendages. Terminology is largely based on Wilson and Wägele (1994). We abbreviated the term ‘unequally bifid’ seta as UB seta.

DNA extraction and amplification

Two females from the type locality were identified without dissection under Olympus SZX 12 stereomicroscope. Before amplification, specimens were transferred into distilled water for 20 minute to remove ethanol and then minced with a small glass stick. Whole specimens were used to isolate genomic DNA with the aid of the LaboPass™ Kit (COSMO Co. Ltd., Korea) following the manufacturer’s protocols. The 16S rDNA gene was amplified with polymerase chain reaction (PCR) using PCR premix (BIONEER. Co) in TaKaRa PCR thermal cycler (TaKaRa Bio Inc., Otsu, Shiga, Japan). The primers used were 16sar-L (5′ – CGC CTG TTT AAC AAA AAC AT–3′) and 16sar-H (5′ – CCG GTC TGA ACT CAG ATC ACG T–3′) (Palumbi et al. 1991). The amplification protocol consisted of initial denaturation 94 °C for 2 min, 35 cycles of denaturation 94 °C for 50 sec, annealing at 50 °C for 50 sec, extension at 72 °C for 1 min 20 sec and final extension at 72 °C for 7 min. The final product was stored at 4 °C. Amplifications were confirmed by electrophoresis in 1% agarose gel. The PCR products were purified for sequencing reactions, using the Labopass PCR Purification Kit (COSMO Co. Ltd., Korea) following the guidelines provided with the kit. DNA was sequenced on an ABI automatic capillary sequencer (Macrogen, Seoul, Korea) using the same set of primers.

Taxonomy

Suborder Asellota Latreille, 1802

Superfamily Janiroidea Sars, 1897

Family Janiridae Sars, 1897

Genus *Caecianiropsis* Menzies & Pettit, 1956

Austroniscus Vanhöffen, 1914: 553; Branch et al. 1991: 28.

Caecianiropsis, Menzies and Pettit 1956: 441; Kensley 1976: 295; Kussakin 1979, 1988: 160; Wilson 1994: 751; Wilson and Wägele 1994: 693.

Type species. *Caecianiropsis psammophila* Menzies & Pettit, 1956

Included species. *C. birsteini* Kussakin, 1979, *C. ectiformis* (Vanhöffen, 1914)

Generic diagnosis. (modified from Wilson and Wägele 1994)

Body six times longer than wide; cephalon with no eye, weakly developed rostrum reaching to middle of antennular article I; pleonite I 0.8 times wider than pereonite VII; pleotelson as wide as pereonite VII; antennula with V–VII articles, antennal article III with

rudimentary scale laterally; mandibular molar process truncate, palp article II medially swollen, with 2–3 robust setae, median setigerous margin slightly depressed; maxillipedal endite two times longer than wide, distomedially pointed; medial lobes of male pleopod I distally rounded, distolateral edge of hyaline lamella projected; exopod of male pleopod II inserting subdistally on sympod, endopod proximally expanded, appendix masculina more than four times longer than sympod, coiled in relaxed position; endopod of pleopod III with three distal broom setae having distinct gap between medial seta and two lateral setae.

Remarks. Wilson and Wägele (1994) provided a simple note on the morphological affinity between *Caecianiropsis* and *Neojaera* emphasizing a coiled, very much elongate stylet of male pleopod II in male. The major differences between *Neojaera* and *Caecianiropsis* are the presence of distinctly developed uropods showing the elongate sympod, and the much longer endopod in *Caecianiropsis*. In addition, the development of rostrum is also a noticeable difference between the two genera, with *Caecianiropsis* having an elongated rostrum reaching to the middle of the antennular article I, and its width is almost the same as antennula article 1, while *Neojaera* has only a weak anterior protrusion on the rostrum. Other morphological differences between the two genera are as follows: 1) cephalon without visual organ; 2) length of the cephalon as long as its width; 3) all pereonites almost same in length; 4) antenna much longer than antennula (more than twice); 5) lateral margin of the male pleopod I extended, angular form; 6) sympod of the male pleopod II, 2.6 times longer than wide. Wilson and Wägele (1994) also found several similarities between *Caecianiropsis* and *Microjaera* Bocquet & Levi, 1955 including the body form, antennal articulation, and male pleopod II with the elongate and coiled stylet. However, the elongation of the body can be a result of adaptation to the interstitial environment and therefore often evolve convergently. In addition, similar antennal articulation can be found in many other isopod groups. This similarity is also only superficial because *Caecianiropsis* shows a rudimentary scale on article III of antenna, which is lacking in *Microjaera*. On the other hand, elongation of the male stylet is one of the most noticeable characters of *Caecianiropsis* within the family Janiridae. Although, Wilson and Wägele (1994) mentioned that *Microjaera anisopoda* Bocquet & Levi, 1955 also possesses a similar morphology of male pleopod II, the original description of this species was limited due to the poor illustration of this particular character. Shimomura (2005) described another species, *Microjaera morii* Shimomura, 2005, but based on non-ovigerous female only; therefore this important male character is missing. Phylogenetic analysis of Janiridae (Wilson 1994) based on 33 morphological characters suggested a close relationship between *Caecianiropsis* and the *Microjaera*. However, this has to be considered with caution because the important male characters are not well described in the latter genus.

***Caecianiropsis goseongensis* sp. n.**

<http://zoobank.org/948FB812-988F-470C-A52D-11B469BEF7F3>

Figures 1–9

Type locality. Shallow water of East Sea (Sea of Japan), Goseong, Gangwondo, Korea, 38°17'43.8"N, 128°33'15.1"E.

Material examined. *Holotype*: adult male, (NIBRIV0000838292) completely dissected and mounted in lactophenol on eight slides; allotype: non-ovigerous female (NIBRIV0000838293) dissected on three slides; *paratype* 1: adult male (NIBRIV0000838294) dissected on three slides; *paratype* 2 adult male (NIBRIV0000838295) dissected on two slides; *paratype* 3: male used for SEM (NIBRIV0000838296).

Diagnosis. Pleotelson 1.45 times longer than wide, 0.23 times of whole body, antennula with seven articles, article VI with one simple seta and two aesthetascs distally, article VII very tiny, with three simple setae and one elongate aesthetasc, male antenna with 29 articles of flagellum, left mandible with five serrate setae in spine low, maxillipedal palp with setal formula as follow: 2:12:7:8:10, pereopod I with setal formula as follow: 5:1:10:13:19:7, pereopod II with setal formula as follow: 8:5:8:11:14:5, distal margin of pleopod I with 38 setae, uropodal exopod 0.5 times of endopod. Female operculum with two setae on medial margin and six setae along distal apex.

Description of the male holotype. *Body* (Fig. 1A): elongate, flattened in dorsal view, color of preserved specimens transparent, total length measured with *paratype* I 2.05 mm, length six times longer than wide, maximal body width in pereonite V 0.91 times of maximal width of pleotelson, setation of pereonites I–VII as follows: 10: 10: 10: 8: 6: 6: 6.

Cephalon (Fig. 1B): 0.94 times longer than wide and 0.16 times of whole body, anterior margin wider than posterior one; rostrum slightly wider than antennular article I.

Pleotelson (Fig. 1C): 1.45 times longer than wide, 0.23 times of whole body, dorsal and lateral margin covered with many setae.

Antennula (Fig. 2A): seven articles, relative length ratios: 1: 0.97: 0.22: 0.17: 0.36: 0.24: 0.03; article I robust, 1.38 times longer than wide, lateral margin with three simple setae, distal margin with three simple and one broom setae; article II 0.48 times wider than article I 1.89 times longer than wide, with three simple and four broom setae; article III 1.1 times longer than wide, with two simple setae distolaterally; article IV 0.89 times longer than wide, with three simple and one broom seta distally; article V 2.58 times longer than wide, with three simple setae distolaterally; article VI 1.72 times longer than wide, one simple seta and two aesthetascs distally; article VII (Fig. 2B) smallest, with three simple and one elongate aesthetasc on distal end.

Antenna (Fig. 2C, D): six peduncle articles and 29 flagellar articles; article I with three mediolateral setae; article II 0.92 longer than wide, with one seta distolaterally; article III twice as long as wide, with four setae distally, rudimentary scale on lateral margin with two setae; article IV 0.71 times longer than wide, with three simple mediolateral setae; article V 3.55 times longer than wide, with 14 simple setae; article VI longest, 5.68 times longer than wide, with 25 simple setae; flagellar article I longest, 2 times longer than wide, with two simple setae distally, setal formula of articles from 2 to 29 as follows: 3: 3: 4: 1: 3: 2: 4: 2: 3: 8: 3: 1: 4: 2: 3: 3: 4: 4: 3: 3: 4: 3: 4: 3: 3: 3.

Maxilliped (Fig. 2E): epipodite, narrow, 3.56 times longer than wide, distal end reaching to palp article II; basis 3.8 times longer than wide; endite width 0.83 times of palp article II, two proximomedial coupling hooks, distal margin covered with numerous fine setae, with six simple setae, three serrated setae, and one fan seta (Fig. 2F); palp relative length ratio: 1: 1.77: 1.14: 1.63: 0.91, article I 0.55 times longer than wide, with two simple setae on both distal corners; article II quadrangular, 0.95 times longer

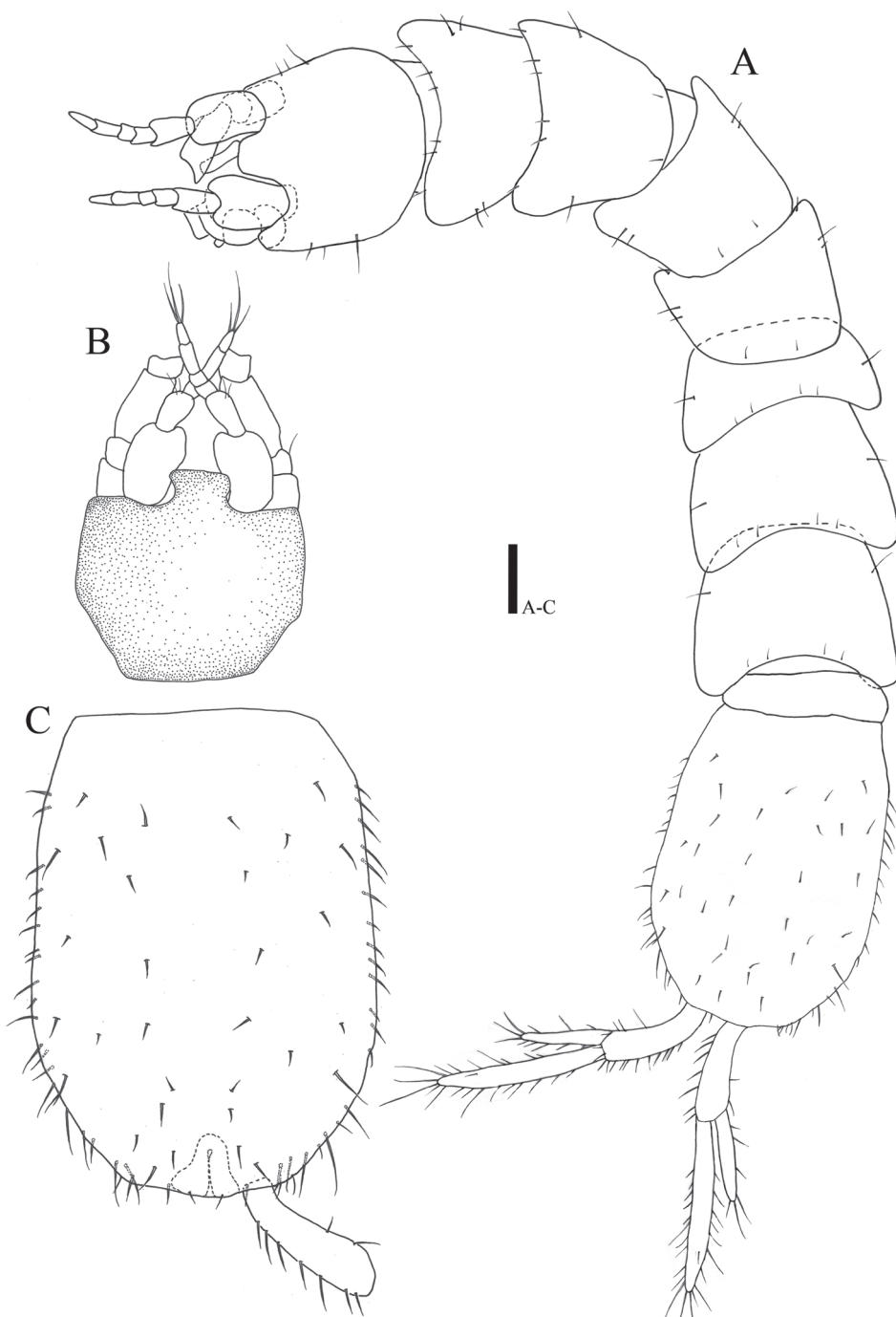


Figure 1. *Caecianiropsis goseongensis* sp. n. **A** Paratype 1, male, habitus, dorsal, $\times 200$ **B** holotype, male, cephalon, dorsal, $\times 200$ **C** holotype, male, pleotelson, dorsal, $\times 200$, uropod, dorsal, $\times 600$. Scale bar: 100 μm .

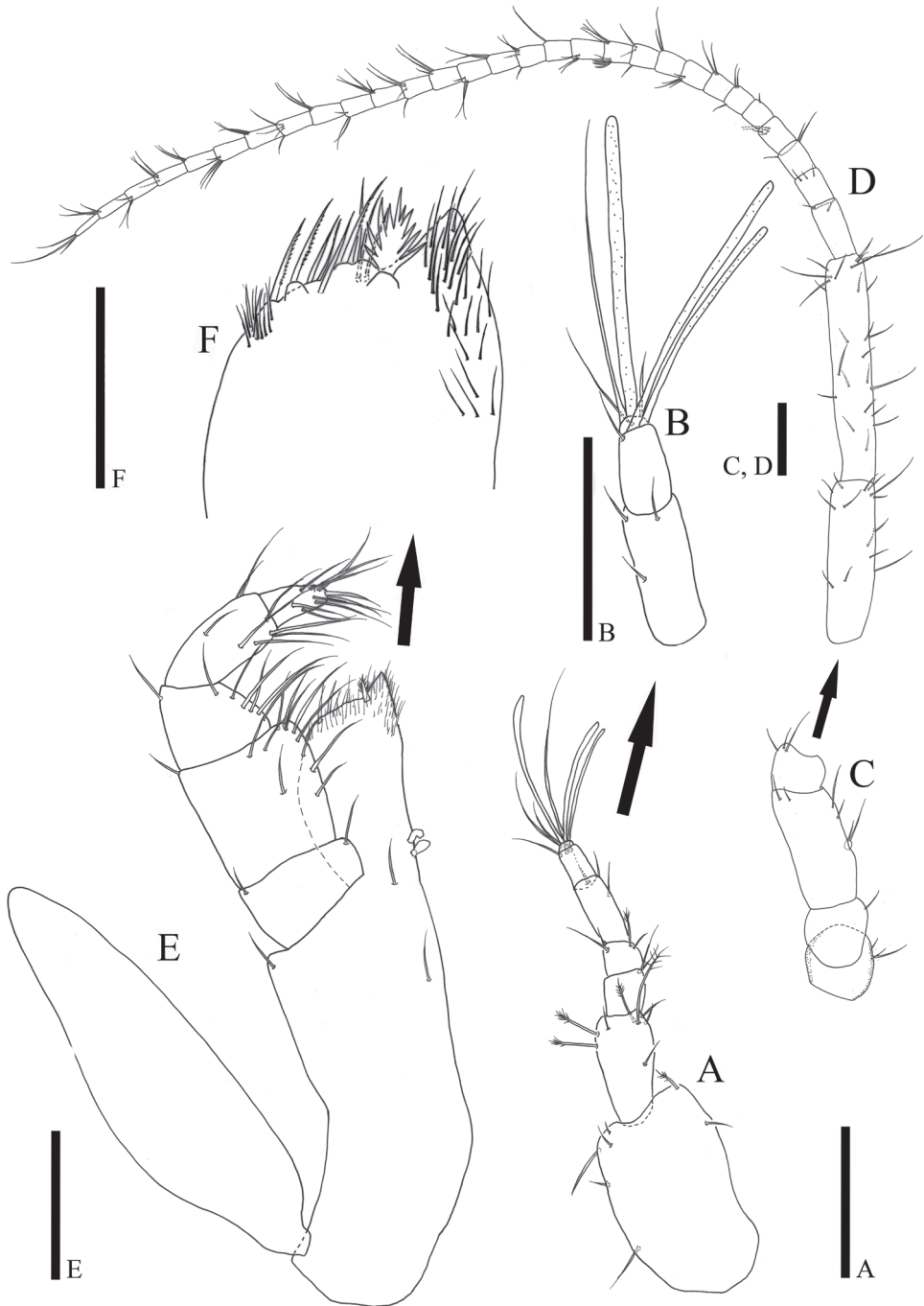


Figure 2. *Caecianiropsis goseongensis* sp. n. holotype, male. **A** Antennula, dorsal, $\times 400$ **B** antennula articles 5–7, dorsal, $\times 600$ **C** antennal article 1–4, dorsal, $\times 400$ **D** antennal article 5, 6 and flagellum, dorsal, $\times 400$ **E** maxilliped, ventral, $\times 400$ **F** paratype 3, male, distal margin of endite, ventral, $\times 1000$. Scale bars: $100\ \mu\text{m}$ (**A–E**), $50\ \mu\text{m}$ (**F**).

than wide, 1.9 times longer than article I, with eleven simple long setae on distomedial margin and one short seta on distolateral corner; article III 1.13 times longer than article I, tapering distally, with six simple long setae along medial margin and one seta on distolateral corner; article IV 2.14 times longer than wide, with eight simple setae on distal margin; article V with eight simple and two robust setae on distal end.

Mandible (Fig. 3A, B): body robust, curved inwardly; left mandible (Fig. 3A) pars incisiva with four cusps; lacinia mobilis much smaller than that of right mandible, with three denticulate, robust spines and three serrate setae (Fig. 3E); pars molaris truncate, missing grinding surface, distal tip blunt, with two apical setae; right mandible (Fig. 3B) pars incisiva with five (Fig. 3H), lacinia mobilis smaller than pars incisiva, with five cusps, five serrate setae located below lacinia mobilis, proximal part of setae covered by fine numerous setae, pars molaris with two apical setae (Fig. 3G); palp (Fig. 3A) 0.97 times of body length, inserted on cuticular projection; article I with one seta distally, 3.58 times longer than wide; article II robust, 1.89 times longer than wide, length 0.83 times of article I, maximal width 1.58 times of article I, median margin swollen, with three serrate setae distolaterally, article III laterally curved with ten serrate setae on inner margin.

Hypopharynx (Fig. 3C): deep medial incision separating two lobes, much of hairs on distal margin of each lobe.

Maxillula (Figs 3D, F): inner endite shorter and more slender than outer one, with one short setae and several hair-like elements on distal apex, along lateral margin with spiny row; outer endite with 12 robust setae on distal margin, most denticulate, some two-sided serrate, along lateral margin with long simple setae.

Maxilla (Fig. 4A): all rami similar in length, with four serrated setae on distal end of each; mesial ramus coalescent with basis, much thicker than others, with eleven strong setae distally and some of them denticulate; median ramus with four pectinate setae and lateral one with three pectinate setae distally, all rami with fine simple setae along medial margin.

Anterior pereopods (Figs 4B, C, 5A, B) inserted on pereon anterolaterally, relative length ratio: 1: 1.12: 1.12: 1.01; width ratio of carpus and propodus: 1: 0.87: 0.87: 0.73/ 1: 0.6: 0.6: 0.55; L/W ratio of articles: basis (2.03: 3.03: 2.97: 2.86), ischium (2.27: 2.57: 2.65: 2.05), merus (1.4: 1.52: 1.75: 1.52), carpus (2.52: 4.21: 3.71: 3.9) propodus (3.21: 6.83: 6.08: 6.17), dactylus (1.5: 1.48: 1.55: 1.45); pereopod I with minute coxa hardly discernable in lateral view (Fig. 9A); coxae of pereopods II–IV clearly visible in lateral view with small simple seta; dactylus of pereopods II–IV partly covered by articular plate projected from distal margin of propodus.

Pereopod I (Fig. 4B) basis with three broom and two simple setae; ischium with one seta on dorsal margin; merus with four setae of different lengths on distodorsal corner and five setae on ventral margin; carpus dorsal margin with four setae, two UB setae on ventral margin with six simple setae of different lengths, ventral margin with hairs; propodus with eight simple setae along dorsal margin, ventral margin with seven simple and four UB setae; dactylus tapering distally, dorsal margin with four setae of different lengths, ventral side with three setae, two claws on distal apex with different lengths.

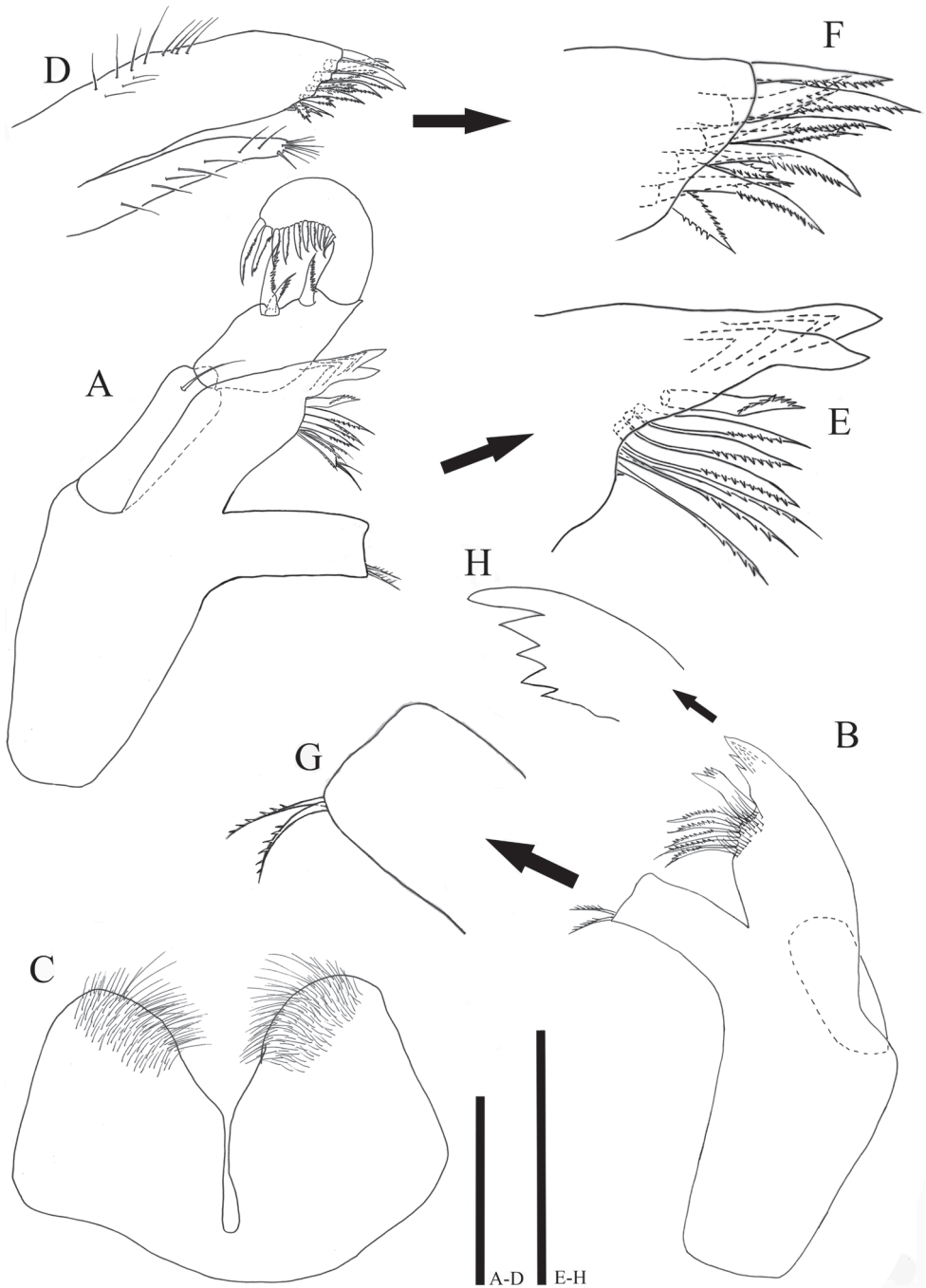


Figure 3. *Caecianiropsis goseongensis* sp. n. holotype, male. **A** Left mandible, ventral, $\times 600$ **B** right mandible, ventral, $\times 600$ **C** hypopharynx, ventral, $\times 600$ **D** maxillula, ventral, $\times 600$ **E** distal end of maxillula, ventral, $\times 1000$, **F** distal end of left mandible, ventral, $\times 1000$, **G** distal end of *pars molaris*, ventral, $\times 1000$, **H** *pars incisiva*, lateral, $\times 1000$. Scale bars: 100 μm (**A–D**), 50 μm (**E–H**).

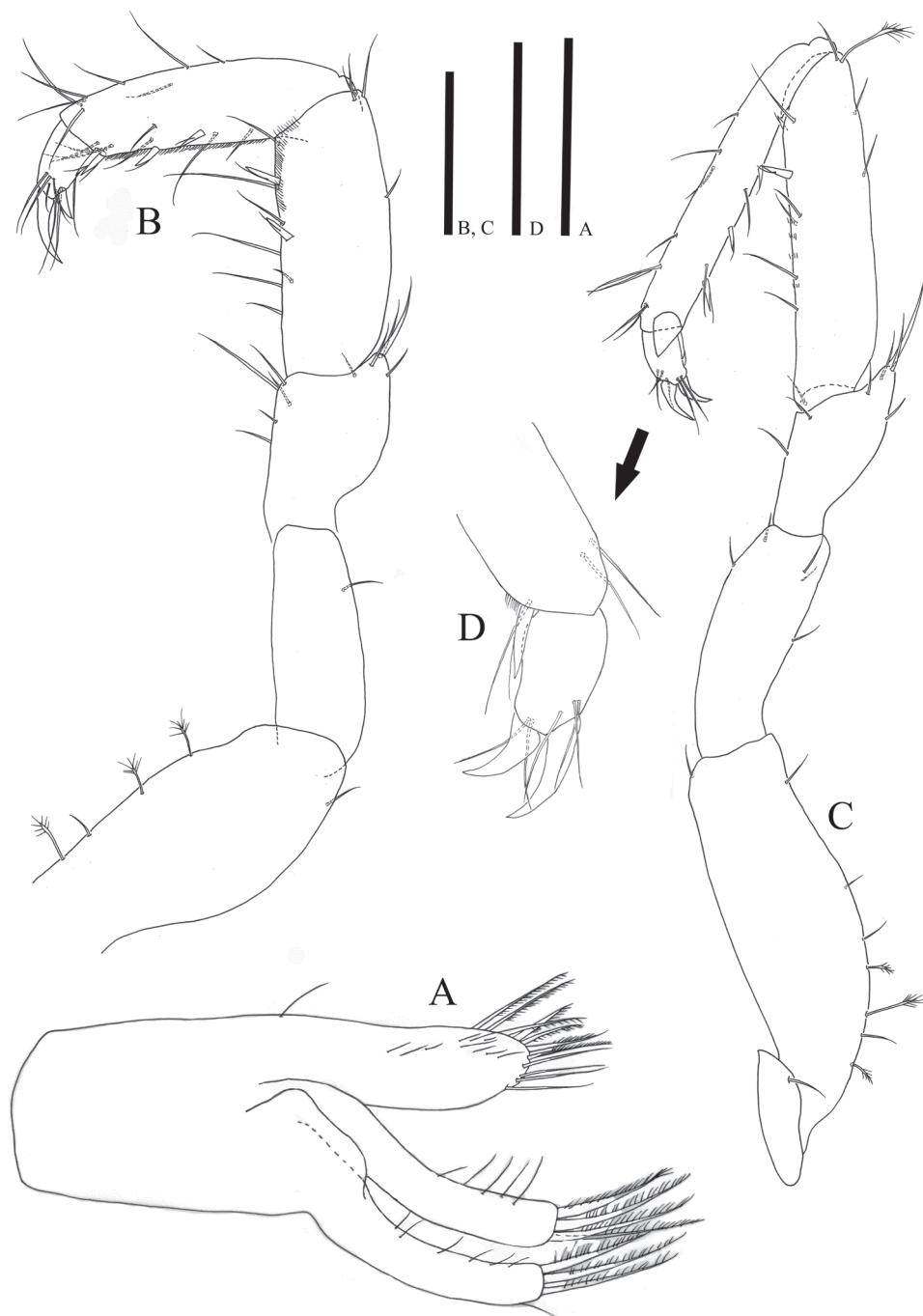


Figure 4. *Caecianiropsis goseongensis* sp. n. **A** Maxilla, paratype 3, male, ventral, $\times 600$; holotype, male, **B** pereopod 1, dorsal, $\times 400$ **C** pereopod 2, dorsal, $\times 400$ **D** propodus of pereopod 2, ventral, $\times 1000$. Scale bars: 50 μm (**A**, **D**), 100 μm (**B**, **C**).

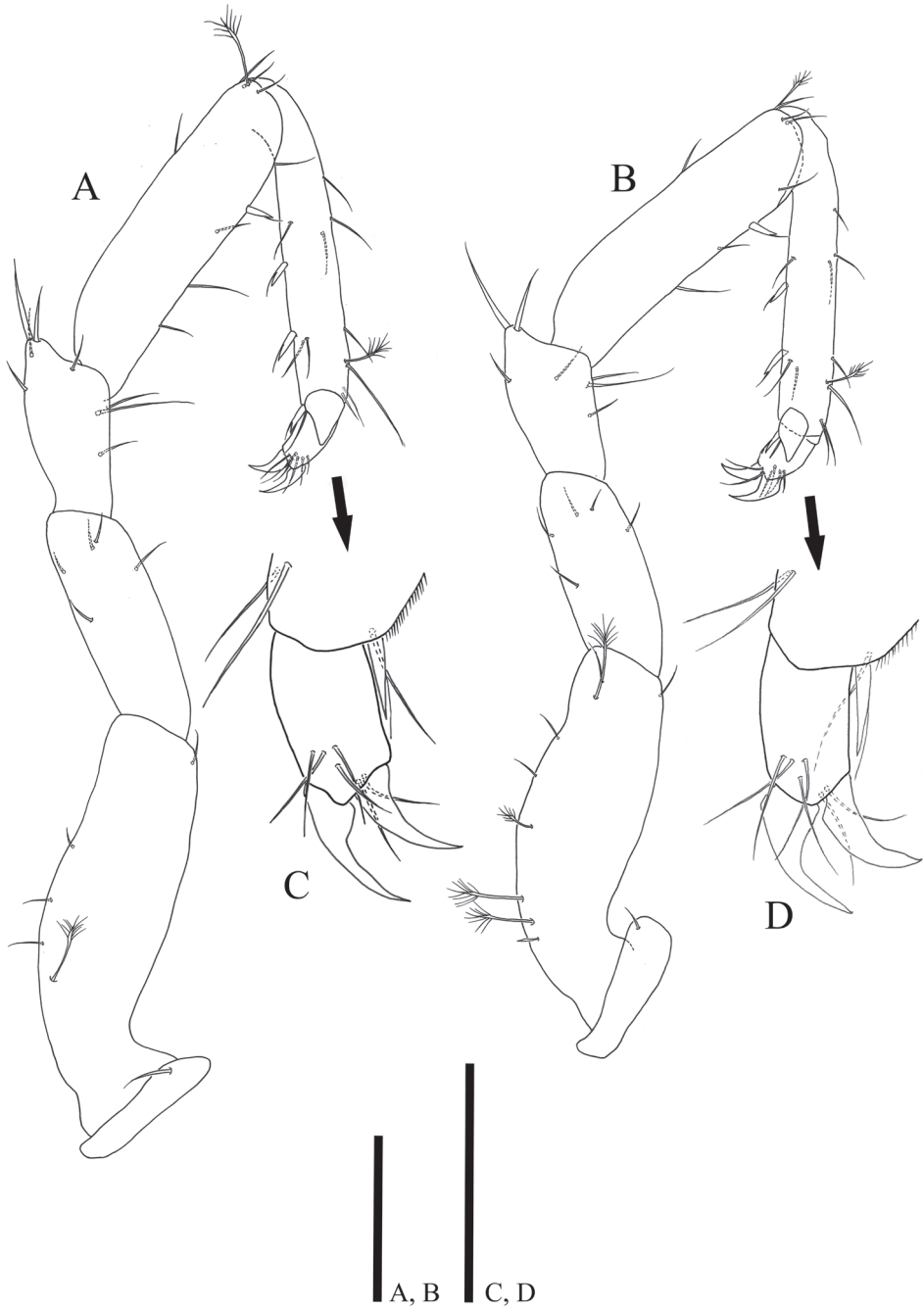


Figure 5. *Caecianiropsis goseongensis*. holotype, male. **A** Pereopod 3, dorsal, $\times 400$ **B** pereopod 4, dorsal, $\times 400$, **C** propodus of pereopod 3, ventral, $\times 1000$ **D** propodus of pereopod 4, ventral, $\times 1000$. Scale bars: 100 μm (**A**, **B**), 50 μm (**C**, **D**).

Pereopod II (Fig. 4C): basis dorsal margin with four simple and three broom setae and with one short seta on distal corner; ischium with five simple setae, merus distal margin with eight setae of different lengths; carpus dorsal margin with three simple and one broom seta, ventral margin with five simple and two UB setae; propodus with eight setae along dorsal margin and two simple and four UB setae along ventral margin; dactylus (Fig. 4D) with two claws and five simple setae.

Pereopod III (Fig. 5A): basis dorsal margin with three simple and one broom setae, and with one short seta on distal corner; ischium with five simple setae; merus distal margin with eight setae of different lengths; carpus dorsal margin with one broom and three simple setae, ventral margin with one UB and four simple setae; propodus dorsal margin with seven simple and one broom setae, four simple and three UB setae along ventral margin; dactylus (Fig. 5C) with two claws and six simple setae.

Pereopod IV (Fig. 5B): basis with three simple and five broom setae on dorsal margin, one short seta on distal corner; ischium with five simple setae on distal margin; merus with seven setae of different length on distal margin; carpus dorsal margin with four simple and one broom seta, ventral margin with three simple and one UB seta; propodus dorsal margin with seven simple and one broom seta, four simple and three UB setae along ventral margin; dactylus (Fig. 5D) with two claws and six simple setae.

Posterior pereopods (Figs 6A, B, 7A) inserted on pereon posterolaterally, relative length ratio in comparison to pereopod I, 1: 1.12: 1.27: 1.31; L/W ratio of articles: basis (3.1: 3.11: 3.08), ischium (2.86: 2.72: 2.72), merus (1.48: 1.67: 1.76), carpus (3.43: 4.17: 4.42) propodus (3.53: 6.69: 5.56), dactylus (1.45: 1.56: 1.45); coxae of pereopods V–VII approximately twice as wide as those of anterior pereopods, clearly visible in lateral view with two simple setae; dactylus partly covered by articular plate projected from distal margin of propodus.

Pereopod V (Fig. 6A): basis dorsal margin with six simple and five broom setae, ventral margin with two setae; ischium with six simple setae; merus with seven setae of different length; carpus with six simple, one broom seta and one UB seta along margin; propodus with relatively bigger UB seta protruding medially, with seven simple setae, one broom seta, and one UB seta; dactylus (Fig. 6C) with two claws and four simple setae distally.

Pereopod VI (Fig. 6B): basis with seven simple three broom setae; ischium with five simple setae; merus with nine simple setae on distal margin; carpus with six simple, one broom, and one UB setae, spiny row along dorsal margin; propodus with seven simple setae, one broom seta, and two UB setae; dactylus (Fig. 6D) with two claws and six simple setae distally.

Pereopod VII (Fig. 7A): basis with six simple three broom setae, ischium with four setae; merus with nine simple setae on distal margin and three of them much elongate; carpus with nine simple, one broom seta, and two UB setae, spiny row along dorsal margin; propodus with six simple, one broom, and three UB setae; dactylus (Fig. 7D) with two claws and six simple setae.



Figure 6. *Caecianiropsis goseongensis* sp. n. holotype, male. **A** Pereopod 5, ventral, $\times 400$ **B** pereopod 6, ventral, $\times 400$, **C** propodus of left pereopod 5, ventral, $\times 1000$ **D** propodus of pereopod 6, ventral, $\times 1000$. Scale bar: $100\ \mu\text{m}$ (**A, B**).

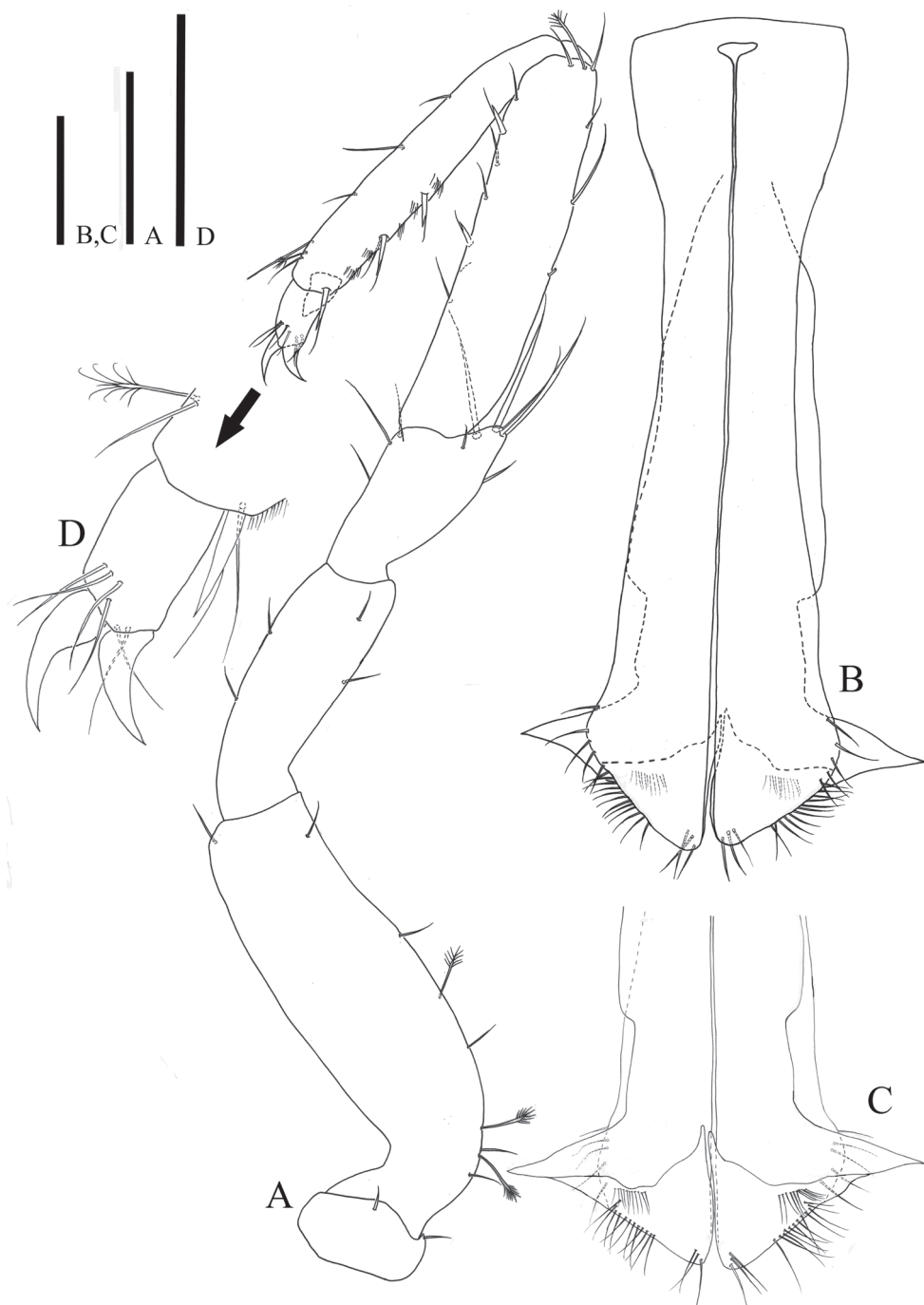


Figure 7. *Caecianiropsis goseongensis* sp. n. holotype, male. **A** Pereopod 7, dorsal, $\times 400$ **B** pleopod 1, ventral, $\times 400$ **C** dorsal side of pleopod 1, $\times 400$ **D** propodus of pereopod 7, ventral, $\times 1000$. Scale bar: 100 μm .

Pleopod I (Fig. 7B): reaching posterior margin of pleotelson, consisting of two coalescent halves, elongate, 3.86 times longer than maximum wide (measured at widest section of proximal part); proximal part enlarged and becoming narrower until half of total length and subsequently broaden distally, 3.86 times longer than maximum wide (measured at widest section of proximal part); separated in half by medial stylet-guiding groove running from triangular opening on proximal part of medial groove, distolateral edge of hyaline lamella angular, projecting laterally; each distomedial lobe tapering distomedially, with ten simple setae distolaterally; distodorsal margin with 22 simple setae; 30 simple setae along distal apex, two distodorsal protrusions developed proximally (Fig. 7C).

Pleopod II (Fig. 8A): sympod elongate, 2.7 times longer than wide; endopodal stylet, elongate, coiled in relaxed position, over 3 times longer than sympod, proximally robust but becoming narrower until distal tip, distal end of sperm duct consisting two rami (Fig. 10H) without ornamentation; exopod distal apex round, located below stylet, distomedially in sympod.

Pleopod III (Fig. 6C): sympod 1.61 times longer than wide; endopod length 0.67 times of sympod, 2.17 times longer than wide, suboval, with ornamentation like turtle shell shape, and with three plumose setae along distal margin; exopod two-segmented, basal segment 5.37 times longer than wide, with hairs along lateral margin, apical segment tapering distally, with one simple seta.

Pleopod IV (Fig. 7B): sympod, pentagonal, exopod 1.6 times longer than endopod, exopod tip not reaching to endopod tip; without ornamentation, exopod proximally curved, with distal seta.

Pleopod V (Fig. 7C): 3.16 times longer than wide, without rami and ornamentation, distal apex rounded.

Uropods (Fig. 7D): length 0.92 times of pleotelson; sympod robust, 3.5 times longer than wide, with 19 simple setae, most of setae on medial margin; endopod length 1.51 times of sympod with 24 simple and nine broom setae; exopod 0.5 times of endopod, with 12 simple setae.

Penial papillae (Fig. 10D, E): pointed distally, located ventrally on posteromedial margin of pereonite VII.

Sexual dimorphism of female. *Body* (Fig. 9A): coxae of pleonites VI and VII visible in dorsal view.

Antennula (Fig. 9A): with five articles.

Antenna (Fig. 9B): with 25 flagellar articles.

Maxillipedal palp (Fig. 9C): article I with one seta distomedially, article II with four setae distomedially, article III with four setae, article IV with five setae, article V with seven setae distally.

Female operculum (Fig. 8D): 1.32 times longer than wide, two simple setae on dorsal surface, six setae along distal apex.

Etymology. The new species is named after the type locality, Goseong, Gangwondo, Korea.

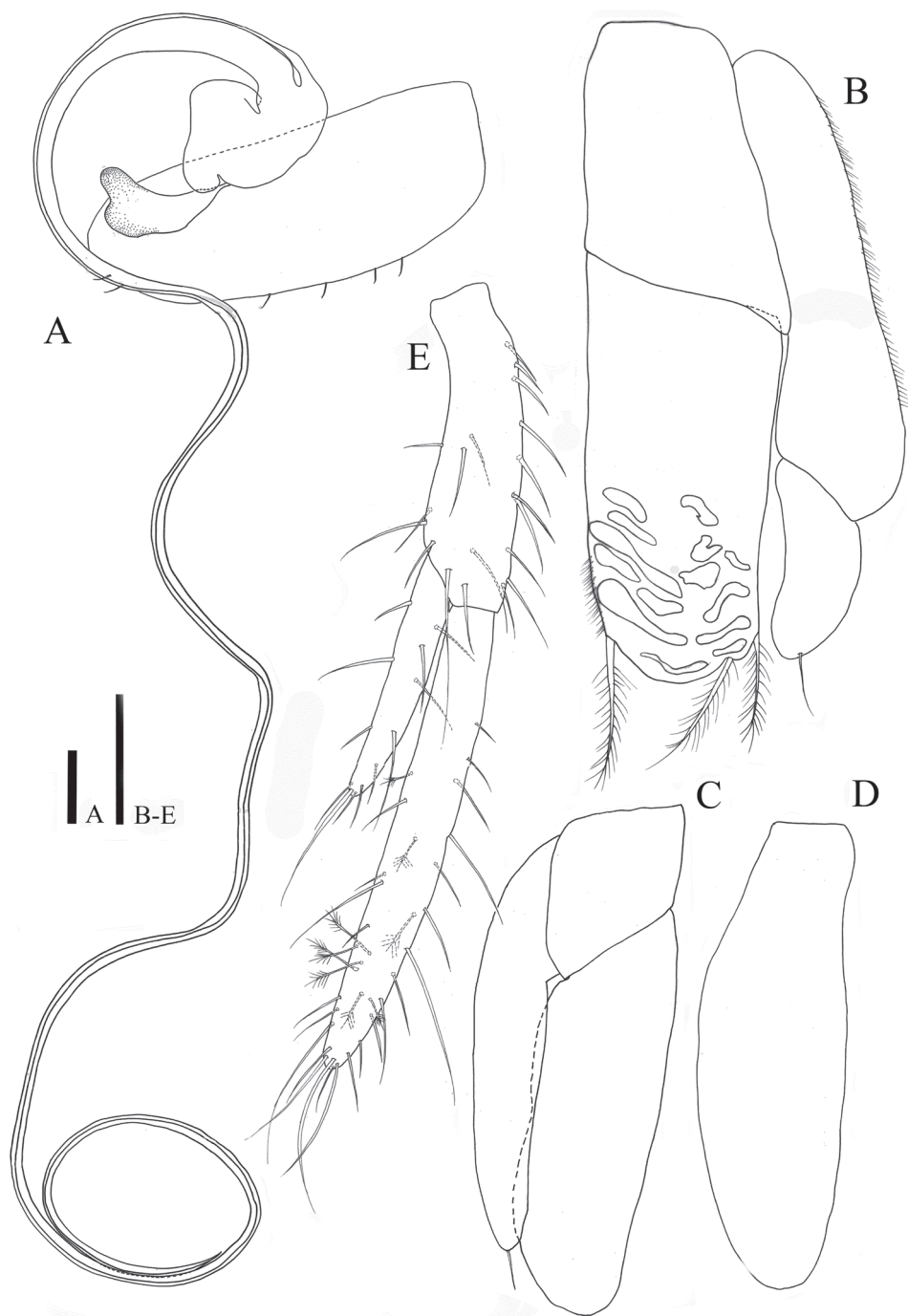


Figure 8. *Caecianiropsis goseongensis* sp. n. holotype, male. **A** Pleopod 2, dorsal, $\times 400$ **B** pleopod 3, dorsal, $\times 400$ **C** pleopod 4, dorsal, $\times 400$ **D** pleopod 5, dorsal, $\times 400$ **E** uroopod, dorsal, $\times 400$. Scale bar: 100 μm .

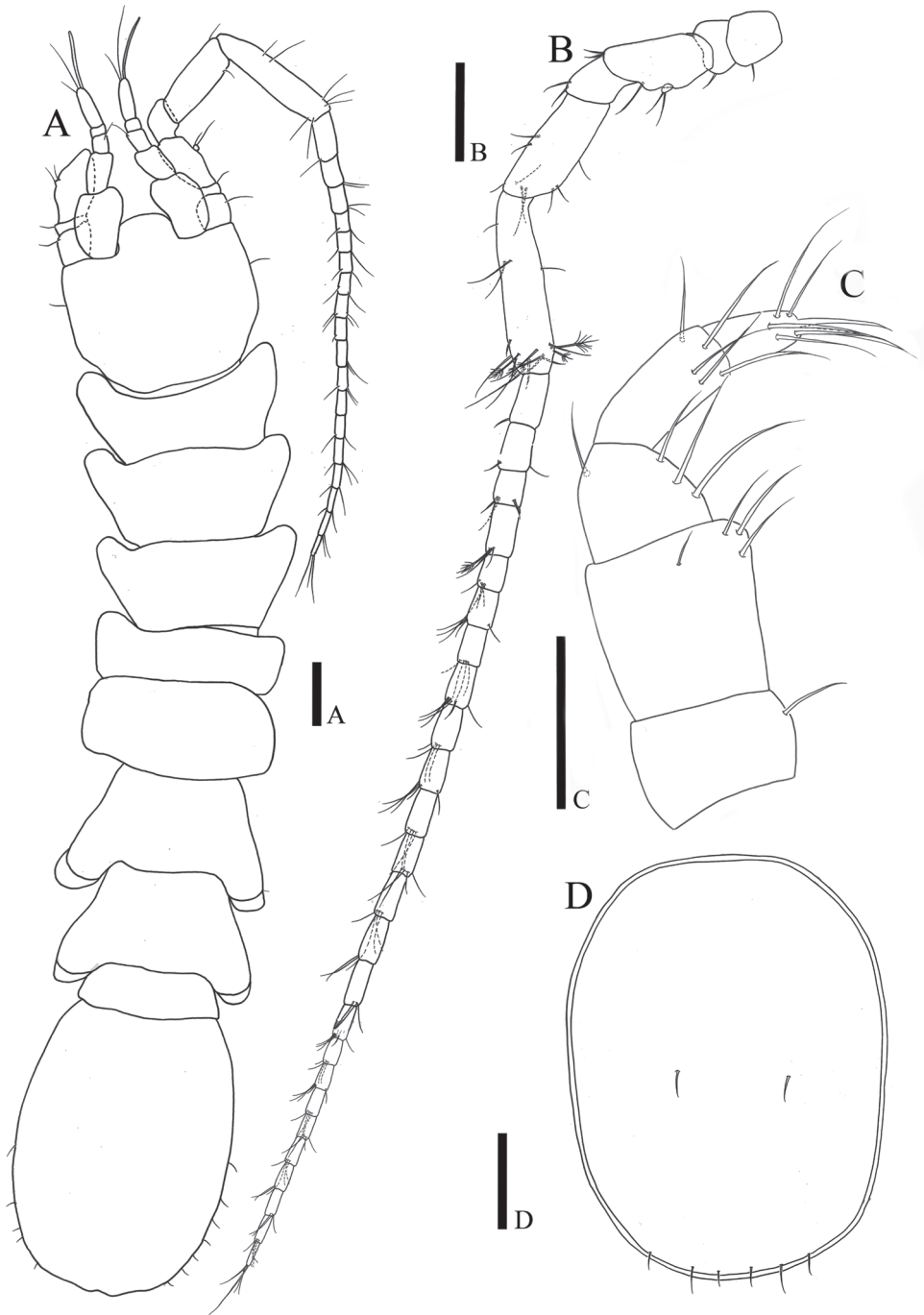


Figure 9. *Caecianiropsis goseongensis* sp. n. allotype, ovigerous female. **A** Habitus, dorsal, $\times 200$ **B** antenna, dorsal, $\times 400$ **C** maxillipedal palp, ventral, $\times 1000$ **D** operculum, dorsal, $\times 600$. Scale bars: 100 μm (**A, B, D**), 50 μm (**C**).

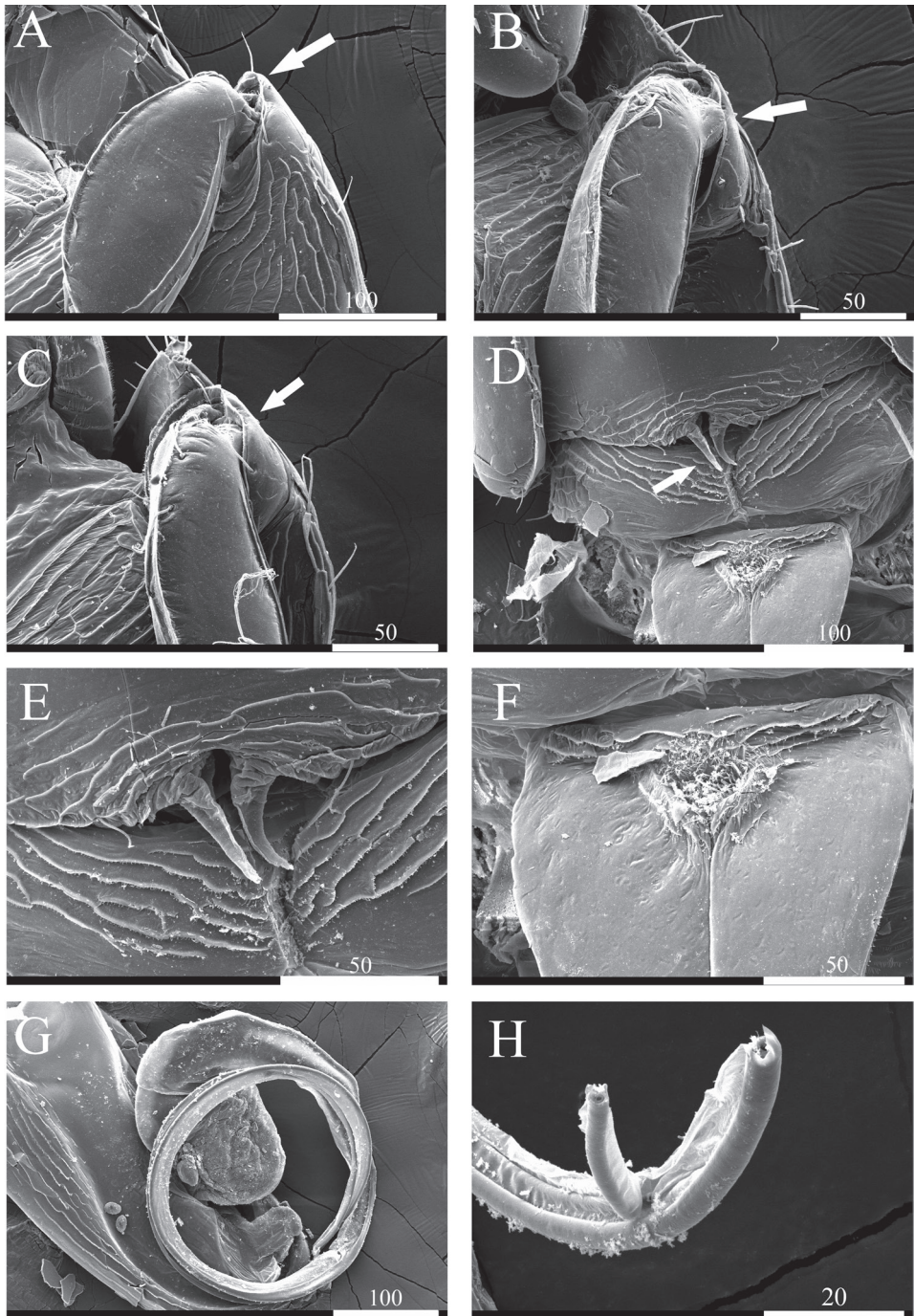


Figure 10. Scanning electron microscope images of *Caecianiropsis goseongensis* sp. n. paratype 2, male. **A** Coxa of pereopod 1, ventral, $\times 450$ **B** coxa of pereopod 2, ventral, $\times 600$ **C** coxa of pereopod 3, ventral, $\times 600$ **D** penial papillae, ventral, $\times 400$ **E** penial papillae, ventral, $\times 900$ **F** basal part of pleopod 1, ventral, $\times 800$ **G** pleopod 2 with stylet, ventral, $\times 300$ **H** distal tip of stylet, ventral, $\times 2000$. Scale bars: 100 μm (**A**, **D**, **G**), 50 μm (**B**, **C**, **F**), 20 μm (**H**).

Key to the species of *Caecianiropsis* (males only)

The key is mainly based on the male characters.

- 1 Rostrum approx. 1.6 times broader than article I of antennula; male pleopod I without distolateral angular projection *C. ectiformis*
- Rostrum as wide as article I of antennula; male pleopod I with distolateral angular projection..... **2**
- 2 Antennula with 7 articles *C. goseongensis* sp. n.
- Antennula with fewer than 7 articles..... **3**
- 3 Endopod of uropod approx. 1.5 times longer than exopod *C. birsteini*
- Endopod of uropod approx. 4 times longer than exopod..... *C. psammophila*

16S rRNA amplification

The final length of the trimmed sequence was comprised of 495 base pairs (GenBank accession numbers MH899013 and MH899014). BLAST (Altschul et al. 1990) of the GenBank database revealed that the obtained sequences were isopod in origin and not contaminated. *Microcharon tanakai* Kim, Lee & Karanovic, 2017 (accession number KY498031.1, Lepidocharontidae) was the most similar sequence to *C. goseongensis* sp. n. resulting from Megablast optimization with 82% identity, $2e-114$ E-value, 99% of query cover, 424 of total, and max score.

Discussion

Morphological comparison

Caecianiropsis goseongensis sp. n. is similar to *C. psammophila* described from northern California in having the second mandibular palp with three serrate setae, truncate molar process, and also in the appearance of the male pleopod I and III. Their differences, however, include: the number of antennular articles (7 vs. 6); the ratio of maxillipedal epipodite (3.56 vs. 2.72), the length ratio of uropodal exopod to endopod (0.8 times of endopod vs. 0.24 times of endopod). *Caecianiropsis birsteini* from Bering and Okhotsk Seas is easily distinguished from the new species by the five segmented antennula, nine apical setae on the maxillula, absence of refraction from the distal margin of male pleopod I, and by the relative length of uropodal exopod to endopod (69% vs. 49 %). Furthermore, differences in setal formula were observed in several appendages including antennula, antennal flagellum, pereopod I, uropodal rami, and the distal margin of pleopod I. The only southern species, *C. ectiformis*, has several distinct characteristics of which the most prominent are: the rostrum broader than antennule article I, the L/W ratio maxillipedal basis (1.4 vs. 3.1), the absence of the distolateral angular projection in the male pleopod I, and, the invisibility of uropodal sympod in dorsal view.

The non-ovigerous female of *C. goseongensis* sp. n. can be distinguished from the male primarily by its smaller body size. The other differences include five segmented antennulae, ratio of the antennular article V (3.27 vs. 2.34), antennula with fewer aesthetascs (1 vs. 3), setal formula of the antennular article I and II, setal formula of the antennal article V and VI, and setal formula of all articles of the maxilliped palp. Although, Menzies and Pettit (1956) and Kussakin (1979) described the female operculum of both *C. psammophila* and *C. birsteini*, there was, however, no more information provided on the sexual dimorphic characters. Unlike all other species of the genus the operculum of *C. goseongensis* sp. n. bears several simple setae on the medial margin of the dorsal surface.

Note on the habitat

The porosity and the volume of water permeating the interstitial space can be influenced by the particle size, which is one of the major factors characterizing the interstice (McLachlan and Turner 1994). Microorganisms inhabiting sediments are dependent on water inflow containing organic substance and minerals, essential for their life (Swedmark 1964). It has also been proven that the composition of many taxa in the sediment is closely related to the certain size or shape of particles (Dahl 1952; Jansson 1967; McLachlan 1996; Strayer et al. 1997; Defeo and Gómez 2005; De troch et al. 2006).

Menzies and Pettit (1956) noted that *C. psammophila* was collected from interstitial water on a coarse sand beach, while the other records of *Caecianiropsis*, including *C. goseongensis* sp. n., are from the shallow sub-littoral zone. Oh et al. (2007) carried out a granulometric analysis to infer the composition of sand particle in Naksan Beach (Gangwondo province, Korea). They found that the average particle size on the beach was much larger than that of the sub-littoral zone. Kim and Song (2012) also obtained similar granulometric results using sand samples from several beaches of Gangwondo province, where the type locality of *C. goseongensis* sp. n. is located. They collected the sand from five stations on each beach with 300 m intervals from the shoreline to the sub-littoral zone and measured the average diameter of particles. According to their results, the composition of sand particles and grain size tend to become smaller with distance, so that the sub-littoral zone has a finer substrate. Based on these results, *C. goseongensis* sp. n. is also distinguished from *C. psammophila* by the size of particles it lives in. This also may indicate that there is an interspecific preference for sand grain size in *Caecianiropsis*. However, data on the specific habitat type for the other *Caecianiropsis* is lacking.

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References

- Branch ML, Griffiths CL, Kensley B, Sieg J (1991) The benthic Crustacea of subantarctic Marion and Prince Edward Islands: illustrated keys to the species and results of the 1982–1989 University of Cape Town surveys. *South African Journal of Antarctic Research* 21: 3–44. <http://alp.lib.sun.ac.za/handle/123456789/7477>
- Dahl E (1952) Some aspects of the ecology and zonation of the fauna on sandy beaches. *Oikos* 4: 1–27. <https://doi.org/10.2307/3565072>
- Defeo O, Gómez J (2005) Morphodynamics and habitat safety in sandy beaches: life-history adaptations in a supralittoral amphipod. *Marine Ecology Progress Series* 293: 143–153. <https://doi.org/10.3354/meps293143>
- De Troch M, Houthoofd L, Chepurinov V, Vanreusel A (2006) Does sediment grain size affect diatom grazing by harpacticoid copepods? *Marine environmental research* 61: 265–277. <https://doi.org/10.1016/j.marenvres.2005.10.004>
- Jansson BO (1967) The significance of grain size and pore water content for the interstitial fauna of sandy beaches. *Oikos* 18: 311–322. <https://doi.org/10.2307/3565107>
- Kensley B (1976) Isopodan and Tanaidacean Crustacea from the St Paul and Amsterdam Islands, Southern Indian Ocean. *Annals of the South African Museum, Capetown* 69: 261–323.
- Kim IH, Song DS (2012) Geographical Distribution of Sand Particle Sizes on Gwang Won Coast. *Korean Society of Surveying, Geodesy, Photogrammetry, and Cartography* 4: 407–410.
- Kussakin OG (1979) The isopod fauna (Crustacea, Isopoda) of the Ochotsk Sea. In: *Investigations of Pelagic and Bottom organisms from the Far Eastern Seas*. Akademii Nauk, SSSR, Vladivostok 15: 106–122.
- Kussakin OG (1988) Marine and brackishwater Crustacea (Isopoda) of cold and temperate waters of northern hemisphere. Vol. 3, Suborder Asellota Part 1. *Academy of Science, USSR* 152, 500 pp.
- McLachlan A (1996) Physical factors in benthic ecology: effects of changing sand particle size on beach fauna. *Marine Ecology Progress Series* 131: 205–217. <https://doi.org/10.3354/meps131205>
- McLachlan A, Turner I (1994) The Interstitial Environment of Sandy Beaches. *Marine Ecology* 15: 177–211. <https://doi.org/10.1111/j.1439-0485.1994.tb00053.x>
- Menzies RJ, Pettit J (1956) A New Genus and Species of Marine Asellote Isopod, *Caecianiropsis psammophila*, from California. *Proceedings of the United States National Museum* 106: 441–446. <https://doi.org/10.5479/si.00963801.106-3376.441>

- Oh JK, Jeong SM, Cho YG (2007) Variations of Grain Textural Parameters of Beaches by Coast Development at East Coast Korea Peninsula. *Journal of the Korean earth science society* 28: 914–924. <https://doi.org/10.5467/JKESS.2007.28.7.914>
- Palumbi SR, Martin A, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR, Version 2.0. <http://palumbi.stanford.edu/SimpleFoolsMaster.pdf>
- Riehl T, Brandt A (2010) Descriptions of two new species in the genus *Macrostylis* Sars, 1864 (Isopoda, Asellota, Macrostylidae) from the Weddell Sea (Southern Ocean), with a synonymisation of the genus *Desmostylis* Brandt, 1992 with *Macrostylis*. *ZooKeys* 57: 9–49. <https://doi.org/10.3897/zookeys.57.310>
- Shimomura M (2005) *Microjaera morii* sp. n., a new interstitial isopod from Japan (Crustacea: Janiridae). *Bulletin of the Kitakyushu Museum of Natural History and Human History* 3: 115–120. http://www.kmnh.jp/wp-content/themes/kmnh_jp/images/pdf/A3-115-E-Shimomura.pdf
- Strayer DL, May SE, Nielsen P, Wollheim W, Hausam S (1997) Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. *Archiv für Hydrobiologie* 140: 131–144. <https://doi.org/10.1127/archiv-hydrobiol/140/1997/131>
- Swedmark B (1964) The interstitial fauna of marine sand. *Biological Reviews* 39: 1–42. <https://doi.org/10.1111/j.1469-185X.1964.tb00948.x>
- Vanhoffen E (1914) Die Isopoden der Deutschen Sddpolar Expedition 1901–1903, Deutschen Sudpolar Expedition. *Zoologie* 15: 47–598.
- Wilson GDF (1994) A phylogenetic analysis of the isopod family Janiridae (Crustacea). *Invertebrate Systematics* 8: 749–766. <https://doi.org/10.1071/IT9940749>
- Wilson GDF, Wägele JW (1994) A systematic review of the family Janiridae (Crustacea: Isopoda: Asellota). *Invertebrate Systematics* 8: 683–747. <https://doi.org/10.1071/IT9940683>

DNA barcoding of British mosquitoes (Diptera, Culicidae) to support species identification, discovery of cryptic genetic diversity and monitoring invasive species

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Abstract

Correct mosquito species identification is essential for mosquito and disease control programs. However, this is complicated by the difficulties in morphologically identifying some mosquito species. In this study, variation of a partial sequence of the cytochrome *c* oxidase unit I (*COI*) gene was used for the molecular identification of British mosquito species and to facilitate the discovery of cryptic diversity, and monitoring invasive species. Three DNA extraction methods were compared to obtain DNA barcodes from adult specimens. In total, we analyzed 42 species belonging to the genera *Aedes* Meigen, 1818 (21 species), *Anopheles* Meigen, 1818 (7 species), *Coquillettidia* Theobald, 1904 (1 species), *Culex* Linnaeus, 1758 (6 species), *Culiseta* Felt, 1904 (7 species), and *Orthopodomyia* Theobald, 1904 (1 species).

Intraspecific genetic divergence ranged from 0% to 5.4%, while higher interspecific divergences were identified between *Aedes geminus* Peus, 1971/*Culiseta litorea* (Shute, 1928) (24.6%) and *Ae. geminus*/*An. plumbeus* Stephens, 1828 (22.5%). Taxonomic discrepancy was shown between *An. daciae* Linton, Nicolescu & Harbach, 2004 and *An. messeae* Falleroni, 1828 indicating the poor resolution of the *COI* DNA barcoding region in separating these taxa. Other species such as *Ae. cantans* (Meigen, 1818)/*Ae. annulipes* (Meigen, 1830) showed similar discrepancies indicating some limitation of this genetic marker to identify certain mosquito species. The combination of morphology and DNA barcoding is an effective approach for the identification of British mosquitoes, for invasive mosquitoes posing a threat to the UK, and for the detection of hidden diversity within species groups.

Keywords

DNA extraction methods, hidden genetic diversity, molecular identification, vector species

Introduction

The family Culicidae includes approximately 112 genera and 3,547 described species (Harbach 2017, 2018). Several species are biting pests playing an important role as vectors of pathogens of humans and livestock (Becker et al. 2010). These include chikungunya, dengue, Japanese encephalitis, yellow fever, West Nile, Rift Valley fever, and Zika viruses, as well as several nematodes and protozoans such as *Plasmodium* Marchiafava & Celli, 1885 (Becker et al. 2010, Medlock et al. 2007). In addition to their medical and veterinary importance, mosquitoes are significant nuisance biters of humans and within the environment may serve additional roles such as key indicators of landscape degradation (Dorvillé 1996, Guedes and Navarro-Silva 2014, Montagner et al. 2018). As a result, mosquitoes are one of the principal target groups within surveillance and control programs worldwide (Hernández-Triana et al. 2017).

Current approaches to species identification still rely heavily upon morphology-based procedures, which typically require substantial training and may not always provide a good resolution on a specimen's identity due to homogeneity between life stages of different species and the presence of species complexes (Cywinska et al. 2006, Hernández-Triana et al. 2012, 2014, 2015, Linton et al. 2005, Packer et al. 2009, Versteirt et al. 2015). To overcome this obstacle, a small region (658 bp) of the mitochondrial cytochrome *c* oxidase unit I (*COI*) gene was proposed as a standardized DNA marker in support of species identification for animal barcodes, in a process commonly referred to as DNA barcoding (Hebert et al. 2003a, b).

Until recently, thirty-four mosquito species have been recorded in the United Kingdom (UK) (Medlock et al. 2015, Medlock and Vaux 2009, 2015). However, Medlock et al. (2017a) detected the presence of *Ae. albopictus* (Skuse, 1895) in southern England, and Dallimore et al. (2017) collected a single male *Ae. aegypti* (Linnaeus, 1762) in Merseyside in north west England, although these two invasive species are not believed to be locally established. Nonetheless, these findings demonstrated that the UK is at risk of introduction by invasive species of *Aedes* (Dallimore et al., 2017). In addition, Harbach et al. (2017) discovered *Ae. nigrinus* (Eckstein, 1918) in the New Forest, southern England, which brings the total count of named species to 37

[35 native species plus two records of invasive species]. In addition, the occurrence of certain species has been very sporadic as in the case of *Ae. vexans* (Meigen, 1830); however, Medlock et al. (2017b) reported a resident population of this species at Marston Marshes, Norwich. Although no mosquito-borne pathogen affecting humans or livestock is presently thought to circulate in the UK, there is potential for future pathogen emergence (Medlock and Leach 2015; Medlock et al. 2017a, b; Vaux et al. 2015) and there remains continuing mosquito nuisance in various parts of the country (Brugman et al. 2017a, b). Collectively, these discoveries highlight the need for continued research on the native mosquito fauna of the UK, taking into account the potential incursion of invasive species.

There is, however, a paucity of data on the utility of molecular methods for species identification of the British mosquito fauna. During the first development of a molecular assay for the identification of hybrids and sibling species within *Culex pipiens* s.l., Smith and Fonseca (2004) used specimens from England and Scotland. Golding et al. (2012) subsequently employed the *COI* marker to compare sequences of *Cx. modestus* Ficalbi, 1890 with other *Culex* Linnaeus, 1758 species in southeast England, and Danabalan et al. (2012) employed a combination of the internal transcribed spacer gene-2 (*ITS-2*) and *COI* markers in their assessment of molecular identification tools to determine the status of *Cx. pipiens* s.l. The same approach was used by Danabalan et al. (2014) to confirm the occurrence of species within the *Anopheles maculipennis* complex Theobald, 1911 in England and Wales. Recently, Hernández-Triana et al. (2015) employed an integrated approach to determine mosquito host feeding preferences (via identification of blood meal origin), as well as the molecular characterization of mosquito species carrying pathogens such as myxoma virus (Brugman et al. 2015, 2017a, b) and *Theileria orientalis* Yakimoff & Soudatschenkoff, 1931 within their bloodmeal (de Marco et al. 2016).

In the present paper, we apply the *COI* DNA barcoding approach in support of the identification of native British mosquitoes and known invasive species in continental Europe. In addition, we assessed the DNA barcode variability using genetic distance methods to detect cryptic diversity across the taxa.

Materials and methods

Collection of specimens

Ten locations were visited between March and October in the years 2012 to 2015 and specimens were collected following the protocols of Brugman et al. (2015, 2017a, b) (see Table 1, Fig. 1). Further samples were obtained by collecting mosquitoes alighting on the collectors and by standard larval dipping procedures followed by laboratory rearing according to Brugman et al. (2015, 2017a, b). All specimens were kept either at -20 °C or dry-pinned, and were morphologically identified using the key of Cranston et al. (1987). We followed the classification of Wilkerson et al. (2015) for taxa in Aedini. The subgeneric placement for all species can be found in Harbach (2017) and Harbach et al. (2017).

Table 1. Description of key collecting sites with reference to habitat and the main livestock present. Further information can be found in Brugman et al. (2017b).

Locality/Farms	County	Coordinates	Habitat	Main livestock types present
1. ADAS Arthur Rickwood	Cambridgeshire	52.422560, -0.098302	Grazing farm	Sheep
2. Church Farm	Oxfordshire	51.715807, -1.380813	Rural area	Cattle, sheep
3. Coombelands Farm	Surrey	51.360241, -0.652256	Mixed farm	Cattle, sheep, pigs, horses
4. Elmley Nature Reserve	Kent	51.377587, 0.783954	Grazing marsh	Cattle, sheep
5. Glendell Livery, Mill Lane	Surrey	51.290499, -0.652256	Mixed woodland	Horses
6. Frimley	Surrey	51.313037, -0.745237	Peri-urban	n/a
7. Mudchute Farm	Greater London	51.491732, -0.009367	City farm	Cattle, sheep, pigs, horses
8. Northney Farm, Hayling Island	Hampshire	50.828166, -0.962151	Arable farm	Cattle
9. White Lodge, Bisley	Surrey	51.322255, -0.637692	Mixed woodland	Cattle
10. Bartley Heath	Hampshire	50.919701, -1.565337	Woodland	Cattle, horses, deer
11. Dee Marsh	Cheshire	52.8322, -3.7656	Salt marsh	n/a

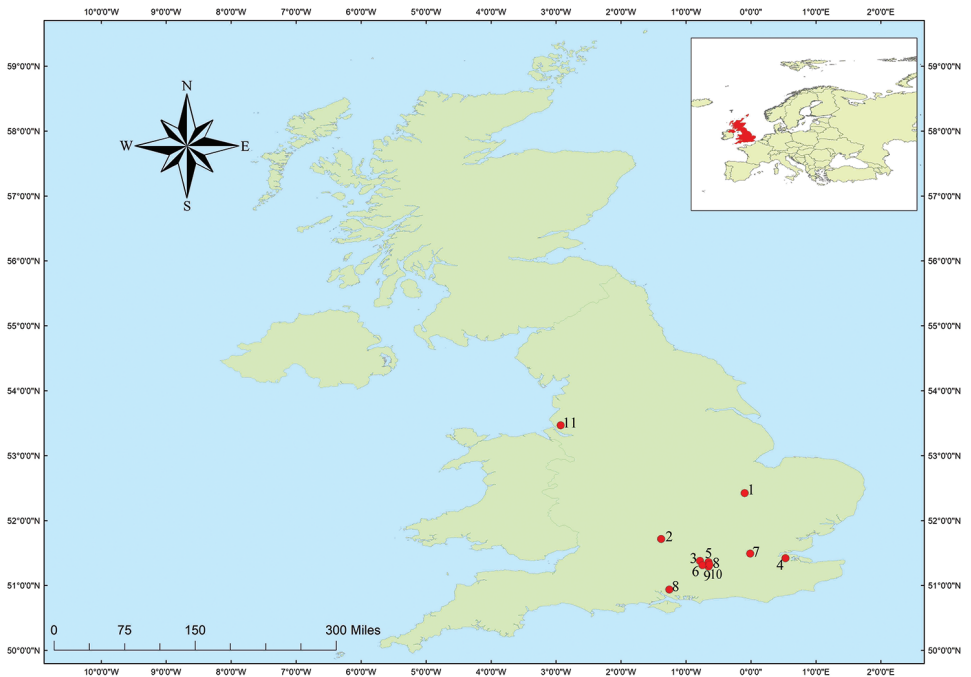


Figure 1. Location of study sites in the United Kingdom. Key: **1** ADAS Arthur Rickwood; **2** Church Farm; **3** Coombelands Farms; **4** Elmley Nature Reserve; **5** Glendell Livery, Mill Lane; **6** Frimley; **7** Mudchute Farm; **8** Northney Farm, Hayling Island; **9** White Lodge, Bisley; **10** Bartley Heath; **11** Dee Marsh.

The source of specimens from invasive species is as follows: *Ae. albopictus* – Luke Alpey, UK (colony from Malaysia); Aleksandra Ignjatović-Ćupina, Serbia (wild caught); *Ae. aegypti* – Shahida Begum, UK (colony from West Africa); *Ae. atropalpus* (Coquillett, 1902), *Ae. japonicus* (Theobald, 1901), *Ae. koreicus* (Edwards, 1917) – Norbert Becker and Daniel Hoffman, Germany, and Ignacio Justicia-Ibáñez, Holland (all wild caught); *Culex tritaeniorhynchus* Giles, 1901, Filiz Gunay, Turkey (wild caught); *Cx. quinquefasciatus* Say, 1823 [for sequences from NCBI and further details Suppl. material 1].

DNA extraction methods

Three methods were used for DNA extraction from two mosquito tissue types (Brugman et al. 2015, 2017). Firstly, 1–2 legs of specimens were placed in 100 µl of molecular grade water in a 96-well plate, which was then sonicated at room temperature for 10 min to release DNA (Hunter et al. 2008). Secondly, we employed a modified Hotshot technique (Montero-Pau et al. 2008). In this case, 1–2 legs were placed directly into 50 µl of alkaline lysis buffer in a 96-well plate, which was then sonicated in a water bath for 10 min. The plate was subsequently incubated in a thermocycler for 30 min at 94 °C, cooled for 5 min at 4 °C, and then centrifuged for 3 min at 3000 rpm, after which 50 µl of the neutralizing buffer was added to each sample. The plate was then centrifuged again for 10 min at 3000 rpm, and stored at -80 °C until analysis. Thirdly, engorged female abdomens were processed using Qiagen DNeasy Blood and Tissue kits following the procedures detailed in Brugman et al. (2015, 2017a, b) and the manufacturer's instructions.

COI DNA barcoding region amplification

For molecular species identification using the *COI* DNA barcoding region, the protocols of Hernández-Triana et al. (2012, 2014) and Hebert et al. (2003a, b) were followed. We used the primers developed by Folmer et al. (1994), which amplify the 658 bp long target region of the *COI* gene. PCR products were obtained using a Qiagen PCR system following the reaction mix of Hernández-Triana et al. (2017).

Data analysis

Paired bi-directional sequence traces were combined to produce a single consensus sequence (i.e., the full-length 658 bp barcode sequence). To achieve this, individual forward and reverse traces were oriented, edited, and aligned using the Sequencer (v.4.5;

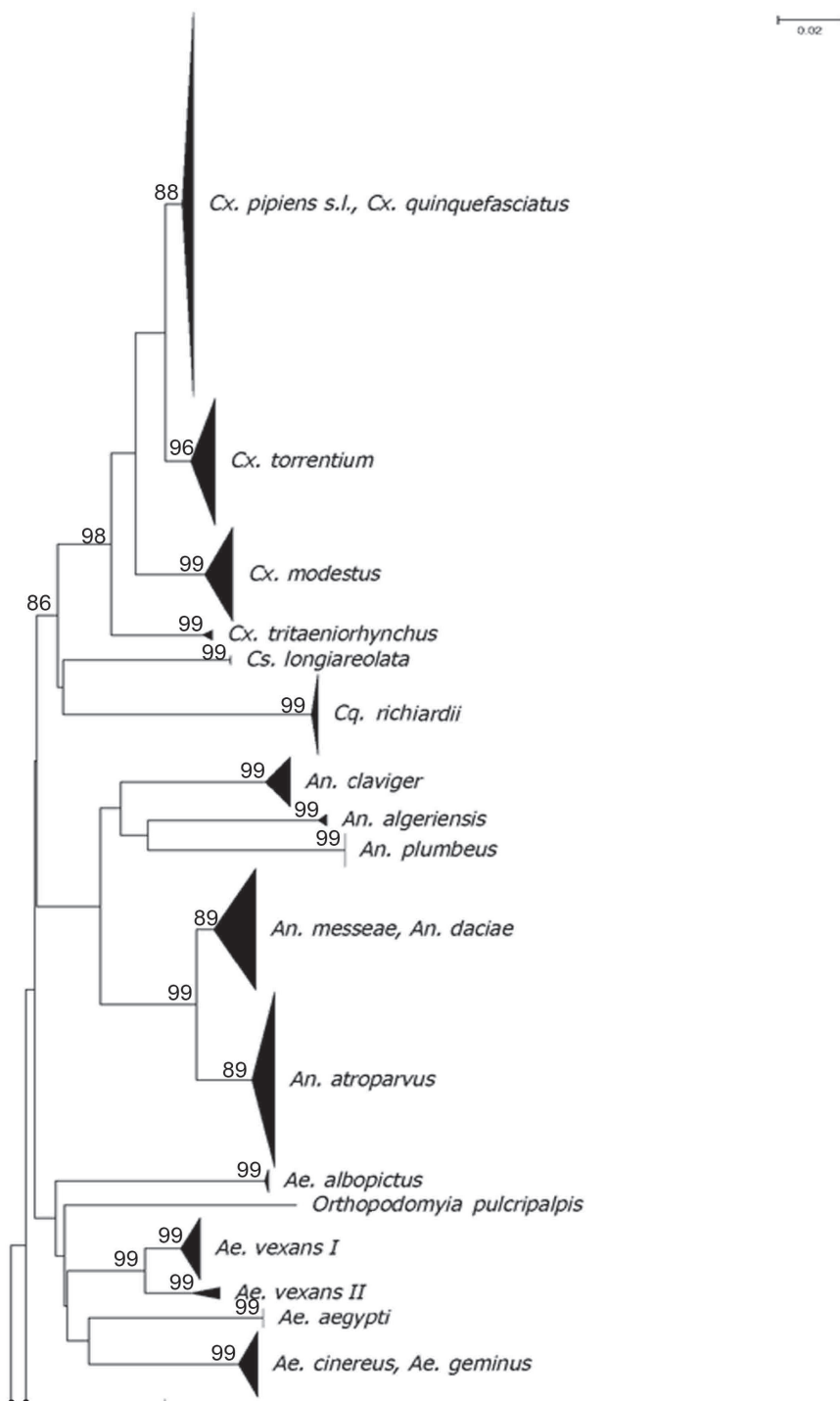


Figure 2. Neighbor joining tree of *COI* DNA barcodes (658 bp) for mosquito species. A divergence of > 2% may be indicative of separate operational taxonomic units. Only bootstrap values higher than 70% are shown.

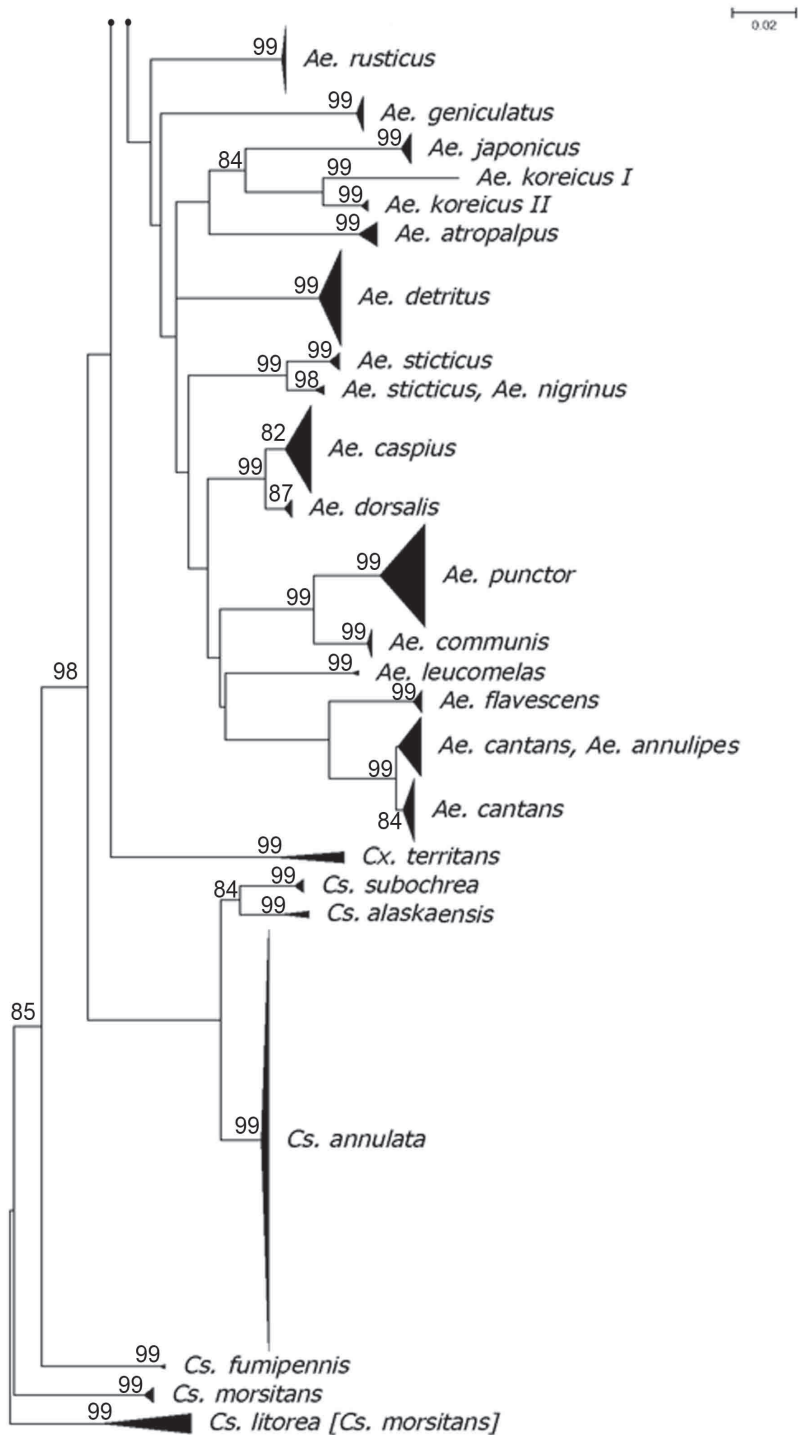


Figure 2. Continued.

Genes Codes Corporation, Ann Harbour, MI), GenDoc (v. 2.6.02) and ClustalX sequence analysis programs (Hernández-Triana et al. 2017). Full details for each specimen and sequence information can be found at the Barcode of Life Database (BOLD) within the “Human Pathogens and Zoonoses Initiative”, Working Group 1.4. The Digital Object Identifier (DOI) for the publically available projects in BOLD is dx.doi.org/10.5883/DS-MQFWUK and dx.doi.org/10.5883/DS-MQIUUV. Accession numbers for all sequences were obtained from NCBI (accession numbers: MK403007–MK403548). For certain species, we used *COI* barcode sequences deposited at NCBI due to the lack of available material from UK populations (Table 3; Suppl. material 1). The dataset was analyzed in MEGA v.6 (Tamura et al. 2007). The Neighbor Joining (NJ) analysis was performed using the Kimura 2-Parameter distance metric to determine their distribution pattern. The tree robustness was measured by the bootstrap approach using 1000 pseudoreplicates (Hernández-Triana et al. 2012, 2014). To barcode sequences larger than 500 bp, a Barcode Index Number (BIN) was assigned and each BIN was mapped according to species (see Fig. 2). The taxonomic discordance in the dataset (Hernández-Triana et al. 2017) was analyzed using BOLD as detailed in Ratnasingham and Hebert (2013).

Results

Assessment of DNA extraction methodologies

In general, adding 1–2 legs to molecular grade water and then sonicating them for 10 min proved to be an effective method for obtaining DNA (30 min total time); however, only 41 barcodes (43.1%) yielded sufficient sequence data for inclusion in our analysis (Table 2). The Hotshot technique also proved to be an efficient approach (1 hour per plate) for processing 1–2 legs with high percentages of target DNA amplification and *COI* DNA barcode sequences (429 sequences, 90.3%). In terms of cost, reagents for the preparation of the Hotshot working stock buffers were only 200 GBP, one purchase of which we estimate can last up to one year. DNA extraction from blood-engorged abdomens processed using the Qiagen DNeasy Blood and Tissue kit also provided barcodes for 306 specimens (64.4%), but this approach was time consuming, with a sample processing rate of 32 specimens per four hour session of DNA extraction. The time for the DNA extraction was also increased by the limitation of the number of wells in the centrifuge available (30 wells). In addition, non-target PCR product was also encountered as vertebrate DNA present was amplified from cows, chicken, sheep, rabbits and birds [169 samples] (see Table 2).

Mosquito species identification using DNA barcoding

In total, we analyzed DNA barcode sequences for 42 species belonging to the genera *Aedes* (21 species), *Anopheles* (7 species), *Coquillettidia* (1 species), *Culex* (6

Table 2. DNA extraction methods and percentage of PCR amplification success in obtaining *COI* DNA barcodes from mosquitoes.

Extraction method	No. plates / samples	Time per plate	Amplification success n (%)	Observations
1. Legs directly into molecular grade water and sonicated for 10 min	1 plate / 95 samples	30 min	41 (43.1%)	High sequencing failure (54 samples)
2. Legs directly into alkaline lysis buffer and sonicated for 10 min (Hotshot)	5 plates / 475 samples	1hr each plate	429 (90.3%)	Target length barcodes obtained
3. Abdomen processed using Qiagen kit	5 plates / 475 samples	Only 32 samples per 4hr session for DNA extraction for each plate	306 (64.4%)	Target length barcodes obtained. Vertebrate DNA amplified

species), *Culiseta* (7 species), and *Orthopodomyia* (1 species) (Table 3). Of these, we analyzed sequences for 23 of the 37 species of mosquito that have been recorded in the UK (62%) (Harbach et al. 2017, Medlock et al. 2007a, b). In addition, we also analyzed representatives of invasive *Aedes* species (*Ae. aegypti*, *Ae. albopictus*, *Ae. japonicus*, *Ae. koreicus*, *Ae. atropalpus*) and two *Culex* species (*Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*), which are of epidemiological relevance in Europe (Medlock et al. 2017a, b). Three or more representatives were available for 38 morphospecies in the dataset (see Table 3). In total, 1198 barcode sequences were analyzed.

Even though in most cases individuals of the same species clustered together, this was not the case for all species. Within the genus *Aedes*, the first incongruence was identified between *Ae. sticticus* (Meigen, 1838) and *Ae. nigrinus*. Although the majority of specimens from Belgium and the two UK specimens identified as *Ae. sticticus* (voucher number APHA-4-2015G06, APHA-4-2015G07) grouped together in a separate cluster with 100% bootstrap support, the only two available *COI* sequences of *Ae. nigrinus* in NCBI (KP942769, KP942770) grouped with the two specimens collected in Belgium, identified as *Ae. sticticus* (CULBE-833009, CULBE-833008) (Fig. 2). To further support our identification of the two UK specimens as *Ae. sticticus*, we obtained *ITS-2* sequences (data not shown) and searched the NCBI database using the BLAST algorithm; both queries retrieved *Ae. sticticus* with 96% match (KF535079) [this relative low percentage could be due to the low coverage of the *ITS-2* sequences we obtained (338 bp and 369 bp, respectively)]. Similar results have been obtained by Versteirt et al. (2015) (see Fig 2). Certain specimens grouped only as *Ae. cantans* or *Ae. annulipes*, but another group was composed of *Ae. cantans* and *Ae. annulipes* (Fig.1) with 100% bootstraps support values. Similarly, no successful identification was reached between *Ae. cinereus* (Meigen, 1818) and *Ae. geminus*, species which are morphologically similar.

Within *Anopheles maculipennis* s.l. (Linton et al. 2002, 2005), no accurate identification was achieved between *An. messeae* [also molecularly identified by *ITS-2* in our laboratory; see also Kronefeld et al. 2012] and *An. daciae* (sensu Nicolescu et al.

Table 3. List of mosquito species (in alphabetical order), country of collection, and number of specimens with DNA barcodes. Mean (%) intraspecific values of sequence divergence (Kimura2-Parameter distance) are shown with missing entries indicating that less than two specimens were analyzed. Asterisks indicate species complexes (*) and taxa with deep splits (**) in the Neighbor Joining tree; (***) taxa with above 2% genetic divergence. Invasive species in Europe are in Bold.

Species	Collection Country	<i>n</i>	mean %
<i>Aedes aegypti</i>	West Africa	10	0
<i>Aedes albopictus</i>	Malaysia; Montenegro	12	0.12
<i>Aedes annulipes</i>	Belgium	12	0.89
<i>Aedes atropalpus</i>	Holland, USA, Canada	11	0.69
<i>Aedes cantans</i>	Belgium; UK	44	0.80
<i>Aedes caspius</i>	Belgium; UK	40	0.78
<i>Aedes cinereus</i>	Sweden; UK	30	0.61
<i>Aedes communis</i>	Belgium	13	0.14
<i>Aedes detritus</i>	Belgium; UK	44	0.66
<i>Aedes dorsalis</i>	USA; Canada	8	0.16
<i>Aedes flavescens</i>	UK	10	0.18
<i>Aedes geminus</i>	Germany	4	0.58
<i>Aedes geniculatus</i>	Belgium	16	0.25
<i>Aedes japonicus</i>	Germany	14	0.32
<i>Aedes koreicus</i> ****	Belgium; Holland; Hungary	6	2.19
<i>Aedes leucomelas</i>	Sweden	2	0.40
<i>Aedes nigritinus</i>	Sweden	2	0.77
<i>Aedes punctator</i>	Belgium; UK	47	0.67
<i>Aedes rusticus</i>	Belgium; UK	31	0.07
<i>Aedes sticticus</i>	Belgium; UK	10	1.29
<i>Aedes vexans</i> **	Belgium; Spain; Holland; Sweden; UK	38	1.46
<i>Anopheles algeriensis</i>	Spain	6	0.41
<i>Anopheles atroparvus</i>	UK; Belgium	91	0.92
<i>Anopheles claviger</i> s.l.	Belgium; UK	26	0.65
<i>Anopheles daciae</i>	Romania; UK	28	0.76
<i>Anopheles messeae</i>	UK	35	1.01
<i>Anopheles plumbeus</i>	Belgium; UK	17	0
<i>Coquillettidia richiardii</i>	Belgium; UK	42	0.07
<i>Culex modestus</i>	Germany; Romania; Turkey; UK	49	0
<i>Culex pipiens</i> s.l.*	Belgium; UK	187	0.06
<i>Culex quinquefasciatus</i>	Pakistan; Turkey	12	0
<i>Culex territans</i>	Belgium; Germany	5	2.05
<i>Culex torrentium</i>	Belgium; Germany; UK	66	0.43
<i>Culex tritaeniorhynchus</i>	Turkey	5	0.65
<i>Culiseta alaskaensis</i>	Canada	3	1.13
<i>Culiseta annulata</i>	Belgium; UK	192	0.05
<i>Culiseta fumipennis</i>	Belgium	2	0.30
<i>Culiseta litorea</i> ***	Spain; UK	9	5.35
<i>Culiseta longiareolata</i>	Spain	5	0.12
<i>Culiseta morsitans</i>	Belgium; UK	7	0.34
<i>Culiseta subochrea</i>	Spain; UK	6	0.34
<i>Orthopodomyia pulcripalpis</i>	Austria	1	n/a

2004), although *An. atroparvus* was clearly separated from the aforementioned species (Fig. 2). This is not surprising as all members of the *An. maculipennis* complex are phylogenetically related, and cannot be readily identified based on adult morphological traits or only using the *COI* genetic marker (Linton et al. 2002, Kronefeld et al. 2014, Ruiz-Arrondo et al. 2017). In the genus *Culex*, *COI* was not able to separate *Culex pipiens* s.l. (including both forms *pipiens* and *molestus*) and *Cx. quinquefasciatus*, in agreement with results by Gunay et al. (2015).

Our DNA barcodes dataset from the genus *Culiseta* separated certain species with high support bootstrap values such as *Cs. alaskaensis* (Ludlow, 1906), *Cs. annulata* (Schränk, 1776), *Cs. longiareolata* (Macquart, 1838) and *Cs. subochrea* (Edwards, 1921) (Fig. 2). However, we could not achieve the same resolution for *Cs. fumipennis* (Stephens, 1825), *Cs. litorea* (Shute, 1928) and *Cs. morsitans* (Theobald, 1901). The specimens identified as *Cs. fumipennis* (Versteirt et al. 2015) from Belgium grouped separately from one specimen (KU748471) collected in the UK (de Marco et al. 2016) previously identified as *Cs. fumipennis*, which clustered with specimens from Belgium identified as *Cs. morsitans* (CULBE-816017, CULBE-816018, CULBE-997001, CULBE-997002, CULBE-9972101, CULBE-972103) with 99% bootstrap values. Therefore, we now consider the UK specimen to be *Cs. morsitans*. In addition, seven specimens from the UK identified as *Cs. morsitans* in de Marco et al. (2016) (KU748440, KU748443, KU748450, KU748453, KU748460, KU748500, KU748488), grouped with 99% bootstrap values with two males recently collected from Spain identified as *Cs. litorea*. Subsequent dissection of the genitalia of these specimens confirmed their identification as *Cs. litorea* based on the key of Becker et al. (2010); therefore, we now considered these seven specimens from the UK as *Cs. litorea*.

The levels of sequence divergence were variable across the taxa, with conspecific individuals collected from a single site often exhibiting zero, or 0.07% to 0.1% divergence values, while other specimens showed higher percentages (see Table 3). The intraspecific genetic divergence measured 1.3%, ranging from 0% to 5.4% (Table 3) (*Ae. aegypti*, *An. plumbeus*, *Cx. modestus* and *Cx. quinquefasciatus*), while the interspecific divergence ranged between 0.19% to 24.6% (Suppl. material 2). Interspecific genetic divergence values were higher between species from different genera. The pairs *Ae. geminus*/*Cs. litorea* (24.6%) and *Ae. geminus*/*An. plumbeus* (22.5%) were the most divergent species. As known, the smallest values of genetic divergence were found among species in the same genus, for example *Cx. pipiens* s.l./*Cx. quinquefasciatus* (0.19%), *Ae. cantans*/*Ae. annulipes* (1.2%) and *Ae. geminus*/*Ae. cinereus* (0.65%) (Suppl. material 2).

In this study, we analyzed three species which are known, or suspected to be, part of species complexes [species which can only be distinguished either by cytotaxonomy or molecular methods (Danabalan et al. 2012, 2014; Linton et al. 2001)]: *An. maculipennis* s.l., *An. claviger* s.l. (Meigen, 1804), and *Cx. pipiens* s.l. All specimens grouped together in either *Cx. pipiens* s.l. or *An. claviger* s.l., and we did not detect high levels of genetic diversity or deep splits in the NJ tree as found in other studies (Gunay et al. 2015, Versteirt et al. 2015). This might be due to specimens originating from localities in relatively close proximity to one another in England (Fig. 1). Specimens of

Cx. pipiens s.l. in this study originated from the study of Brugman et al. (2015, 2017), in which the *CQ11* PCR assay was conducted to separate the forms *molestus* and *pipiens*. Only specimens from the typical *pipiens* form of *Cx. pipiens* were detected in the aforementioned studies, with 0.06% genetic diversity in our dataset. Nonetheless, not all morphologically identified species clustered as expected. Certain species exhibited higher levels of divergence above 2% (see Table 1) and other showed deep splits in the NJ tree (Fig. 2). For example, intraspecific genetic divergence averaged 1.46% in *Ae. vexans*, but the specimens separated into two defined clusters (I and II) (Fig. 2). Similarly, *Ae. koreicus* showed a deep split in the NJ with 2.19% genetic divergence.

The BIN counts in our dataset in BOLD of 721 full length barcode sequences from 1006 records in BOLD datasets found 21 BINs. The BIN analysis did not include sequences downloaded from the NCBI database. In general, 487 barcodes were assigned a BIN number, which represented 14 concordant BINs, three BINs were singletons (*Cs. fumipennis* BOLD:AAR2210, *Ae. geniculatus* BOLD:AAM5898, and *Cs. subochrea* BOLD:AAV90 75), and only four BINs (231 records) were discordant. The discordant BINs occurred at the species level, mainly because of the discrepancy in taxonomic information assigned to certain specimens, for example *Ae. cinereus* versus *Ae. nr. cinereus*, and *Ae. caspius* versus *Ae. nr. caspius*. Another discordance was in a single specimen identified as *Cx. torrentium*, which appears to be close to a BIN within *Cx. pipiens* s.l. (BOLD:AAA4751; Process ID:MSEMV855-15); however, morphological traits in the male genitalia and other analysis (*CQ11* PCR) showed that it does belong to *Cx. torrentium* (Manley et al. 2015), and it did cluster with this species when further 66 barcode sequences of *Cx. torrentium* were added to the dataset.

Discussion

This study assessed minimally destructive approaches that retained a significant part of the sample as a referenced voucher and the development of a *COI* DNA barcoding library in mosquitoes, and assessed the use of the variability within *COI* in support of species identification. Overall, the three extraction methods used provided sufficient DNA for subsequent analysis; however the modified Hotshot technique of Montero-Pau et al. (2008) proved to be the most efficient and inexpensive method for obtaining *COI* DNA barcode sequences. This method has been applied to other groups such as the Hymenoptera (Guzmán-Larralde et al. 2016) with good results as assessed by DNA yield and PCR amplification success. The amplification of vertebrate DNA from engorged abdomens using the Qiagen DNeasy Blood and Tissue extraction kit highlights the need to use insect specific primers, for example LepF/LepR (see www.boldsystems.org/index.php/Public_Primer_PrimerSearch) instead of the standard Folmer primers (Folmer et al. 1994). In terms of cost, considering that we did not have to buy a DNA extraction kit to perform the DNA extraction for processing the legs, the Hotshot technique represented savings of around 500 GBP per 96-well plate to our laboratory, making it a cost-effective method for performing DNA extractions.

The majority of morphologically identified species in this study formed defined groups in the NJ analysis based on DNA barcodes (Fig. 2), supporting the use of *COI* DNA barcoding in combination with morphological observation as a suitable approach for species identification. Genetic divergence between morphospecies ranged from 0.19% to 24.6%, whereas intraspecific genetic divergences within distinct species ranged from 0% to 5.4% (average 1.30%; Table 1, Suppl. material 2). Most of the specimens within a morphospecies were resolved in the NJ tree (Fig. 2). However, some individuals in certain taxa such as *Ae. annulipes*/*Ae. cantans*, *Ae. cinereus*/*Ae. geminus*, *Ae. sticticus*/*Ae. nigrinus*, *An. daciae*/*An. messeae*, and *Cx. quinquefasciatus*/*Cx. pipiens* s.l. clustered together (Fig. 2), indicating some limitations of the *COI* gene as a marker to separate these species. This finding is not surprising as these taxa are phylogenetically, as it has been highlighted in the literature (Harbach 2017, Harbach et al. 2017).

With regard to *Anopheles maculipennis* s.l., although some morphological traits in egg structure provide an effective method to separate some members of this group, there is some dispute regarding the taxonomic status of *An. daciae* (e.g. Linton et al. 2002, 2005, Kronefeld et al. 2012). This species was described by Nicolescu et al. (2004) based on all life stages collected from the Black Sea region in Romania. The authors stated that *An. daciae* and *An. messeae* have been misidentified in the past because of similar morphology. However, they showed that *An. daciae* eggs are generally smaller than those of *An. messeae*, with patches of larger dark tubercles that contrast more sharply with patches of smaller tubercles to impart greater definition to the mottled surface of the deck (see Nicolescu et al. 2004; fig. 3 A, C). In contrast, the deck of *An. messeae* eggs has a more diffuse or weakly mottled appearance (see Nicolescu et al. 2004; fig. 3 B, D). In the same study, molecular analysis of *ITS-2* sequences identified single nucleotide polymorphisms and unique haplotypes which, in the authors' views, confirmed the specific status of *An. daciae*. However, other authors have queried the specific status of *An. daciae*, and stated that *COI* offered poor resolution and advocated for further work to determine the status of *An. daciae*. Similarly, in their study of Belgian mosquitoes, Versteirt et al. (2015) reported that specimens identified as *Ae. annulipes* and *Ae. cantans* grouped together in their NJ tree, and stated that *COI* cannot separate these two species; the same results have been obtained in our study (Fig. 2).

Moreover, *COI* DNA barcoding highlighted mis-identifications within the genus *Culiseta* (*Cs. fumipennis*, *Cs. litorea* and *Cs. morsitans*). These species are placed in the subgenus *Culicella*, and the females are difficult to identify because of their morphological similarity and absence of reliable diagnostic characteristics (Becker 2010, Cranston et al. 1987, Medlock and Vaux 2010). All of our females were collected in traps and were in relatively poor condition. In addition, no *COI* sequences of reliably identified material of *Cs. litorea* were available in the NCBI or BOLD databases to compare with our specimens (Ruiz-Arrondo et al. 2017). Because the sequences of females of *Cs. morsitans* matched the two males identified as *Cs. litorea* from Spain, we considered all our specimens to be *Cs. litorea*. In the literature (Medlock et al. 2007), *Cs. morsitans* is considered a widespread species in the UK, found in permanent waters, while *Cs. litorea* has a more restricted distribution in coastal regions of southern England. Both

species feed mainly on birds, but can also bite humans, and they are considered bridge vectors of arboviruses (Medlock et al. 2007). Our findings highlight the need for careful examination of material obtained from traps in combination with the application of molecular techniques for a reliable identification of these species. Even though in our dataset *Ae. vexans* showed a low genetic divergence of approximately 2%, specimens of this species separated into two distinct groups in the NJ tree (Fig. 2), which may be an indication of some genetic differentiation within the population. This agrees with the findings of Lilja et al. (2018) in which the authors reported a distinct genotype of *Ae. vexans* in Europe.

Regarding non-indigenous mosquito species, although the adults of certain species are easily identified using morphological keys, for example *Ae. aegypti* and *Ae. albopictus* (Becker 2010, Cranston et al. 1987), the development of a molecular library for species identification is important, in particular when specimens are found in a poor stage of preservation. This is an essential step for the establishment of control measures (Versteirt et al. 2015) in the event of a recent introduction, as in the case of the detection of *Ae. albopictus* in the UK (see Medlock et al. 2017a, b).

In our dataset, *Ae. koreicus* and *Cs. litorea* showed higher intraspecific genetic divergences (Table 1, Fig. 2), which may indicate the presence of cryptic diversity. For all other species, the variation in intra- and interspecific genetic values reported in this study fall within the range for DNA barcoding studies of European mosquitoes (Engdahl et al. 2014, Gunay et al. 2015, Versteirt et al. 2015) or other zoogeographical regions such as the Nearctic and Oriental Regions (Cywinska et al. 2006, Kumar et al. 2007, Murugan et al. 2016). Nonetheless, we advocate the combination of the *COI* DNA barcoding with other genetic markers such as the Elongator Complex Protein 1 gene (*ECPI*) (Low et al. 2016, Pangjanda and Pramual 2016, Senatore et al. 2014) and *ITS-2* sequences from a larger number of specimens across the species distribution range in order to resolve some of the taxonomic problems highlighted in this study.

Conclusions

This study provides *COI* DNA barcoding data to support the molecular identification of mosquito species in the UK as well as invasive mosquito species, many of which are currently expanding their geographical range in continental Europe. We augment the barcoding data for anthropophilic species such as *Ae. cinereus*, *Ae. detritus*, *Ae. sticticus*, *Ae. vexans*, and *Cx. modestus*, as well as other species of veterinary importance such as the bridge vector *Cs. annulata*. Even though the majority of specimens were separated by *COI*, certain taxa could not be distinguished using this genetic marker within the genera *Aedes*, *Anopheles* and *Culex*. The use of *COI* also underlined identification problems in *Culiseta* species (*Cs. fumipennis*, *Cs. litorea* and *Cs. morsitans*) within the BOLD and NCBI databases. This finding supports the need for continuing research combining the use of molecular methodologies with morphological traits for species delineation in the Culicidae.

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References

- Becker N, Petrić D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A (2010) Mosquitoes and their control. 2nd edn. Springer-Verlag, Berlin Heidelberg, 577 pp. <https://doi.org/10.1007/978-3-540-92874-4>
- Brugman VA, England ME, Stoner J, Tugwell L, Harrup LE, Wilson JW, Medlock JM, Logan JG, Fooks AR, Mertens PPC, Johnson N, Carpenter S (2017a) How often do mosquitoes bite humans in southern England? A standardised summer trial at four sites reveals spatial, temporal and site-related variation in biting rates. *Parasites & Vectors* 10: 420. <https://doi.org/10.1186/s13071-017-2360-9>

- Brugman VA, Hernández-Triana LM, England ME, Medlock JL, Logan JG, Wilson AJ, Fooks AR, Johnson N, Carpenter S (2017b) Host-feeding patterns and insights into the potential role of native mosquitoes as pathogen vectors in the United Kingdom. *Parasites & Vectors* 10: 163. <https://doi.org/10.1186/s13071-017-2098-4>
- Brugman VA, Hernández-Triana LM, Prosser SW, Weland C, Westcott DG, Fooks AR, Johnson N (2015) Molecular species identification, host preference and detection of myxoma virus in the *Anopheles maculipennis* complex (Diptera: Culicidae) in southern England, UK. *Parasites & Vectors* 8: 1–8. <https://doi.org/10.1186/s13071-015-1034-8>
- Cranston PS, Ramsdale CD, Snow KR, White GB (1987) Keys to the adults, male hypopygia, fourth-instar larvae and pupae of the British mosquitoes (Culicidae) with notes on their ecology and medical importance. Freshwater Biological Association Scientific Publication 48: 1–152.
- Cywinska A, Hunter FF, Hebert PDN (2006) Identifying Canadian mosquitoes species through DNA barcodes. *Medical and Veterinary Entomology* 20: 413–24. <https://doi.org/10.1111/j.1365-2915.2006.00653.x>
- Dallimore T, Hunter T, Medlock JM, Vaux GC, Harbach RR, Strode C (2017) Discovery of a single male *Aedes aegypti* (L.) in Merseyside, England. *Parasites & Vectors* 10: 239.
- Danabalan R, Monaghan MT, Ponsonby DJ, Linton Y-M (2014) Occurrence and host preferences of *Anopheles maculipennis* group mosquitoes in England and Wales. *Medical and Veterinary Entomology* 28: 169–78. <https://doi.org/10.1111/mve.12023>
- Danabalan R, Ponsonby DJ, Linton Y-M (2012) A critical assessment of available molecular identification tools for determining the status of *Culex pipiens* s.l. in the United Kingdom. *Journal of American Mosquito Control Association* 28: 68–74. <https://doi.org/10.2987/8756-971X-28.0.68>
- de Marco MF, Brugman VA, Hernández-Triana LM, Nikolova NI, Fooks AR, Johnson N (2016) Detection of *Theileria orientalis* in the blood meals of *Culiseta annulata* in southern England, United Kingdom. *Veterinary Parasitology* 229: 31–36. <https://doi.org/10.1016/j.vetpar.2016.09.012>
- Dorvillé LFM (1996) Mosquitoes as bioindicators of forest degradation in southeastern Brazil, a statistical evaluation of published data in the literature. *Studies Neotropical Environment* 31: 68–78. <https://doi.org/10.1076/snfe.31.2.68.13331>
- Engdahl C, Larsson P, Näslund J, Bravo M, Evander M, Lundström JO (2014) Identification of Swedish mosquitoes based on molecular barcoding of the COI gene and SNP analysis. *Molecular Ecology Resources* 140: 478–88. <https://doi.org/10.1111/1755-0998.12202>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. *Molecular Microbiology and Biological Biotechnology* 3: 294–299.
- Golding N, Nunn MA, Medlock JM, Purse VB, Vaux AGC, Schäfer SM (2012) West Nile virus vector *Culex modestus* established in southern England. *Parasites & Vectors* 5: 32. <https://doi.org/10.1186/1756-3305-5-32>
- Guedes MLP, Navarro-Silva MA (2014) Mosquito community composition in dynamic landscapes from the Atlantic Forest biome (Diptera, Culicidae). *Revista Brasileira de Entomologia* 58: 88–94. <https://doi.org/10.1590/S0085-56262014000100014>

- Gunay F, Alten B, Simsek F, Aldemir A, Linton T-M (2015) Barcoding Turkish *Culex* mosquitoes to facilitate arbovirus vector incrimination studies reveals hidden diversity and new potential vectors. *Acta Tropica* 143: 112–120. <https://doi.org/10.1016/j.actatropica.2014.10.013>
- Guzmán-Larralde AJ, Suaste-Dzul AP, Gallou A, Peña-Carrillo KI, González-Hernández A (2016) DNA recovery from microhymenopterans with six non-destructive methodologies and considerations for museum slides preparations. *Genome* 260: 85–91. <https://doi.org/10.1139/gen-2015-0172>
- Harbach RE (2017) Mosquito Taxonomic Inventory. <http://mosquito-taxonomic-inventory.info> [Accessed on 23 January 2018]
- Harbach RE (2018) Culicopedia: species-group, genus-group and family-group names in Culicidae (Diptera). CABI, United Kingdom, 396 pp. <https://doi.org/10.1079/9781786399052.0000>
- Harbach RE, Dallimore T, Briscoe AG, Culverwell CL, Vaux AGC, Medloch JM (2017) *Aedes nigrinus* (Eckstein, 1918) (Diptera, Culicidae) a new country record for England, contrasted with *Aedes sticticus* (Meigen, 1838). *ZooKeys* 671: 119–130. <https://doi.org/10.3897/zookeys.671.12447>
- Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270: 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Hebert PDN, Ratnasingham S, de Waard JR (2003b) Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* 270: S96–S99. <https://doi.org/10.1098/rsbl.2003.0025>
- Hernández-Triana LM, Brugman VA, Prosser SWJ, Weland C, Nikilova N, Thorne L, de Marco MF, Fooks AR, Johnson N (2017) Molecular approaches for blood meal analysis and species identification of mosquitoes (Insecta: Diptera: Culicidae) in rural locations in southern England, United Kingdom. *Zootaxa* 4250: 067–076. <https://doi.org/10.11646/zootaxa.4250.1.5>
- Hernández-Triana LM, Chaverri LG, Rodríguez-Pérez, MA, Prosser SWJ, Hebert PDN, Ryan-Gregory T, Johnson N (2015) DNA barcoding of Neotropical black flies (Diptera: Simuliidae): Species identification and discovery of cryptic diversity in Mesoamerica. *Zootaxa* 3936: 93–114. <https://doi.org/10.11646/zootaxa.3936.1.5>
- Hernández-Triana LM, Crainey JL, Hall A, Fatih F, Mackenzie-Dodds J, Shelley AJ, Zhou X, Post RJ, Gregory RT, Hebert DN (2012) The utility of DNA barcoding for species identification within the blackfly Subgenus *Trichodagmia* Enderlein (Diptera: Simuliidae: *Simulium*) and related taxa in the New World. *Zootaxa* 3154: 43–69.
- Hernández-Triana LM, Prosser SW, Rodríguez-Pérez MA, Chaverri LG, Hebert PDN, Gregory TR (2014) Recovery of DNA barcodes from blackfly museum specimens (Diptera: Simuliidae) using primer sets that target a variety of sequence lengths. *Molecular Ecology Resources* 4: 508–18. <https://doi.org/10.1111/1755-0998.12208>
- Hunter SJ, Goodall TI, Walsh KA, Owen R, Day JC (2008) Nondestructive DNA extraction from blackflies Diptera: Simuliidae: retaining voucher specimens from DNA barcoding projects. *Molecular Ecology Resources* 8: 56–61. <https://doi.org/10.1111/j.1471-8286.2007.01879.x>
- Kronefeld M, Werner D, Kampen H (2014) PCR identification and distribution of *Anopheles daciae* (Diptera, Culicidae) in Germany. *Parasitological Resources* 113: 2079–2086. <https://doi.org/10.1007/s00436-014-3857-1>

- Kronefeld M, Dittmann M, Zielke D, Werner D, Kampen H (2012) Molecular confirmation of the occurrence in Germany of *Anopheles daciae* (Diptera, Culicidae). *Parasites and Vectors* 5: 250. <https://doi.org/10.1186/1756-3305-5-250>
- Kumar NP, Rajavel AR, Natarajan R, Jambulingam P (2007) DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology* 44: 1–7. <https://doi.org/10.1093/jmedent/41.5.01>
- Lilja T, Troell K, Kirik H, Lindström A (2018) A distinct group of north European *Aedes vexans* as determined by mitochondrial and nuclear markers. *Journal and Veterinary Entomology* 32: 282–289. <https://doi.org/10.1111/mve.12294>
- Linton Y, Lee A, Curtis C (2005) Discovery of a third member of the Maculipennis group in SW England. *European Mosquito Bulletin* 2005: 19:5–9.
- Linton Y-M, Samanidou-Voyadjoglou A, Harbach RE (2002) Ribosomal ITS2 sequence data for *Anopheles maculipennis* and *An. messeae* in northern Greece, with a critical assessment of previously published sequences. *Institute of Molecular Biology* 11: 379–383. <https://doi.org/10.1046/j.1365-2583.2002.00338.x>
- Linton Y-M, Harbach RE, Seng CM, Anthony TG, Matusop A (2001) Morphological and molecular identity of *Anopheles (Cellia) sundaicus* (Diptera: Culicidae), the nominotypical member of a malaria vector species complex in Southeast Asia. *Systematic Entomology* 26: 357–366.
- Low VL, Pramual P, Adler PH, Ya'cob Z, Huang YT, Da Pham X, Sofian-Azirun M (2016) Delineating taxonomic boundaries in the largest species complex of black flies (Simuliidae) in the Oriental Region. *Scientific Reports* 6: 1–8. <https://doi.org/10.1038/srep20346>
- Manley R, Harrup LE, Veronesi E, Stubbins F, Stoner J, Gubbins S, Gubbins S, Wilson A, Batten C, Koenraad HM, Barber J, Carpenter S (2015) Testing of UK populations of *Culex pipiens* L. for Schmallenberg virus vector competence and their colonization. *PLoS ONE* 10: 0134453. <https://doi.org/10.1371/journal.pone.0134453>
- Medlock JM, Leach SA (2015) Effect of climate change on vector-borne disease risk in the UK. *The Lancet Infectious Diseases* 15: 721–730. [https://doi.org/10.1016/S1473-3099\(15\)70091-5](https://doi.org/10.1016/S1473-3099(15)70091-5)
- Medlock JM, Vaux AGC (2009) *Aedes (Aedes) geminus* Peus (Diptera, Culicidae) – an addition to the British mosquito fauna. *Diptera Digest* 16: 1–4.
- Medlock JM, Vaux AGC (2010) Morphological separation of the European members of the genus *Culiseta* (Diptera, Culicidae). *Dipterists Digest* 17: 1–6.
- Medlock JM, Vaux AGC (2015) Seasonal dynamics and habitat specificity of mosquitoes in an English wetland: implications for UK wetland management and restoration. *Journal of Vector Ecology* 40: 90–106. <https://doi.org/10.1111/jvec.12137>
- Medlock JM, Snow KR, Leach SA (2007) Possible ecology and epidemiology of medically important mosquito-borne arboviruses in Great Britain. *Epidemiology and Infection* 135: 466–82. <https://doi.org/10.1017/S0950268806007047>
- Medlock JM, Cull B, Vaux AGC, Irwin AG (2017b) The mosquito *Aedes vexans* in England. *Veterinary Record* 181: 243. <https://doi.org/10.1136/vr.j4048>
- Medlock JM, Vaux AGC, Cull B, Schaffner F, Gillingham E, Pfluger V, Leach S (2017a) Detection of the invasive mosquito species *Aedes albopictus* in southern England. *Lancet Infectious Diseases* 2017. [https://doi.org/10.1016/S1473-3099\(17\)30024-5](https://doi.org/10.1016/S1473-3099(17)30024-5)

- Montagner FRG, Silva OS, Jahnke SM (2018) Mosquito species occurrence in association with landscape composition in green urban areas. *Brazilian Journal of Biology* 78: 233–239. <https://doi.org/10.1590/1519-6984.04416>
- Montero-Pau J, Gómez A, Muñoz J (2008) Application of an inexpensive and high throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography: Methods* 6: 218–222. <https://doi.org/10.4319/lom.2008.6.218>
- Murugan K, Vadivalagan C, Karthika P, Panneerselvam C, Paulpandi M, Subramaniam J et al. (2016) DNA barcoding and molecular evolution of mosquito vectors of medical and veterinary importance. *Parasitology Resources* 115: 107–21. <https://doi.org/10.1007/s00436-015-4726-2>
- Niculescu G, Linton YM, Vladimirescu A, Howard TM, Harbach RE (2004) Mosquitoes of the *Anopheles maculipennis* group (Diptera: Culicidae) in Romania, with the discovery and formal recognition of a new species based on molecular and morphological evidence. *Bulletin of Entomological Research* 94: 525–535. <https://doi.org/10.1079/BER2004330>
- Packer L, Gibbs J, Sheffield C, Hanner R (2009) DNA barcoding and the mediocrity of morphology. *Molecular Ecology Resources*, Special issue on Barcoding Life, supplement 1: 42–50. <https://doi.org/10.1111/j.1755-0998.2009.02631.x>
- Pangjanda S, Pramual P (2016) Tests of conspecificity for closely related black fly (Diptera: Simuliidae) species of *Simulium multistriatum* group in Thailand. *Zootaxa* 4231(3): 421–430. <https://doi.org/10.11646/zootaxa.4231.3.8>
- Ratnasingham S, Hebert PDN (2013) A DNA-Based registry for all animal species: The Barcode Index Number (BIN) system. *PLoS ONE* 8(8): e66213. <https://doi.org/10.1371/journal.pone.0066213>
- Ruiz-Arrondo I, Hernández-Triana LM, Oteo J (2017) Fauna de mosquitos (Diptera, Culicidae) presentes en el humedal de La Grajera (Logroño) y sus implicaciones en salud pública. *Zubía, Instituto de Estudios Riojanos* 35: 123–140.
- Senatore GL, Alexander EA, Adler PH, Moulton JK (2014) Molecular systematics of the *Simulium jenningsi* species group (Diptera: Simuliidae), with three new fast-evolving nuclear genes for phylogenetic inference. *Molecular Phylogenetics and Evolution* 75: 138–148. <https://doi.org/10.1016/j.ympev.2014.02.018>
- Smith JL, Fonseca DM (2004) Rapid assays for identification of members of the *Culex* (*Culex*) *pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). *American Journal of Tropical Medicine and Hygiene* 70: 339–345. <https://doi.org/10.4269/ajtmh.2004.70.339>
- Tamura K, Stoecher G, Peterson N, Filipski A, Kumar S (2007) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Vaux AGC, Gibson G, Hernández-Triana LM, Cheke RA, McCracken F, Jeffries, CL, Horton DL, Springate S, Johnson N, Fooks AR, Leach S, Medlock JM (2015) Enhanced West Nile virus surveillance in the North Kent marshes, UK. *Parasites & Vectors* 8: 91. <https://doi.org/10.1186/s13071-015-0705-9>

- Versteirt V, Nagy ZT, Roelants P, Denis L, Breman FC, Damiens D, Dekoninck W, Backeljau T, Coosemans M, Van Bortel W (2015) Identification of Belgian mosquito species (Diptera: Culicidae) by DNA barcoding. *Molecular Ecology Resources* 15: 449–457. <https://doi.org/10.1111/1755-0998.12318>
- Wilkerson RC, Linton Y-M, Fonseca DM, Schultz TR, Price DC, Strickman DA (2015) Making mosquito taxonomy useful: A stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PloS ONE* 10: e0133602. <https://doi.org/10.1371/journal.pone.0133602>

Supplementary material 1

Accession number(s) of COI DNA barcode sequences used in this study downloaded from the NCBI database or provided by colleagues

Authors: Luis M. Hernández-Triana, Victor A. Brugman, Nadya I. Nikolova, Ignacio Ruiz-Arrondo, Elsa Barrero, Leigh Thorne, Mar Fernández de Marco, Andreas Krüger, Sarah Lumley, Nicholas Johnson, Anthony R. Fooks

Data type: molecular data

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Link: <https://doi.org/10.3897/zookeys.832.32257.suppl1>

Supplementary material 2

Percentage of Interspecific (between groups) pairwise K2P genetic divergence of unique DNA barcodes (658 bp), representing 42 species of mosquitoes

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Data type: molecular data

Explanation note: Highest pairwise distances (most divergent taxa) and lowest pairwise distances (most closely related taxa) are highlighted in yellow/bold, and green, respectively.

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A new species and a new record of the Southeast Asian millipede genus *Antheromorpha* Jeekel, 1968 (Polydesmida, Paradoxosomatidae) from Vietnam

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Abstract

Antheromorpha nguyenii sp. n. is described and illustrated from Kon Ka Kinh National Park, southern Vietnam. The new species is distinguished by a peculiar colour pattern showing a uniformly black-brown body contrasting with yellow-brown paraterga and epiproct, as well as in the pointed gonopodal process being unusually short, only approximately half as long as the solenophore. In addition, an identification key to all 13 presently known species, all mapped, is given. A new record of *A. festiva* is provided from southern Vietnam.

Keywords

Antheromorpha, millipede, new species, Orthomorphiini, taxonomy, Vietnam

Introduction

The Southeast Asian millipede genus *Antheromorpha* Jeekel, 1968 was established to replace *Brachytropis* Silvestri, 1896 which had been preoccupied by *Brachytropis* Fieber, 1858, a genus of Hemiptera (Jeekel 1968). It was later redefined, especially against the similarly large-bodied, even more species-rich, and mostly sympatric genus *Orthomorpha* Bollman, 1893, with some Burmese species revised and a few new synonymies proposed based on type material (Jeekel 1980). *Antheromorpha* has since been reviewed and rediagnosed, with even more synonymies established, and most species likewise redescribed, based both on type and fresh material (Likhitrakarn et al. 2016).

It was Attems (1937) who described the first member of this genus from Vietnam, *Orthomorpha harpaga* Attems, 1937, from Hon Ba Mountain in the south-central part of the country. Jeekel (1968, 1980) correctly transferred it to *Antheromorpha*. Likhitrakarn et al. (2016) have since redescribed this species from the types, while Golovatch and Semenyuk (2018) have freshly documented and illustrated it from Kon Ka Kinh National Park, Gia Lai Province, central highlands of Vietnam. Nguyen et al. (2018) have recently reviewed *Antheromorpha* in the scope of the Vietnamese fauna, described a new species, *A. pumatensis* Nguyen, Nguyen & Le, 2018 from Nghe An Province, north-central Vietnam, and provided additional records of *A. paviei* (Brölemann, 1896) from Ba Na National Park, Da Nang Province, and of *A. festiva* (Brölemann, 1896) from Kien Giang, Dak Lak and Tay Ninh provinces (Fig. 5).

The present paper adds to the record another new species of this genus from Vietnam, the fifth to be found in that country. This brings the diversity of *Antheromorpha* to a total of 13 recognised species that occur only in mainland Southeast Asia: Myanmar (6 species: *A. bistrata* (Pocock, 1895), *A. comotti* (Pocock, 1895), *A. mediovirgata* (Carl, 1941), *A. minlana* (Pocock, 1895), *A. miranda* (Pocock, 1895), *A. pardalis* (Pocock, 1895)), Vietnam (5 species: *A. festiva* (Brölemann, 1896), *A. harpaga* (Attems, 1937), *A. nguyeni* sp. n., *A. paviei* (Brölemann, 1896), *A. pumatensis* Nguyen, Nguyen & Le, 2018), and Thailand (3 species: *A. rosea* Golovatch, 2013, *A. festiva* (Brölemann, 1896), *A. uncinata* (Attems, 1931)). Southern China (*A. rosea* Golovatch, 2013), Laos (*A. paviei* (Brölemann, 1896)), and Western Malaysia (*A. festiva* (Brölemann, 1896)) currently support only a single species each (Fig. 5). Because a full catalogue of all previously described species and their distributions are available elsewhere (Likhitrakarn et al. 2016; Nguyen et al. 2018), we simply list them below in alphabetic order:

Antheromorpha bistrata (Pocock, 1895)
Antheromorpha comotti (Pocock, 1895)
Antheromorpha festiva (Brölemann, 1896)
Antheromorpha harpaga (Attems, 1937)
Antheromorpha mediovirgata (Carl, 1941)
Antheromorpha minlana (Pocock, 1895)
Antheromorpha miranda (Pocock, 1895)
Antheromorpha nguyeni sp. n.

- Antheromorpha pardalis* (Pocock, 1895)
Antheromorpha paviei (Brölemann, 1896)
Antheromorpha pumatensis Nguyen, Nguyen & Le, 2018
Antheromorpha rosea Golovatch, 2013
Antheromorpha uncinata (Attems, 1931)

Materials and methods

The material documented below was collected by one of us (Irina Semenyuk abbreviated IS), according to Agreements 432/TCLN-BTTN and 142/SNgV-VP between the Kon Ka Kinh National Park and the Joint Russian-Vietnamese Tropical Center, as part of IS's research project on the diversity, biology, and ecology of millipedes in Vietnam.

Live animals were photographed in their habitats using a Canon 70D digital camera with a Canon PowerShot A4000IS 16.0 MP Digital Camera. Specimens were preserved in 75% ethanol, and morphological investigations were carried out in the laboratory using an Olympus stereo microscope. Scanning electron micrographs (SEM) of gold-coated gonopods were taken using a JEOL, JSM-5410 LV microscope. Specimens were also photographed and the images stacked in the laboratory using the "CellD" automontage software of the Olympus Soft Imaging Solution package, while the gonopods of a paratype were dissected and illustrated under Euromex iScope microscopes. Almost all material is housed in the Zoological Museum, State University of Moscow (ZMUM), Russia, with one paratype donated to the collection of Chulalongkorn University's Museum of Zoology (CUMZ), Bangkok, Thailand.

In the synonymy section given below, **D** stands for a description or descriptive notes, **R** for a new record or records, while **M** for a mere mention. Other abbreviations are:

- | | |
|-------------|--|
| d | gonopod process d , a distinct lobe on middle part of lamina lateralis, seen in mesal view |
| m | gonopod process m , a small lower lobe on distal part of gonopod, clearly seen in mesal view |
| s | lateral sulcus, a distinct sulcus distally on femur, visible on femur in lateral view |
| sl | solenomere, a usually long, flagellum-like appendage, originating on base of solenophore |
| sph | solenophore (= tibiotarsus), apical part of telopodite, consisting of lamina lateralis and lamina medialis |
| v | gonopod process v , a small upper lobe on distal part of gonopod, clearly seen in mesal view |
| CUMZ | Chulalongkorn University Museum of Zoology, Bangkok, Thailand |
| ZMUM | Zoological Museum, University of Moscow, Russia |
| a.s.l. | above sea level |
| ca. | approximately, around (circa) |

The Animal Care and Use Protocol Review No. 1723018 was applied.

Coordinates and elevation were recorded by Garmin GPSMAP 60 CSx and Garmin eTrex 30 using the WGS84 datum and subsequently double-checked with Google Earth.

Taxonomy

Family Paradoxosomatidae Daday, 1889

Subfamily Paradoxosomatinae Daday, 1889

Tribe Orthomorphini Brölemann, 1916

Genus *Antheromorpha* Jeekel, 1968

***Antheromorpha festiva* (Brölemann, 1896)**

Fig. 1

Orthomorpha festiva Brölemann, 1896: 1 (D).

Orthomorpha festiva: Attems 1898: 339 (M); 1914: 194 (D); 1930: 131 (D); Brölemann 1904: 4 (D, R).

Orthomorpha (*Orthomorpha*) *festiva*: Attems 1936: 199 (M); 1937: 60 (D).

“*Orthomorpha*” *festiva* Jeekel, 1963: 269 (M).

Antheromorpha festiva Jeekel, 1968: 57 (M); 1980: 85 (M); Golovatch 1983: 181 (M); Enghoff et al. 2004: 37 (M); Enghoff 2005: 95 (R); Nguyen and Sierwald 2013: 1234 (M); Likhitrakarn et al. 2016: 45 (D); Nguyen et al. 2018: 98 (D, R).

Material examined. 3 ♂ (ZMUM), Vietnam, Dong Nai Province, Cat Tien National Park, 11°25'37"N, 107°25'39"E, 140 m a.s.l., secondary monsoon lowland forest with dominating *Lagerstroemia calyculata*, on forest floor, 10.V.2015; 1 ♂ (ZMUM), same locality, on ground road in grasslands, 11°24'20"N, 107°24'17"E, 120 m a.s.l., 14.XI.2014, all leg. I Semenyuk.

Remarks. The new specimens fully agree with the detailed and beautifully illustrated redescrptions of the species as given by Brölemann (1904) and Likhitrakarn et al. (2016). This is the first formal record of *A. festiva* in Cat Tien National Park. In that park, *A. festiva* shows a pronounced seasonal rhythm. According to several years of observation by one of us (IS), juveniles of different stages (from the 4th to the last instar) start swarming in the autumn, just before the dry season. Swarms contain hundreds of millipedes slowly moving around and feeding. One swarm patch usually contains mainly same-age individuals (Fig. 1). Even though swarms of different instars may appear next to each other, they normally do not mix. Patches of swarming are usually localized in secondary forest with dominating bamboo and in grasslands. Swarms of individuals of the latest instars are looser than those of earlier stages. Adults appear during the same season, but do not swarm. In the dry season, the abundance of *A. festiva* abruptly declines and then, in the early rainy season in summer, it gradually grows again to abruptly stop in the middle of the rainy season.



Figure 1. Swarming later instars of *Antheromorpha festiva* (Brölemann, 1896) in Cat Tien National Park. Photographs by I Semenyuk.

***Antheromorpha nguyenii* sp. n.**

<http://zoobank.org/98A9247E-F3B0-4D4A-B1E2-08DF101ABF4F>

Figs 2–4

Type material. Holotype. ♂ (ZMUM), Vietnam, Gia Lai Province, Kon Ka Kinh National Park, 14°13'08"N, 108°19'31"E, 1200 m a.s.l., tropical forest with *Lithocarpus* spp. abundant on hill slopes, on forest floor, daytime, 24.V.2017, leg. I Semenyuk.

Paratypes. 1 ♂ (ZMUM), same locality, together with holotype; 1 ♂ (ZMUM), same locality as holotype, but on top of a hill with cloud forest, 14°13'12"N,

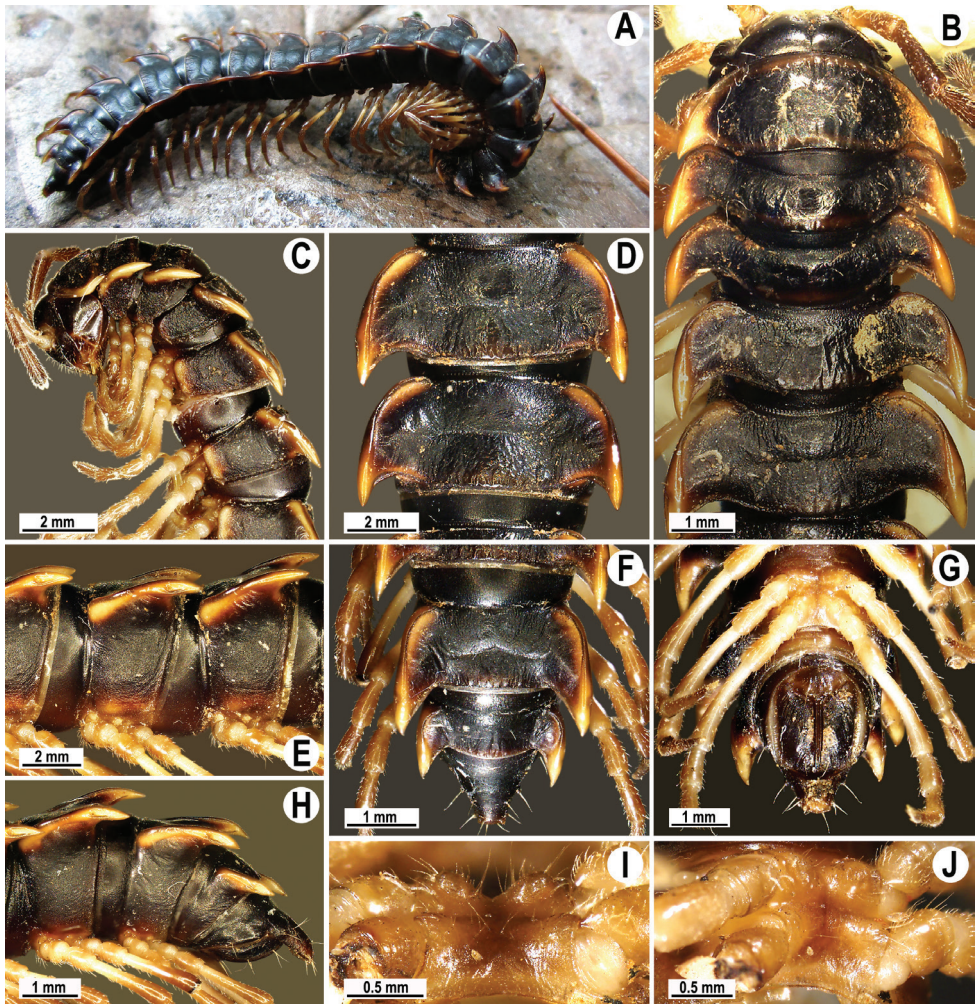


Figure 2. *Antheromorpha nguyeni* sp. n., ♂ holotype. **A** habitus, live coloration; **B**, **C** anterior part of body, dorsal and lateral views, respectively **D**, **E** segments 10 and 11, dorsal and lateral views, respectively **F**, **G**, **H** posterior part of body, dorsal, ventral and lateral views, respectively **I**, **J** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

108°19'54"E, 1500 m a.s.l., on log, daytime, 26.V.2017; 1 ♂ (CUMZ), same locality as holotype, broadleaved tropical forest in river valley, on log, 800 m a.s.l., 14°12'46"N, 108°18'55"E, night time 22.V.2017, all leg. I Semenyuk.

Name. To honour Nguyen Duc Anh, the leading Vietnamese myriapologist.

Diagnosis. Differs from congeners mainly in the colour pattern (a uniformly black-brown body with contrasting yellow-brown paraterga and epiproct), as well as in gonopod process **d** being unusually short, approximately half as long as the solenophore.

Description. Length of holotype 41.5 mm, width of midbody pro- and metazonae 3.2 and 4.7 mm, respectively. Paratypes 39.5–42.5 mm long, 2.9–3.8 and 4.5–5.1 mm wide on midbody pro- and metazonae, respectively (♂).

Colouration of live animals blackish (Fig. 2A), edges of paraterga dark to light brown; antennae dark brownish, legs and venter dark to light brown (Fig. 2A); colouration in alcohol, after one-year-long preservation, faded to dark brownish (Fig. 2B–H), edge of paraterga faded to brownish or pale brown, antennae legs and venter light brown to pale yellowish (Fig. 2B–J).

Clypeolabral region sparsely setose; epicranial suture distinct. Antennae long (Fig. 2C), extending behind metaterga 3 when stretched dorsally (♂). In width, head < segment 4 < 3 < collum < segment 2 < 5 < 6–17 (♂), body gently and gradually tapering thereafter. Collum with three transverse rows of setae: 3+3 in anterior, 2+2 in intermediate, and 3+3 in posterior row; paraterga very broad (Fig. 2B), slightly upturned, anterior edge angular, lateral edge smooth, caudal corner almost or fully pointed, extending behind rear tergal margin (Fig. 2B, C).

Tegument generally smooth and poorly shining, prozonae finely shagreened, metaterga leathery and rugulose (Fig. 2A, B, D, F), surface below paraterga finely microgranulate (Fig. 2C, E, H). Postcollum metaterga with two transverse rows of setae traceable at least as insertion points when setae broken off: 2+2 in anterior (pre-sulcus) and 3+3 in posterior (post-sulcus) row. Tergal setae simple, slender, ca. 1/3 of metatergal length. Axial line visible only on metazonae, starting with collum. Paraterga very strongly developed (Fig. 1B–H), mostly clearly upturned above dorsum; only paraterga 2–4 slightly upturned, all lying below dorsum, set at ca. upper 1/3 (segments 2 and 3) or 1/4 (segment 4 and following ones) of body height, moderately enlarged in lateral view on pore-bearing segments, thinner on poreless ones; anterior edge broadly rounded and narrowly bordered, fused to callus; calluses delimited by a sulcus only dorsally on segments 2–4, following segments delimited by a sulcus both dorsally, and, albeit more poorly so, ventrally, in dorsal view narrower on poreless segments than on pore-bearing ones; lateral edge without incisions, caudal corners narrowly rounded to fully pointed, always extending behind rear tergal margin, posterior edge oblique to clearly concave, especially well so in segments 16–19 (Fig. 1B, D, F). Ozopores evident, lateral, lying in an ovoid groove at ca. 1/4 of metatergal length in front of caudal corner. Transverse sulcus usually distinct (Fig. 2B, D, F), complete on metaterga 5–18, shallow, reaching the bases of paraterga, very faintly beaded at bottom, incomplete and nearly wanting on segments 5 and 19. Stricture between pro- and metazona rather wide, deep, beaded at bottom down to base of paraterga (Fig. 2B–D, F). Pleurosternal carinae complete crests with a sharp caudal tooth on segments 2–4, thereafter crests bulged anteriorly and with a small, sharp, caudal tooth on segments 5–7, with only a small, sharp, caudal tooth on segments 8–10, and a very small denticle on segments 11–16 (Fig. 2C, E, H). Epiproct (Fig. 2H) long, stout, conical, flattened dorsoventrally, with two evident, caudoventrally curved, apical papillae; tip subtruncate; pre-apical papillae small, but evident, lying rather close to tip. Hypoproct subtriangular, caudal margin round, setiferous knobs at caudal edge evident and clearly separated.

Sterna sparsely setose, shining, cross-impressions shallow, without modifications; but with two rounded, low, fully separated, setose cones between ♂ coxae 4 (Fig. 2I, J). A conspicuous ridge present in front of gonopod aperture. Legs moderately long and

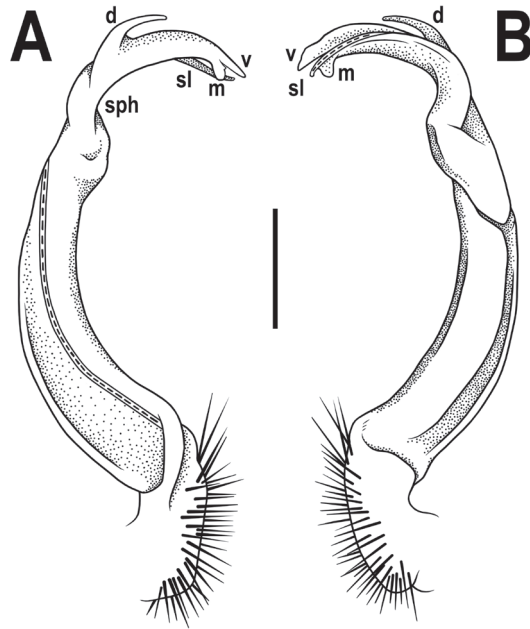


Figure 3. *Antheromorpha nguyenii* sp. n., ♂ holotype. **A, B** left gonopod, mesal and lateral views, respectively. Scale bar: 0.5 mm.

slender, midbody ones ca. 1.2–1.5 times as long as body height, prefemora without modifications, ♂ tarsal brushes present until segment 17.

Gonopods (Figs 3, 4) simple, with femorite ca. 3 times as long as prefemoral (= strongly setose) part. Femorite moderately curved caudad, postfemoral portion demarcated by an oblique lateral sulcus; solenomere flagelliform, almost fully sheathed by solenophore, tip of solenophore very deeply bifid; with a rather long, slender, fully pointed process **d**; process **m** with a narrowly rounded terminal lobule, longer than a small, rounded process **v** with a very small, middle, spiniform prong.

Remarks. Adults of the new species were found in May during a short expedition to a small area within Kon Ka Kinh National Park near its headquarters. A prospected forest with a similar forest structure within the same park near the village of Krong (N14°17', E108°26', 700–1000 m a.s.l.), ca. 14 km NE of the type locality, failed to reveal this species. It co-exists at the type locality together with *Orthomorpha scabra* Jeekel, 1967 and three other *Orthomorpha* species, all apparently undescribed. According to IS' observations, the five species share the same microhabitats. Given the conspicuously large and similar sizes of the adults of *Antheromorpha* and *Orthomorpha*, an ecological study of this syntopy in Kon Ka Kinh National Park would be worthwhile.

Although our new species is superficially very similar to *Orthomorpha* species such as *O. elevata* Likhitrakarn, Golovatch & Panha, 2011, from Perak State, Malaysia, it is clearly different in the shape of the sternal lamina between ♂ coxae 4 and in gonopodal structure (Likhitrakarn et al. 2011).

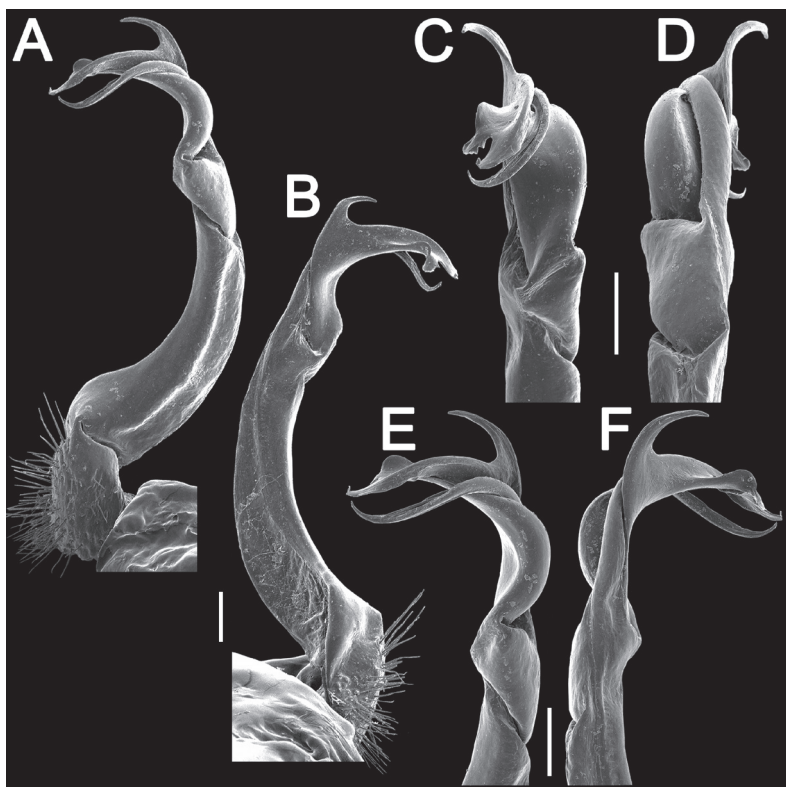


Figure 4. *Antheromorpha nguyeni* sp. n., ♂ paratype. **A, B** left gonopod, lateral and mesal views, respectively **C–F** distal part of right gonopods, suboral, subcaudal, lateral and mesal views, respectively. Scale bar: 0.2 mm.

Key to the known species of *Antheromorpha*, chiefly based on the male characters (modified mostly after Likhitrakarn et al. 2016)

- | | | |
|---|---|-------------------------------|
| 1 | Colour pattern of metaterga: yellowish paramedian spots in front of transverse sulcus, the latter visible starting with segment 2 | <i>A. pardalis</i> |
| – | Colour pattern of metaterga otherwise. Transverse sulcus present starting with segment 4 or 5 | 2 |
| 2 | Colour pattern of metaterga: yellowish or brownish paramedian stripes | 3 |
| – | Colour pattern of metaterga otherwise | 9 |
| 3 | Gonopod femorite relatively short..... | 4 |
| – | Gonopod femorite long (Figs 3, 4A, B)..... | 6 |
| 4 | Midbody metazonae ca. 2.0 mm wide. Pleurosternal carinae poorly-developed, in ♂ slightly projecting caudad behind rear tergal margin only until segment 5 | <i>A. mediovirgata</i> |
| – | Midbody metazonae ≥ 2.9 mm wide. Pleurosternal carinae well-developed, in ♂ slightly projecting caudad behind rear tergal margin at least until segment 10..... | 5 |

- 5 Sternal lamina between ♂ coxae 4 with a paramedian pair of evident, high, nearly pointed, fully separated, setose cones. Gonopod process **d** very long..... *A. festiva*
- Sternal lamina between ♂ coxae 4 with only a single small cone. Gonopod process **d** shorter..... *A. bistrata*
- 6 Sternal lamina between ♂ coxae 4 with a paramedian pair of separated lobes... 7
- Sternal lamina between ♂ coxae 4 a simple, rounded, conical knob **8**
- 7 Paraterga narrow. Sternal lamina between ♂ coxae 4 a large, cordiform, ventrally evidently concave lobe. Gonopod solenophore clearly curved *A. comotti*
- Paraterga broad. Sternal lamina between ♂ coxae 4 a median pair of rather small and rounded lobes. Gonopod solenophore suberect and nearly straight *A. pumatensis*
- 8 Metazonae ca. 2.6 mm (♂) or ca. 3.0 mm wide (♀). Gonopod femorite nearly straight, process **d** longer than solenophore *A. minlana*
- Metazonae 3.2–3.7 mm (♂) or 3.6–4.6 mm wide (♀). Gonopod femorite strongly curved caudad, process **d** shorter than solenophore *A. miranda*
- 9 Sternum between ♂ coxae 4 with a single lamina or cone **10**
- Sternum between ♂ coxae 4 with a paramedian pair of separated cones..... **11**
- 10 Colour pattern: a light axial stripe flanked on each side by a dark stripe on collum to epiproct. Epiproct simple, not particularly large, with two small, but evident apical papillae. Tip of gonopod split rather deeply, but process **d** shorter *A. uncinata*
- Colour pattern indistinct, with a pale yellowish median stripe against a uniformly brown background. Epiproct particularly large, with two apical papillae curved remarkably ventrad, claw-shaped. Tip of gonopod split deeper, process **d** very long *A. harpaga*
- 11 Colour pattern: uniform with contrasting yellow or yellow-brown paraterga and epiproct (Fig. 1A–H). Gonopod process **d** simple, narrowly rounded apically (Figs 3, 4)..... **12**
- Colour pattern: a dark band present only on pinkish or pale yellowish posterior halves of proterga and posterior halves of metaterga. Gonopod process **d** clavate apically, rounded and significantly longer than process **m** *A. rosea*
- 12 Sternum between ♂ coxae 4 with a pair of evident, high, separated cones. ♂ tarsal brushes present until legs of segment 8. Gonopod process **d** simple, as long as solenophore *A. paviei*
- Sternum between ♂ coxae 4 with a pair of small, low, separated cones. ♂ tarsal brushes present until segment 18. Gonopod process **d** very short, approx. half as long as solenophore..... *A. nguyenii* sp. n.

Conclusions

The genus *Antheromorpha* belongs to the tribe Orthomorphini which currently contains 25 genera (Nguyen and Sierwald 2013, Srisonchai et al. 2018a, b, c), this allocation being supported not only morphologically, but also by molecular evidence (Nguyen et al. 2018). *Antheromorpha* species range from southern China in the north, through My-

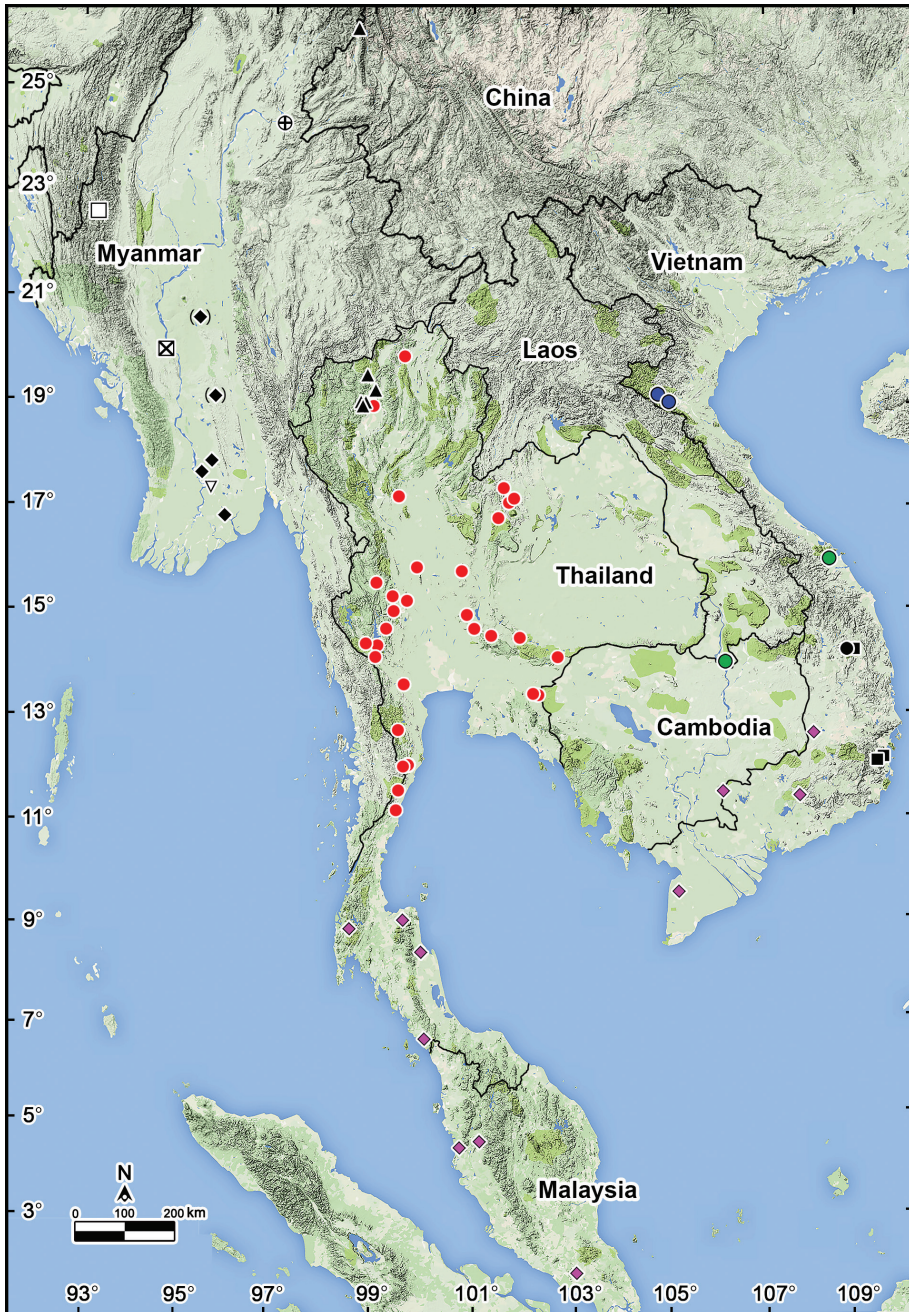


Figure 5. Distribution of *Antheromorpha* species (13) Key: Black triangle *A. rosea* Golovatch, 2013 Crossed circle *A. bistrata* (Pocock, 1895) Open square *A. comotti* (Pocock, 1895) and *A. mediovirgata* (Carl, 1941) Black diamond *A. miranda* (Pocock, 1895) Crossed square *A. comotti* (Pocock, 1895), *A. miranda* (Pocock, 1895) and *A. minlana* (Pocock, 1895) Inverted open triangle *A. pardalis* (Pocock, 1895) Red circle *A. uncinata* (Attems, 1931) Blue circle *A. pumatensis* Nguyen, Nguyen & Le, 2018 Green circle *A. paviei* (Brölemann, 1896) Black circle *A. nguyenii* sp. n. Black square *A. harpaga* (Attems, 1938) Pink diamond *A. festiva* (Brölemann, 1896).

anmar, Thailand, Laos, and Vietnam, to Western Malaysia in the south (Fig. 5). Four species seem to be particularly widespread; thus, *A. uncinata* occurs all over Thailand and it has repeatedly been reported swarming in the northern parts of the country (Likhitrakarn et al. 2016). A similarly vast distribution is also characteristic of *A. festiva*, which has been recorded across the southern half of the Malay Peninsula (southern Thailand and Western Malaysia) to southern Vietnam. As the range of *A. rosea* covers southern China and northern Thailand, most probably it occurs also in eastern Myanmar. Finally, since *A. paviei* is known from southern Laos and Vietnam, it seems very likely that it should exist in Cambodia as well (Likhitrakarn et al. 2014) (Fig. 5). Moreover, further *Antheromorpha* species may well be revealed with additional collecting efforts, especially in poorly prospected places so numerous in the huge region involved (Likhitrakarn et al. 2016).

Acknowledgements

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References

- Attems C (1898) System der Polydesmiden. I. Theil. Denkschriften der Kaiserlichen Akademie der Wissenschaften zu Wien, mathematisch-naturwissenschaftliche Classe 67: 221–482. <https://biodiversitylibrary.org/page/27925127>
- Attems C (1914) Die indo-australischen Myriopoden. Archiv für Naturgeschichte 80A: 1–398. <https://biodiversitylibrary.org/page/46057676>
- Attems C (1930) Myriopoden der Kleinen Sunda-Inseln, gesammelt von der Expedition Dr. Rensch. Mitteilungen aus dem Zoologischen Museum in Berlin 16(1): 117–184. <https://doi.org/10.1002/mmzn.19300160103>
- Attems C (1936) Diplopoda of India. Memoirs of the Indian Museum 11(4): 133–323.
- Attems C (1937) Myriapoda 3. Polydesmoidea I. Fam. Strongylosomidae. Das Tierreich 68: 1–300.
- Brölemann HW (1896) Myriapodes recueillis en Indo-Chine par M Pavie et offerts au Muséum d'Histoire Naturelle de Paris. Bulletin du Muséum national d'Histoire naturelle 7: 1–2. <https://biodiversitylibrary.org/page/42894685>
- Brölemann HW (1904) Myriapodes recueillis par MA Pavie en Indo-Chine. In: Pavie A (Ed.) Mission Pavie Indochine 1879–1895. Recherches sur l'Histoire Naturelle en Indochine Orientale. Ernest Leroux Ed, Paris, T 3, 1–13 (296–309).

- Enghoff H (2005) The millipedes of Thailand (Diplopoda). *Steenstrupia* 29(1): 87–103. <http://www.zmuc.dk/commonweb/JOURNALS/PDF/Vol29-1/Enghoff.pdf>
- Enghoff H, Golovatch SI, Nguyen AD (2004) A review of the millipede fauna of Vietnam (Diplopoda). *Arthropoda Selecta* 13(1–2): 29–43.
- Golovatch SI (1983) [Millipedes (Diplopoda) of the fauna of Vietnam]. In: Medvedev LN (Ed.) [Fauna and ecology of the animals of Vietnam]. Nauka, Moscow, 207 pp. [In Russian]
- Golovatch SI, Semenyuk II (2018) On several new or poorly-known Oriental Paradoxosomatidae (Diplopoda: Polydesmida), XXIV. *Arthropoda Selecta* 27(3): 187–200. http://kmkjournals.com/upload/PDF/ArthropodaSelecta/27/27_3_187_200_Golovatch_Semenyuk_for_Inet.pdf
- Jeekel CAW (1963) Paradoxosomatidae from Borneo (Diplopoda, Polydesmida). *Tijdschrift voor Entomologie* 106: 205–283.
- Jeekel CAW (1968) On the classification and geographical distribution of the family Paradoxosomatidae (Diplopoda, Polydesmida). *Academisch Proefschrift*, Rotterdam, 162 pp.
- Jeekel CAW (1980) A revision of the Burmese Paradoxosomatidae (Diplopoda, Polydesmida) in the Museo Civico di Storia Naturale at Genoa (Part III). *Tijdschrift voor Entomologie* 123: 71–88.
- Likhitrakarn N, Golovatch SI, Panha S (2011) Revision of the Southeast Asian millipede genus *Orthomorpha* Bollman, 1893, with the proposal of a new genus (Diplopoda, Polydesmida, Paradoxosomatidae). *ZooKeys* 131: 1–161. <https://doi.org/10.3897/zookeys.131.1921>
- Likhitrakarn N, Golovatch SI, Panha S (2014) The millipede genus *Orthomorpha* Bollman, 1893 in Laos (Diplopoda, Polydesmida, Paradoxosomatidae), with descriptions of new species. *ZooKeys* 374: 1–22. <https://doi.org/10.3897/zookeys.374.6711>
- Likhitrakarn N, Golovatch SI, Panha S (2016) Review of the Southeast Asian millipede genus *Antheromorpha* Jeekel, 1968 (Diplopoda, Polydesmida, Paradoxosomatidae). *ZooKeys* 571: 21–57. <https://doi.org/10.3897/zookeys.571.7566>
- Nguyen AD, Nguyen SG, Le SX (2018) On the millipede genus *Antheromorpha* Jeekel, 1968 (Diplopoda: Polydesmida: Paradoxosomatidae) from Vietnam, with a description of a new species. *Raffles Bulletin of Zoology* 66: 96–109. <https://lkcnhm.nus.edu.sg/app/uploads/2018/01/66rbz096-109.pdf>
- Nguyen AD, Sierwald P (2013) A worldwide catalog of the family Paradoxosomatidae Daday, 1889 (Diplopoda: Polydesmida). *Check List* 9(6): 1132–1353. <https://doi.org/10.15560/9.6.1132>
- Srisonchai R, Enghoff H, Likhitrakarn N, Panha S (2018a) A revision of dragon millipedes II: The new genus *Nagaxytes*, with the description of three new species (Diplopoda, Polydesmida, Paradoxosomatidae). *European Journal of Taxonomy* 462: 1–44. <https://doi.org/10.5852/ejt.2018.462>
- Srisonchai R, Enghoff H, Likhitrakarn N, Panha S (2018b) A revision of dragon millipedes III: The new genus *Gigaxytes*, with the description of three new species (Diplopoda, Polydesmida, Paradoxosomatidae). *European Journal of Taxonomy* 463: 1–43. <https://doi.org/10.5852/ejt.2018.463>
- Srisonchai R, Enghoff H, Likhitrakarn N, Panha S (2018c) A revision of dragon millipedes IV: The new genus *Spinaxytes*, with the description of nine new species (Diplopoda, Polydesmida, Paradoxosomatidae). *ZooKeys* 797: 19–69. <https://doi.org/10.3897/zookeys.797.29510>

The Trichopterygini (Lepidoptera, Geometridae) of Austral South America: description of new species from Chile

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Abstract

Four new species belonging to the genera *Hoplosauris* Butler, *Butleriana* Parra, *Warrenaria* Parra, and *Fueguina* Parra from south-central Chile are described. The species are *H. morenoi* Ramos-González & Parra, **sp. n.**, *B. phoenix* Ramos-González & Parra, **sp. n.**, *W. onca* Ramos-González & Parra, **sp. n.**, and *F. araucana* Ramos-González & Parra, **sp. n.** The genus *Aloba* Warren is reassigned to tribe Trichopterygini and *A. carolinae* Ramos-González & Parra, **sp. n.** is described. Comparative diagnosis for all new species are provided, and illustrations of genitalia and the wing venation of the males for all new described species are given.

Keywords

Andean region, *Aloba*, *Butleriana*, *Fueguina*, *Hoplosauris*, Larentiinae, taxonomy, *Warrenaria*

Introduction

Geometridae is the second largest family within Lepidoptera, with approximately 23000 species worldwide (Scoble 1999; Scoble and Hausmann 2007; Van Nieukerken et al. 2011). More than 280 geometrid species are known from Chile, 252 of which are endemic (*sensu* Parra and Villagrán-Mella 2008). However, Parra (1995) estimated the diversity of Chilean geometrids to be at least 450 species.

Larentiinae is the second largest subfamily within Geometridae (Gaston et al. 1995; Scoble et al. 1995), its members occur in a wide variety of habitats, and is particularly abundant at great altitude in the tropics and in temperate forests (Holloway 1997), like those in south-central Chile (Hausmann and Parra 2009; Zamora-Manzur et al. 2011). Despite their high species-richness in Chile (i.e., around half of known Chilean geometrids are larentiines), there are relatively few studies related to these moths as compared to the Ennominae. So far, most research efforts focused on the revision of the genus *Eupithecia* Curtis (Vojnits 1985, 1992, 1994; Rindge 1987, 1991) and the tribe Trichopterygini (Parra 1991, 1996; Parra and Santos-Salas 1991, 1992; Parra et al. 2009, 2017).

Phylogenetically, Trichopterygini is a group at the base of Larentiinae, sister to all other larentiines, along with Chesiadini and Dyspteridini (Viidalepp 2011; Sihvonen et al. 2011; Öunap et al. 2016). The characteristics that distinguish Trichopterygini are the reduced size of the anal area of male hindwing to a fold, crevice, vesicle, flap or lobe, and the presence of a sternal pouch that does not occlude the tympanal opening (Dugdale 1980; Parra et al. 2017). In Chile, there are 14 genera and 39 species of trichopterygines. A phylogenetic hypothesis at the genus level was formulated by Parra (1991) and Parra et al. (2017). Despite this, no information regarding the natural history of most species is available and there are several undescribed taxa. The aim of this article is to describe five new species for the Chilean fauna and reassign one genus to Trichopterygini.

Methods

Specimens from the Museum of Zoology of the Universidad de Concepción, Chile (MZUC-UCCC) and Zoologische Staatssammlung München, Germany (ZSM) were examined, as well specimens from field surveys, which were collected using a UV light trap and net sweeping. Activity period (i.e., flight times) and geographic distribution were obtained from each specimen label. All species were assigned to biogeographic provinces proposed by Morrone (2015).

The Barcode Index Number (BIN) of each species is reported which was obtained from the BOLDSystems v4 database (Ratnasingham and Hebert 2007). BINs represent a species-level taxonomic registry of the animal kingdom based on the analysis of nucleotide variation patterns in the barcode region of the cytochrome c oxidase I (COI) gene (Ratnasingham and Hebert 2013). Genetic distances (when available) were calculated using the Kimura 2-parameter (K2P) distance model, using the analytical tools provided by BOLDSystems v4 platform. Intra-specific and inter-specific

genetic distances were reported as maximum and minimum distances, respectively. This genetic information facilitates the species delimitation and form the basis of future phylogenetic works (Brehm 2015, 2018).

The generic assignment of new taxa is based primarily on male genitalia and hindwing venation, which are important characters for the delimitation of species and genera within Trichopterygini (Parra et al. 2017). Species descriptions were made based on external morphological characteristics and genital armature from males and in some cases females. Wing and genitalia slides were prepared according to Parra (1991). Nomenclature for genitalia and external characteristics follow Klots (1970) and Scoble (1995) respectively.

Taxonomy

Aloba Warren, 1895

Aloba Warren 1895: 105.

Type species. *Hoplosauris cinereus* Bartlett-Calvert, 1893, by original designation.

Diagnosis. Palpi short, slightly tilted up. Male: Hindwing subtriangular, valvae with brush-like setal tuft with accessory undulated individual bristles. Female: ductus bursae half the length of corpus bursae. Posterior third of corpus bursae with longitudinal striation; the remaining two thirds with microspines.

Redescription. Antennae filiform in both sexes, but subapically broadened in males. Thorax and abdomen with brownish scales, varying in color from greyish to yellowish shades. Forewings with wide and dark antemedial and postmedial bands; apical spot subquadrate and discal spot always present. In males, hindwings are reduced, subtriangular and whitish; its apex can be extended or not, and there is no visible modification in anal margin. Wing venation in males: forewing with two accessory cells; hindwing with $Sc+R_1$ and $Rs+M_1$ separated, M_2 is free and M_3 and Cu_1 are pedunculated near the angle of discal cell, which is polygonal and it extends for one third of wing surface. Tibial formula 0-2-4 in both sexes. Abdomen is longer and narrower in males than in females. Male genitalia: ensiform valvae with cucullus projected apically, setal tuft is brush-like with accessory and undulated individual bristles, juxta with sclerotized S-shaped lateral processes. Female genitalia: corpus bursae sub-pyriform with longitudinal striation on the posterior third; the remaining two-thirds with microspines on its surface.

Aloba carolinae Ramos-González & Parra, sp. n.

<http://zoobank.org/7C4B292A-F1BE-4EA7-9572-688B20CE23A3>

BIN: BOLD:AAD7992

Figures 1, 2, 9, 10, 17

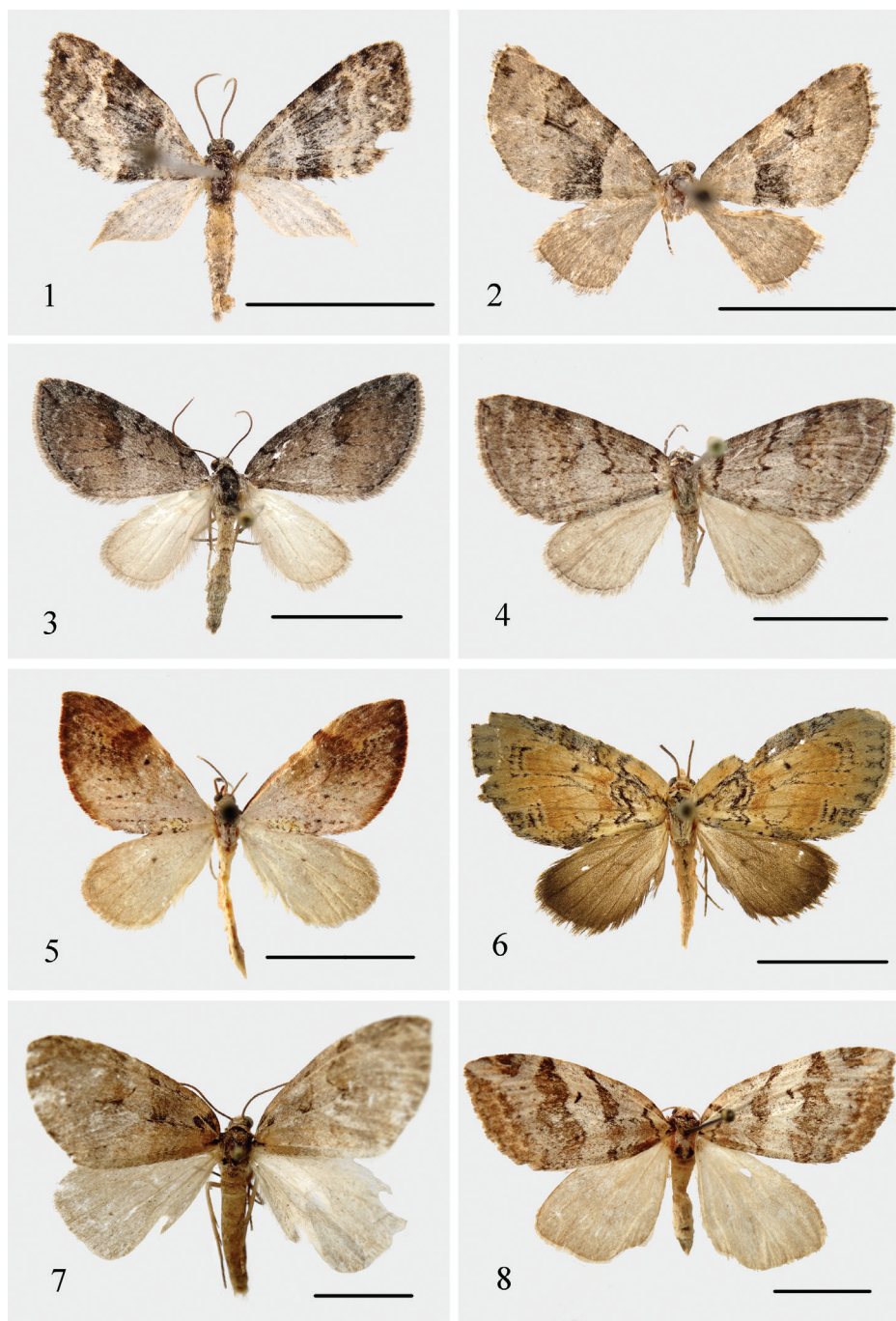
Diagnosis. This species is distinguished from *A. cinereus* (Bartlett-Calvert) by the following characteristics: saccus-vinculum broader, accessorial bristles in setal tuft apically

undulated, and corpus bursae with the inner surface of its anterior half completely covered with microspines. Externally, this species stands out for its reduced wingspan and for the feather-like extended hindwing apex in males.

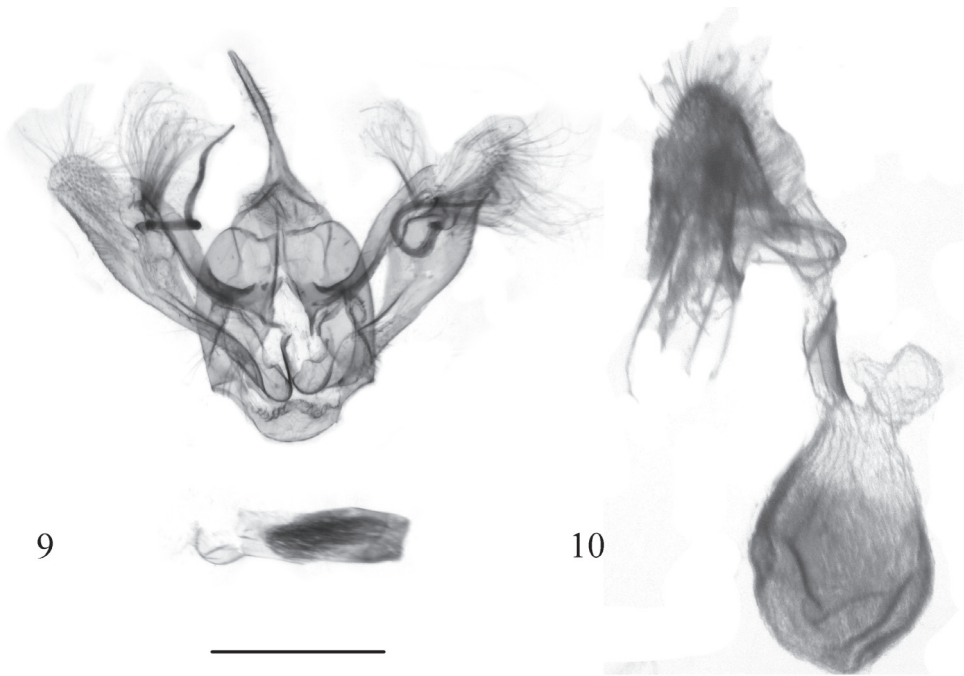
Description. Male (Fig. 1). Head: antennae filiform, subapically broadened; palpi short, subequal to eye diameter and slightly tilted upwards. Thorax: Patagia and tegulae covered by piliform grayish scales. Forewings: background color ashy gray; termen rounded, with piliform ashy scales; basal band blackish brown; antemedial band slender, blackish brown, and surrounded by two stripes of ashy-white scales; medial band blackish brown, with a small and subrounded ashy spot on the costal third, medial band with proximal margin arcuate and distal margin with five undulations. Some specimens, in both sexes, with a blackish spot near half of the band and the subrounded ashy spot on the costal third is absent or located in the anal third; postmedial band slender blackish brown and surrounded with two stripes of ashy-white scales; subterminal band zigzagging of whitish scales; apical spot on the wing apex subquadrate and blackish. This spot connects with subterminal band; terminal band formed by a dashed stripe of short blackish spots; distal spot present and blackish. Hindwings: ashy-white, reduced, one-third the length of forewings, triangular with prolonged apex, anal margin with no visible modification; discal spot not visible. Wing venation in males (Fig. 17): same as the genus. Male genitalia (Fig. 9): valvae ensiform, cucullus apically projected, sclerotized costa, subapical setal tuft brush-like with thick, large and undulated individual accessory bristles; saccus subrounded; juxta with quadrate base and posterior apex indented, with two sclerotized and disjointed S-shaped lateral processes, which extend to the height of the transtilla; socius triangular; transtilla projected in a Y-shaped, with apices equal in length. Aedeagus tubular; cornuti arranged as a longitudinal group in the vesica. Female (Fig. 2). Similar to male, but with filiform antennae slighter and hindwings not reduced, quadrangular and ashy-grey. Female genitalia (Fig. 10): ductus bursae half the length of corpus bursae; corpus bursae membranous, subpyriform, with straight longitudinal striation that does not exceed one-third of corpus bursae; anterior region of corpus bursae with microspines on its entire inner surface; cestum present; posterior apophyses larger than anterior apophyses.

Type material. Holotype: 1 ♂, pinned, CHILE, Concepción, Fundo El Guindo Point 1A, 36°50.18'S, 73°1.40'W, 20-X-2014, leg. M. Ramos & C. Rose, "Holotype *Aloba carolinae*" [red handwritten label] (MZUC-UCCC); allotype: 1 ♀, pinned, with genitalia in microscope slide, Chile, Concepción, Fundo El Guindo Point 1B, 36°50.21'S, 73°1.39'W, 26-X-2014, leg. M. Ramos & C. Rose, "FGCR LP 109" [genitalia slide] "Allotype *Aloba carolinae*" [red handwritten label] (UCCC-MZUC).

Paratypes: 46 males, 5 females. CHILE: **Curicó:** Los Queñes, 34°59.65'S, 70°48.78'W, 721 m, 10-II-2016, leg. M. Ramos & M. Astrosa (1 ♂) (MZUC-UCCC); P.N. Radal Siete Tazas, 35°28'S, 71°W, 1100 m, 19-XII-2000, leg. Gielis [ID BC ZSM Lep 07419, barcode sequence 658 bp; ID BC ZSM Lep 07433, barcode sequence 658 bp] (2 ♂) (ZSM). **Diguillín:** Termas de Chillán, 05/11-II-2010, leg. G. Moreno (3 ♂) (MZUC-UCCC); Las Trancas, 01/08-II-2011, leg. G. Moreno (1 ♂) (MZUC-UCCC); Las Trancas, 03/10-I-2011, leg. G. Moreno (3 ♂) (MZUC-UCCC); Las Trancas, 14/20-I-2012, leg. G. Moreno (1 ♂) (MZUC-UCCC); Las



Figures 1–8. Adults. **1–2** *Aloba carolinae* Ramos-González & Parra, sp. n. **1** male (holotype) **2** female (paratype). **3, 4** *Hoplosauris morenoi* Ramos-González & Parra, sp. n. **3** male (holotype) **4** female (allotype). **5** *Butleriana phoenix* Ramos-González & Parra, sp. n., male (holotype). **6** *Warrenaria onca* Ramos-González & Parra, sp. n., male (holotype). **7, 8** *Fueguina araucana* Ramos-González & Parra, sp. n. **7** male (holotype) **8** female (allotype). Scale bar: 1 cm.



Figures 9, 10. Genitalia of *Aloba carolinae* Ramos-González & Parra, sp. n. **9** male genitalia (paratype, MZUC-UCCC, slide No. FGCR LP 103) **10** female genitalia (allotype, MZUC-UCCC, slide no. FGCR LP 109). Scale bar: 1 mm.

Trancas, 16-I-1996, leg. unknown (3 ♂, 1 ♀) (MZUC-UCCC); Las Trancas, 11-I-1996, leg. unknown (1 ♂) (MZUC-UCCC); Las Trancas, 12-I-2017, leg. P. Bocaz (4 ♂); Las Trancas, Cabañas Bordenieve (IX FH), 36°54.83'S, 71°29.69'W, 1236 m, 13-I-2017, "Hoplo-005" [wing slide], leg. L. Parra, M. Ramos & C. Zamora-Manzur (1 ♂) (MZUC-UCCC); Las Trancas, 36°54'S, 71°28'W, 1400 m, 14-I-2001, leg. Gielis & Wolf [ID BC ZSM Lep 07435, barcode sequence 658 bp; ID BC ZSM Lep 07431, barcode sequence 658 bp; ID BC ZSM Lep 07417, barcode sequence 658 bp] (2 ♂, 1 ♀) (ZSM). **Concepción:** Concepción, 15-XII-1961 (1 ♂) (MZUC-UCCC); same as holotype but "FGCR LP 011", "FGCR LP 012" and "FGCR LP 013" [genitalia slides] (4 ♂) (MZUC-UCCC); same as holotype but 26-X-2014, female with "FGCR LP 096" [genitalia slide] (1 ♂, 1 ♀) (MZUC-UCCC); same as allotype but "FGCR LP 101" [genitalia slide] (1 ♂) (MZUC-UCCC); same as holotype but 03-XI-2014 (4 ♂) (MZUC-UCCC); same as allotype but 03-XI-2014, "FGCR LP 133" [genitalia slide] and "AML P 103" [wing slide] (1 ♂) (MZUC-UCCC); same as holotype but Point 1C 36°50.23'S, 73°1.39'W, 26-X-2014, "FGCR LP 103" [genitalia slide] and "AML P 0088" [wing slide] (3 ♂) (MZUC-UCCC); same as holotype but Point 2A 36°50.23'S, 73°1.47'W, 21-X-2014, "FGCR LP 110", "FGCR LP 132" [genitalia slide] and "AML P 0102" [wing slide] (3 ♂) (MZUC-UCCC); same as holotype but Point 2A 36°50.23'S, 73°1.47'W, 17-XI-2014 (2 ♂) (MZUC-UCCC); Chiguayante,

06-III-2002, leg. P. Bocaz (1 ♀) (MZUC-UCCC). **Cautín:** 15 km NE from Colico Lake, 39°3'S, 71°49.02'W, 400 m, 03-XII-2000, leg. Gielis [ID BC ZSM Lep 03051, barcode sequence 613 bp] (1 ♂) (ZSM). **Palena:** Fiordo Comau, San Ignacio del Huinay, 42°22.82'S, 72°24.8'W, 35 m, 20-II-2008, leg. T. Roy [ID BC ZSM Lep 16933, barcode sequence 658 bp; ID BC ZSM Lep 16922, barcode sequence 658 bp; ID BC ZSM Lep 16936, barcode sequence 658 bp; ID BC ZSM Lep 16923, barcode sequence 658 bp] (3 ♂, 1 ♀) (ZSM).

Distribution. This species occurs between Curicó and Palena provinces. It is distributed in parts of Santiago, Maule and Valdivian Forest biogeographic provinces, Central Chilean and Subantarctic subregions, Andean region.

Flight period. Specimens were captured from October to March.

Molecular data. BOLD:AAD7992. Ten available sequences of DNA barcode: BC ZSM Lep 07419 (Molina), BC ZSM Lep 07433 (Molina), BC ZSM Lep 07431 (Pinto), BC ZSM Lep 07417 (Pinto), BC ZSM Lep 07435 (Pinto), BC ZSM Lep 03051 (Cunco), BC ZSM Lep 16933 (Huinay), BC ZSM Lep 16922 (Huinay), BC ZSM Lep 16936 (Huinay), BC ZSM Lep 16923 (Huinay). Maximum intraspecific distance: 0.76%; Minimum genetic distance with *A. cinereus*: 9.35%.

Etymology. The species name is dedicated to the collector and biologist Carolina Rose Garrido, Concepción, Chile.

***Hoplosauris morenoi* Ramos-González & Parra, sp. n.**

<http://zoobank.org/BCE65057-D13A-4829-80BA-C9E73B9EB258>

BIN: BOLD:AAH6701

Figures 3, 4, 11, 12, 18

Diagnosis. This species and *H. heliconoides* Butler share the following characters: valvae with sclerotized costa and apically rounded; in females, two-thirds (or more) of corpus bursae with longitudinal striation. However, in the case of *H. morenoi* there are microspines on the sclerotized longitudinal striation only in the mid-ventral region (autapomorphy). The external morphology is highlighted by the grayish forewing, which is crossed by coppery-brown bands.

Description. Male (Fig. 3). Head: antennae filiform, subapically broadened; palpi porrect and subequal to eye diameter. Thorax: Patagia and tegulae covered by piliform ashy and brown scales. Tibial formula 0-2-4. Forewings: background color dark gray; termen rounded, with piliform dark-gray scales; basal band straight coppery-brown; antemedial band coppery-brown, slightly zigzagging; postmedial band coppery-brown, straight, twice as wide as the basal and antemedial bands; subterminal band whitish, zigzagging; apical spot slender and blackish which connects with subterminal band; terminal band formed by a dashed stripe of short coppery-brown spots; discal spot present and blackish. Hindwings: reduced, half the length of forewings, subrounded, pale ashy, with no visible modification at the base of anal margin; without discal spot. Wing venation in males (Fig. 18): forewing with two accessory

cells; hindwing with $Sc+R_1$ and R_s connected by a weak transverse vein, one third before the end of the cell; R_s and M_1 pedunculated; M_2 free and M_3 and Cu_1 pedunculated; discal cell triangular and extends for a quarter of wing surface; anal cell present, formed by a weak transverse vein towards the middle of the discal cell that connects cubital stem with anal margin. Male genitalia (Fig. 11): valvae ensiform, costa sclerotized and rounded, cucullus apically extended, subapical setal tuft brush-like; saccus subrounded; juxta with subquadrangular base and indented posterior apex, with two disjointed lateral processes that have subtriangular apex, these processes extend to the height of the transtilla; uncus setose and curved; socius triangular; transtilla projected in a Y-shaped, with apices unequal in length. Aedeagus tubular; cornuti arranged as two longitudinal groups in the vesica. Female (Fig. 4). Similar to males but with filiform antennae slighter and hindwings not reduced, subquadrangular and pale ashy. Female genitalia (Fig. 12): ductus bursae one-sixth the length of corpus bursae; corpus bursae subpyriform, sclerotized, with straight longitudinal striations that exceed two-thirds of corpus bursae and mid-ventral region with rows of microspines; posterior apophyses larger than anterior apophyses.

Type material. Holotype: 1 ♂, pinned, CHILE, Icalma, 02-II-2017, leg. H. Torres, “Holotype *Hoplosauris morenoi*” [red handwritten label] (MZUC-UCCC); allotype: 1 ♀, pinned, Chile, Malalcahuello, 20-I-2017, leg. C. Zamora-Manzur, “Allotype *Hoplosauris morenoi*” [red handwritten label] (MZUC-UCCC).

Paratypes: 17 males, 7 females. CHILE: **Diguillín:** Volcán Chillán, 03-III-1979, coll. light traps (1 ♂) (MZUC-UCCC); Las Trancas, 7-I-1987, leg. M. Beéche, “AMLP 0030” [wing slide] (1 ♂) (MZUC-UCCC); Las Trancas, 03/10-I-2011, leg. G. Moreno, “AMLP 0122” [female genitalia slide] (1 ♂, 3 ♀) (MZUC-UCCC); Las Trancas, 08-I-1996, leg. M. Beéche (1 ♂); Las Trancas, 16-I-1996, coll. Phototropic trap (1 ♂) (MZUC-UCCC); Las Trancas, 14/20-I-2012, leg. G. Moreno, “UCCC_MZUC_Lep_0388” [male ID code] (1 ♂, 1 ♀) (MZUC-UCCC). **Malleco:** Curacautín, 20-II-2008, leg. O. Vergara & J. Guzmán, “BC LP 0039” [Barcode voucher] (1 ♀) (MZUC-UCCC); same as holotype but 21-II-2017, “AMLP 0300” [genitalia slide] (1 ♂) (MZUC-UCCC); Curacautín, Río Blanco, 38°12'S, 71°55.99'W, 28-II-1995, leg. H. Thoeny [ID BC ZSM Lep 07781, barcode sequence 530 bp; ID BC ZSM Lep 07779, barcode sequence 570 bp; ID BC ZSM Lep 07628, barcode sequence 577 bp] (1 ♂, 2 ♀) (ZSM); Pino Hachado, 38°12'S, 71°55.99'W, 18-II-1995, leg. H. Thoeny [ID BC ZSM Lep 07634, barcode sequence 582 bp] (1 ♂) (ZSM); Contulmo, Palo botado, 02-II-1953, leg. L.E. Peña (1 ♂) (MZUC-UCCC). **Cautín:** Termas de Río Blanco, III-1951, leg. L.E. Peña (2 ♂) (MZUC-UCCC). **Coyhaique:** Laguna Azul, 23-I-2008, leg. L.E. Parra, “Genitalia 0258” [genitalia in microvial] (1 ♂) (MZUC-UCCC). **Capitán Prat:** Cochrane, Balsa Baker, 27-I-2008, “Genitalia 0245”, “Genitalia 0246”, “Genitalia 0257” [genitalia slides] leg. Muñoz-Escobar (4 ♂) (MZUC-UCCC).

Distribution. This species occurs between Diguillín and Capitán Prat provinces. It is distributed in parts of Santiago, Maule and Valdivian Forest biogeographic provinces, Central Chilean and Subantarctic subregions, Andean region.

Flight period. Specimens were captured from January to March.



Figures 11, 12. Genitalia of *Hoplosauris morenoi* Ramos-González & Parra, sp. n. **11** male genitalia (paratype, MZUC-UCCC, slide No. AMLP 0300) **12** female genitalia (paratype, MZUC-UCCC, slide No. AMLP 0122). Scale bar: 1 mm.

Molecular data. BOLD:AAH6701. Five available sequences of DNA barcode: BC LP 0039 (Curacautín), BC ZSM Lep 07781 (Curacautín), BC ZSM Lep 07779 (Curacautín), BC ZSM Lep 07628 (Curacautín), BC ZSM Lep 07634 (Lonquimay). Maximum intraspecific distance: 1.15%; Minimum genetic distance with *H. pachrophylloides* Parra: 7.74%.

Etymology. The species name is dedicated to the naturalist and great collector Sr Guillermo Moreno Crisóstomo, Chillán, Chile.

***Butleriana phoenix* Ramos-González & Parra, sp. n.**

<http://zoobank.org/AE5952C6-72B0-423A-B065-2F0DA3EBEB2C>

BIN: BOLD:AAD7597

Figures 5, 13, 19

Diagnosis. This species has a characteristic maculation pattern that easily distinguishes it from congeners: background color of forewings ashy-white, splashed with violaceous-red scales and crossed by dark violaceous-red antemedial and postmedial bands, which are more noticeable towards the costa. *Butleriana phoenix* differs from *B. minor* (Butler, 1882), *B. oculata* (Mabille, 1885), *B. fumosa* (Butler, 1882), and *B. fasciata*

(Butler, 1882) by the presence of free Rs and M1 veins on the hindwings of males. Additionally, *B. phoenix* shares with *B. fasciata* by having the A_1 vein insinuated only at the base, but both species differ in male genitalia, as *B. phoenix* presents a strongly sclerotized costa, which exceeds the apex of cucullus, thereby forming a L-shaped notch at the apex of the valva.

Description. Male (Fig. 5). Head: antennae filiform, subapically broadened; palpi subequal to eye diameter, covered by erect piliform violaceous-red scales with third segment slightly curved down; frons covered with flattened reddish scales. Thorax: patagia covered by silvery-white and violaceous-red scales; tegulae covered by flattened scales, violaceous-red at proximal area and whitish towards its distal area. Tibial formula 0-2-4. Forewings: background color ashy-white, splashed with violaceous-red scales, with two irregular spots of golden-olive scales: one subapical the other in a post-basal position, on the anal margin; medial, cubital and anal veins framed by blackish scales, which are interspersed with the background color; termen rounded with piliform reddish scales; antemedial band dark violaceous-red, slightly arcuate; postmedial band dark violaceous-red, extended laterally towards the wing's apex at the height of the two accessory cells; subterminal band diffuse, formed by two slender violaceous-red stripes; presence of an oblique blackish apical spot, which connects with postmedial band; discal spot blackish. Hindwings: reduced, three-quarters the length of forewings, subrounded, ashy-white, with an extended, narrow and subtriangular lobe at the base of the anal margin; discal spot blackish. Wing venation (Fig. 19): forewing with two accessory cells; hindwing with $Sc+R_1$ and Rs anastomosed as far as one-third before the end of radial trunk; Rs, M_1 , M_2 , M_3 , Cu_1 and Cu_2 are free and located on the vertices of discal cell; Rs closer to M_1 than to $Sc+R_1$; M_2 closer to M_3 ; Cu_2 originating from the middle of cubital trunk; in anal lobe only with A_2 present, which is curved; A_1 only insinuated at base of lobe; discal cell polygonal and it is extended for half of wing surface. Male genitalia (Fig. 13): valvae subrectangular, with a bulbous projection in the central area of anterior edge, costa strongly sclerotized, exceeding the apex of cucullus, apical notch L-shaped; saccus subrounded; juxta with subquadrangular base and forked posterior apex also with two lateral processes having a setose subtriangular apex connected each other in the midventral region, at the height of transtilla; uncus glabrous and curved; transtilla simple. Aedeagus tubular; cornuti arranged as a longitudinal group in the vesica. Female unknown.

Type material. Holotype: 1 ♂, pinned, CHILE, Chiloé, Quellón, 21-II-1951, leg. J.C. Vargas, "Museo", "AMLP 0141" [genitalia slide] "Holotype *Butleriana phoenix*" [red handwritten label] (MZUC-UCCC).

Paratypes: 4 males. CHILE: **Chiloé:** Mocopulli, Ruta 5 Sur km 1170, 42°22.08'S, 73°43.73'W, 182 m, 03-II-2017, leg. M. Ramos-G, M. Ramos-SM & C. Rose (1 ♂) (MZUC-UCCC); Ancud, Pauldeo, 23-I-2005, "Colección Numhauser 2013", "AMLP 0100" [wing slide], leg. Numhauser (1 ♂) (MZUC-UCCC). **Palena:** Fiordo Comau, San Ignacio del Huinay, pasture, 42°22.8'S, 72°24.78'W, 35 m, 04-I-2008, leg. A. Hausmann (1 ♂) [ID BC ZSM Lep 11682, barcode sequence 658 bp] (ZSM);

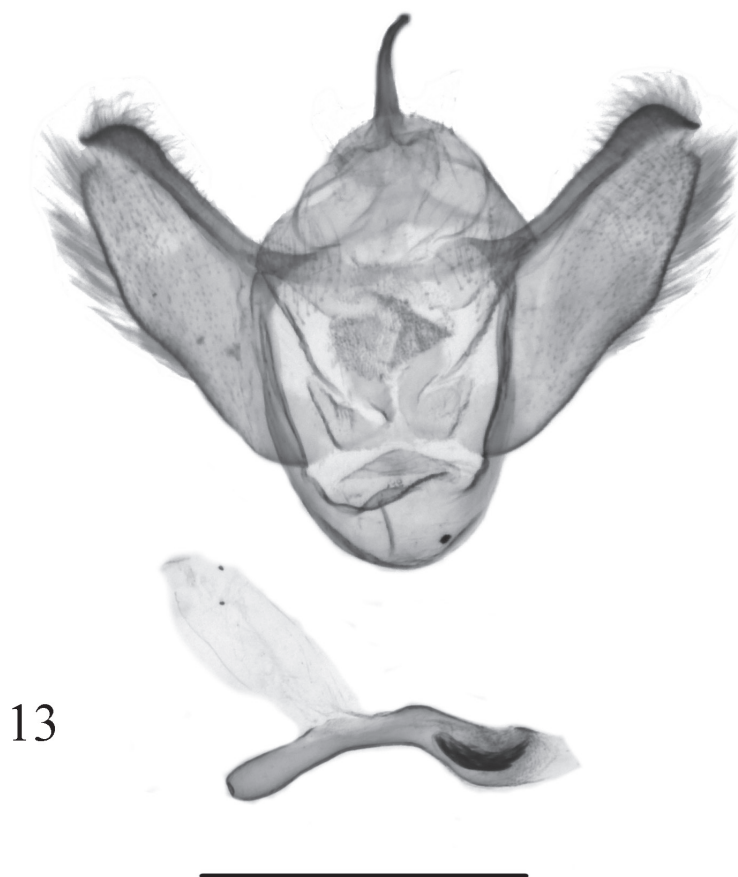


Figure 13. Male genitalia of *Butleriana phoenix* Ramos-González & Parra, sp. n., male, holotype, MZUC-UCCC, slide No. AMLP 0141. Scale bar: 1 mm.

Fiordo Comau, San Ignacio del Huinay, buildings, 42°22.86'S, 72°24.9'W, 20 m, 09-I-2008, leg. A. Hausmann, T. Greifenstein & L. Parra [ID BC ZSM Lep 11236, barcode sequence 632 bp] (1 ♂) (ZSM).

Distribution. This species occurs in Chiloé and Palena provinces. It is distributed in a part of the Valdivian Forest biogeographic province, Subantarctic subregion, Andean region.

Flight period. Specimens were captured from January to March.

Molecular data. BOLD:AAD7597. Two available sequences of DNA barcode: BC ZSM Lep 11682 (Huinay), BC ZSM Lep 11236 (Huinay). Maximum intraspecific distance: 0.79%; Minimum genetic distance with *B. minor*: 10.59%.

Etymology. The species name is a noun in the apposition, referring to the Phoenix (a mythical firebird), for the red/purple that is present in the moth's forewing coloration pattern.

***Warrenaria onca* Ramos-González & Parra, sp. n.**

<http://zoobank.org/D02F7D34-4754-437E-9BB5-7E49B2539D20>

BIN not assigned

Figures 6, 14, 20

Diagnosis. This species can be easily distinguished from *W. martha* (Butler) by the presence of ashy-brown forewings, with less evident antemedial and postmedial bands, which have a ferruginous tone. Both species have an U-shaped posterior apex of the juxta in male genitalia but differs in the shape of the juxta's base: subquadrangular in *Warrenaria onca* but subtriangular in *W. martha*.

Description. Male (Fig. 6). Head: antennae filiform, subapically broadened; palpi twice as long as eye diameter, covered by piliform straight light-brown scales; frons covered with imbricated flattened ashy-brown scales. Thorax: patagia covered by juxtaposed flattened ashy-brown scales; tegulae covered by piliform whitish, blackish and ashy-brown scales. Tibial formula 0-2-4. Forewings: background color ashy-brown splashed with blackish scales, slightly darker and with olivaceous tinge towards the costa and termen; M_3 and Cu_1 framed by blackish scales that cross the postmedial band; termen rounded, with dark piliform olivaceous-brown scales; basal region crossed by three wavy subcircular lines: proximal line light brown and diffuse, distal lines blackish and better defined than proximal one; costal margin of basal region only with a small subquadrate blackish spot, splashed with ferruginous-orange scales; antemedial band ferruginous-orange, slightly diffuse, zigzagging; postmedial band wavy, diffuse and composed of three slender ferruginous-orange stripes; costa of medial region mottled with blackish scales; subterminal band formed by two interrupted slender blackish stripes; adterminal band formed by rectangular interveinal spots; terminal band formed by blackish semicircles that are weakly connected with adterminal band; discal spot present and blackish. Hindwings: reduced, three-quarters the length of forewings, subrounded, dark brown, with an extended and subrounded lobe at the base of anal margin; discal spot blackish. Wing venation (Fig. 20): forewing with two accessory cells; hindwing with $Sc+R_1$ and R_s linked by a transverse vein a quarter before of the end of the cell; R_s , M_1 , M_2 , M_3 are free and located on the vertices of discal cell; Cu_1 slightly arched, near the angle of cell; Cu_2 inconspicuous, one-fifth before the angle of the cell; lobe crossed by sub-straight A_1 and curved A_2 ; discal cell polygonal and extend for half of wing surface. Male genitalia (Fig. 14): valvae subrectangular, costa strongly sclerotized, rounded apical notch with a small indentation, about 1/16 the length of valvae; saccus subquadrate; juxta with subquadrangular base and U-shaped posterior apex, with two lateral processes that have a setose triangular apex and are connected in the midventral region, at height of transtilla; uncus simple and slightly setose; transtilla simple. Aedeagus tubular; cornuti arranged as two longitudinal groups in the vesica. Female unknown.

Type material. Holotype: 1 ♂, pinned, CHILE, Nahuelbuta, Río Picoiquen, 22-XII-1965, leg. Fetis, "AMLP 0137" [genitalia slide], "Holotype *Warrenaria onca*" [red handwritten label] (MZUC-UCCC).

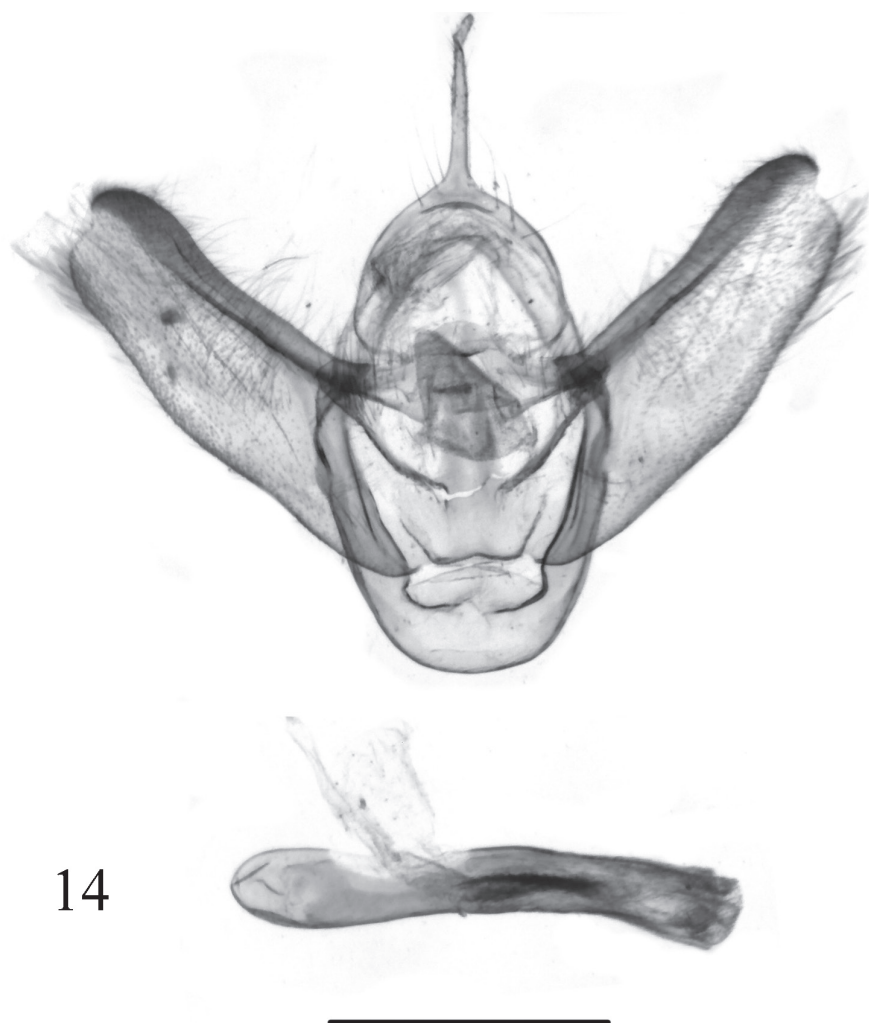


Figure 14. Male genitalia of *Warrenaria onca* Ramos-González & Parra, sp. n., male, holotype, MZUC-UCCC, slide No. AMLP 0137. Scale bar: 1 mm.

Distribution. This species is only known from the type locality: Chile, Araucanía, Malleco, Angol, Nahuelbuta, Río Picoiquen. This locality belongs to Maule biogeographic province, Central Chilean subregion, Andean region.

Flight period. The single specimen was captured in December.

Etymology. The species name is a noun in apposition and is in reference to the jaguar (*Panthera onca*), a feline that inhabited the forests of southern South America until the end of the 19th century and which gives its name to the type locality (Nahuelbuta) in Mapudungun language (*nawel*: jaguar; *füta*: big).

***Fueguina araucana* Ramos-González & Parra, sp. n.**

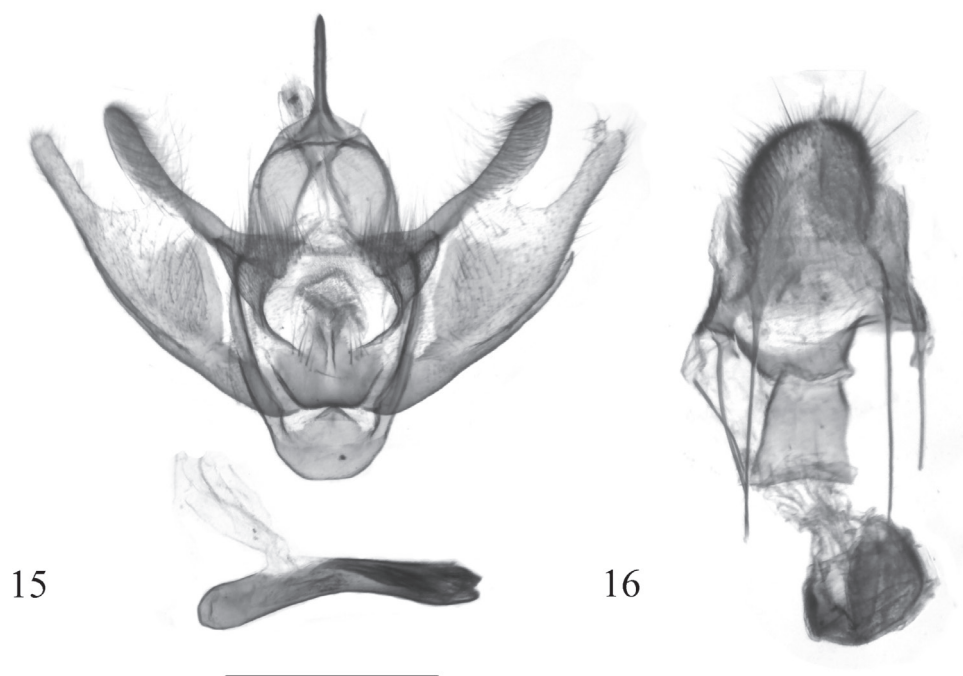
<http://zoobank.org/EA416114-32CA-4D92-BCEB-2F15171006F8>

BIN not assigned

Figures 7, 8, 15, 16, 21

Diagnosis. This species can be easily distinguished from *F. varians* (Butler) and *F. celovalva* Parra by its ashy forewings, crossed by dark-brown stripes, and a less-developed saccular process. Externally, it differs from *F. magallanica* Parra by its antemedial and postmedial bands, which are less angular in *F. araucana*. Can be distinguished from congeners by three other characters: the presence of disjointed subtriangular lateral processes in the juxta, the large subrounded apical indentation, which extends approximately through half of valva, and having a globular corpus bursae which is short and subequal to the length of ductus bursae.

Description. Male (Fig. 7). Head: antennae filiform, subapically broadened; palpi correct, slightly tilted up covered by straight piliform dark-brown scales and 1.5 times larger than eye diameter; frons and vertex covered with imbricated flattened whitish and dark-brown scales. Thorax: patagia covered by juxtaposed flattened whitish and dark-brown scales; tegulae covered by dark-brown scales splashed with black scales, piliform scales on the posterior region. Tibial formula 0-2-4. Forewings: background color ashy; medial and Cu₁ veins framed by three elongated blackish spots, between postmedial and subterminal bands; termen rounded, with piliform light-brown scales; basal band blackish, curved, slightly zigzagging towards the inner margin; antemedial band dark brown, sinuous, which is thinner towards the costa and inner margin than in its medial sector; postmedial band sinuous and wide, formed by two brown-orange stripes mottled with dark brown and framed with blackish-brown scales; subterminal band dark brown, diffuse, cut off on its costal third by an ashy apical spot; discal spot present and blackish. Hindwings: same size as in females, subrectangular, ashy-brown, with a digitiform lobe extended over the base of anal margin; discal spot not visible. Wing venation (Fig. 21): forewing with two accessory cells; hindwing with Sc+R₁ and Rs connected by a transverse vein towards one-third before the end of the cell; Rs and M₁ pedunculated; M₂ absent and M₃ near Cu₁; Cu₁ is near the angle of the cell; Cu₂ weak, one-fifth before the angle of the cell; lobe crossed by straight A₁ and slightly curved A₂; discal cell polygonal and extend for half of wing surface. Male genitalia (Fig. 15): valvae subrectangular, costa strongly sclerotized with rounded and setose apex, deep subrectangular apical notch, approximately half the length of valvae; cucullus projected in the apex of anterior edge, sacculus present and spine-like; saccus rounded; juxta with subquadrangular base and M-shaped posterior apex, with two disjointed lateral processes that have setose subtriangular apex and extends at the height of transtilla; uncus glabrous and straight. Aedeagus tubular; vesica armed with three cornutus. Female (Fig. 8): similar to males, but with filiform antennae slighter and subrectangular hindwings without lobe on the anal margin. Female genitalia (Fig. 16): ductus bursae striated and subequal in length to corpus bursae; corpus bursae



Figures 15, 16. Genitalia of *Fueguina araucana* Ramos-González & Parra, sp. n. **15** male genitalia (holotype, MZUC-UCCC, slide No. AMLP 0139) **16** female genitalia (allotype, MZUC-UCCC, slide No. AMLP 0138). Scale bar: 1 mm.

globular, membranous; cestum present, subrectangular and strongly sclerotized; posterior apophyses longer than anterior ones.

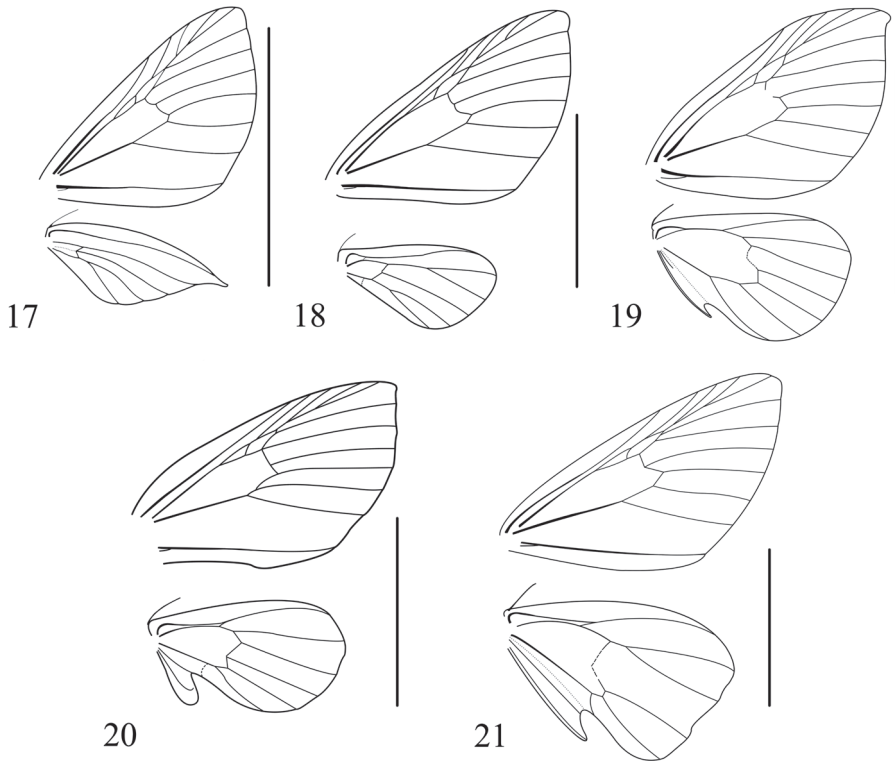
Type material. Holotype: 1 ♂, pinned, CHILE, Araucanía, Malleco, R.N. Malalcahuello-Nalcas, Corralco, 09-XII-2014, leg. L.E. Parra, “AMLP 0139” [genitalia slide], “UCCC_MZUC_Lep_0031” [ID code], “Holotype *Fueguina araucana*” [red handwritten label] (MZUC-UCCC); Allotype: 1 ♀, pinned, CHILE, Malleco, Río Blanco III-1951, leg. L.E. Peña, “Especie 23 H” [ID code, female], “AMLP 0138” [genitalia slide], “Allotype *Fueguina araucana*” [red handwritten label] (MZUC-UCCC).

Paratypes: 1 male, 3 females. CHILE: **Malleco:** Curacautín, Termas de Río Blanco, 1050-1300 m, 21/24-II-1954, leg. L.E. Peña (1 ♀) (MZUC-UCCC). **Cautín:** Pucón, Termas de Río Blanco, II-1951, leg. L.E. Peña, “Especie 23 M” [ID code, male], “AMLP 0093” [wing slide] (1 ♂, 1 ♀) (MZUC-UCCC); Pucón, Termas de Río Blanco, III-1951, leg. L.E. Peña (1 ♀) (MZUC-UCCC).

Distribution. This species occurs between Malleco and Cautín provinces. It is distributed in parts of Maule and Valdivian Forest biogeographic provinces, Subantarctic subregion, Andean region.

Flight period. Specimens were captured in December, February and March.

Etymology. The species name is dedicated to the Araucanía region, Chile, the locality where all specimens were collected.



Figures 17–21. Wing venation of males **17** *Aloba carolinae* Ramos-González & Parra, sp. n. **18** *Hoplosauris morenoi* Ramos-González & Parra, sp. n. **19** *Butleriana phoenix* Ramos-González & Parra, sp. n. **20** *Warrenaria onca* Ramos-González & Parra, sp. n. **21** *Fueguina araucana* Ramos-González & Parra, sp. n. Scale bar: 1 cm

Discussion

The genus *Hoplosauris* was proposed by Butler (1882) and currently is the most species-rich Chilean trichopterygine genus with eight valid species (Parra et al. 2009, 2017). The species are: *H. granitata* (Fletcher, 1953), *H. heliconoides* Butler (1882), *H. indistincta* (Butler, 1882), *H. macarenae* Parra (2009), *H. mabillei* Parra (2009), *H. pachrophylloides* Parra (2009), *H. schausi* (Warren, 1908), and *H. valeria* Butler (1893). The genus can be recognized by three synapomorphies: a small flap, vesicle and/or tuft of piliform scales in the anal margin of the hindwing in males; a setal tuft in the subapical region of valvae; and microspines and striated areas in the internal surface of corpus bursae (Parra et al. 2009, 2017).

It is possible to include *H. morenoi* in this genus, due to the low genetic distance between this species and *H. pachrophylloides* (< 8%; Hausmann and Hebert 2009; Hausmann et al. 2011) and also because of the large number of characters shared with

H. heliconoides, the type species. Some of these characters are the presence of short and porrect palpi; the connection of the Sc+R₁ and Rs veins by a weak transverse vein; and the pedunculated M₃ and Cu₁ veins; the absence of Cu₂ and anal veins; the presence of an anal cell; the short and triangular discal cell in the forewings of males; the valvae with brush-like subapical setal tuft and apically projected cucullus; the ductus bursae which is one-sixth the length of corpus bursae; and the subpyriform completely sclerotized corpus bursae with longitudinal striation and rows of microspines. Thus, the number of species belonging to *Hoplosauris* increases to nine.

Several Chilean Trichopterygini (e.g., *Butleriana*, *Warrenaria*, *Fueguina*, *Tomopteryx* Philippi, *Triptila* Warren, *Triptiloides* Parra & Santos-Salas, *Pachrophylla* Blanchard, and *Parapachrophylla* Parra) share ancestral characters in the male genitalia, e.g. valvae with indented posterior apex and juxta with a pair of lateral processes joined each other at transtilla height (Viidalepp 2011). This means that the venation pattern of the hindwings is particularly important for the determination of Chilean genera, especially in males (Parra 1991, 1996; Parra and Santos-Salas 1991, 1992).

Males of the genera *Butleriana* and *Llampidken* Parra have in common the shape of the lobes on the hindwing. However, venation of lobes is different in these genera, as well as some structures in the male genitalia (e.g., hooked socius, presence of saccular processes and costal arm in *Llampidken*). *Butleriana* is characterized by the presence of a single anal vein (A₂) crossing the lobe (a synapomorphy that defines *Butleriana*). A₁, when present, is only a remnant vein, slightly visible at the base of the hindwing. This is different in *Llampidken* in which no anal veins go across the lobe (an autapomorphy) (Parra and Santos-Salas 1992; Parra et al. 2017). Although the genetic divergence between *B. phoenix* and the type species (*B. minor*) is high (approximately 11%), it is possible to assign *B. phoenix* to the genus *Butleriana* because of the consistency in males of the hindwing and genital morphology, i.e., both species have similar valvae and a single anal vein through the subtriangular lobe, with the A₁ vein slightly visible at its base.

It is possible to distinguish *Warrenaria* by its reddish-brown coloration, rectangular valvae, and the shape of the uncus (Parra et al. 2017). *Warrenaria onca* is included in this genus because it shares with *W. martha* (type species) the maculation and wing venation general patterns. Other similarities are: the length of apical indentation in the valvae, the shape of valva and socius, and the U-shaped juxta. All these characters, combined, are unique of *Warrenaria* and do not occur in other Chilean Trichopterygini.

Fueguina comprises three species: *F. varians*, *F. celovalva*, and *F. magallanica*. This genus can be distinguished by the presence of three features in males: a lobe at the hindwing base with two anal veins, a spiniform saccular process, and a deep indentation on the cucullus region (Parra 1991; Parra et al. 2017). It is possible to include *F. araucana* in this taxon because of the shape and venation of hindwing lobe, the presence of a costal process, and the presence of a spiniform saccular process with a deep apical indentation. *Fueguina araucana* and *F. magallanica* share the following characters: general wing venation pattern, general shape of valvae and juxta, and the similar maculation pattern. However, there are distinctive characters in *F. araucana*: distinctive lateral processes of juxta; a deeper apical indentation of valvae (in this sense, similar to *F. varians*

and *F. celoalva* but more rounded, as in *F. magallanica*); and the shape of bursa copulatrix. The large number of common characters between *F. magallanica* and *F. araucana* suggests that both species are closely related, placing them as the sister-species.

Regarding *Aloba*, Warren (1895) was the first to placed this genus in the tribe Trichopterygini and included only one species. Nevertheless, this species was not considered to belong to the Trichopterygini in later works (e.g., Parra et al. 2017). After analyzing the anatomical features of this species, it is possible to recognize it as a member of the Trichopterygini and re-assign it to the tribe. This species shares with the Trichopterygini the diagnostic characters that defines the tribe, such as the reduction in the anal margin of male hindwings with subsequent simplification of venation and the presence of a sternal pouch in the tympanic opening (Dugdale 1980; Parra et al. 2017). *Aloba* can be considered as the taxon morphologically closest to *Hoplosauris*, based on the absence of lobe and anal veins in the hindwings of males, the presence of a cucullus projected apically, and a setal tuft. This taxonomic relationship is supported by molecular phylogenetic analyses (Ramos-González et al. unpublished data; Brehm et al. submitted).

Finally, considering all these new findings, the number of Chilean Trichopterygini increases to 15 genera and 45 species.

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References

- Bartlett-Calvert W (1893) Nuevos lepidópteros de Chile. Anales de la Universidad de Chile 84: 813–834. <https://anales.uchile.cl/index.php/ANUC/article/view/20691/21857>
- Brehm G (2015) Three new species of *Hagnagora* Druce, 1885 (Lepidoptera, Geometridae, Larentiinae) from Ecuador and Costa Rica and a concise revision of the genus. Zookeys 537: 131–156. <https://doi.org/10.3897/zookeys.537.6090>
- Brehm G (2018) Revision of the genus *Callipia* Guenée, 1858 (Lepidoptera, Geometridae), with the description of 15 new taxa. European Journal of Taxonomy 404: 1–54. <https://doi.org/10.5852/ejt.2018.404>

- Butler AG (1882) Heterocerous Lepidoptera collected in Chili by Thomas Edmonds, Esq. Part III—Geometrites. Transactions of the Entomological Society of London 30(3): 339–427. <https://doi.org/10.1111/j.1365-2311.1882.tb01580.x>
- Butler AG (1893) On a small collection of Lepidoptera from Chili. The Annals and Magazine of Natural History, including Zoology Botany, and Geology 12(6): 457–467. <https://doi.org/10.1080/00222939308677653>
- Dugdale JS (1980) Australian Trichopterygini (Lepidoptera: Geometridae) with descriptions of eight new taxa. Australian Journal of Zoology 28: 301–340. <https://doi.org/10.1071/ZO9800301>
- Fletcher DS (1953) Some new species of Geometridae from Argentina and Chile. Acta Zoológica Lilloana 12: 367–380.
- Gaston KJ, Scoble MJ, Cook A (1995) Patterns in species descriptions: a case study using the Geometridae (Lepidoptera). Biological Journal Linnean Society 55(3): 225–237. <https://doi.org/10.1111/j.1095-8312.1995.tb01061.x>
- Hausmann A, Haszprunar G, Hebert P (2011) DNA barcoding the geometrid fauna of Bavaria (Lepidoptera): successes, surprises, and questions. PLoS ONE 6(2): e17134. <https://doi.org/10.1371/journal.pone.0017134>
- Hausmann A, Hebert P (2009) Order Lepidoptera, family Geometridae (part 2): the Geometridae of the UAE revised in the light of mtDNA data. In: Van Harten T (Ed.) Arthropod Fauna of the UAE 2. Dar Al Ummah Printing, Publishing, Distribution & Advertising, Adu Dhabi, United Arab Emirates, 468–479.
- Hausmann A, Parra LE (2009) An unexpected hotspot of moth biodiversity in Chilean northern Patagonia (Lepidoptera, Geometridae). Zootaxa 1989: 23–38.
- Holloway JD (1997) The moths of Borneo: family Geometridae, subfamilies Sterrhinae and Larentiinae. Malayan Nature Journal 51: 1–242.
- Klots AB (1970) Lepidoptera. In: Tuxen SL (Ed.) Taxonomist's Glossary of Genitalia in Insects. Second Enlarged Edition. Ejnar Munksgaard, Copenhagen, 115–130.
- Morrone JJ (2015) Biogeographical regionalisation of the Andean region. Zootaxa 3936(2): 207–236. <https://doi.org/10.11646/zootaxa.3936.2.3>
- Öunap E, Viidalepp J, Truuvverk A (2016) Phylogeny of the subfamily Larentiinae (Lepidoptera: Geometridae): integrating molecular data and traditional classifications. Systematic Entomology 41(4): 824–843. <https://doi.org/10.1111/syen.12195>
- Parra LE (1991) Revisión y filogenia del género *Pachrophylla* Blanchard, 1852 (sensu auctorum) (Geometridae: Larentiinae: Trichopterygini). Gayana Zoología 55(2): 145–199.
- Parra LE (1995) Lepidoptera. In: Simonetti JA, Arroyo MTK, Spotorno AE, Lozada E (Eds) Diversidad Biológica de Chile. CONICYT, Santiago, Chile, 269–279.
- Parra LE (1996) Trichopterygini Neotropicales IV: descripción de nuevos géneros y especies de Chile (Lepidoptera: Geometridae). SHILAP Revista de Lepidopterología 24(93): 37–54.
- Parra LE, Jiménez-Urrutia MC, Zamora-Manzur C (2009) Revision of the genus *Hoplosauris* Butler, 1882 (Lepidoptera: Geometridae). Zootaxa 1989: 39–54.
- Parra LE, Santos-Salas CP (1991) Trichopterygini Neotropicales II (Lepidoptera: Geometridae): El complejo *Rhopalodes* Guenée, 1857. Gayana Zoología 55: 267–303.

- Parra LE, Santos-Salas CP (1992) Trichopterygini Neotropicales III: género y especie nuevos para Chile (Lepidoptera: Geometridae). Boletín de la Sociedad de Biología de Concepción 63: 151–156.
- Parra LE, Vargas HA, Sanzana MJ, Hernández CE (2017) Phylogenetic study of the genera of Trichopterygini from austral South America (Lepidoptera: Geometridae): a new classification. Gayana 81(2): 64–99. <https://doi.org/10.4067/S0717-65382017000200064>
- Parra LE, Villagrán-Mella R (2008) Orden Lepidoptera (mariposas). In: CONAMA (Ed.) Biodiversidad de Chile, Patrimonio y Desafíos, 2° Edición. Ocho Libros Editores, Santiago, Chile, 159–165.
- Ratnasingham S, Hebert P (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7(3): 355–364. <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Ratnasingham S, Hebert P (2013) A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. PLoS ONE 8(7): e66213. <https://doi.org/10.1371/journal.pone.0066213>
- Rindge FH (1987) The *Eupithecia* (Lepidoptera: Geometridae) of Chile. Bulletin of the American Museum of Natural History 186(3): 269–363. <http://hdl.handle.net/2246/912>
- Rindge FH (1991) The *Eupithecia* (Lepidoptera: Geometridae) of Chile II. American Museum Novitates 3020: 1–14. <http://hdl.handle.net/2246/5049>
- Scoble MJ (1995) The Lepidoptera: Form, Function and Diversity. Oxford University Press, Oxford, 404 pp.
- Scoble MJ (1999) Geometrid Moths of the world: a Catalogue. Vols 1 and 2. CSIRO Publishing, Collingwood, 1016 pp. <https://doi.org/10.1071/9780643101050>
- Scoble MJ, Gaston KJ, Crook A (1995) Using taxonomic data to estimate species richness in Geometridae. Journal of the Lepidopterists' Society 49: 136–147.
- Scoble MJ, Hausmann A (2007) Online list of valid and available names of the Geometridae of the World. Lepidoptera Barcode of Life, iBOL. <http://www.lepbarcoding.org> [Accessed on 2018-9-19]
- Sihvonen P, Mutanen M, Kaila L, Brehm G, Hausmann A, Staude HS (2011) Comprehensive molecular sampling yields a robust phylogeny for geometrid moths (Lepidoptera: Geometridae). PLoS ONE 6(6): e20356. <https://doi.org/10.1371/journal.pone.0020356> <https://doi.org/10.1371/journal.pone.0020356>
- Van Nieukerken EJ, Kaila L, Kitching IJ, Kristensen NP, Lees DC, Minet J, Mitter C, Mutanen M, Regier JC, Simonsen TJ, Wahlberg N, Yen SH, Zahir R, Adamski D, Baixeras J, Bartsch D, Bengtsson BA, Brown JW, Bucheli SR, Davis DR, Prins JD, Prins WD, Epsteina ME, Gentili-Poole P, Gielis C, Hättenschwiler P, Hausmann A, Holloway JD, Kallies A, Karsholt O, Kawahara AY, Koster S, Kozlov MV, Lafontaine D, Lamas G, Landry JF, Lee S, Nuss M, Park KT, Penz C, Rota J, Schintlmeister A, Schmidt BC, Sohn JC, Solis MA, Tarmann GM, Warren AD, Weller S, Yakovlev RV, Zolotuhin VV, Zwick A (2011) Order Lepidoptera Linnaeus. In: Zhang Z-Q (Ed.) Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 212–221.
- Viidalepp J (2011) A Morphological review of tribes in Larentiinae (Lepidoptera: Geometridae). Zootaxa 3136: 1–44.

- Vojnits AM (1985) New South American genera and species of Eupithicini (Lepidoptera: Geometridae). *Acta Zoologica Hungarica* 31: 405–418.
- Vojnits AM (1992) New data to the *Eupithecia* fauna of Chile and Peru (Lepidoptera: Geometridae). *Acta Zoologica Hungarica* 38(1–2): 125–144.
- Vojnits AM (1994) New data on the *Eupithecia* fauna of Chile (Lepidoptera, Geometridae). *Entomofauna* 15(32): 361–376.
- Warren W (1895) New species and genera of Geometridae in the Tring Museum. *Novitates Zoologicae* 2: 82–159. <https://biodiversitylibrary.org/page/3859357>
- Warren W (1908) Descriptions of new species of South American geometrid moths. *Proceedings of the United States National Museum* 34: 91–110. <https://doi.org/10.5479/si.00963801.1601.91>
- Zamora-Manzur C, Parra LE, Jaque E (2011) Patrones de distribución de los geométridos de la Región del Biobío, Chile: una aproximación para su conservación. *Revista Chilena de Historia Natural* 84: 465–480. <https://doi.org/10.4067/S0716-078X2011000400001>

A new species of terrestrial frog *Pristimantis* (Strabomantidae) from the upper basin of the Pastaza River, Ecuador

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Abstract

We describe a new species of *Pristimantis* from the montane forest of the Río Zuñag Ecological Reserve, upper basin of the Pastaza River, Ecuador. *Pristimantis mallii* sp. n. is characterized by a snout-vent length of 11.6–21.3 mm in adult males ($n = 12$), 22.6–34.3 mm in adult females ($n = 8$), and is compared morphologically and genetically with *Pristimantis miktos* and with other relevant species of *Pristimantis*. The new species is characterized by having skin on dorsum and flanks shagreen, distinctive scapular folds, snout broadly rounded in dorsal view, upper eyelid bearing one or two subconical tubercles and some rounded tubercles, dorsum and flanks light brown to brown, with irregular dark brown marks bounded by dirty cream and groin with irregular yellowish marks.

Keywords

Montane forest, *Pristimantis mallii* sp. n., Río Zuñag Reserve, Terrarana

Resumen

Describimos una nueva especie de *Pristimantis* del bosque montano de la Reserva Ecológica Río Zuñag, cuenca alta del río Pastaza, Ecuador. *Pristimantis mallii* sp. n. es caracterizada por una longitud rostro-cloacal de 11.6–21.3 mm en machos adultos ($n = 12$), 22.6–34.3 mm en hembras adultas ($n = 8$), y es comparada morfológica y genéticamente con *Pristimantis miktos* y con otras especies relevantes de *Pristimantis*. La especie nueva se caracteriza por tener la piel del dorso y flancos finamente granular, pliegues escapulares distintivos, hocico redondeado en vista dorsal, párpado superior con uno dos tubérculos subcónicos y algunos redondeados, dorso y flancos café claro a café, con marcas irregulares café oscuras bordeadas de crema sucio y marcas irregulares amarillentas en las ingles.

Palabras clave

Bosque montano, *Pristimantis mallii* sp. n., Reserva Río Zuñag, Terrarana

Introduction

The genus *Pristimantis* is an endemic group of terrestrial frogs of the Neotropical region; with more than 525 species, it is the largest genus of all vertebrates (Frost 2018). Its highest diversity is found in the Andean montane forests of Colombia, Ecuador, and Peru (Heinicke et al. 2007; Frost 2018). In Ecuador, this genus represents 38.5% of the amphibians, with 230 species currently described, of which 125 are endemic (Ron et al. 2018). In the last five years, 37 species of *Pristimantis* in Ecuador have been described (Ron et al. 2018). This rapid and continuous increase of the known species suggests that this number will keep rising, considering the many regions that still remain unexplored in the Ecuadorian Andes. Presumably, the high diversity of this terrestrial group is explained by the success of their direct development, which allows individuals to be independent of water and to colonize new terrestrial niches (Hedges et al. 2008). Most of the species of this genus are characterized by having small distributions (Lynch and Duellman 1980; Terán-Valdez and Guayasamin 2010; Yáñez-Muñoz et al. 2016). This could explain the high rate of discovery in the eastern Andes of Ecuador, where recently several species have been described (e.g. Reyes-Puig et al. 2010, 2013, 2014; Reyes-Puig and Yáñez-Muñoz 2012; Batallas and Brito 2014; Brito et al. 2014, 2016, 2017a, b; Yáñez-Muñoz et al. 2014; Navarrete et al. 2016).

The upper basin of the Pastaza River is an important endemic region, mainly because the Río Pastaza is a major Ecuadorian tributary of the Amazon (i.e. biogeographic barrier), with a rugged topography of volcanic and granitic origin (Kennerly and Bromley 1971; Gradstein et al. 2004; Sánchez et al. 2018). These characteristics have allowed the presence of flora and fauna distributed only in this restricted region (e.g. Gradstein et al. 2004; Reyes-Puig et al. 2010, 2013, 2014, 2015; Jost and Shepard 2017). Here we describe a new species of direct-developing frog of the genus *Pristimantis* from the montane forest of the Pastaza River basin, with morphological and phylogenetic analyzes based on lab and field work executed by several institutions.

Materials and methods

DNA extraction, amplification and sequencing

DNA extraction and amplification processes took place at the Laboratorio de Biología Molecular of the Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ). Total DNA was extracted from liver and muscular tissue preserved in 95% ethanol by applying the Guanidinium thiocyanate (GITC) protocol (Esselstyn et al. 2008). Samples were quantified on a nanodrop (Thermo Scientific) and diluted by aliquots at a 20 ng/μl concentration. Standard PCR procedures were used to amplify the mitochondrial gene 16S rRNA (16S) and the nuclear recombination-activating genes (RAG1). The primers used were 16L19 and 16H36E for 16S (Heinicke et al. 2007) as well as RAG1FF2 and RAG1FR2 for RAG (Heinicke et al. 2007). The amplified results were purified by the ExoSap tool and sent to the Macrogen company (Macrogen Inc., Seoul, Korea) for sequencing. Additionally, the genetic sample included various 12S rRNA (12S) mitochondrial gene sequences obtained from the GenBank database.

The sequences generated de novo were assembled and edited manually on the GeneiousPro 5.4.6 software (Biomatters Ltd). Both ends of the sequence were cut during editing to avoid low quality base pairs. GenBank Access codes were assigned to new sequences presented in this study are MK391384, RAG1; MK391386, 16S, tRNAs, ND1 for QCAZ 52473 (*Pristimantis mallii* sp. n.). MK391383, RAG1; MK391385, 16S, tRNAs, ND1 for QCAZ 55445 (*Pristimantis miktos*).

Phylogenetic Analysis

New sequences were compared to the GenBank sequences using the BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) in order to confirm their genetic identity and determine similar species that allow the evaluation of the phylogenetic position of the new taxon. The search showed a high likeness between the new species and *Pristimantis riveti*. Therefore, we have included comparisons with *P. riveti* and other closely related species (sensu Padial et al. 2014), as well as other representative species from the *Pristimantis* clade. Sequences from *Diasporus*, *Eleutherodactylus*, *Holoaden*, *Hypodactylus*, *Ichnocnema*, *Lynchius*, *Oreobates*, and *Strabomantis* were also used as external groups. GenBank sequences employed correspond to data previously reported by Darst and Cannatella (2004), Faivovich et al. (2005), Elmer et al. (2007), Heinicke et al. (2007, 2009, 2015), Hedges et al. (2008), Arteaga-Navarr and Guayasamin (2011), Fouquet et al. (2012), Kok et al. (2012, 2018), Lehr et al. (2012, 2017), Lehr and Von May (2017), Pinto-Sánchez et al. (2012), Arteaga et al. (2013, 2016), Barrio-Amorós et al. (2013), Crawford et al. (2013), Zhang et al. (2013), Ortega-Andrade and Venegas (2014), Rivera-Prieto et al. (2014), Hutter and Guayasamin (2015), Rivera-Correa and Daza (2015), Chávez and Catenazzi (2016), de Oliveira et al. (2017), García-R. et al. (2012), Shepack et al. (2016), Székely et al. (2016), González-Durán et al. (2017), Guayasamin et al.

2015, Guayasamin et al. 2017, Jablonski et al. (2017), von May et al. (2017), Mahecha et al. (unpub.).

Multiple sequence alignment was done on the GeneiousPro 5.4.6 software under the MUSCLE algorithm (Robert 2004). Revision and manual correction of the matrix was performed on the Mesquite v2.75 software (Maddison and Maddison 2011). The codifying loci (RAG) were translated in amino acids to evaluate and avoid the presence of stop codons. In total, the combined DNA matrix showed 2968 base pairs. The best model for trait evolution and the best partition outline for our data were estimated simultaneously in the PartitionFinder v1.1.1 software (Lanfear et al. 2012), by means of five partitions of the *a priori* configured matrix: one for 12S, one for 16S and one partition for each RAG codon.

Phylogenetic trees were rebuilt based on Bayesian inference and maximum likelihood estimation (MLE). For the MLE analyses, 4 independent searches of one replica each were performed, two of them under the systematized starting command stepwise (streefname = stepwise) and the remaining two were configured under the alternative command random (streefname = random). Phylogenetic searches ended after 2000000 degenerations with no improvement in the tree's topology (genthreshfortopoterm = 2000000). The support of each branch was estimated considering 200 bootstrap replicas obtained under the same configuration parameters used to determine the best tree. The consensus tree was estimated in the Mesquite v2.75 software by a 50% majority consensus rule. Bayesian inference analyses took place on the MrBayes v3.2 software (Ronquist et al. 2012) available online on the CIPRES Science Gateway portal (Miller et al. 2010). The search consisted of five parallel runs of Markov Monte Carlo chain, each one configured at 20×106 search generations, four chains at standard temperature values. 50% of the generations were removed as burn-in. By using the TRACER v1.6 (Rambaut and Drummond 2007) software we confirmed the convergence in our searches, with an effective stationary distribution and sample size (ESS > 200). Finally, non-corrected *p* genetic distances were estimated based on the 16S gene for the new species and related clades using the Mega 6 (Tamura et al. 2013) software. The reference threshold for genetic separation used in the present study is 3% to determine different species (Fouquet et al. 2007).

Morphological data

Description, measurements and terminology follow the standardized format of Lynch and Duellman (1997). The diagnostic characters follow the definitions of Duellman and Lehr (2009). The collected specimens were sacrificed with lidocaine, fixed in 10% formalin and preserved in 70% ethanol. The sex and age of the specimens were determined by secondary sexual characteristics (nuptial pads, vocal slits and size) and direct inspection of the gonads through a dorsolateral incision. The following measurements were taken by the same person at least three times and were averaged with calipers to the nearest 0.1 mm: snout-vent length (SVL), tibia length (TL), foot length (FL), head

width (HW), head length (HL), interorbital distance (IOD), width of the upper eyelid (EW), internarial distance (IND), eye-nostril distance (EN), tympanum diameter (TD), eye diameter (ED). The life coloration pattern of the specimens was recorded with field notes and in-field color photography. The localities, coordinates and elevations were determined from field notes of the collectors and taken with a GPS receiver. The examined specimens were deposited in the Museo de Zoología, Universidad San Francisco de Quito (ZSFQ); Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ); and Sección de Herpetología, Instituto Nacional de Biodiversidad (DHMECN). All institutions are located in Quito, Ecuador.

Results

Phylogenetic relationships and genetic distances (Fig. 1)

Placement of *Pristimantis mallii* sp. n. in the genus *Pristimantis* was strongly supported, and according to the available information, the new species is sister to *Pristimantis miktos*. Both species form a clade with high support (Fig. 1) sister to a clade composed of *Pristimantis cryophilus*, *P. spinosus*, *P. phoxocephalus*, *P. riveti*, *P. versicolor*, and *P. hampatusami*. The uncorrected *p*-genetic distance between the new species and *P. miktos* is 11.9% (gene16S).

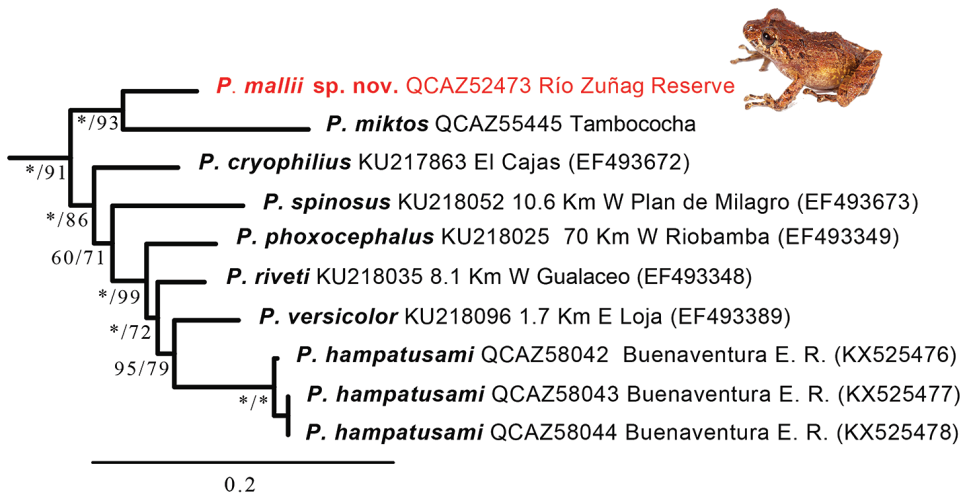


Figure 1. Phylogeny of *Pristimantis* showing the relationships of *Pristimantis mallii* sp. n. (red). The phylogram was derived from analysis of 2968 bp of mitochondrial (gene fragments 12S and 16S) and nuclear (gene fragment RAG) DNA sequences. Branch support is presented for each clade as Bayesian posterior probabilities $\times 100$ (left of the slash) and non-parametric bootstrap (right of the slash). Asterisks indicate support values of 100. The external group is not shown. For each specimen, museum catalog number, locality, and GenBank accession number (in parentheses) are reported. Abbreviations: E. R. = Ecological Reserve.

Systematic accounts

Pristimantis mallii sp. n.

<http://zoobank.org/6B898DBA-743A-470A-ABC3-7B123648DFB5>

Figures 2–6

Proposed standard English name: Malli's Rain Frog

Proposed standard Spanish name: Cutín de Malli

Holotype. QCAZ 52473 (field no. SC-PUCE 35222; Figs 3, 4), adult female, collected by Fernando Ayala, Diego Paucar, Yerka Sagredo, Juan Pablo Reyes-Puig, Fausto Recalde, Luis Recalde and Santiago Recalde on January 17, 2012 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.36740S, 78.14573W; 2140 m elev.).

Paratypes (7 females, 12 males). QCAZ 39777, adult female, collected by Diego Páez on January 1, 2009 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.349399S, 78.15870W; 2127 m elev.). QCAZ 52476, 52477, adult females, collected by Fernando Ayala, Diego Paucar, Yerka Sagredo, Juan Pablo Reyes-Puig, Fausto Recalde, Luis Recalde and Santiago Recalde on January 17, 2012 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.36761S, 78.14584W; 2153 m elev.). QCAZ 52494, adult female, collected by Fernando Ayala, Diego Paucar, Yerka Sagredo, Juan Pablo Reyes-Puig, Fausto Recalde, Luis Recalde and Santiago Recalde on January 17, 2012 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.37220S, 78.15386W; 1823 m elev.). DHMECN 5236, 5264, adult females, collected by Mario Yáñez-Muñoz, Miguel Urgilés and Andrés Laguna on May, 2008 at Reserva Río Zuñag, Baños, Province of Tungurahua, Ecuador (1.40045S, 78.186776W; 1300 m). ZSFQ 1305, adult female, collected by Carolina Reyes-Puig, Nicolás Dávalos, Daniel Velarde and Emilio Mancero on October 7, 2017 at at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.36761S, 78.14583W; 2190 m elev.). QCAZ 52512, subadult male, collected by Fernando Ayala, Diego Paucar, Yerka Sagredo, Juan Pablo Reyes-Puig, Fausto Recalde, Luis Recalde and Santiago Recalde on January 20, 2012 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.37513S, 78.16363W; 1532 m elev.). QCAZ 52471, 52474, adult males with the same data of the holotype. QCAZ 52478, adult male, collected by Fernando Ayala, Diego Paucar, Yerka Sagredo, Juan Pablo Reyes-Puig, Fausto Recalde, Luis Recalde and Santiago Recalde on January 17, 2012 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.36761S, 78.14583W; 2146 m elev.). QCAZ 52480, 52481, adult males, collected by Fernando Ayala, Diego Paucar, Yerka Sagredo, Juan Pablo Reyes-Puig, Fausto Recalde, Luis Recalde and Santiago Recalde on January 17, 2012 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.36765S, 78.14594W; 2135 m elev.). DHMECN 5233–5235, 5238, adult males, collected by Mario Yáñez-Muñoz, Miguel Urgiles and Andrés Laguna on May, 2008 at Reserva Río Zuñag, Baños, Province of Tungurahua,

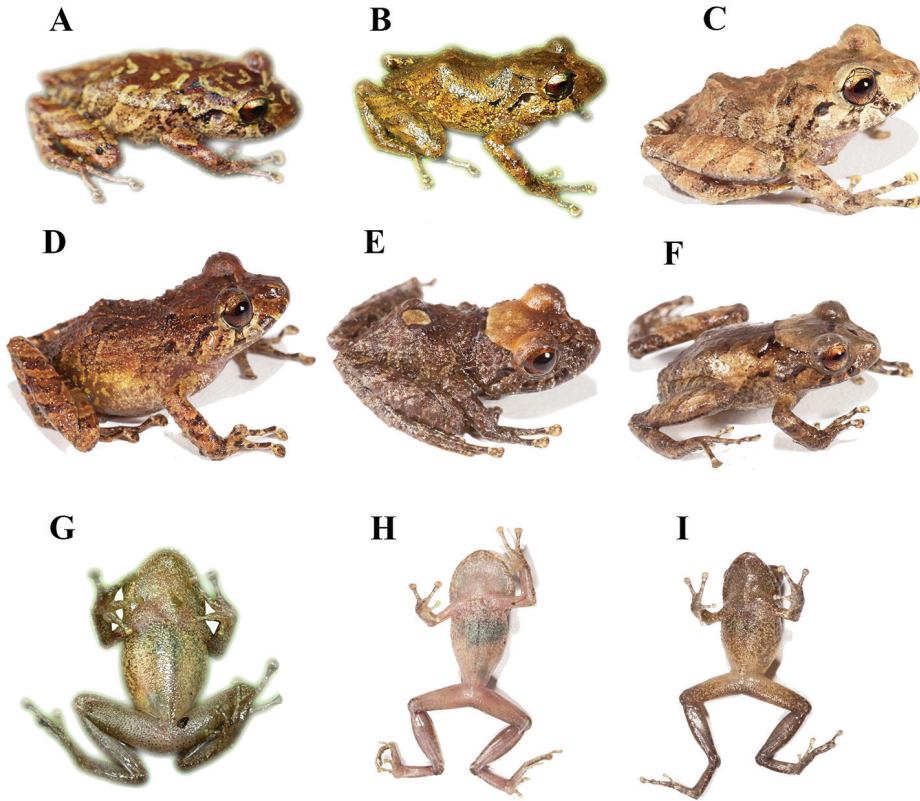


Figure 2. Coloration in life of *Pristimantis mallii* sp. n. Dorsal view. **A** ZSFQ 1305, SVL = 34.3 mm, adult female **B** DHMECN 5236, SVL = 30.9 mm, adult female **C** QCAZ 52473, SVL = 28.8 mm, holotype, adult female; Second line from left to right **D** QCAZ 52494, SVL = 29.3 mm, adult female **E** QCAZ 52512, SVL = 10.3 mm, subadult male **F** QCAZ 52474, SVL = 11.6 mm, adult male. Ventral view **G** DHMECN 5236, SVL = 30.9 mm, adult female **H** QCAZ 52473, SVL = 28.8 mm, holotype, adult female **I** QCAZ 52474, SVL = 11.6 mm, adult male. Pictures are not to scale.

Ecuador (1.40045S, 78.186776W; 1269 m elev.). ZSFQ 1306, 1327, adult males, collected by Carolina Reyes-Puig, Nicolás Dávalos, Daniel Velarde and Emilio Mancero on October 7, 2017 at Reserva Río Zuñag, Fundación EcoMinga, Baños, province of Tungurahua, Ecuador (1.36761S, 78.14583W; 2190 m elev.).

Generic placement. We assign the new species in *Pristimantis* based on our molecular data (Fig. 1).

Diagnosis. A new species of *Pristimantis* having the following combination of characters: (1) skin on dorsum and flanks shagreen, with rounded tubercles scattered towards the axillary region, with “) (” shaped scapular folds (evident in life); dorsolateral folds absent; skin on venter areolate; discoidal fold slightly defined; (2) tympanic membrane and tympanic annulus present, round, equivalent to 45% of ED; supratym-

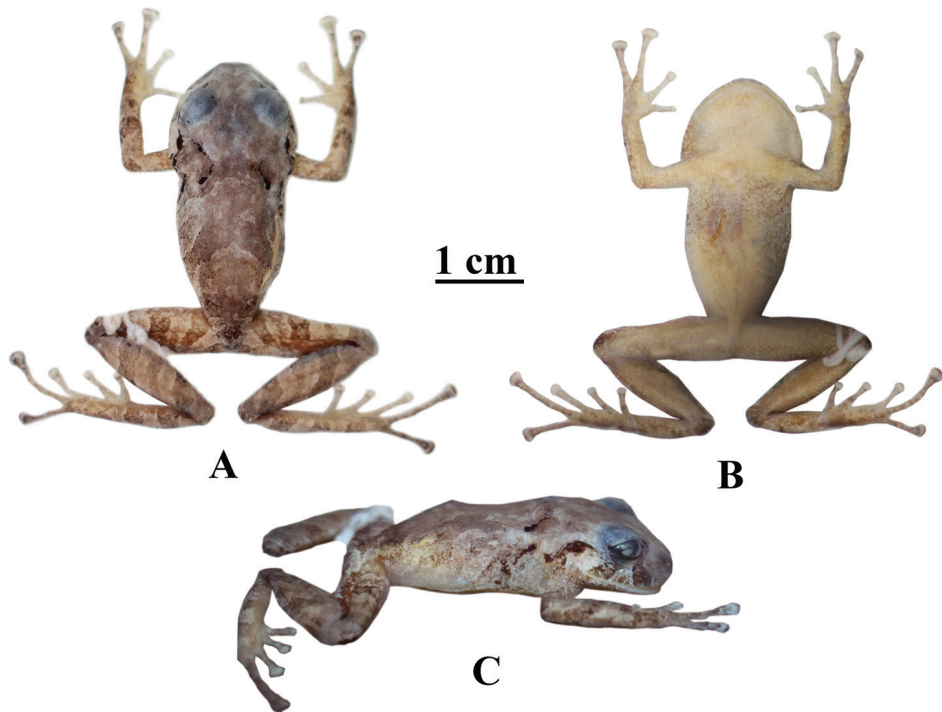


Figure 3. Preserved holotype of *Pristimantis mallii* sp. n., QCAZ 52473, adult female, SVL = 28.8 mm
A dorsal view **B** ventral view **C** lateral view.

panic fold present; (3) snout broadly rounded in dorsal view, moderate in length and rounded in lateral view; (4) upper eyelid with one or two subconical tubercles on the center of eyelid and some rounded tubercles (less evident in preserved specimens); EW 100% of IOD; cranial crests absent (5) dentigerous processes of vomers oblique in outline, with five to seven teeth, moderately separated, posteromedial to choanae; (6) vocals slits and nuptial pads present; (7) Finger I shorter than Finger II; discs of digits expanded, truncate; two times the width of the digits on Fingers III and IV; (8) fingers with lateral fringes; (9) ulnar tubercles present, rounded; (10) heel bearing one or two subconical tubercles (less evident in preserved specimens) surrounded by few lower rounded tubercles; inner tarsal fold present, it extends up to 1/4 of the tarsus; (11) inner metatarsal tubercle oval, 5–6× as large as outer metatarsal tubercle that is subconical; supernumerary plantar tubercles indistinct; (12) toes with slightly defined lateral fringes; webbing absent; Toe V longer than Toe III, disc on Toe V reach the distal sub-articular tubercle on Toe IV; (13) in life, dorsum and flanks light brown to brown, with irregular dark brown marks bounded by dirty cream, light brown or greenish cream; hidden surfaces of thighs brown splashed with dirty cream; groin with irregular yellowish marks; venter light gray or cream, spotted to densely spotted with brown. Golden coppery iris with black reticulations and a reddish-brown horizontal stripe; (14) SVL in adult males 16.7 ± 4.5 (11.6–21.3 mm); females with 27.6 ± 3.9 (22.6–34.3).

Comparisons with other species. *Pristimantis mallii* is most similar to its sister species *P. miktos* (Ortega-Andrade and Venegas 2014) (characters in parentheses) from the eastern lowlands of Ecuador. However, *P. mallii* can be easily distinguished for having “) (” shaped scapular folds (W- or X-shaped scapular fold); snout broadly rounded in dorsal view (subacuminate); upper eyelid bearing one or two subconical tubercles and some rounded tubercles (one small non-conical tubercle); dentigerous processes of vomers with 5–7 teeth (2 or 3 teeth); vocal slits in males present (absent); lateral fringes present (absent); dorsum and flanks light brown to brown, with irregular dark brown marks bounded by dirty cream, light brown or greenish cream (dorsum reddish-brown with some greenish-orange stains in scapular region, with or without yellowish-pale spots); golden coppery iris with black reticulations and a reddish-brown horizontal stripe (deep orange finely reticulated with black).

Other species of *Pristimantis* from the eastern lowlands of Ecuador, that can be confused with the new species by having dermal ridges in the scapular region, are *P. kichwarum* (Elmer and Cannatella 2008) and *P. luscombei* (Duellman and Mendelson 1995). Nonetheless, these two species have W-shaped dermal ridges (“) (” shaped fold in *P. mallii*); snout subacuminate in dorsal view (broadly rounded in *P. mallii*); ulnar tubercles absent or low (present, rounded in *P. mallii*); and nuptial pads in males absent (present in *P. mallii*). Other species of rain frogs from the eastern Andean slopes of Ecuador that are morphologically similar to *Pristimantis mallii* are *P. marcoreyesi* (Reyes-Puig et al., 2014), *P. yanezi* (Navarrete et al., 2016) and *P. spinosus* (Lynch, 1979). In males of *Pristimantis marcoreyesi*, *P. yanezi* and *P. spinosus* the vocal slits and nuptial pads are absent (present in *P. mallii*). The snout in *P. marcoreyesi* and *P. spinosus* is subacuminate in dorsal view (broadly rounded in *P. mallii*); furthermore, *P. marcoreyesi* has dorsolateral folds slightly defined (absent in *P. mallii*), *P. spinosus* has the skin of dorsum finely tuberculate (shagreen in *P. mallii*), and the groin is black enclosing white spots (groin with irregular yellowish marks in *P. mallii*). Besides, *P. yanezi* can be distinguished by having one conical tubercle on the upper eyelid (one or two subconical in *P. mallii*); discoidal fold absent (present, slightly defined in *P. mallii*); fingers and toes without lateral fringes (present in *P. mallii*); dorsum yellowish brown to dark brown with scattered pale brown or orange blotches and black flecks, bearing a faint mid-dorsal hourglass-shaped band (dorsum and flanks light brown to brown, with irregular dark brown marks bounded by dirty cream, light brown or greenish cream).

Description of the holotype. Adult female. Measurements (in mm): SVL 28.7; tibia length 15.6; foot length 15.2; head length 11.7; head width 12.8; eye diameter 4.7; tympanum diameter 2.5; interorbital distance 3.6; upper eyelid width 3.7; internarial distance 3.5; eye–nostril distance 4.6. Head slightly wider than long (12.8 mm vs 11.7); head width 44.4% of SVL; head length 41% of SVL; snout broadly rounded in dorsal view, moderate in length and rounded in lateral view (Fig. 4); eye–nostril distance 16% of SVL; canthus rostralis slightly concave in lateral view, nostrils directed laterally; interorbital area flat, as wide as the upper eyelid; cranial crests absent; upper eyelid with one or two subconical tubercles (reduced by preservation effects), and some rounded tubercles; upper eyelid width 100% of IOD; tympanic membrane differen-

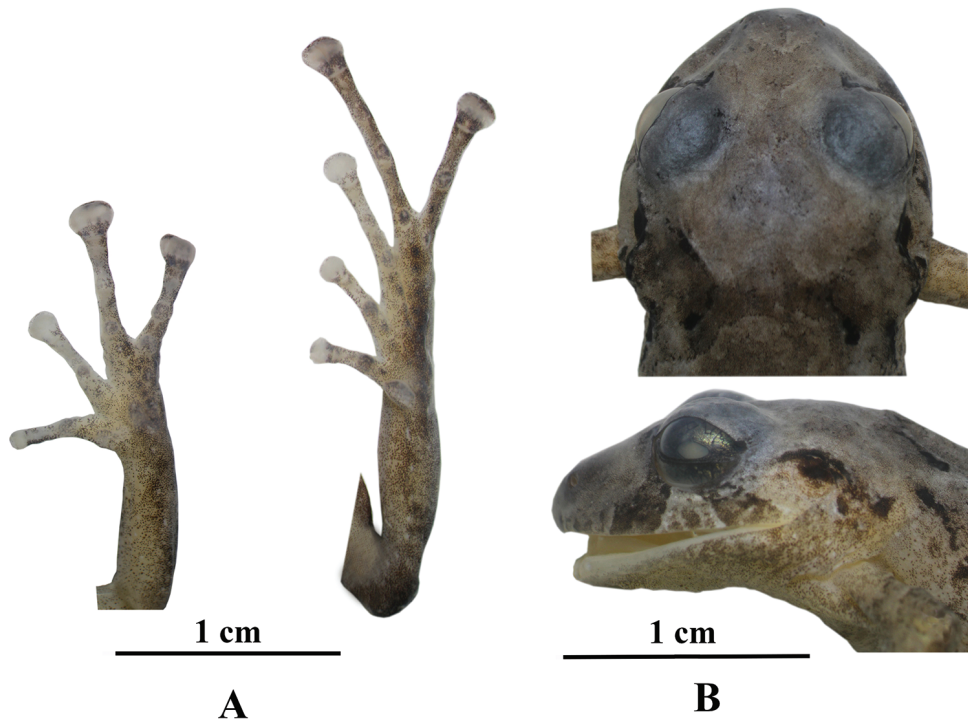


Figure 4. Preserved holotype of *Pristimantis mallii* sp. n., QCAZ 52473, adult female, SVL = 28.8 mm **A** palmar and plantar surfaces **B** dorsal and lateral views of the head.

tiated, tympanic annulus present, with upper margins covered by a supratympanic fold; tympanum diameter 54% of eye diameter; three subconical postrictal tubercles. Choanae moderately in size, with a drop-shaped outline, not concealed by palatal shelf of maxilla; dentigerous processes of vomer oblique in outline, moderately separated, posteromedial to choanae, with six to seven teeth; tongue wider than long, notched posteriorly, approximately 40% of it fixed to the mouth floor.

Skin on dorsum and flanks shagreen, with rounded tubercles scattered towards the axillary region, with “) (” shaped scapular folds (evident in life); dorsolateral folds absent; skin on venter areolate; discoidal fold slightly defined; cloaca with rounded tubercles on the inferoposterior margin. Forearms slender, ulnar tubercles present, rounded; palmar tubercle heart-shaped, bilobed, approximately twice the size of oval thenar tubercle (the tubercles are slightly defined); subarticular tubercles rounded, defined, few supernumerary tubercles, indistinct; fingers with narrow lateral fringes; Finger I shorter than Finger II; disc on Finger I rounded and on Finger II expanded, twice the width of the digits on Fingers III and IV, truncate; pads on fingers well defined by circumferential grooves on all fingers (Fig. 4).

Hindlimbs slender, tibia length 54% of SVL; foot length 53% of SVL heel; upper surfaces of hindlimbs shagreen; posterior surfaces of thighs smooth, ventral surfaces areolate; heel bearing one or two subconical tubercles (less evident by preservation

Table 1. Measurements (in mm) of type series of *Pristimantis mallii* sp. n. Ranges followed by mean and standard deviation in parentheses.

Characters	Females (n = 8)	Males (n = 12)
SVL	22.6–34.3 (27.6±3.9)	10.2–21.3 (16.7±4.5)
TL	13.1–16.0 (15.3±1.0)	8.8–11.4 (10.7±1.0)
FL	12.2–15.0 (14.3±1.2)	8.2–11.4 (9.6±1.1)
HW	9.4–14 (11.8±1.4)	5.8–8.9 (7.4±0.9)
HL	9.5–14.2 (12.1±1.4)	6.2–8.2 (8.1±0.9)
IOD	2.7–4.2 (3.6±0.4)	1.7–2.8 (2.3±0.3)
EW	3.0–4.0 (3.5±0.3)	1.9–3.2 (2.6±0.3)
IND	1.8–3.7 (3.1±0.6)	1.1–2.9 (2.1±0.5)
EN	3.1–4.6 (4.0±0.5)	2.0–2.8 (2.4±0.2)
TD	1.5–3.0 (2.1±0.5)	1.0–1.7 (1.2±0.2)
ED	3.6–5.0 (4.4±0.5)	2.7–4.1 (3.4±0.4)

effect) surrounded by few lower rounded tubercles; inner tarsal fold present, it extends up to 1/4 of the tarsus; inner metatarsal tubercle oval, 5–6× as large as outer metatarsal tubercle that is subconical; supernumerary plantar tubercles indistinct; toes with slightly defined lateral fringes; webbing absent; discs on Fingers I and II rounded, discs in Fingers III, IV and V expanded, twice the width of the digit; all toes with ventral pads well defined by circumferential grooves; Toe V longer than Toe III, disc on Toe V reach the distal subarticular tubercle on Toe IV (Fig. 4).

Color of holotype in life. (based on digital photographs, Fig. 2) Dorsum light brown with irregular brown marks bounded by dirty cream; black “)” shaped scapular fold; head with dirty cream marks, one located behind the nostrils; flanks with brown oblique stripes delineated of dirty cream, with marbled brown marks concentrated towards axillary region; with two diagonal dark brown subocular stripes. Ventral areas of body, limbs and palms cream spotted with minute brown dots; throat cream spotted with brown dots, aggregates towards the outer edge of the jaw; forearms and hindlimbs with transversal brown bars separated by light brown interspaces; posterior surfaces of thigh dark brown; groin with irregular pale-yellowish marks. Golden coppery iris with black reticulations and a reddish-brown horizontal stripe.

Color of holotype in ethanol 70%. (Fig. 3) Dorsum light brown with irregular brown marks slightly bounded by cream; black “)” shaped scapular fold; with two black longitudinal lines above and behind the tympanum; with two diagonal brown subocular stripes; dorsal surfaces limbs, fingers and toes with transversal brown bars separated by dirty cream; the anterior surfaces of flanks light brown, with marbled brown marks concentrated towards axillary region; posterior surfaces of flanks and groin grayish cream. Ventral areas of body, limbs and palms cream spotted with minute brown dots aggregates towards the outer edge of the jaw, carpus, ulnar surfaces, flanks, posterior surfaces of thigh and tarsus. Golden olive iris.

Variation. Preserved individuals (Figs 5, 6). In the type series, adult males (10.2–21.3 mm) are smaller than females (22.6–34.3). See Table 1 for measurements of the type specimens. Males have vocal slits located in the posteromedial region of the floor

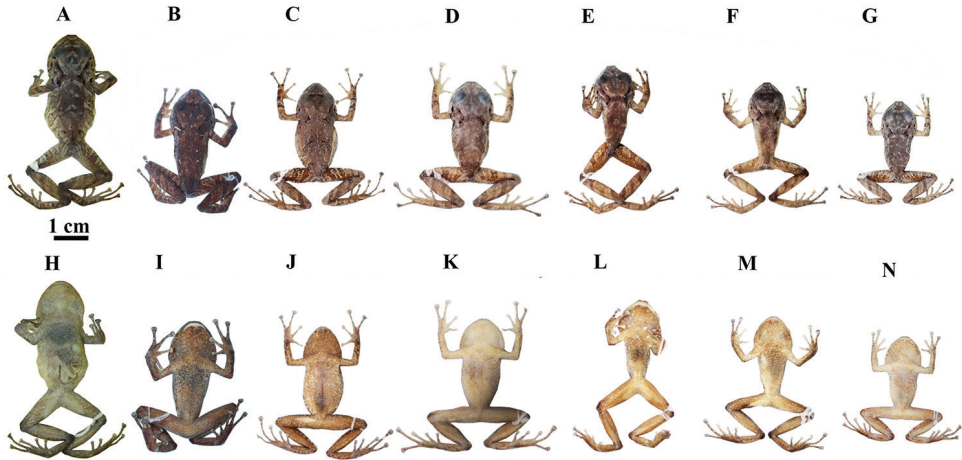


Figure 5. Preserved individuals of *Pristimantis mallii* sp. n. showing dorsal and ventral variation in adult females **A–G** dorsal view **H–N** ventral view. **A, H** ZSFQ 1305, SVL = 34.3 mm **B, I** DHMECN 5236, SVL = 30.9 mm **C, J** QCAZ 52494, SVL = 29.3 mm **D, K** QCAZ 52473, SVL = 28.8 mm, holotype **E, L** QCAZ 39777, SVL = 26.5 mm **F, M** QCAZ 52477, SVL = 24.7 mm **G, N** QCAZ 52476, SVL = 24.0 mm.

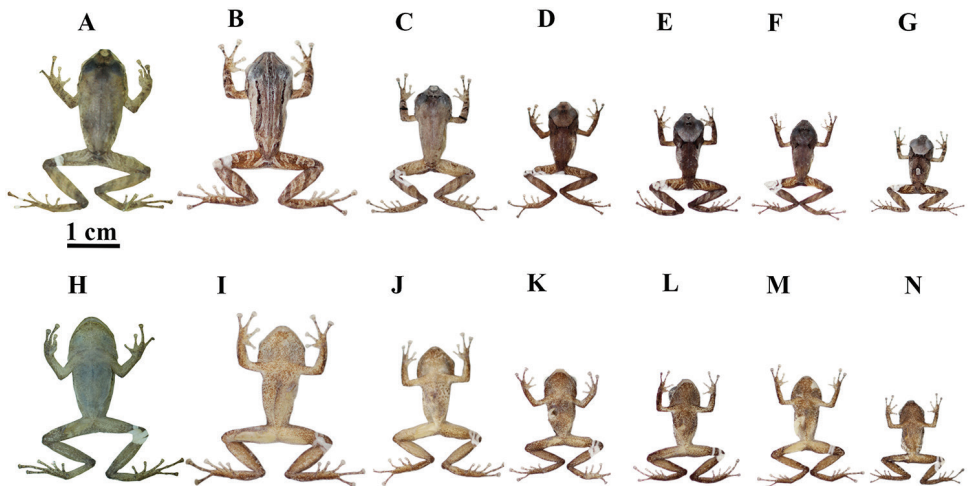


Figure 6. Preserved individuals of *Pristimantis mallii* sp. n. showing dorsal and ventral variation in males **A–G** dorsal view **H–N** ventral view. **A, H** ZSFQ 1306, SVL = 21.3 mm **B, I** QCAZ 52480, SVL = 21.1 mm **C, J** QCAZ 52481, SVL 15.6 = mm **D, K** QCAZ 52471, SVL = 12.9 mm **E, L** QCAZ 52474, SVL = 11.6 mm **F, M** QCAZ 52478, SVL = 12.2 mm **G, N** QCAZ 52512, SVL = 10.3 mm.

of the mouth; and nuptial pads located in the lower external portion of the Finger I. The “)” shaped scapular fold is present in all individuals, but is black in all females, while it is not in some males (ZSFQ 1306, QCAZ 52481) (Figs 5, 6). Background coloration varies from gray or light brown to brown. Marks on dorsum and flanks are similar in all the type series, except for the adult males (ZSFQ 1306, QCAZ 52481) that

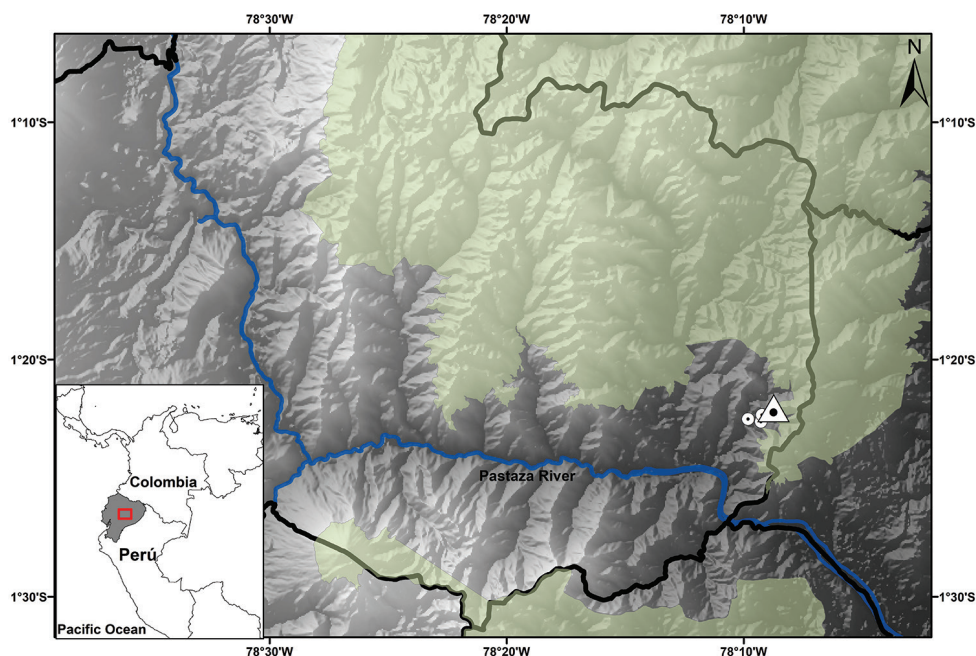


Figure 7. Map showing the four known localities for *Pristimantis mallii* sp. n. Localities are based on type specimens deposited at the QCAZ, DHMECN and ZSFQ collections. Triangle represents the holotype locality; circles represents the paratypes localities.

have a dorsum without irregular marks and exhibit an internarial cream brand (Fig. 6). Some males present a gray patch on the head between the narinal and postorbital region (QCAZ 52471, QCAZ 52474, QCAZ 52478, and QCAZ 52512); this pattern is not present in any female (Figs 5, 6). The males (QCAZ 52512 and DHMECN 5234) have a gray spot in the middorsal region (Fig. 6). One male presents a dorsal pattern with longitudinal stripes on the dorsum (QCAZ 52480) (Fig. 6). In general, males have more variable dorsal patterns than females. Ventral coloration varies from cream to light brown; from slightly spotted (ZSFQ 1305, QCAZ 52473) to roughly spotted with brown (DHMECN 5236, QCAZ 52474, QCAZ 52212) (Figs 5, 6).

Coloration in life. (based on digital photographs of the type specimens, Fig. 2).

Dorsum and flanks light brown (QCAZ 52473) to brown (QCAZ 52494, QCAZ 52512), with irregular dark brown marks bounded by dirty cream (QCAZ 52473), light brown (DHMECN 5236) or greenish cream (ZSFQ 1305) (Fig. 2); hidden surfaces of thighs brown splashed with dirty cream; groin with irregular yellowish marks; venter light gray (QCAZ 52474) or cream (QCAZ 52473) spotted to densely spotted (QCAZ 52474, DHMECN 5236, QCAZ 5212) with brown. Golden coppery iris with black reticulations and a reddish horizontal stripe (Fig. 2).

Distribution and natural history. *Pristimantis mallii* is only known from Fundación EcoMinga's Río Zuñag Ecological Reserve, which is located in the southeastern buffer zone of the Llanganates National Park, in Baños, Río Negro, Tungurahua

province, in the upper basin of the Pastaza River, on the east-central slope of the Andes in Ecuador. This locality comprises montane cloud forest (MAE 2012). The elevation range is 1300–2190 m above sea level.

All specimens were found on herbaceous and shrub vegetation inside mature forest, where they perched on herbs, shrubs, palms, ferns, bromeliads and Araceae between 100 and 450 cm above the ground. A couple in amplexus was found in January 2012, and the female deposited an egg clutch in a field bag, in the time passed between being collected in the field and reaching the base camp. Additionally, two couples in amplexus and an adult female were found in October 2017.

Etymology. The new species is named in honor of the late Dr V. N. Mallikarjuna “Malli” Rao, of Wilmington, Delaware, USA. A winner of the Lavosier Medal at DuPont, he helped develop an environmentally safe alternative to the fluorocarbons that were depleting the ozone layer. His donation to EcoMinga in 2007 started the Río Zuñag Reserve, the type locality of *P. mallii*.

Discussion

Pristimantis mallii is part of a clade of *Pristimantis* distributed in the Andes of central and southern Ecuador. The only non-Andean species of the group is *P. miktos*, which occurs in the Amazon basin below 300 m. We refrain from assigning the new species to a named species group. Most species groups in *Pristimantis* have been shown to be non-monophyletic, especially the large *P. unistrigatus* group (sensu Hedges et al. 2008). Of note, the clade presented in this paper is distributed in Andean forests, has a medium-sized SVL, lives in shrubby habits, and the majority of species have cryptic dorsal colorations and irregular diffuse flash marks on the hidden surfaces of the groin (Lynch 1979; Ortega-Andrade and Venegas 2014; Yáñez-Muñoz et al. 2015; Ron et al. 2018).

The upper basin of the Pastaza River has proven to be a priority area for the conservation of *Pristimantis* due to its high alpha and beta diversity and high endemism (Reyes-Puig et al. 2013, 2014). In the last decade, nine species of *Pristimantis* have been described in this important region (Reyes-Puig et al. 2010, 2013, 2014, 2015; Yáñez-Muñoz et al. 2010; Reyes-Puig and Yáñez-Muñoz 2012). The discovery of *P. mallii*, represents the tenth species of *Pristimantis* discovered and described from the upper basin of the Pastaza River after one decade of herpetological research by the Instituto Nacional de Biodiversidad and the EcoMinga Foundation. These discoveries have helped biodiversity conservation outside government protected areas. The upper basin of the Pastaza River is a region with high diversity and endemism of several vertebrates (Reyes-Puig et al. 2013; Ríos-Alvear and Reyes-Puig 2015; Rodríguez-Galarza et al. 2017), but also of plants (e.g. Jost and Shepard 2017). The EcoMinga Foundation’s reserves function as a corridor between two large National Parks (i.e. Llanganates and Sangay) and contribute to the protection and connectivity of this important area. Coincidentally, this tenth new species of *Pristimantis* corresponds to ten years of herpetological research by EcoMinga which now manages ten ecological reserves. The total area protected within those reserves approximates 10,000 hectares.

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References

- Arteaga-Navarro AF, Guayasamin JM (2011) A new frog of the genus *Pristimantis* (Amphibia: Strabomantidae) from the high Andes of southeastern Ecuador, discovered using morphological and molecular data. *Zootaxa* 2876(1): 17–29. <https://doi.org/10.11646/zootaxa.2876.1.2>
- Arteaga A, Bustamante L, Guayasamin JM (2013) The Amphibians and Reptiles of Mindo: Life in the Cloudforest. Universidad Tecnológica Indoamérica, Quito, 258 pp.
- Arteaga A, Pyron RA, Peñafiel N, Romero-Barreto P, Culebras J, Bustamante L, Yanez-Muñoz MH, Guayasamin JM (2016) Comparative phylogeography reveals cryptic diversity and repeated patterns of cladogenesis for amphibians and reptiles in northwestern Ecuador. *Plos ONE* 11(4): e0151746. <https://doi.org/10.1371/journal.pone.0151746>
- Barrio-Amorós CL, Heinicke MP, Hedges SB (2013) A new tuberculated *Pristimantis* (Anura, Terrarana, Strabomantidae) from the Venezuelan Andes, redescription of *Pristimantis pleurostriatus*, and variation within *Pristimantis vanadisae*. *Zootaxa* 3647(1): 43–62. <https://doi.org/10.11646/zootaxa.3647.1.2>
- Batallas D, Brito J (2014) Nueva especie de rana del género *Pristimantis* del grupo lacrimosus (Amphibia: Craugastoridae) del Parque Nacional Sangay, Ecuador. *Papéis Avulsos de Zoologia* 54(5): 51–62. <https://doi.org/10.1590/0031-1049.2014.54.05>
- Brito J, Batallas D, Velalcázar D (2014) Nueva especie de rana terrestre del género *Pristimantis* (Amphibia: Craugastoridae), meseta de la Dordillera del Cóndor. *Papéis Avulsos de Zoología* 54(30): 435–466. <https://doi.org/10.1590/0031-1049.2014.54.30>

- Brito J, Ojala-Barbour R, Batallas RD, Almendáriz CA (2016) A new species of *Pristimantis* (Amphibia: Strabomantidae) from the cloud forest of Sangay National Park, Ecuador. *Journal of Herpetology* 50 (2): 337–344. <https://doi.org/10.1670/13-103>
- Brito J, Almendariz AC, Batallas D, Ron SR (2017a) Nueva especie de rana bromelícola del género *Pristimantis* (Amphibia: Craugastoridae), meseta de la Cordillera del Cóndor, Ecuador. *Papéis Avulsos de Zoología* 57 (15): 177–195. <https://doi.org/10.11606/0031-1049.2017.57.15>
- Brito J, Batallas D, Yáñez-Muñoz MH (2017b) Ranas terrestres *Pristimantis* (Anura: Craugastoridae) de los bosques montanos del río Upano, Ecuador: lista anotada, patrones de diversidad y descripción de cuatro especies nuevas. *Neotropical Biodiversity* 3(1): 125–156. <https://doi.org/10.1080/23766808.2017.1299529>
- Chávez G, Catenazzi A (2016) A new species of frog of the genus *Pristimantis* from Tingo María National Park, Huánuco Department, central Peru (Anura, Craugastoridae). *ZooKeys* 610: 113–130. <https://doi.org/10.3897/zookeys.610.8507>
- Crawford AJ, Cruz C, Griffith E, Ross H, Ibáñez R, Lips KR, Driskell AC, Bermingham E, Crump P (2013) DNA barcoding applied to ex situ tropical amphibian conservation programme reveals cryptic diversity in captive populations. *Molecular Ecology Resources* 13(6): 1005–1018. <https://doi.org/10.1111/1755-0998.12054>
- Darst CR, Cannatella DC (2004) Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 31(2): 462–475. <https://doi.org/10.1016/j.ympev.2003.09.003>
- De Oliveira EA, Rodrigues LR, Kaefer IL, Pinto KC, Hernández-Ruz EJ (2017) A new species of *Pristimantis* from eastern Brazilian Amazonia (Anura, Craugastoridae). *ZooKeys* 687: 101–129. <https://doi.org/10.3897/zookeys.687.13221>
- Duellman WE, Mendelson JR (1995) Amphibians and reptiles from northern Departamento Loreto, Peru: taxonomy and biogeography. *The University of Kansas Science Bulletin* 55: 329–376. <https://doi.org/10.5962/bhl.part.779>
- Duellman WE, Lehr E (2009) Terrestrial-breeding Frogs (Strabomantidae) in Peru. *Natur Und Tier-Verlag, Norwich*, 384pp.
- Elmer KR, Dávila JA, y Loughheed SC (2007) Cryptic diversity and deep divergence in an upper Amazonian leaf litter frog, *Eleutherodactylus ockendeni*. *BMC Evolutionary Biology* 7(1): 247. <https://doi.org/10.1186/1471-2148-7-247>
- Elmer KR, Cannatella DC (2008) Three new species of leaf litter frogs from the upper Amazon forests: cryptic diversity within *Pristimantis* “ockendeni” (Anura: Strabomantidae) in Ecuador. *Zootaxa* 1784: 11–38.
- Esselstyn JA, García HJD, Saulog MG, Heaney LR (2008) A new species of *Desmalopex* (Pteropodidae) from the Philippines, with a phylogenetic analysis of the Pteropodini. *Journal of Mammalogy* 89(4): 815–825. <https://doi.org/10.1644/07-MAMM-A-285.1>
- Faivovich J, Haddad CFB, Garcia PC, Frost DR, Campbell JA, Wheeler WC (2005) Systematic review of the frog family Hylidae, with special reference to Hyalinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History* 294(1): 240. [https://doi.org/10.1206/0003-0090\(2005\)294\[0001:SROTF\]2.0.CO;2](https://doi.org/10.1206/0003-0090(2005)294[0001:SROTF]2.0.CO;2)
- Fouquet A, Gilles A, Vences M, Marty C, Blanc M (2007) Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. *PLoS ONE* 2(10): e1109. <https://doi.org/10.1371/journal.pone.0001109>

- Fouquet A, Noonan BP, Rodrigues MT, Pech N, Gilles A, Gemmell NJ (2012) Multiple Quaternary refugia in the Eastern Guiana Shield revealed by comparative phylogeography of 12 frog species. *Systematic Biology* 61: 461–489. <https://doi.org/10.1093/sysbio/syr130>
- Frost DR (2018) *Amphibian Species of the World: an Online Reference*. Version 6.0. American Museum of Natural History, New York. <http://research.amnh.org/herpetology/amphibia> [Accessed on: 2017-2-11]
- García-R JC, Crawford AJ, Mendoza ÁM, Ospina O, Cárdenas H, Castro F (2012) Comparative phylogeography of direct-developing frogs (Anura: Craugastoridae: *Pristimantis*) in the southern Andes of Colombia. *PLoS ONE* 7(9): 1–9. <https://doi.org/10.1371/journal.pone.0046077>
- González-Durán GA, Targino M, Rada M, Grant T (2017) Phylogenetic relationships and morphology of the *Pristimantis leptolophus* species group (Amphibia: Anura: Brachycephaloidea), with the recognition of a new species group in *Pristimantis* Jiménez de la Espada, 1870. *Zootaxa* 4243(1): 42–74. <https://doi.org/10.11646/zootaxa.4243.1.2>
- Gradstein SR, Reiner-Drehwald ME, Jost L (2004) The systematic position and distribution of *Myriocolea irrorata* (Lejeuneaceae), an endangered liverwort of the Ecuadorian Andes. *The Journal of the Hattori Botanical Laboratory* 95: 235–248.
- Guayasamin JM, Krynak T, Krynak K, Culebras J, Hutter CR (2015) Phenotypic plasticity raises questions for taxonomically important traits: a remarkable new Andean rainfrog (*Pristimantis*) with the ability to change skin texture. *Zoological Journal of the Linnean Society* 173(4): 913–928. <https://doi.org/10.1111/zoj.12222>
- Guayasamin JM, Hutter CR, Tapia EE, Culebras J, Peñafiel N, Pyron RA, Morochz C, Funk C, Arteaga A (2017) Diversification of the rainfrog *Pristimantis ornatissimus* in the lowlands and Andean foothills of Ecuador. *PLoS ONE* 12(3): e0172615. <https://doi.org/10.1371/journal.pone.0172615>
- Hedges SB, Duellman WE, Heinicke MP (2008) New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737(1): 1–182.
- Heinicke MP, Duellman WE, Hedges SB (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academy of Sciences* 104(24): 10092–10097. <https://doi.org/10.1073/pnas.0611051104>
- Heinicke MP, Duellman WE, Trueb L, Means BD, Macculloch RD, Hedges SB (2009) A new frog family (Anura: Terrarana) from South America and an expanded direct-developing clade revealed by molecular phylogeny. *Zootaxa* 2211: 1–35.
- Heinicke MP, Barrio-Amorós CL, Hedges SB (2015) Molecular and morphological data support recognition of a new genus of New World direct-developing frog (Anura: Terrarana) from an under-sampled region of South America. *Zootaxa* 3986(2): 151–172. <https://doi.org/10.11646/zootaxa.3986.2.1>
- Hutter CR, Guayasamin JM (2015) Cryptic diversity concealed in the Andean cloud forests: two new species of rainfrogs (*Pristimantis*) uncovered by molecular and bioacoustic data. *Neotropical Biodiversity* 1(1): 36–59. <https://doi.org/10.1080/23766808.2015.1100376>
- Jablonski D, Gruľa D, Barrio-amorós CL, Kok PJR (2017) Correspondence Molecular phylogenetic relationships among *Pristimantis* summit populations in the eastern tepui chain: insights from *P. aureoventris* (Anura : Craugastoridae). *Salamandra* 53(3): 473–478.

- Jost L, Shepard A (2017) Four new *Teagueia* (Orchidaceae: Pleurothallidinae) from the upper río Pastaza watershed of east-central Ecuador. *Lankesteriana* 17(2): 261–278. <https://doi.org/10.15517/lank.v17i2.30159>
- Kennerly J, Bromley R (1971) Geology and Geomorphology of the Llanganati Mountains, Ecuador. Editorial Minerva, Quito, 16 pp.
- Kok PJR, Dezfoulan R, Means DB, Fouquet A, Barrio-Amorós CL (2018) Amended diagnosis and redescription of *Pristimantis marmoratus* (Boulenger, 1900) (Amphibia: Craugastoridae), with a description of its advertisement call and notes on its breeding ecology and phylogenetic relationships. *European Journal of Taxonomy* 397: 1–30. <https://doi.org/10.5852/ejt.2018.397>
- Kok PJR, MacCulloch RD, Means DB, Roelants K, Van Boclaer I, Bossuyt F (2012) Low genetic diversity in tepui summit vertebrates. *Current Biology* 22(15): R589–R590. <https://doi.org/10.1016/j.cub.2012.06.034>
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Lehr E, Moravec J, Cusi JC (2012) Two new species of *Phrynopus* (Anura, Strabomantidae) from high elevations in the Yanachaga-Chemillén National park in Peru (Departamento de Pasco). *ZooKeys* 235: 51–71. <https://doi.org/10.3897/zookeys.235.3885>
- Lehr E, Moravec J, Cusi JC, Gvoždík V (2017) A new minute species of *Pristimantis* (Amphibia: Anura: Craugastoridae) with a large head from the Yanachaga-Chemillén National Park in central Peru, with comments on the phylogenetic diversity of *Pristimantis* occurring in the Cordillera Yanachaga. *European Journal of Taxonomy* 325: 1–22. <https://doi.org/10.5852/ejt.2017.325>
- Lehr E, Von May R (2017) A new species of terrestrial-breeding frog (Amphibia, Craugastoridae, *Pristimantis*) from high elevations of the Pui Pui protected forest in Central Peru. *ZooKeys* 660: 17–42. <https://doi.org/10.3897/zookeys.660.11394>
- Lynch JD (1979) Leptodactylid frogs of the genus *Eleutherodactylus* from the Andes of southern Ecuador. Lawrence: Kansas University. <https://doi.org/10.5962/bhl.title.16268>
- Lynch JD, Duellman WE (1980) The *Eleutherodactylus* of the Amazonian slopes of the Ecuadorian Andes (Anura: Leptodactylidae). *Miscellaneous Publication* (University of Kansas, Museum of Natural History) 69: 1–86. <https://doi.org/10.5962/bhl.title.16222>
- Lynch JD, Duellman WE (1997) Frogs of the Genus *Eleutherodactylus* in Western Ecuador. University of Kansas, Natural History Museum, Special Publication 23: 1–236. <https://doi.org/10.5962/bhl.title.7951>
- Maddison WP, Maddison DR (2011) Mesquite: a Modular System for Evolutionary Analysis. Version 3.10. <http://mesquiteproject.org> [Accessed on: 2017-05-01]
- MAE [Ministerio del Ambiente del Ecuador] (2012) Sistema de clasificación de los ecosistemas del Ecuador continental. Subsecretaría de Patrimonio Natural, Quito, 32 pp.
- Navarrete MJ, Venegas PJ, Ron SR (2016) Two new species of frogs of the genus *Pristimantis* from Llanganates National Park in Ecuador with comments on the regional diversity of Ecuadorian *Pristimantis* (Anura, Craugastoridae). *ZooKeys* 593: 139–162. <https://doi.org/10.3897/zookeys.593.8063>
- Ortega-Andrade HM, Venegas PJ (2014) A new synonym for *Pristimantis luscombei* (Duellman and Mendelson 1995) and the description of a new species of *Pristimantis* from the up-

- per Amazon basin (Amphibia: Craugastoridae). *Zootaxa* 3895(1): 31–57. <https://doi.org/10.11646/zootaxa.3895.1.2>
- Padial JM, Grant T, Frost DR (2014) Molecular systematic of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa* 3825: 1–132. <https://doi.org/10.11646/zootaxa.3825.1.1>
- Pinto-Sánchez NR, Ibáñez R, Madrián S, Sanjur OI, Bermingham E, Crawford AJ (2012) The Great American Biotic Interchange in frogs: multiple and early colonization of Central America by the South American genus *Pristimantis* (Anura: Craugastoridae). *Molecular Phylogenetics and Evolution* 62(3): 954–972. <https://doi.org/10.1016/j.ympev.2011.11.022>
- Rambaut A, Drummond AJ (2007) Tracer v1.6. University of Edinburgh. <http://beast.bio.ed.ac.uk/Tracer>
- Reyes-Puig JP, Yáñez-Muñoz MH, Cisneros-Heredia DF, Ramírez-Jaramillo SR (2010) Una nueva especie de rana *Pristimantis* (Terrarana: Strabomantidae) de los bosques nublados de la cuenca alta del río Pastaza, Ecuador. *ACI Avances en Ciencias e Ingenierías* 2(3): B78–B82. <https://doi.org/10.18272/aci.v2i3.48>
- Reyes-Puig JP, Yáñez-Muñoz MH (2012) Una nueva especie de *Pristimantis* (Anura: Craugastoridae) del corredor ecológico Llangantes-Sangay, Andes de Ecuador. *Papéis Avulsos de Zoología* 52(6): 81–91. <https://doi.org/10.1590/S0031-10492012000600001>
- Reyes-Puig MM, Reyes-Puig JP, Yáñez-Muñoz MH (2013) Ranas terrestres del género *Pristimantis* (Anura: Craugastoridae) de la Reserva Ecológica Río Zúñag, Tungurahua, Ecuador: Lista anotada y descripción de una especie nueva. *ACI Avances en Ciencias e Ingenierías* 5(2): B5–B13. <https://doi.org/10.18272/aci.v5i2.133>
- Reyes-Puig JP, Reyes-Puig CP, Ramírez-Jaramillo SR, Pérez-Lara MB, Yáñez-Muñoz MH (2014) Tres nuevas especies de ranas terrestres *Pristimantis* (Anura: Craugastoridae) de la cuenca alta del Río Pastaza, Ecuador. *ACI Avances en Ciencias e Ingenierías* 6(2): B51–B62. <https://doi.org/10.18272/aci.v6i2.179>
- Reyes-Puig JP, Reyes-Puig CP, Pérez-Lara MB, Yáñez-Muñoz MH (2015) Dos nuevas especies de ranas *Pristimantis* (Craugastoridae) de la cordillera de los Sacha Llanganatis, vertiente oriental de los Andes de Ecuador. *ACI Avances en Ciencias e Ingenierías* 7(2): B61–B74. <https://doi.org/10.18272/aci.v7i2.258>
- Ríos-Alvear GR, Reyes-Puig C (2015) Corredor ecológico Llanganates-Sangay: un acercamiento hacia su manejo y funcionalidad. *Yachana Revista Científica* 4(2):11–21.
- Rivera-Correa M, Daza JM (2015) Molecular phylogenetics of the *Pristimantis lacrimosus* species group (Anura: Craugastoridae) with the description of a new species from Colombia. *Acta Herpetologica* 10(2): 129–134. https://doi.org/10.13128/Acta_Herpetol-16434
- Rivera-Prieto DA, Rivera-Correa M, Daza JM (2014) A new colorful species of *Pristimantis* (Anura: Craugastoridae) from the eastern flank of the Cordillera Central in Colombia. *Zootaxa* 3900(2): 223–242. <https://doi.org/10.11646/zootaxa.3900.2.3>
- Robert EC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–97. <https://doi.org/10.1093/nar/gkh340>
- Rodríguez-Galarza FE, Valdiviezo-Rivera J, Reyes-Puig JP, Yáñez-Cajo DJ (2017) Ictiofauna de los ríos Zúñag y Anzu en el Corredor Ecológico Llanganates–Sangay, Provincias de Pastaza y Tungurahua, Ecuador. *Serie Zoológica* 12(13): 33–52.

- Ron SR, Yanez-Muñoz MH, MerinoViteri A, Ortiz DA (2018) Anfibios del Ecuador. Version 2018.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <https://bioweb.bio/faunaweb/amphibiaweb> [Accessed on: 2018-7]
- Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Sánchez D, Merlo J, Haro R, Acosta M, Bernal G (2018) Soils from the Amazonia. In: Espinosa J, et al. (Eds) *The Soils of Ecuador – World Soils Book Series*. Springer, Cham, 113–138. https://doi.org/10.1007/978-3-319-25319-0_4
- Shepack A, Von May R, Tito A, Catenazzi A (2016) A new species of *Pristimantis* (Amphibia, anura, craugastoridae) from the foothills of the Andes in Manu National Park, Southeastern Peru. *ZooKeys* 594: 143–164. <https://doi.org/10.3897/zookeys.594.8295>
- Székely P, Cogălniceanu D, Székely D, Páez N, Ron SR (2016) A new species of *Pristimantis* from southern Ecuador (Anura, Craugastoridae). *ZooKeys* 606: 77–97. <https://doi.org/10.3897/zookeys.606.9121>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Terán-Valdez A, Guayasamin JM (2010) The smallest terrestrial vertebrate of Ecuador: A new frog of the genus *Pristimantis* (Amphibia: Strabomantidae) from the Cordillera del Cóndor. *Zootaxa* 2447: 53–68. <https://doi.org/10.11646/zootaxa.2447.1.2>
- von May R, Catenazzi A, Corl A, Santa-Cruz R, Carnaval AC, Moritz C (2017) Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecology and Evolution* 7(9): 3257–3267. <https://doi.org/10.1002/ece3.2929>
- Yáñez-Muñoz MH, Cisneros-Heredia DF, Reyes-Puig JP (2010) Una nueva especie de rana terrestre *Pristimantis* (Anura: Terrarana: Strabomantidae) de la cuenca alta del Río Pastaza, Ecuador. *ACI Avances en Ciencias e Ingenierías* 2(3): 28–32. <https://doi.org/10.18272/aci.v2i3.41>
- Yáñez-Muñoz MH, Bejarano-Muñoz P, Brito J, Batallas D (2014) Ranas terrestres de los Andes Surorientales de Ecuador II: Una nueva especie de *Pristimantis* verde espinosa de los bosques montanos del Parque Nacional Sangay (Anura: Craugastoridae). *ACI Avances en Ciencias e Ingenierías* 6(2): B63–B77. <https://doi.org/10.18272/aci.v6i2.180>
- Yáñez-Muñoz M, Sánchez-Nivicela JC, Reyes-Puig C (2016) Tres nuevas especies de ranas terrestres *Pristimantis* (Anura: Craugastoridae) de la Provincia de El Oro, Ecuador. *ACI Avances en Ciencias e Ingenierías* 8(1): 5–25. <https://doi.org/10.18272/aci.v8i1.455>
- Zhang P, Liang D, Mao RL, Hillis DM, Wake DB, Cannatella DC (2013) Efficient sequencing of anuran mtDNAs and a mitogenomic exploration of the phylogeny and evolution of frogs. *Molecular Biology and Evolution* 30(8): 1899–1915. <https://doi.org/10.1093/molbev/mst091>

Appendix I

Additional specimens examined

***Pristimantis kichwarum*: Ecuador, Orellana:** QCAZ 22679, 22680, Parque Nacional Yasuní, 265 m; QCAZ 54894, Parque Nacional Yasuní, 290 m; QCAZ 20447, Parque Nacional Yasuní, vía Pompeya sur, 230 m; QCAZ 56572, Parque Nacional Ysauní, Tambococha, 203 m. ***Pristimantis luscombei*: Ecuador, Pastaza:** QCAZ 25456–25463, Kapawi Lodge, 239 m; QCAZ 54019, 54021– 54023, Curaray, 240 m; **Perú, Loreto:** QCAZ 55640, 55649, 55650, Curaray Paiche, 200 m. ***Pristimantis marcoreyesi*: Ecuador, Tungurahua:** DHMECN 11343m Patate, Río Alisal, 3131 m; DHMECN 4816, 4819, 4822, Baños, Nahuazo Runtún, 2720 m. DHMECN 4825, 4830, San Antonio, Río Pucayacu, 2500 m; DHMECN 4818, 4823, 4824, 5084, Bosque Protector Cerro La Candelaria, 2700 m. ***Pristimantis miktos*: Ecuador, Morona Santiago:** QCAZ 53272, 53273, Tukupi, 211 m; Orellana: QCAZ 55445, Parque Nacional Yasuní, 175 m; QCAZ 49228– 49229, Parque Nacional Yasuní, 230 m; **Pastaza:** QCAZ 53581, 53582, Juyuintza, 200 m; QCAZ 54987, Lorocachi, 200 m; **Perú, Loreto:** QCAZ 55639, 55644, 55646, Curaray Paiche, 200 m. ***Pristimantis yanezi*: Ecuador, Napo:** QCAZ 46156, 46229, Tena, Carretera Salcedo Tena, 2253 m; QCAZ 46257–46259, Tena, Vía Salcedo Tena km 60, 2095 m; QCAZ 70089–70090, Tena Parque Nacional Llanganates, 2347 m; **Pastaza:** 66385, 66541, 66546, 66549, Mera, Reserva Comunitaria Ankaku, 2216 m.

Two new species in the genus *Kuvera* Distant, 1906 (Hemiptera, Cixiidae, Cixiinae) from China

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Abstract

Two new species (*Kuvera huoditangensis* sp. n. and *Kuvera longwangshanensis* sp. n.) in the family Cixiidae from China are described and illustrated. The generic characteristics are redefined. A checklist to all species of *Kuvera* worldwide and an identification key to the Chinese species are provided. A map of the geographic distribution of *Kuvera* species is also provided.

Keywords

Auchenorrhyncha, Fulgoroidea, morphology, new species, planthopper, taxonomy

Introduction

Cixiidae is the largest family of planthoppers in the world (slightly larger than the Delphacidae), with nearly 2500 described species (Bourgoin 2018). Some cixiids are economically important pests that feed on crops and vector plant pathogens such as: *Hyalosthes obsoletus* Signoret, 1865, *Reptalus (Proreptalus) quinquecostatus* (Dufour, 1833) and *Myndus taffini* Bonfild, 1983, causing serious economic losses (Julia 1982; Sforza et al. 2010; Pinzauti et al. 2010). Even though this family is very large and important, Cixiidae from the Oriental Region has not been studied extensively.

The genus *Kuvera* Distant, 1906 is a member of the tribe Semonini of the subfamily Cixiinae (Hemiptera: Cixiidae). Semonini are characterized by a swollen postclypeus, a convex clypeofrontal suture, and the median carina of frons is incomplete or obscure (Holzinger et al. 2002). Currently, this genus contains 21 species worldwide (Bourgoin 2018). Members of this genus are distributed in China, Korea, Japan, Russia, India, Myanmar and Afghanistan (Distant 1906; Matsumura 1914; Tsaur et al. 1991; Lee and Kwon 1977; Anufriev 2009; Rahman et al. 2017). Previously, nine species in this genus have been recorded from China with eight of them occurring in Taiwan (Matsumura 1914; Tsaur et al. 1991). The Chinese species include: *K. communis* (Tsaur & Hsu, 1991), *K. hama* (Tsaur & Hsu, 1991), *K. laticeps* (Metcalf, 1936), *K. longipennis* (Matsumura, 1914), *K. similis* (Tsaur & Hsu, 1991), *K. taiwana* (Tsaur & Hsu, 1991), *K. tappanella* (Matsumura, 1914), *K. toroensis* (Matsumura, 1914) and *K. transversa* (Tsaur & Hsu, 1991). Since the 1991 study, no further taxonomic work has been done on the genus *Kuvera* in China.

In this paper, we describe and illustrate two new Chinese species of the genus *Kuvera*: *K. huoditangensis* sp. n. and *K. longwangshanensis* sp. n., and we found *K. vilbastei* Anufriev, 1987 for the first time in Tibet, China. We also have provided an amended genus description. A checklist to all worldwide species of *Kuvera* is provided as well as a map of their geographic distribution. We also have developed a key for the Chinese species of *Kuvera*. Differences between *K. flaviceps* (Matsumura, 1900) and *K. longwangshanensis* sp. n. are briefly described.

Materials and methods

All materials, including holotypes of the new species, were deposited in the Entomological Museum of Northwest A&F University (NWAUFU), Yangling, Shaanxi Province, China. Most of their geographical distribution data is based on the localities recorded in the literature, and the rest of the data is based on the collection localities of the specimens examined, which are deposited in Entomological Museum of NWAUFU. The updated distribution data is presented in the checklist and on the map.

The morphological terminology and measurements follow Bourgoin et al. (2015) for the venation patterns of the tegmen and Tsaur et al. (1988), Löcker et al. (2006) and Bourgoin (1993) for male and female genitalia.

Measurements of external body length are the distance between the apex of the vertex to the tip of the forewing. Measurements of the vertex length are the distance between the apical transverse carina to the most caudal limits of the vertex.

External morphology was observed using a light LEICA Zoom 2000 microscope. To prepare male genitalia for dissection, specimens were softened for 12h in a humid glass cylinder. The genital segments of specimens were then dissected and macerated in hot 10% NaOH solution overnight or by boiling for 3 to 5 min. The genital segments were then rinsed in distilled water and transferred into PVC microvials containing glycerol. Tissues were immersed in glycerin on slides for drawing. The anal segment

and pygofer were drawn. Images were made using a LEICA MZ12.5 stereoscope fitted with a drawing tube and mirror. Photographs of specimens were taken with a Scientific Digital micrography system equipped with an Auto-montage imaging system and a QIMAGING 4000R digital camera (CCD) and imported into Adobe Photoshop CC for labeling and plate composition.

Taxonomy

Family Cixiidae Spinola, 1839

Subfamily Cixiinae Spinola, 1839

Tribe Semonini Emeljanov, 2002

Genus *Kuvera* Distant, 1906

Kuvera Distant, 1906: 261.

Type species. *Kuvera semihyalina* Distant, 1906.

Diagnosis. Total length varies from 4.7–7.3mm. Body coloration black to yellowish brown. Head including eyes narrower than pronotum. Vertex brown with yellow carinae and borders. Vertex short, wider than long, anterior margin of vertex obscure, with only residual traces. Vertex narrowest at subapical carina, widening towards anterior and posterior margins. Anterior and posterior margins wide and parabolic, almost parallel (Figs 1, 4, 23, 26). Frons prominent, median carina only distinct on basal portion, not reaching the anterior margin of vertex. Both sides of frons usually with yellow to brown stripe, lateral carina slightly elevated, median ocellus small. Frontoclypeal suture sub-semicircular curved upward. Clypeus swollen, postclypeus with prominent median carina, anteclypeus carina sharp or arcuate. Rostrum just reaching hind coxae, apically black (Figs 2, 24). Pronotum small, tapered with obvious carinae and distinct lateral carinae, strongly incised in middle. Mesonotum with three distinct carinate (Figs 1, 4, 23, 26). Tegmina hyaline to semi-hyaline with small granules, slender and longer than abdomen, tectiform. Forewings with a small irregular, roundish spot on anterior branch of Y-vein. Venation pattern: Scp+R usually forked distad of CuA. RP 3-branched, MP with 4 or 5 terminals, CuA 2 or 3-branched, with 10–11 apical cells (Figs 1, 3, 23, 25). Legs yellow, generally 2–4 tibial lateral spines. Hind tibia with 6 apical spines; chaetotaxy of hind tarsi: 7/ (7–8), 2nd tarsal segment with many platellae.

Male terminalia. Pygofer with a triangular medioventral process (Figs 5, 14, 27, 36). Anal segment with a rounded or concave posterior margin (Figs 7, 16, 29, 38, 46). Aedeagus with 2 spinose processes arising near base of flagellum, and flagellum with 1–2 spinose processes. Periandrium almost flat and widened at base. In ventral view, caudal margin of basal segment of periandrium convex, lateral apical angle with two teeth near distal portion (Figs 13, 19, 32, 41).

Female terminalia. Structurally variable among the included species. Ovipositor elongate, orthopteroid and apically curved upwards. 7th sternite (pre-genital sternite) small. Abdominal 9th tergite with a distinct and elliptic wax plate.

Remarks. This genus is similar to the genus *Betacixius* Matsumura, 1914, but can be separated by the following features: Forewings with a small irregular, roundish spot on the anterior branch of the Y-vein, but in *Betacixius*, forewings with a stripe on the anterior branch of the CuA to the posterior portion of A₂, and a dark long stripe on the nodal lines; one sharp process at about the mid-length of the aedeagal flagellum, but in *Betacixius*, the apex of the flagellum with a sharp process.

Distribution. China (Tibet, Shaanxi, Sichuan, Zhejiang, Taiwan), Korea, Japan, Russia, India, Myanmar, Afghanistan.

Checklist and distributions of the species of *Kuvera* Distant, 1906

- K. amurensis* Anufriev, 1987; Russia (Primorsky Krai).
K. basarukini Emeljanov, 1998; Russia (Sakhalin).
K. brunettii Muir, 1922; India (Eastern Himalayas: Darjeeling).
K. brunnea (Dlabola, 1957); Afghanistan (Hindu Kush).
K. communis Tsaour & Hsu, 1991; China (Taiwan).
K. flaviceps (Matsumura, 1900); Japan (Chishima Islands, Hokkaido, Honshu, Shikoku, Kyushu, Tsushima Island), Korea, Russia (Kuril: Iturups, Kunashir, Shikotan).
K. hagilsanensis Rahman, Kwon & Suh, 2017; Korea (Central, South, Jeju-do).
K. hallasanensis Rahman, Kwon & Suh, 2017; Korea (Central, South, Jeju-do).
K. hama Tsaour & Hsu, 1991; China (Taiwan).
K. huoditangensis, sp. n.; China (Shaanxi).
K. kurilensis Anufriev, 1987; Russia (Kuriles: Kunashir).
K. laticeps (Metcalf, 1936); China (Sichuan).
K. ligustri Matsumura, 1914; Japan (Honshu: Hakone, Shikoku, Kyushu, Tsushima Island), Korea.
K. longipennis Matsumura, 1914; China (Taiwan).
K. longwangshanensis sp. n.; China (Zhejiang).
K. pallidula Matsumura, 1914; Russia (Kuriles: Kunashir, Shikotan), Japan (Hokkaido, Honshu).
K. semihyalina Distant, 1906; Myanmar (Ruby Mines), India.
K. similis Tsaour & Hsu, 1991; China (Taiwan).
K. taiwana Tsaour & Hsu, 1991; China (Taiwan).
K. tappanella Matsumura, 1914; China (Taiwan).
K. toroensis Matsumura, 1914; China (Taiwan).
K. transversa Tsaour & Hsu, 1991; China (Taiwan).
K. ussuriensis (Vilbaste, 1968); Russia (Khabarovsk), Japan (Hokkaido), China (Sichuan).
K. vilbastei Anufriev, 1987; Russia (Primorsky Krai), China (Tibet).
K. yecheonensis Rahman, Kwon & Suh, 2017; Korea (Gyeongangbuk-do).

Key to the known species (males) of *Kuvera* from China

- 1 Tegmina with 11 apical cells2
- Tegmina with 10 apical cells6
- 2 Vertex about 3 times wider than long (Tsaour et al. 1991: fig. 32)
..... *K. longipennis* Matsumura, 1914
- Vertex more than 3 times as wide as long3
- 3 Periandrium with 2 spinose processes; left process longer than right process4
- Periandrium with 2 spinose processes; right process longer than left process; in dorsal view, 2 processes cross near middle of periandrium (Tsaour et al. 1991: fig. 28) *K. transversa* Tsaour & Hsu, 1991
- 4 Left process of periandrium S-shaped curve; right process of periandrium hook-shaped curve5
- Left process of periandrium curved 60 degrees, directed cephalad at apex; right process of periandrium sickle-shaped; middle portion curved outward (Tsaour et al. 1991: fig. 27) *K. similis* Tsaour & Hsu, 1991
- 5 Apex of left process reaching base of periandrium, flagellum with a small and short spine, reaching apex of sclerotized portion of flagellum (Figs 10, 22)
..... *K. huoditangensis*, sp. n.
- Apex of left process not reaching base of periandrium, flagellum with a stout and long spine, reaching middle of membranous portion of flagellum (Fig. 45)
..... *K. vilbastei* Anufriev, 1987
- 6 Periandrium with 2 unequally long spinose processes7
- Periandrium with 2 nearly equally long spinose processes, approximately equal to half length of periandrium, left process of periandrium curved outward (Emeljanov 2015: fig. 95) *K. ussuriensis* (Vilbasate, 1968)
- 7 Periandrium with 2 spinose processes; left process longer than right process8
- Periandrium with 2 spinose processes; right process longer than left process10
- 8 Left process of periandrium S-shaped curve; right process of periandrium sickle-shaped and curved (Tsaour et al. 1991: fig. 30) *K. hama* Tsaour & Hsu, 1991
- Left process of periandrium not S-shaped curve; right process of periandrium not sickle-shaped and curved9
- 9 Left process of periandrium curved 60 degrees, only directed cephalad at apex; most portions of right process parallel with shaft, apex slightly curved (Tsaour et al. 1991: fig. 29) *K. communis* Tsaour & Hsu, 1991
- Left process of periandrium gently curved from left side to right side, apex curved over shaft and towards the right side; right process of periandrium, touching shaft apically, apex curved and directed ventrally (Figs 35, 44)
..... *K. longwangshanensis*, sp. n.
- 10 Left process of periandrium not curved across shaft11
- Left process of periandrium curved across the shaft, apex curved and directed cephalad; right process of periandrium almost straight, directed outward (Anufriev 1987: figs 13–16) *K. laticeps* (Metcalf, 1936)

- 11 Left process of periandrium not S-shaped curve; right process of periandrium slightly semi-orbicularly curved..... **12**
- Left process of periandrium S-shaped curved; right process of periandrium hook-shaped, curved; in dorsal view, 2 processes are close and near middle of periandrium (Anufriev 1987: figs 68–71) ***K. toroensis* Matsumura, 1914**
- 12 In ventral view, 2 spinose processes of periandrium almost straight (Tsaour et al. 1991: fig. 26) ***K. tappanella* Matsumura, 1914**
- In ventral view, 2 spinose processes of periandrium slightly arched (Tsaour et al. 1991: fig. 25) ***K. taiwana* Tsaour & Hsu, 1991**

***Kuvera huoditangensis* sp. n.**

<http://zoobank.org/6C717862-1D4D-4D36-9B30-227CEB9A973C>

Figs 1–22

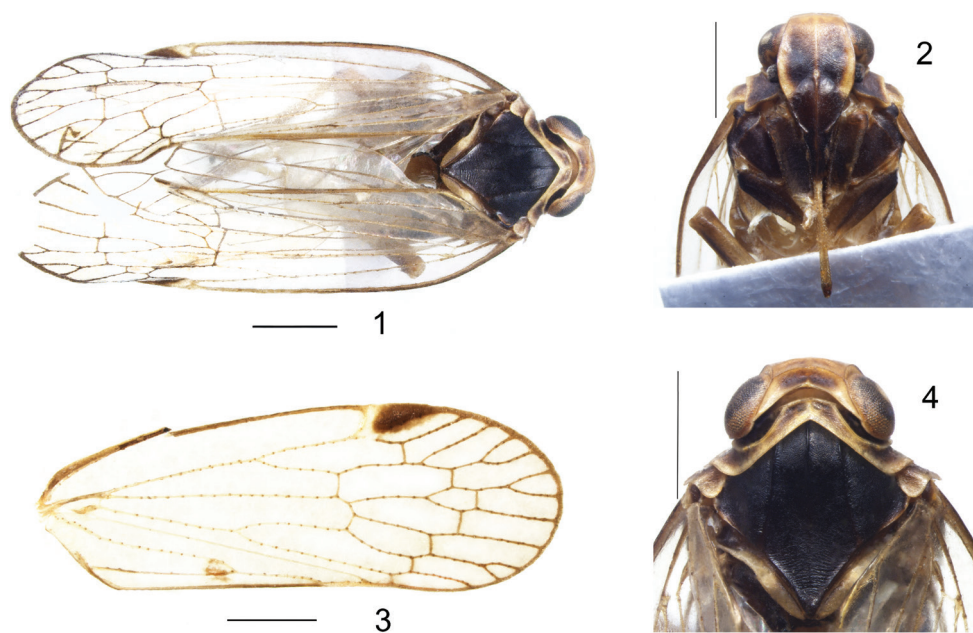
Type material. Holotype: male, **China:** Shaanxi, Ningshan County, Huoditang (33°22'N, 108°33'E), 1400–1500m a.s.l., 21.VI.1985, Lan Liu (NWAUFU). Paratypes: 1 male, China, Shaanxi, Ningshan County, Huoditang (33°22'N, 108°33'E), 1500m a.s.l., 15.VI.1985, Lan Liu (NWAUFU).

Description. Body length: male 6.7–7.0 mm (n=2), forewing length: male 5.8–6.0 mm (n=2).

Coloration. General color black. Body slightly covered with powdery wax (Fig. 1). Eyes dark brown, ocelli milky white. Antenna and rostrum generally dark brown (Fig. 2). Vertex brown with yellow carinae. Frons dark brown near base with lateral carinae yellow brown to pale brown from latero-basal angles to ends of frontoclypeal suture, clypeus black (Fig. 4). Pronotum shallow brown with darker areas. Mesonotum black with 3 dark brown carinae (Figs 1, 4). Tegmina hyaline with veins yellowish and dark brown granules, pterostigma blackish brown. Forewings with a small irregular, roundish spot on anterior branch of Y-vein (Figs 1, 3). Legs and abdomen yellowish brown.

Head and thorax. Vertex about 3.4 times wider than long. Anterior margin of vertex obscure, with only residual traces, subapical transverse carina parabolic, median carina reaching transverse carinae (Fig. 4). Frons slightly swollen, median carina only distinct on basal portion, frontoclypeal suture strongly arcuate. Middle ocelli present. Clypeus swollen, with a visible median carina. Rostrum, just reaching hind coxae (Fig. 2). Pronotum tapered with obvious carinae and distinct lateral carinae, strongly incised in middle. Meso-notum with 3 distinct con-colourous carinae (Fig. 4). Tegmina slender, venation pattern: Scp+R usually forked distad of CuA. RP 3-branched, MP with 4 terminals: MP₁, MP₂, MP₃, and MP₄, CuA 3-branched, with 11 apical cells (Figs 1,3). Legs with 3 tibial lateral spines. Hind tibia with 6 apical spines; chaetotaxy of hind tarsi: 7/8, 2nd tarsal segment with 3 platellae.

Male terminalia. Pygofer with a sub-triangular lateral margin; in dorsal view, asymmetrical, with a triangular medioventral process (Figs 5, 6, 14, 15). Anal segment in lateral view slender, straight at basal part; in dorsal view asymmetrical, longer than



Figures 1–4. *K. huoditangensis* sp. n. **1**, habitus, dorsal view; **2** frons and clypeus; **3** forewing; **4** head and thorax. Scale bars: 1mm.

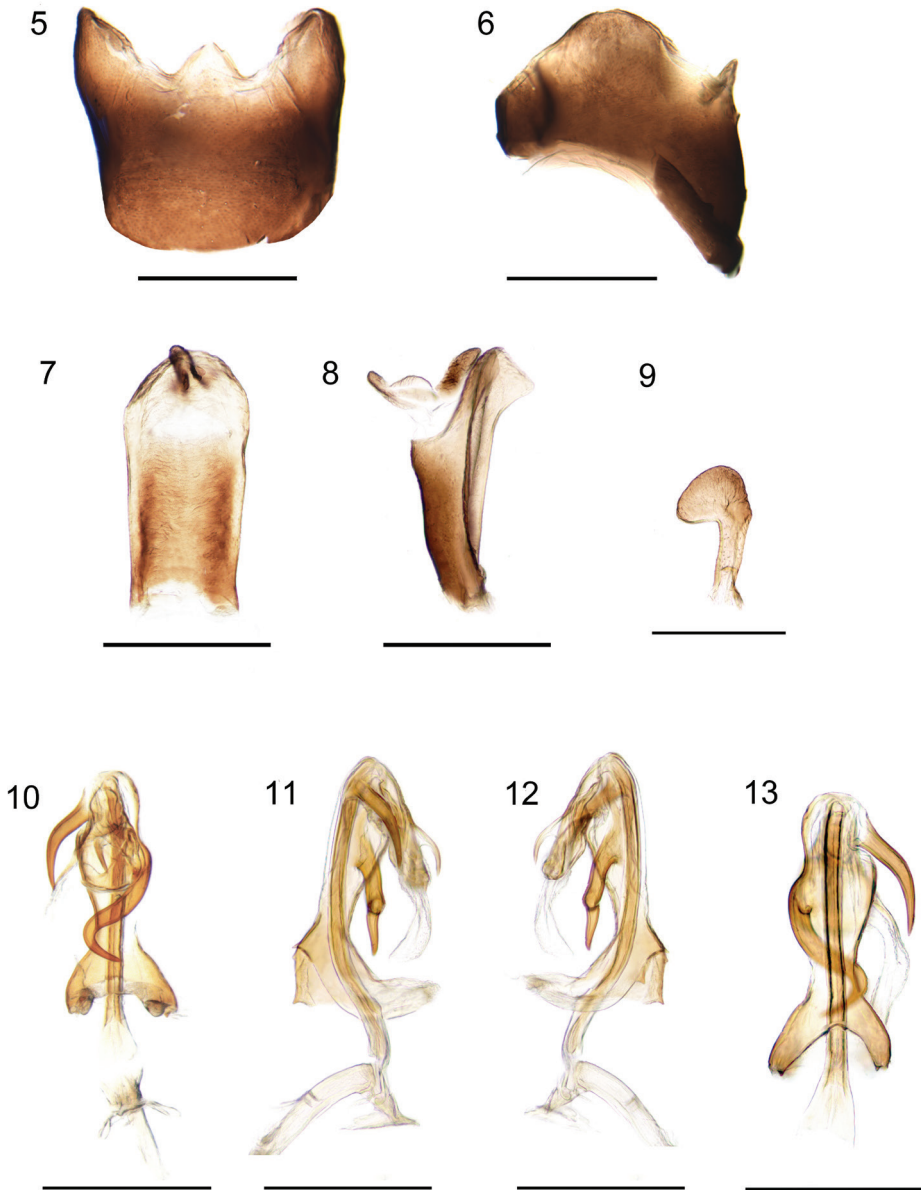
broad, widening to middle then narrowing, rounded to apex. Anal style sits subapically (Figs 7, 8, 16, 17). Genital styles symmetrical, in lateral view with hook-shaped apex, parallel-sided at basal half (Figs 9, 18). Aedeagus with 3 spinose processes, in ventral view, periandrium narrow near middle, with 2 spinose processes, one comparatively short, arising near base of flagellum, apex curved and directed cephalad. Another process comparatively long, implanted on left side near mid-length of periandrium, S-shaped, curved from left side to right side and then to middle of periandrium, apex curved 120 degrees and directed left-cephalad (Figs 13, 19). In dorsal view, flagellum with a small and short spine extending from middle, reaching apex of the sclerotized portion of flagellum, directed cephalad. Tip of flagellum near base of periandrium (Figs 10, 22). Periandrium asymmetrically widened at base, slightly curving to left. In ventral view, caudal margin of basal segment of periandrium convex, medially with two teeth, lateral apical angle with two teeth near distal portion (Figs 13, 19).

Female terminalia. Unknown.

Etymology. This species epithet is named after the type locality Huoditang.

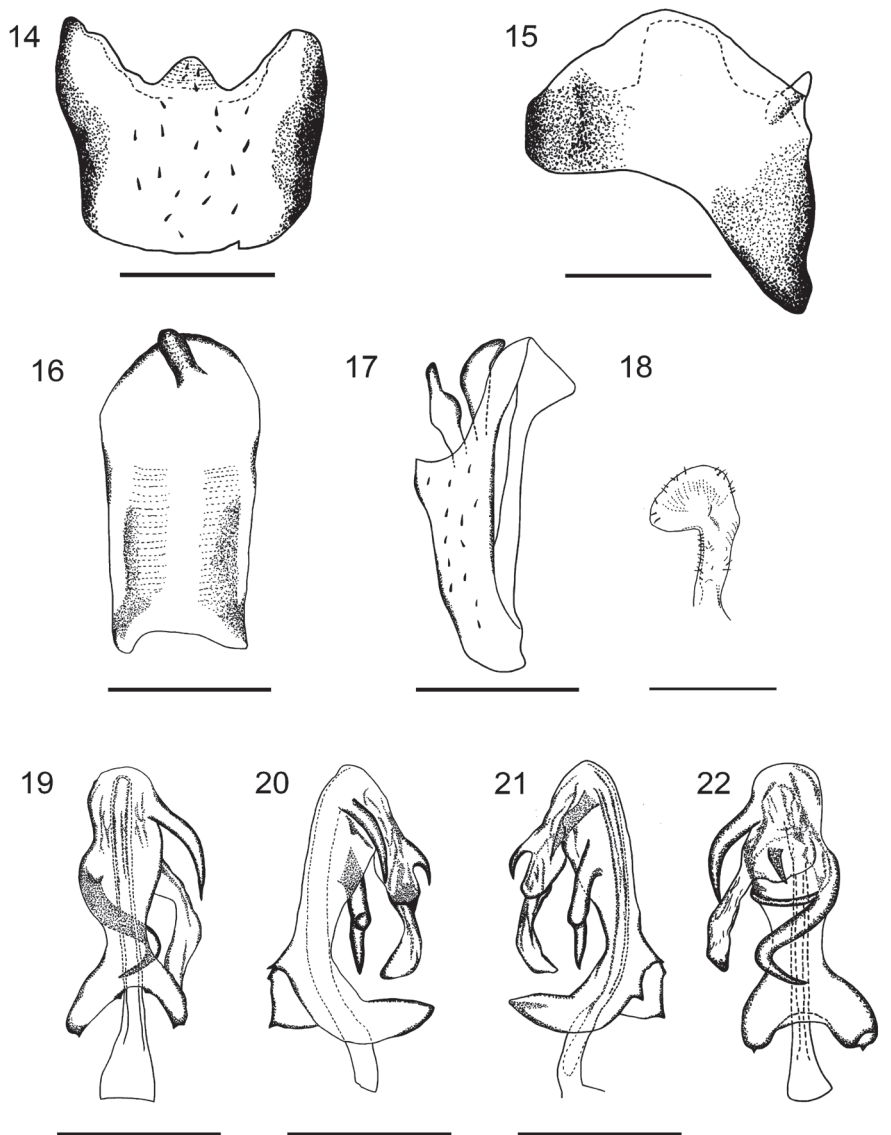
Distribution. China (Shaanxi).

Remarks. This new species is similar to *K. vilbastei* but can be separated by the following characteristics: (1) the process implanted on the left side near the mid-length of periandrium (*K. huoditangensis* has a long and S-shaped spinose process, curved from the left side to the right side and then to the middle of the periandrium, apex curved 120 degrees and directed left-cephalad, but *K. vilbastei* has a long spinose process,



Figures 5–13. *K. huoditangensis* sp. n. **5** pygofer, ventral view; **6** pygofer, lateral view; **7** anal segment, dorsal view; **8** anal segment, lateral view; **9** genital style, dorsal view; **10** aedeagus, dorsal view; **11** aedeagus, right lateral view; **12** aedeagus, left lateral view; **13** aedeagus, ventral view. Scale bars: 0.5mm.

curved from the left side to the right side, across the shaft sub-apically, apex curved 90 degrees and directed cephalad, not reaching the base of the perianthrium); (2) the process extending from the middle of the flagellum (*K. huoditangensis* has a small and short spine, reaching the apex of the sclerotized portion of flagellum, but *K. vilbastei*



Figures 14–22. *K. huoditangensis* sp. n. **14** pygofer, ventral view; **15** pygofer, lateral view; **16** anal segment, dorsal view; **17** anal segment, lateral view; **18** genital style, dorsal view; **19** aedeagus, ventral view; **20** aedeagus, right lateral view; **21** aedeagus, left lateral view; **22** aedeagus, dorsal view. Scale bars: 0.5mm.

has a stout and long spine, reaching the middle of the membranous portion of flagellum); and (3) the basal segment of perianthrium (*K. huoditangensis* asymmetrically widens in dorsal view, slightly curving to the left, caudal margin of the basal segment of the perianthrium convex, medially with two teeth, but *K. vilbastei* symmetrically widens, in ventral view, caudal margin of the basal segment of perianthrium convex and serrated).

***Kuvera longwangshanensis* sp. n.**

<http://zoobank.org/C4EFC153-B71B-4716-97A8-988089BCBEAA>

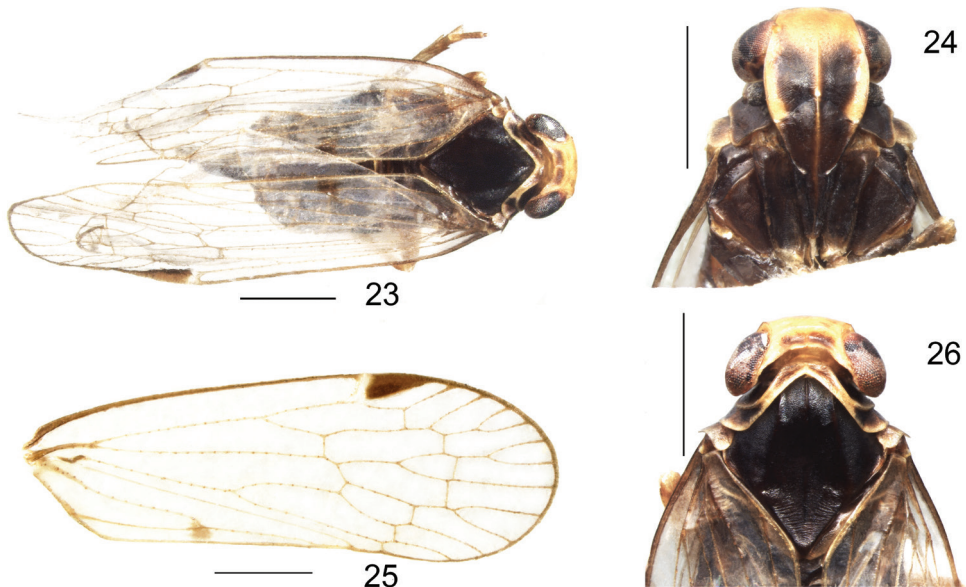
Figs 23–44

Type material. Holotype: male. **China:** Zhejiang, Anji County, Longwangshan (30°23'N, 119°23'E), 1000–1200m a.s.l., 6/8.VIII.2000, Wu Dai & Cong Wei (NWAUFU). Paratypes: 2 males, same data as holotype.

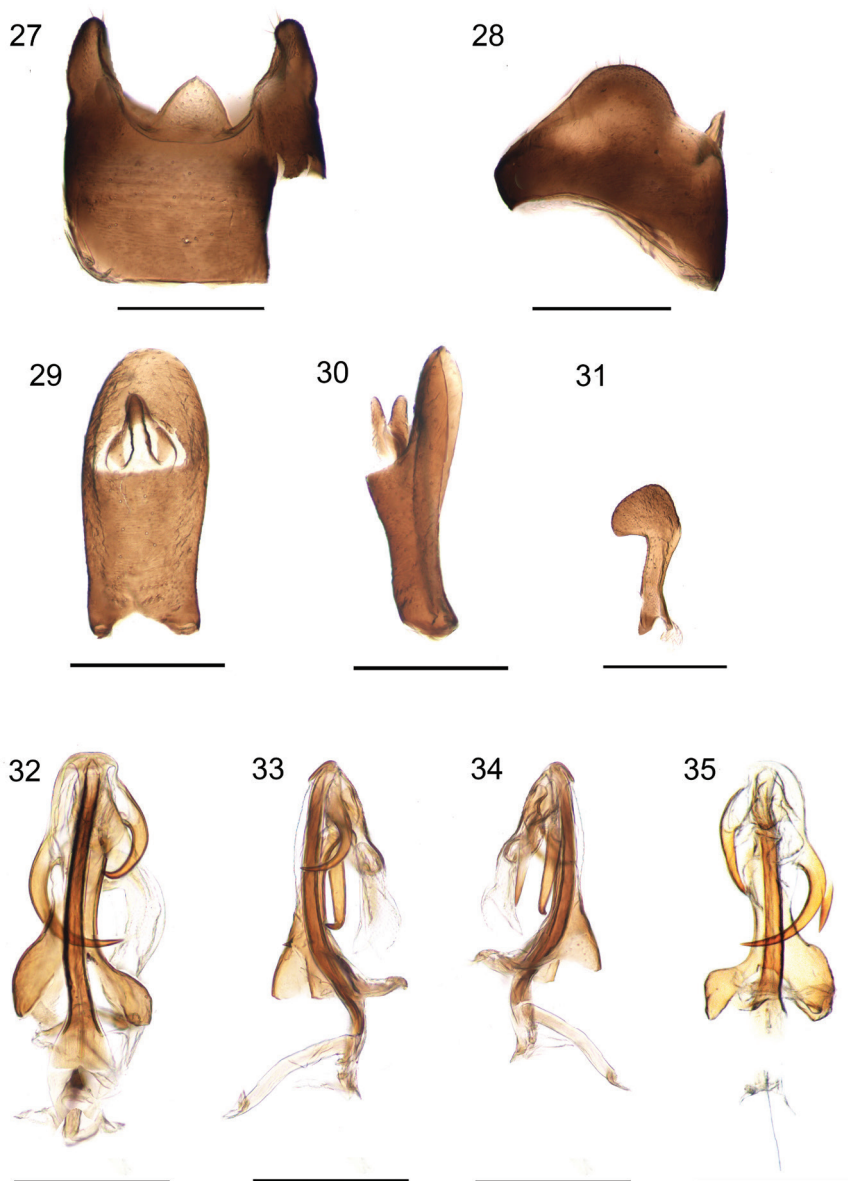
Description. Body length: male 5.1–5.6 mm (n=3), forewing length: male 5.2–5.3 (n=3).

Coloration. General color black. Body slightly covered with powdery wax (Fig. 23). Eyes dark brown, ocelli white. Antenna and rostrum generally dark brown (Fig. 24). Vertex brown, apical margin of vertex and surroundings yellow (Fig. 26). Frons dark brown, apical and lateral margins of frons yellowish brown, adjacent area of middle carinae near middle to frontoclypeal suture dark, and V-shaped, frontoclypeal suture and clypeus blackish brown, median carina yellowish (Fig. 24). Pronotum dark brown with yellowish areas. Mesonotum black with 3 dark brown carinae (Figs 23, 26). Tegmina hyaline with veins brown and yellow brown granules, pterostigma blackish brown. Forewings with a small irregular, roundish spot on anterior branch of Y-vein (Figs 23, 25). Legs brown, abdomen dark brown.

Head and thorax. Vertex about 3.8 times wider than long. Lateral and transvers carinae slightly elevated, sub-apical transverse carina parabolic, median carina reaching

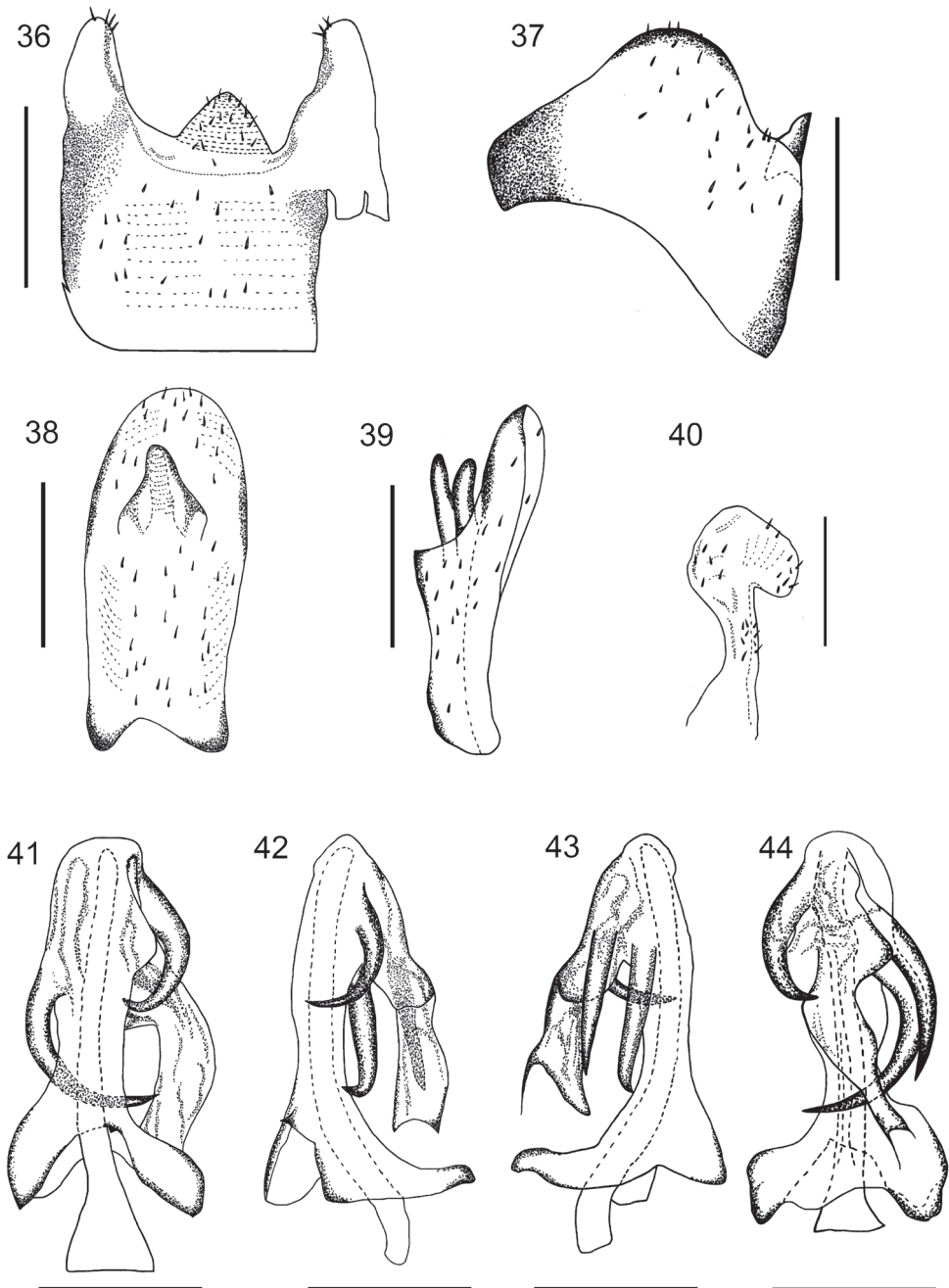


Figures 23–26. *K. longwangshanensis* sp. n. **23** habitus, dorsal view; **24** frons and clypeus; **25** forewing; **26** head and thorax. Scale bars: 1mm.



Figures 27–35. *K. longwangshanensis* sp. n. **27** pygofer, ventral view; **28** pygofer, lateral view; **29** anal segment, dorsal view; **30** anal segment, lateral view; **31** genital style, dorsal view; **32** aedeagus, ventral view; **33** aedeagus, right lateral view; **34** aedeagus, left lateral view; **35** aedeagus, dorsal view. Scale bars: 0.5mm.

transverse carinae (Fig. 26). Frons slightly swollen, median carina only distinct on basal portion, frontoclypeal suture strongly arcuate. Middle ocelli present. Clypeus swollen, with a visible median carina. Rostrum, just reaching hind coxae (Fig. 24). Pronotum tapered with obvious carinae and distinct lateral carinae, strongly incised in middle.



Figures 36–44. *K. longwangshanensis* sp. n. **36** pygofer, ventral view; **37** pygofer, lateral view; **38** anal segment, dorsal view; **39** anal segment, lateral view; **40** genital style, dorsal view; **41** aedeagus, ventral view; **42** aedeagus, right lateral view; **43** aedeagus, left lateral view; **44** aedeagus, dorsal view. Scale bars: 0.5mm.

Mesonotum with 3 distinct con-colourous carinae (Figs 23, 26). Tegmina slender, venation pattern: Scp+R usually forked distad of CuA. RP 3-branched, MP with 5 terminals: MP_{11} , MP_{12} , MP_2 , MP_3 , and MP_4 , CuA 2-branched, with 10 apical cells (Figs 23, 25). Legs with 3 tibial lateral spines. Hind tibia 6 apical spines; chaetotaxy of hind tarsi: 7/8, 2nd tarsal segment with 4 platellae.

Male terminalia. Pygofer with lateral margin sub-triangular in outline; in dorsal view, asymmetrical, wider than long, with a triangular medioventral process (Figs 27, 28, 36, 37). Anal segment in lateral view slender, widening in the middle and then narrowing, rounded at the apex; in dorsal view asymmetrical, longer than broad, narrow near base, expanded sub-apically (Figs 29, 30, 38, 39). Genital styles symmetrical, in lateral view with hook-shaped apex, inner margin deeply concave but outer margin rounded (Figs 31, 40). Aedeagus with 3 spinose processes, in ventral view, periandrium narrow near middle, with 2 spinose processes, the length of shorter spinose process about two-thirds of the longer spinose process. The longer one implanted on the left side near the mid-length of periandrium, gently curved from left to right side, apex curved over shaft and towards the right side. The shorter one arising near base of flagellum, touching shaft apically, apex curved and directed ventrally (Figs 32, 41). In dorsal view, flagellum with a stout and long spine extending nearly one-third the length of flagellum, the length of this spine more than two-thirds that of the longest spinose process, directed cephalad. The tip of flagellum reaches the base of the periandrium (Figs 35, 44). Periandrium asymmetrically widens at base, slightly curving to left. In ventral view, caudal margin of the basal segment of the periandrium convex, medially with a tooth, lateral apical angle with two teeth near the distal portion (Figs 32, 41).

Female terminalia. Unknown.

Etymology. This species epithet is named after the type locality Longwangshan.

Distribution. China (Zhejiang).

Remarks. This new species is similar to *K. flaviceps*, but can be separated by the following characteristics: (1) in dorsal view, the process implanted on the left side near the mid-length of the periandrium (*K. longwangshanensis* has a long spine, gently curved from left side to right side, apex curved over the shaft and to the right side, but *K. flaviceps* has a long spine, gently curved from the left to right side, apex not reaching the right lateral margin of the periandrium); (2) the process arising near the base of the flagellum (*K. longwangshanensis* has a shorter spine, touching the shaft apically, apex strongly curved mesad and directed ventrally, but *K. flaviceps* has a spine not touching the shaft apically, apex slightly curved and directed cephalad); and (3) the process of the flagellum (*K. longwangshanensis* has a stout and long spine extending nearly one-third the length of flagellum, the length of this spine is more than two-thirds of the longest spinose process, directed cephalad; but *K. flaviceps* has a thinner and shorter spine extending from the middle of flagellum, this spine is about half the length of the spinose process).

***Kuvera vilbastei* Anufriev, 1987**

Figs 45–50

Kuvera vilbastei Anufriev, 1987: 7, figs 17–22.

Type material. 1 male, China, Tibet Autonomous Region, Bomi Country, Yigong (29°85'N, 95°79'E), 2300m a.s.l, 29.VII.1978, Fa-Sheng Li (NWAFU); 1 male, China, Tibet Autonomous Region, Yadong Country (27°55'N, 88°93'E), 2800m a.s.l, 24.VIII.1978, Fa-Sheng Li (NWAFU).

Distribution. Russia (Primorsk), China (Tibet).

Plants associations. Cedar (*Cedrus deodara* (Roxb.) G. Don).

Remarks. Based on the description and figures by Anufriev (1987), this species can be distinguished from other species in this genus by following characters: Pygofer with subtriangular lateral margin; with a triangular medioventral process. Anal segment in lateral view slender, straight at basal part; in dorsal view, asymmetrical, about 3 times longer than broad, slightly widening at middle, rounded at apex. Anal style sits subapically (Figs 46, 47). Genital styles symmetrical, in lateral view with hook-shaped apex (Fig. 48). Aedeagus with 3 spinose processes, in dorsal view, periandrium narrow near middle, with 2 spinose processes, one comparatively short, arising near the base of flagellum, apex curved and directed cephalad. Another one comparatively long, implanted on the left side near the mid-length of periandrium, curved from left to right side, curving across the shaft subapically, apex curved 90 degrees and directed cephalad, not reaching the base of the periandrium. Flagellum with a stout and long spine extending from the middle, reaching the middle of the membranous portion of flagellum, directed cephalad. Flagellum reaching the base of the periandrium (Fig. 45). Periandrium symmetrically widened at base, caudal margin of the basal segment of the periandrium convex and serrated, lateral apical angle with two teeth near the distal portion (Figs 49, 50).

***Kuvera flaviceps* (Matsumura, 1900)**

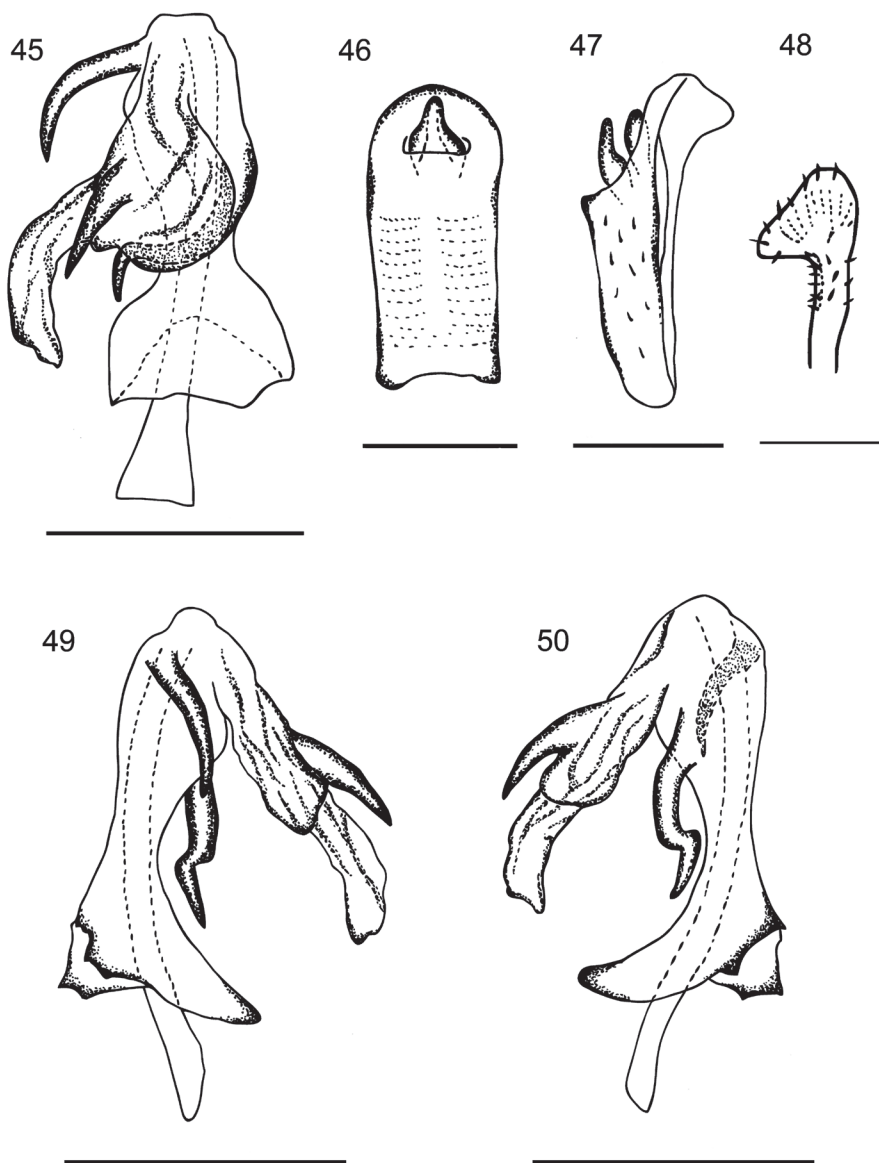
Oliarus flaviceps Matsumura, 1900: 208.

Kuvera flaviceps Matsumura, 1914: 407 (Fig. 2).

Distribution. Japan (Chishima Islands, Hokkaido, Honshu, Shikoku, Kyushu, Tsushima Island), Korea, Russia (Kuril: Iturups, Kunashir, Shikotan).

Plants associations. Birches (*Betula platyphylla* Suk.) and hairy alder (*Alnus japonica* (Thunb.) Steud.).

Remarks. Based on the description and figures by Matsumura (1914) and Anufriev (1987), this species can be distinguished from other species in this genus by the following characters: Pygofer has a lateral margin with a subtriangular outline; in dorsal view, asymmetrical, wider than long, with a triangular medioventral process. Anal segment in lateral view slender; in dorsal view asymmetrical, longer than broad, with convex lateral



Figures 45–50. *Kuvera vilbastei* Anufriev, 1987. **45** aedeagus, dorsal view; **46** anal segment, dorsal view; **47** anal segment, lateral view; **48** genital style, dorsal view; **49** aedeagus, right lateral view; **50** aedeagus, left lateral view. Scale bars: 0.5mm.

margins, rounded apically. Genital styles symmetrical, in lateral view with hook-shaped apex. Aedeagus has 3 spinose processes, in dorsal view, periandrium narrows near middle, with 2 spinose processes. A spine is implanted on the left side near the mid-length of the periandrium, which gently curves from the left side to right side, apex not reaching the right lateral margin of the periandrium. Another spine arises near the base of flagellum,

not touching the shaft apically, apex curved and directed cephalad. Flagellum with a stout and long spine extending from the middle, this spine is about half as long as the longest spinose process, directed cephalad. Flagellum reaches the base of the periandrium.

Discussion

The biology of *Kuvera* species throughout the world have not been extensively studied. According to our collection surveys, these species are primarily found on grass, trees, shrubs and forbs, ranging in altitude from 0 to 3000 m a.s.l. The plant associations of *Kuvera* have been described in several previous studies. Anufriev (1987) described cedar and birches as the primary host plants of *K. vilbastei* and *K. pallidula*. Emeljanov (2015) listed the following host plants for *K. ussuriensis* and *K. flaviceps*: myrica (*Myrica* L.), Nanking cherry (*Cerasus tomentosa* (Thunb.) Wall.), birches (*Betula platyphylla* Suk.) and alder (*Alnus japonica* (Thunb.) Steud.). We also found members of this genus on the cedar (*Cedrus deodara* (Roxb.) G. Don).

As part of ongoing monitoring studies in Chinese agroecosystems, we collected specimens of Cixiidae from crop plants, trees, forbs, shrubs and weeds in locations primarily in Southern China. We found that *K. huoditangensis* sp. n. occurs in Ningshan County, which is on the southern slope in the middle of the Qingling Mountain range. The specimens were collected in Huoditang Teaching and Experimental Forest Farm of Northwest A&F University of Ningshan County at an elevation between 1400 to 1500 m. *Kuvera longwangshanensis* sp. n. occurs in the Longwang Mountain National Nature Reserve (LNNR) of Anji County in the northwest of Zhejiang Province at 200 to 1500 m a.s.l. LNNR is located in the hinterland of the Yangtze River Delta and is covered by virgin forests. In the LNNR, the specimens were collected on Longwang Mountain at an elevation between 1000m and 1200m.

The *Kuvera* genus is distributed in eastern Asia, central Asia and the Indo-Malayan region (Fig. 51). Most *Kuvera* species occur in the Oriental region of the world: China (Sichuan, Zhejiang, Taiwan, south of Qinling Mountain in Shaanxi), India and Myanmar. Some species of *Kuvera* mainly occur in adjacent regions in the northeast Palaearctic such as Primorsk, Khabarovsk, and the Kuriles (eastern Russia); Hokkaido (northern Japan); and the Korean Peninsula. A few species of *Kuvera* occur in the southwest Palaearctic such as the Tibet Autonomous Region (western China) and the Hindu Kush (eastern Afghanistan). We anticipate that additional species of *Kuvera* will be found in countries throughout the primary distribution range of this genus.

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We are very grateful to Prof. A. Emeljanov (Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia) for providing older related papers. We wish to express our sincere thanks to Prof. W. H. Reissig (New York State Agriculture Station, Cornell University, USA) for his critical comments on this manuscript. And we sincerely thank



Figure 51. Geographic distribution of *Kuvera* species: *K. amurensis* (●); *K. basarukini* (●); *K. brunettii* (●); *K. brunnea* (●); *K. communis* (●); *K. flaviceps* (●); *K. hagilsanensis* (●); *K. hallasanensis* (●); *K. hama* (▲); *K. huoditangensis* sp.n. (■); *K. kurilensis* (▲); *K. laticeps* (▲); *K. ligustri* (▲); *K. longipennis* (▲); *K. longwangshanensis* sp. n. (■); *K. pallidula* (▲); *K. semihyalina* (★); *K. similis* (★); *K. taiwana* (★); *K. tappanella* (★); *K. toroensis* (★); *K. transversa* (★); *K. ussuriensis* (■); *K. vilbastei* (■); *K. yecheonensis* (■); distribution range of *Kuvera* species (—).

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References

- Anufriev GA (1987) Review of the cixiid genus *Kuvera* Distant (Homoptera, Auchenorrhyncha, Cixiidae). In: Kapustina OG (1987) [Taxonomy of the insects of Siberia and Soviet Far East], Taksonomiia nasekomykh Sibiri I Dal'nego Vostoka SSR., Vladivostok Dalnauka, Russia, 4–21.
- Anufriev GA (2009) Cicadina. In: Storozhenko SYu (Ed.) 2009-insects of Lazovsky Nature Reserve, Russian Academy of science, Far Eastern Branch, Institute of Biology and soil Science. Vladivostok: Dalnauka, 52–69.
- Anufriev GA, Emeljanov AF (1988) Suborder Cicadinea (Auchenorrhyncha) In: Lehr, PA (Ed.), Keys to the Insects of the Far East of the USSR Volume 2 Homoptera and Heteroptera Nauka Publishing House, Leningrad, 441–465.
- Bourgoin T (1993) Female genitalia in Hemiptera Fulgoromorpha, morphological and phylogenetic data. Annales De La Societe Entomologique De France 29(3): 225–244.
- Bourgoin T (2018) FLOW (Fulgoromorpha Lists On the Web): a world knowledge base dedicated to Fulgoromorpha. Version 8, updated 15 June. 2018. <http://hemiptra-databases.org/flow> [accessed 17 September 2018]

- Bourgoin T, Wang RR, Asche M, Hoch H, Soulier-Perkins A, Stroiński A (2015) From micropterism to hyperpterism: recognition strategy and standardized homology-driven terminology of the forewing venation patterns in planthoppers (Hemiptera: Fulgoromorpha). *Zoomorphology* 134(1): 63–77. <https://doi.org/10.1007/s00435-014-0243-6>
- Distant WL (1906) The Fauna of British India including Ceylon and Burma, Rhynchota 3 (Heteroptera-Homoptera). Taylor & Francis, London, 503pp.
- Emeljanov AF (1998) Contribution to the knowledge of the genus *Kuvera* Distant (Homoptera: Cixiidae). *Zoosystematica Rossica* 7(1): 133–137.
- Emeljanov AF (2002) Contribution to classification and phylogeny of the family Cixiidae (Hemiptera, Fulgoromorpha) *Denisia* 4: 103–112.
- Emeljanov AF (2015) Planthoppers of the family Cixiidae of Russia and adjacent territories. Key to the fauna of Russia 177: 1–252.
- Hayashi M, Fujinuma S (2016) Part Fulgoromorpha. In: Entomological Society of Japan 2016 – Catalogue of the insects of Japan. Volume 4 Paraneoptera (Psocodea, Thysanoptera, Hemiptera), 4. Editorial Committee of Catalogue of the Insects of Japan, 323–355.
- Holzinger WE, Emeljanov AF, Kammerlander I (2002) The family Cixiidae Spinola 1839 (Hemiptera: Fulgoromorpha) – a review. *Denisia* 4: 113–138.
- Julia JF (1982) *Myndus taffini* (Homoptera: Cixiidae), a vector of foliar decay of coconut in Vanuatu. *Oleagineux* 37(8): 409–414.
- Lee CE, Kwon YJ (1977) Studies on the spittlebugs, leafhoppers and planthoppers (Auchenorrhyncha, Homoptera, Hemiptera,). *Nature and Life* 7: 55–111.
- Löcker B, Fletcher MJ, Larivière MC, Gurr GM (2006) The Australian Pentastirini (Hemiptera: Fulgoromorpha: Cixiidae). *Zootaxa* 1290: 1–138.
- Matsumura S (1900) Uebersicht der Fulgoriden Japans. *Entomologische Nachrichten*. Berlin 26: 205–213.
- Matsumura S (1914) Die Cixiinen Japans. *Annotationes Zoologicae Japonenses* 8: 393–434.
- Metcalf ZP (1936) Part 2. Cixiidae. In: Metcalf ZP (Ed.) General Catalogue of the Homoptera. Fascicule IV, North Carolina State College, Raleigh 180: 1–267.
- Nast J (1972) Palaearctic auchenorrhyncha (homoptera) an annotated check list. Palaearctic Auchenorrhyncha An Annotated Check List, 550 pp.
- Pinzauti F, Trivellone V, Bagnoli B (2010) Ability of *Reptalus quinquecostatus* (Hemiptera: Cixiidae) to inoculate stolbur phytoplasma to artificial feeding medium. *Annals of Applied Biology* 153(3): 299–305. <https://doi.org/10.1111/j.1744-7348.2008.00261.x>
- Rahman MA, Yong JK, Sang JS (2017) Three new species of the genus *Kuvera* Distant (Hemiptera: Fulgoromorpha: Cixiidae) from Korea. *Oriental Insects*, 1–13. <https://doi.org/10.1080/00305316.2017.1344741>
- Sforza R, Clair D, Daire X, Larrue J, Boudonpadieu E (2010) The Role of *Hyalesthes obsoletus* (Hemiptera: Cixiidae) in the Occurrence of Bois noir of Grapevines in France. *Journal of Phytopathology* 146(11–12): 549–556. <https://doi.org/10.1111/j.1439-0434.1998.tb04753.x>
- Tsaur SC, Hsu TC, Van Stalle J (1988) Cixiidae of Taiwan, Part (I) Pentastirini. *Journal of Taiwan Museum* 41(1): 35–47.
- Tsaur SC, Hsu TC, Van Stalle J (1991) Cixiidae of Taiwan, Part (V) Cixiini except *Cixius*. *Journal of Taiwan Museum* 44(1): 1–78.

First description of the male of *Barylestis saaristoi* Jäger, 2008 (Araneae, Sparassidae) from China

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Abstract

The male of *Barylestis saaristoi* Jäger, 2008 is described for the first time from Menglun Town, Yunnan Province, China. This is the first record of this genus from China. An illustrated morphological description of this species is given.

Keywords

biodiversity, Yunnan, huntsman spiders, taxonomy

Introduction

The genus *Barylestis* was established by Simon (1910). Currently, of all 11 known *Barylestis* species, 10 from tropical Africa (Cameroon, Congo, Central Africa, Equatorial Guinea, Gabon, Nigeria, Rwanda, Sudan, Uganda, West Africa) and one from South-east Asia (Thailand, Myanmar) have been recorded (Jäger 2002; World Spider Catalog 2018). This genus had long been recognized as a purely African genus by Jäger (2002) until *Barylestis saaristoi* Jäger, 2008 was first described based on female specimens from Thailand and Myanmar. Jäger and his colleagues tried to collect the male of *B. saaristoi* specifically because of its potential contribution to zoogeographic and phylogenetic rela-

tionships of this genus, but the search was not successful (Jäger 2008). Recently, the authors examined specimens collected from Yunnan Province and found three females and one male belonging to this species. This is also the first record of this genus from China.

Materials and methods

Specimens were examined and measured with a Leica M205C stereomicroscope. Epigynes were examined and illustrated after dissection from the spider bodies. All photos were taken with a Leica DFC450 digital camera attached to a Leica M205C stereomicroscope, with 10–20 photographs taken in different focal planes and combined using image stacking software (Leica LAS). Photographic images were edited using Adobe Photoshop. Left palps are depicted. Most hairs and macrosetae are omitted in the palp drawings. All specimens examined in this study are deposited in the College of Life Sciences, Hubei University.

Leg measurements are shown as: total length (femur, patella, tibia, metatarsus, tarsus). Number of spines is listed for each segment in the following order: prolateral, dorsal, retrolateral, ventral (in femora and patellae ventral spines are absent and the fourth digit is omitted in the spination formula). Abbreviations follow Zhong et al. (2017, 2018):

ALE	anterior lateral eyes;	Pa	patella;
AME	anterior median eyes;	PL	prosoma length;
AW	anterior width of prosoma;	PLE	posterior lateral eyes;
CH	clypeus height;	PME	posterior median eyes;
FE	femur;	Pp	palp;
Mt	metatarsus;	PW	prosoma width;
OL	opisthosoma length;	Ta	tarsus;
OW	opisthosoma width;	Ti	tibia I, II, III, IV—legs I to IV.

Abbreviations for the collection depositories:

HBU	Hubei University, Wuhan, China;
SMF	Research Institute Senckenberg, Frankfurt, Germany

Taxonomy

Family Sparassidae Bertkau, 1872

Genus *Barylestis* Simon, 1910

***Barylestis saaristoi* Jäger, 2008**

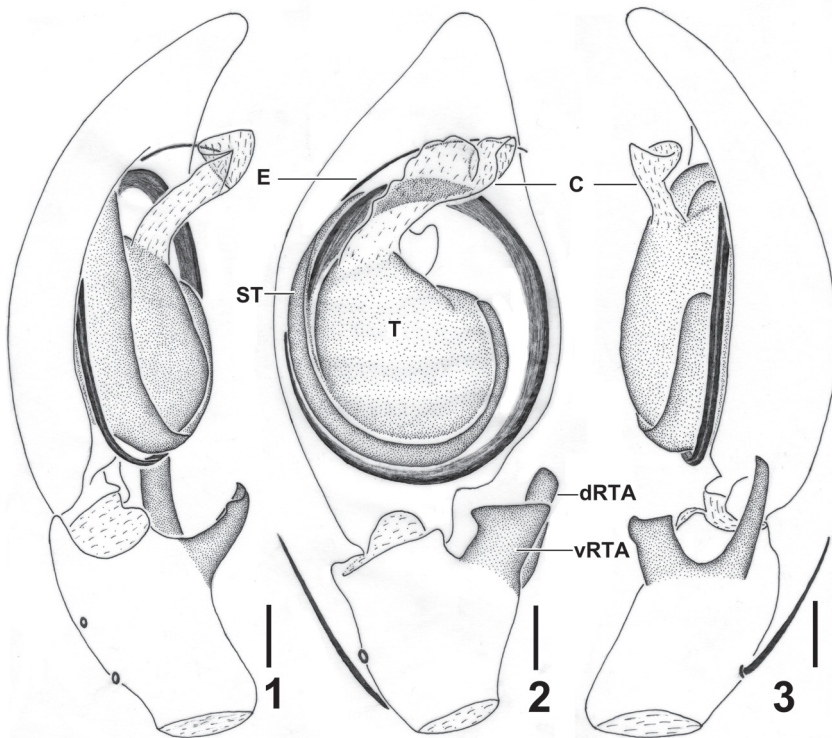
Figures 1–26

Barylestis saaristoi Jäger, 2008: 106, figs 1–14 (holotype female from Mae Hong Son Province, Thailand, deposited in SMF 58342).

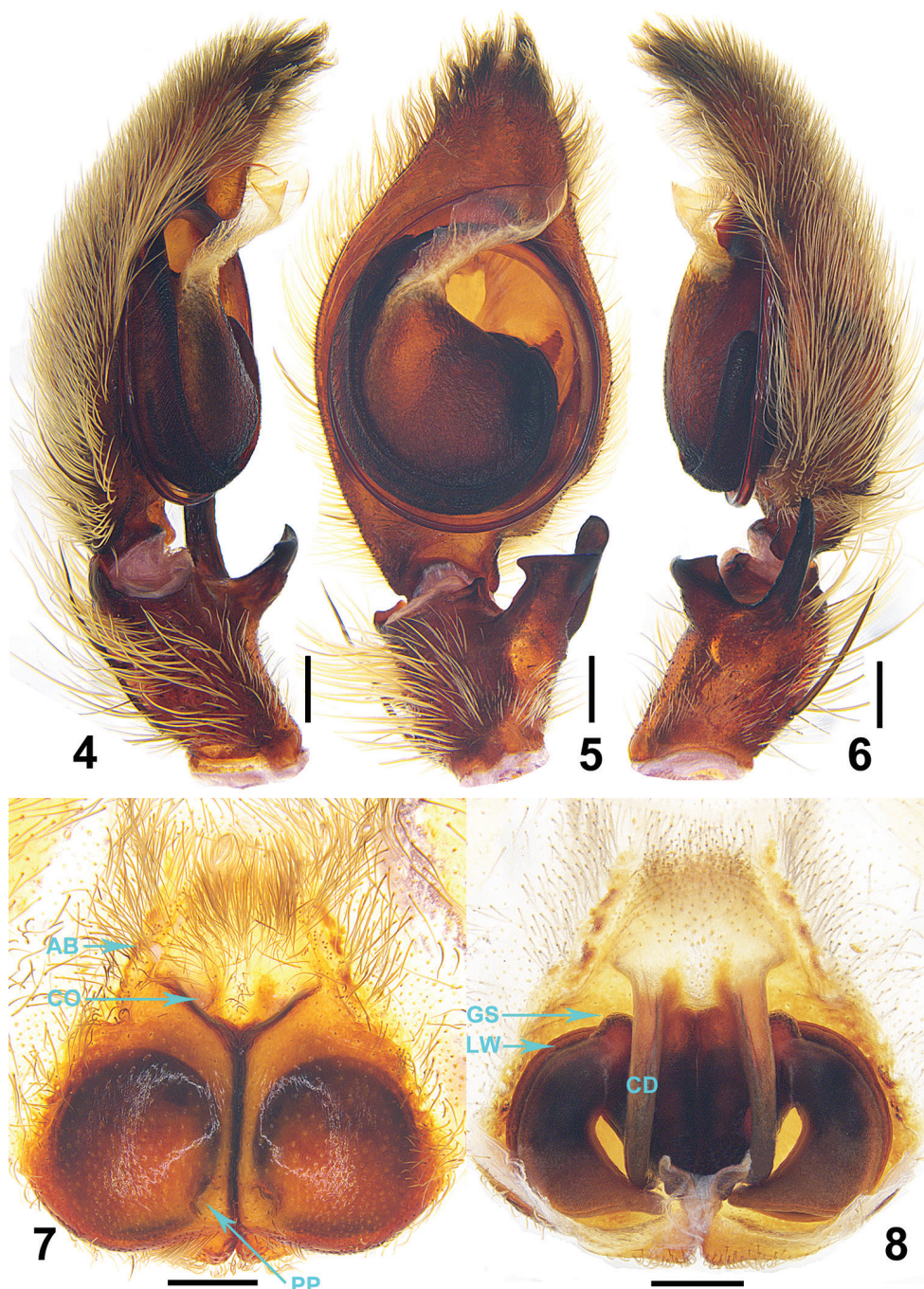
Material examined. 1 male and 1 female (HBU), Mengxin Farm [21.89°N, 101.36°E, 736m], Dai Autonomous Prefecture of Xishuangbanna, China, 4 May 2018, Yiwu Zhu leg.; 2 females (HBU), Xishuangbanna Tropical Botanical Garden [21.96°N, 101.22°E, 757m] Dai Autonomous Prefecture of Xishuangbanna, China, 30 May 2015, Wancheng Li leg.

Diagnosis. Male of *B. saaristoi* can be separated from *B. montandoni* (Lessert, 1929) and *B. occidentalis* (Simon, 1887) by embolus arising from tegulum in an 11-o'clock-position (3-o'clock-position in *B. montandoni* and *B. occidentalis*, Figs 2, 5), separated from *B. fagei* (Lessert, 1929) and *B. variatus* (Pocock, 1900) by the long and slender dRNA (short and wide in *B. fagei* and *B. variatus*, Figs 3, 6), separated from *B. scutatus* (Pocock, 1903) by tegulum partly covered embolic base (wholly covered in *B. scutatus*). Females of this species can be recognised as this particular species by the following combination of characters: 1. Epigyne with V-shaped pit; 2. Vulva with tips of lateral coils pointing mediad and first part of copulatory ducts slender, running parallel (Jäger 2008).

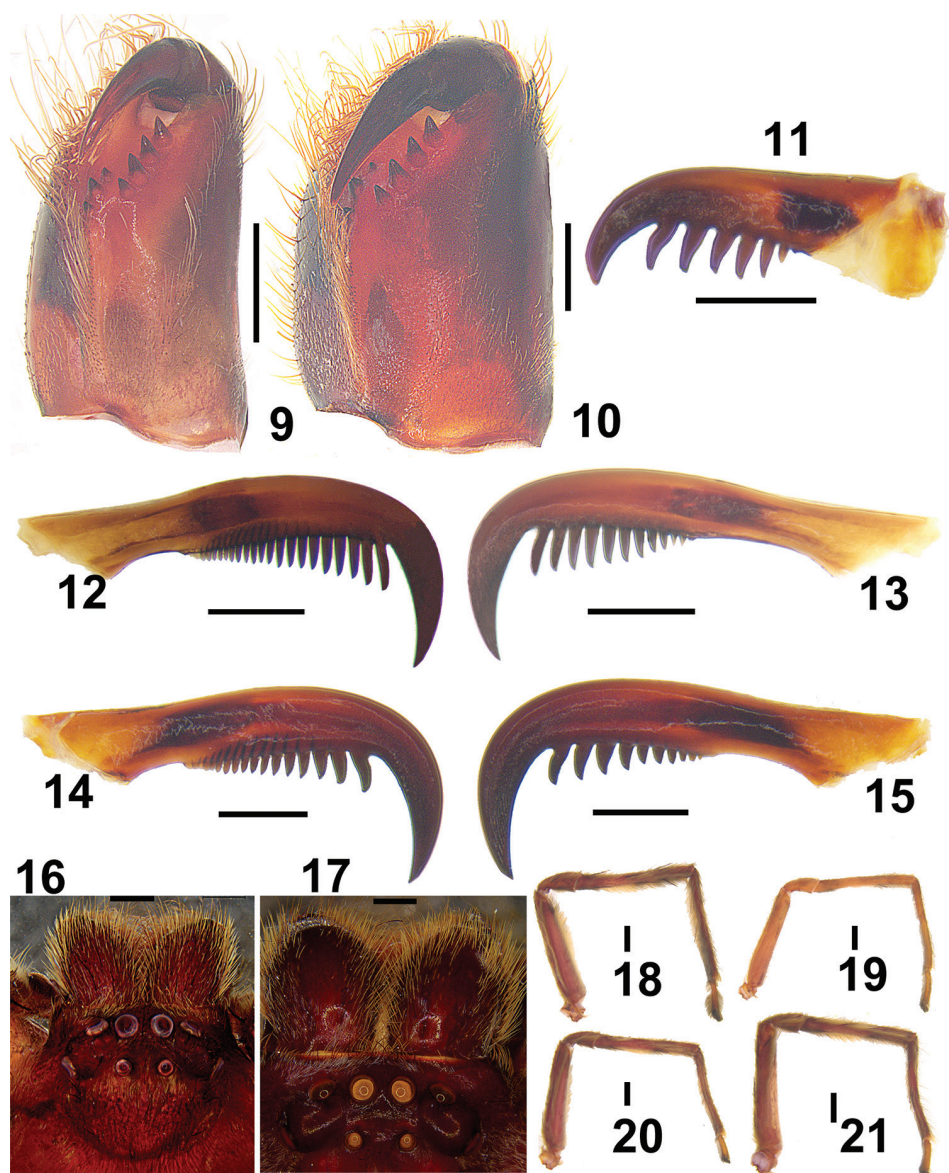
Description. Male. PL 6.4, PW 7.5, AW 4.0, OL 5.4, OW 3.9. Eyes: AME 0.34, ALE 0.43, PME 0.26, PLE 0.44, AME–AME 0.26, AME–ALE 0.15, PME–PME 0.59, PME–PLE 1.03, AME–PME 0.55, ALE–PLE 0.97, CH AME 0.47, CH ALE



Figures 1–3. *Barylestis saaristoi* Jäger, 2008. 1–3 Left male palp (1 prolateral 2 ventral 3 retrolateral). Abbreviations: C—conductor; dRTA—dorsal retrolateral tibial apophysis; E—embolus; ST—subtegulum; T—tegulum, vRTA—ventral retrolateral tibial apophysis. Scale bar: 0.5 mm.



Figures 4–8. *Barylestis saaristoi* Jäger, 2008. **4–6** Left male palp (**4** prolateral **5** ventral **6** retrolateral) **7** epigyne, ventral **8** vulva, dorsal. Abbreviations: AB—anterior bands, CD—copulatory ducts, CO—copulatory opening, GS—glandular structures of internal duct system, LW—lateral winding of internal duct system, PP—posterior pits of lateral lobes. Scale bar: 0.5 mm.



Figures 9–21. *Barylestis saaristoi* Jäger, 2008. **9, 10** Cheliceral dentition, ventral view (**9** male **10** female) **11** palpal claw of female, retrolateral view **12–15** leg I prolateral and retrolateral view of two claws (**12, 13** male **14, 15** female) **16, 17** eye arrangement, dorsal view (**16** male **17** female) **18–21** Leg I–IV (male, right). Scale bars: 1 mm (**9, 10, 16, 17**); 0.2 mm (**11–15**); 2 mm (**18–21**).

0.62. Spination: Palp: 131, 101, 2021; Fe: I 333, II 000, III 333, IV 331; Pa: I 101, II 000, III 101, IV 000; Ti: I 2226, II 0004, III 2116, IV 2014; Mt: I 1014, II 0004, III 1014, IV 1016. Measurements of palp and legs: Palp 9.5 (2.9, 1.2, 1.6, –, 3.8), I 32.5 (8.6, 3.2, 8.7, 9.4, 2.6), II 27.1 (7.5, 2.3, 7.5, 7.1, 2.7), III 29.0 (9.1, 2.4, 8.4,



Figures 22–25. *Barylestis saaristoi* Jäger, 2008. **22, 23** Male (**22** dorsal **23** ventral) **24, 25** female (**24** dorsal **25** ventral). Scale bar: 2 mm.

7.1, 2.0), IV 29.1 (9.3, 2.5, 8.1, 6.8, 2.4). Leg formula: I-IV-III-II (second leg may have fractured before collection, as it is very tiny; Figs 18–21). Cheliceral furrow with 3 anterior and 4 posterior teeth, without denticles. Claws of leg I with long and slightly curved teeth in both male and female. Female palpal claws with seven long teeth, almost same size as those of leg I (Figs 9–15). Dorsal carapace reddish-brown, posterior margins dark. Chelicerae, sternum, gnathocoxae and labium deep reddish-brown to black. Legs reddish-brown without spots and patches. Dorsal opisthosoma covered by long and dense hairs. Ventral opisthosoma uniformly yellowish-brown (Figs 22, 23). Cymbium significantly longer than tibia. Conductor membranous, arising from direction of tegulum 11:30. Embolus running 1.25 coils around tegulum, with tip situated

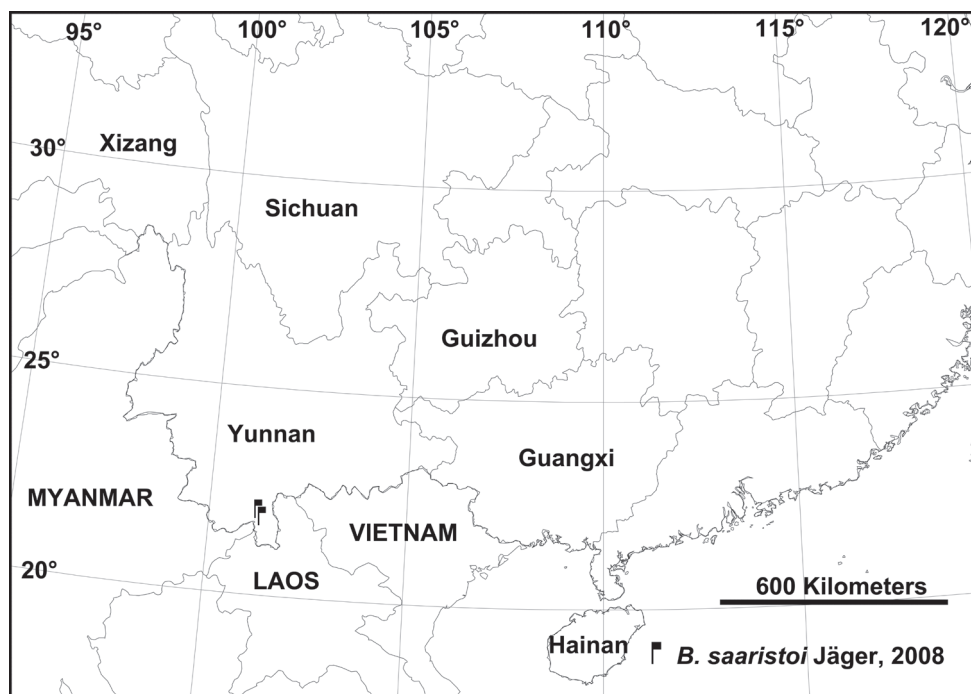


Figure 26. Collection localities of *Barylestis saaristoi* in Yunnan Province, China.

near conductor. RTA arising medially from tibia, vRTA developed, almost rectangle-shaped and dRTA finger-shaped in ventral view (Figs 1–6).

Female. For details see Jäger (2008).

Distribution. China (Yunnan Province, new record) (Fig. 26), Thailand (Mae Hong Son Province), Myanmar (Karen, Kayin State).

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References

- Jäger P (2002) Heteropodinae: transfers and synonymies (Arachnida: Araneae: Sparassidae). *Acta Arachnologica* 51: 33–61. <https://doi.org/10.2476/asjaa.51.33>
- Jäger P (2008) *Barylestis* Simon, 1910 – first record in Asia with comments on its zoogeography (Araneae: Sparassidae: Heteropodinae). *Arthropoda Selecta* 17: 105–110.

- Simon E (1910) Arachnides recueillis par L. Fea sur la côte occidentale d'Afrique. 2^e partie. Annali del Museo Civico di Storia Naturale di Genova 44: 335–449.
- World Spider Catalog (2018) World Spider Catalog. Version 19.5. Natural History Museum Bern. <http://wsc.nmbe.ch> [Accessed on 17 September 2018]
- Zhong Y, Cao XW, Liu J (2017) Six *Sinopoda* species (Araneae: Sparassidae) from Fujian and Yunnan Provinces in China. Zootaxa 4227: 151–172. <https://doi.org/10.11646/zootaxa.4227.2.1>
- Zhong Y, Jäger P, Chen J, Liu J (2018) Taxonomic review of the *Sinopoda okinawana*-group (Araneae: Sparassidae) in China. Zootaxa 4388(3): 328–346. <https://doi.org/10.11646/zootaxa.4388.3.2>