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# The alien ascidian *Styela clava* now invading the Sea of Marmara (Tunicata: Ascidiacea)

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### Abstract
During the implementation of a large project aimed to investigate the benthic community structures of the Sea of Marmara, specimens of the invasive ascidian species *Styela clava* were collected on natural substrata (rocks) at 10 m depth at one locality (Karamürsel) in İzmit Bay. The specimens were mature, containing gametes, indicating that the species had become established in the area. The Sea of Marmara seems to provide suitable conditions for this species to survive and form proliferating populations.

### Keywords
Invasive alien species, *Styela clava*, Ascidiacea, Tunicata, Sea of Marmara

## Introduction

The Sea of Marmara is unique in having two stratified water layers separated by a halocline, generally developing at 20-25 m depths (Besiktepe et al. 1994). The upper layer is composed of brackish water originating from the Black Sea, while, the lower layer comprises marine water from the Aegean Sea. This sea has been under great anthropogenic pressures, mainly due to crowded cities situated along its coastlines (including İstanbul), and the presence of many industrialized regions, in particular, İzmit Bay. Pollution from different sources has caused hyper eutrophication (Aral 1992) and occasionally anoxia (Basturk et al. 1990) in some areas. Moreover, the establishment of some invasive alien species [e.g. the ctenophore *Mnemiopsis leidyi* Agassiz, 1865, the asteroid *Asterias rubens* Linnaeus, 1758 and the gastropod *Rapana venosa* (Valenciennes, 1846)] in the basin has made conditions worse (Çinar et al. 2011). The mussel and oyster beds in the sea have been largely destroyed by the aforementioned asteroid and gastropod.

The invasive alien species are known to have great impacts on native communities and often make complete changes to ecosystems that cannot be rectified (Ruiz et al. 1997). The eastern Mediterranean Sea is one of the known regions that hosts high numbers of alien species, due to its proximity to the Suez Canal and high rate of maritime traffic (Çinar et al. 2012). This region includes 75% of the total number of alien species reported for the whole of the Mediterranean Sea (Zenetos et al. 2012). Seventeen alien ascidian species were reported from the Mediterranean Sea (see Zenetos et al. 2012), some of which, such as *Distaplia bermudensis* Van Name, 1902, *Microcosmus squamiger* Michaelsen, 1927 *Botrylloides violaceus* Oka, 1927 and *Didemnum vexillum* Kott, 2002, have become invasive in some areas, especially in the western Mediterranean and Adriatic Sea (Mastrototaro and Brunetti 2006; Occhipinti-Ambrogi 2000; Turon et al. 2007; Tagliapietra et al. 2012). The lessepsian invaders such as *Symplegma brakenhielmi* (Michaelsen, 1904), *Herdmania momus* (Savigny, 1816), *Microcosmus exasperatus* Heller, 1878 and *Phallusia nigra* Savigny, 1816 densely colonize both natural habitats and man-made structures in coastal regions of the eastern Mediterranean (in Levantine Sea), gradually extending their distributions to the north and west, including the Aegean Sea (Çinar et al. 2006; Kondilatos et al. 2010; Thessalou-Legaki 2012; Evans et al. 2013; Ramos-Esplá et al. 2013). Shenkar and Loya (2009) reported 7 alien species along the Mediterranean coast of Israel, including *Microcosmus exasperatus* Heller, 1878. A total of 4 alien ascidian species [*P. nigra*, *H. momus*, *M. exasperatus* and *S. brakenhielmi*] have been reported along the Levantine and Aegean coasts of Turkey up to date, but no alien ascidian species have been encountered in the Sea of Marmara and the Black Sea coasts of Turkey (Çinar 2014).

During a TUBITAK project (number 111Y268), specimens of *Styela clava* Herdman, 1881 were encountered and photographed in one locality, Karamürsel, located in Izmit Bay. This sessile and solitary ascidian species is native to the north-western Pacific but now occurs worldwide, due to anthropogenic transport (Carlisle 1954; Millar 1960; Holmes 1976; Christiansen and Thomsen 1981; Berman et al. 1992; Cohen et al. 1998; Lambert and Lambert 1998; Minchin et al. 2006; Davis and Davis 2007; Ross et al. 2007; Hayward and Morley 2009). It is mainly characterized by its tunic shape and long stalk. This species was first reported in the Mediterranean Sea in June 2005, in Bassin de Thau (France) and was thought to have been transferred to the area by shellfish transfer (Davis and Davis 2008). This species was also recorded in the Black Sea in a species list of the macro-zoobenthos associated with a mussel facies inside the Constanta Sud-Agigea Seaport situated on the coast of Romania (Micu and Micu 2004). The species generally colonizes areas of shallow water and is especially abundant 10-200 cm below the sea surface, occasionally inhabiting hard substrate at depths of 15-40 m (Lützen 1999). However, Kott (2008) found it at 100 m depth in Shark Bay (Western Australia).

The aim of this paper is to report this species in the Sea of Marmara and to give additional information regarding its morphological and ecological characteristics.

## Material and methods

Specimens of *Styela clava* were collected at one locality (Karamürsel, K15, İzmit Bay, 40°41'38"N-29°36'26"E) in the Sea of Marmara at 10 m depth on rocks via scuba-diving on 01 October 2012 (Figure 1). The animals were randomly sampled and fixed with 4% formaldehyde in the field. In the laboratory, specimens were rinsed with tap water and preserved in 70% ethanol. Specimens were deposited at the Museum of Faculty of Fisheries, Ege University (ESFM).

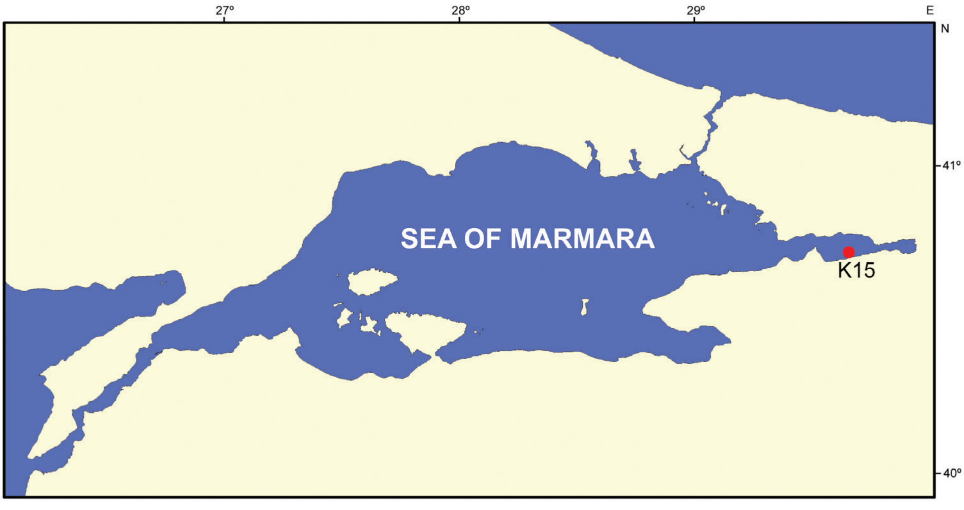


**Figure 1.** Map of the sampling site.

## Results and discussion

### The description of *Styela clava*

Four specimens (registration code: ESFM-TUN/2012-1) were collected in the Sea of Marmara from station K15 at 10 m depth on rocks. Specimens are stalked and sessile. The body is more or less cylindrical, tapering to stalk. The body of the largest specimen is 5.5 cm long and 2 cm wide. The smallest specimen is 3.2 cm long and 1.8 mm wide. The specimen stalk reaches 3.5 cm long (Figure 2A–C). The siphons are short and placed anteriorly; the branchial siphon is more obvious than the atrial siphon. The external body surface is leathery, wrinkled, with irregular rounded conical warts (Figure 2B, C). The body color is white in fixed specimens (Figure 2C), but is chocolate-brown when alive (Figure 2A, B). Apertures have alternate longitudinal pale brown and dark brown stripes (Figure 2A).

The branchial tentacles are simple. There are four branchial folds curved inwards on each side of the posterior part of the body. The branchial sac has numerous rows of

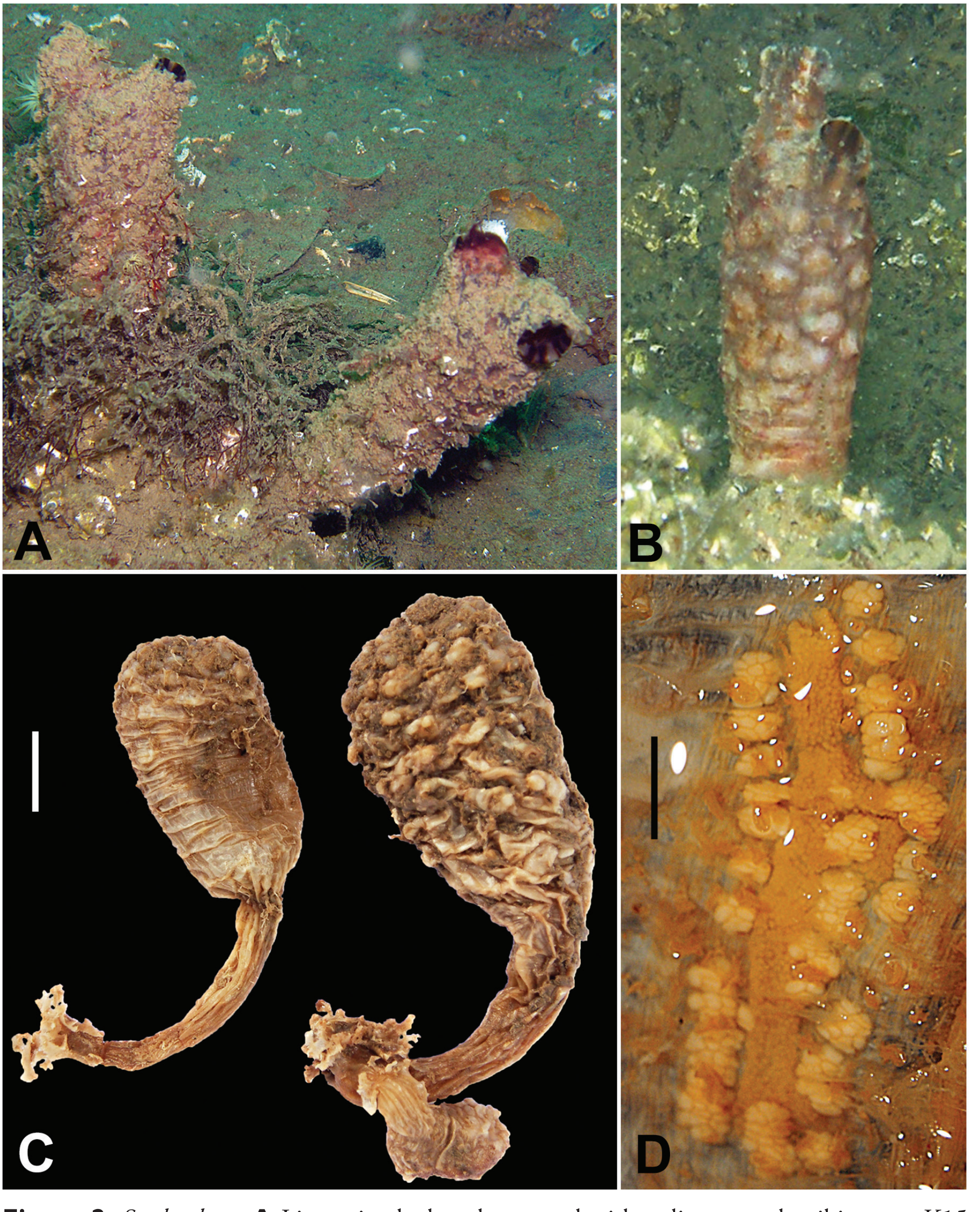


**Figure 2.** *Styela clava*, **A** Live animals densely covered with sediment and epibionts at K15 **B** Live animal almost bare at same station **C** Fixed specimens **D** Gonads. Scale bars: **C** = 1 cm, **D** = 2 mm.

straight stigmata. The gut is placed on the left side of the branchial sac, like a simple vertical loop. Gonads are long, parallel to each other, consisting of a central ovarian tube with testis follicles on the body wall along the each side of the ovary (Figure 2D). In the largest specimen, gonads are placed on both sides (2 on the left side and 4 on the right side), consisting of a long ovary surrounded by male follicles (Figure 2D).

## The epibionts of *Styela clava*

The specimens of *Styela clava* from the Sea of Marmara were generally covered by sediment and some epibionts, such as *Diadumene cincta* Stephenson, 1925, *Spirobranchus triqueter* (Linnaeus, 1758) and green algae. The former species is known also to be an alien species, probably transferred to the area by shipping from the north-east Atlantic (Çinar et al. 2014). Lützen (1999) reported various epibionts on *S. clava* in the North Sea, from tufts of red or green algae to ascidians including smaller specimens of the same species as well as *Ascidiella aspersa* (Müller, 1776) and *Botryllus schlosseri* (Pallas, 1766).

## The density of *Styela clava*

During many scuba dives and snorkeling trips performed along the Sea of Marmara in September and October 2012 (30 stations), this species was only encountered at station K15 (İzmit Bay, Karamürsel) and only 10 specimens were observed at a depth of 10 m on natural habitats (on rocks). The density of the species was approximately 1 ind.$m^{-2}$. The dominant macrozoobenthic species sharing the same habitat with *S. clava* were *Mytilus galloprovincialis* Lamarck, 1819, *S. triqueter*, *D. cincta*, *R. venosa* and *A. rubens*. The latter three species are also invasive alien species in the Sea of Marmara. *Styela clava* has been known to become extremely dominant in some areas, attaining a density of 1000 ind.$m^{-2}$ in European waters (Lützen 1999; Minchin et al. 2006). Micu and Micu (2004) reported a density of 4 ind.$m^{-2}$ and biomass (dry-weight) of 22.8 g.$m^{-2}$ in the Agigea seaport in the Black Sea (Romanian coast).

## The survival requirements of *Styela clava*

This species has a club-shaped body that can reach a length of 200 mm and attaches to hard substrata by an expanded membranous plate (Minchin et al. 2006). It reaches maturity at a size of 5 to 7.5 cm after ten months of settlement (Davis and Davis 2007). It is a hermaphroditic species and has a pelagic lecithotrophic larva that rarely travels more than a few centimeters according to Davis et al. (2007), but probably travels much father due to currents. It is known to tolerate temperatures ranging from -2 to +23 °C and salinity from 20 to 32 psu (Davis and Davis 2007). At this particular station within the Sea of Marmara, the temperature was near the maximum known tolerance limit of the species (22.6 °C) and the salinity was 23 psu. Similar environmental conditions were also encountered at different sampling stations in the Sea of Marmara, but no animals of this species were found at those stations. These findings might suggest that the species was found at the area of first establishment. In the Sea of Marmara, the summer surface water temperatures and salinity at the sampling site (İzmit Bay) were reported to be 25 °C and 23 psu, respectively (Isinibilir et al. 2008). Winter surface water temperature of Izmit Bay is around 7 °C. This suggests that there are no physico-chemical barriers in the region to hinder the population spread of *S. clava*. The Sea of Marmara's specimens had ripe gonads, indicating its successful reproductive capacity in the area. At this stage it could be concluded that this species is established and has formed a proliferating population in the area.

## The vector for introduction of *Styela clava*

This species has been introduced to different parts of the world's oceans, including the east Atlantic coast (see Minchin et al. 2006), Australia (Hewitt et al. 1999), New Zealand (Davis and Davis 2006), both coasts of North America (Osman et al. 1989; Lambert and Lambert 1998; Lambert 2003; Wonham and Carlton 2005), Mediterranean Sea (Davis and Davis 2008) and the Black Sea (Micu and Micu 2004). As Davis and Davis (2008) summarized, there are two possible mechanisms of ascidian introduction; shellfish transportation (juvenile ascidians) or via ship's hulls and sea chests (mature ascidians) (Coutts and Dodgshun 2007). As there is no shellfish farming in the Sea of Marmara, the only possible vector for the introduction of this species to the area was via shipping. The sampling station (K15, Karamursel) is located in İzmit Bay, which is one of the most industrialized areas in Turkey, with intense international ship traffic. The donor area for the Sea of Marmara's population of this species is unknown at present. It might have been transferred from an area in the Black Sea or/the Mediterranean, or from outside the Mediterranean. Molecular analysis to be performed on the specimens might shed more light on from where this population was originated.

## Impacts of *Styela clava*

The effects of *Styela clava* on soft bottom sediment assemblages in Port Philip Bay were reported to be negligible (Ross et al. 2007). However, Bourque et al. (2007) reported that it caused a decline in mussel production in Canada, as it densely covered mussel lines. It was reported to be an aggressive invader, affecting native fauna by replacing the native competitive dominants in the benthic community (Clarke and Therriault 2007). The economic impact of this species on shellfish production in Canada alone was estimated at between \$34–88,000 million (Canadian) per year (Colautti et al. 2006). Experiments made by Osman et al. (1989) indicated that *S. clava* is capable of greatly reducing the local settlement rate of oysters by preying on their planktonic larvae. The introduction and dense establishment of *S. clava* in England occurred simultaneously to a sharp decline in the population of the local ascidian, *Ciona intestinalis* (Linnaeus, 1767) (Lützen 1999). *Styela clava* has effectively replaced the indigenous *Pyura haustor* (Stimpson, 1864) and *Ascidia ceratodes* (Huntsman, 1912), which were the dominant ascidian species in southern California (Lambert and Lambert 1998). However, in the Mediterranean Sea, the population level of this species has not increased in the Bassin de Thau in the three years since its discovery in the area and has not affected the shellfish industry greatly. It is thought that summer water temperatures (max. 29.1 °C) and salinity (max. 40.4 psu) in the area might kill off large proportions of the population (Davis and Davis 2009).

## Conclusions

As the Sea of Marmara's hydrographical conditions conform with the survival requirements of *Styela clava*, it has a great potential to invade the coastal habitats of the Sea of Marmara. In order to stop, or at least mitigate the effects of this invasion, an eradication program should be urgently planned and implemented while the population is still confined to a very small area.

## Acknowledgments

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# First description of the female of the jumping spider *Balmaceda nigrosecta* Mello-Leitão (Salticidae, Dendryphantini, Marpissina)

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### Abstract
The female of *Balmaceda nigrosecta* Mello-Leitão, 1945 is described and illustrated for the first time. In addition, this paper further illustrates the male, and provides the first known observations on the natural history of this species, including habitat, cohabitation, and prey capturedata.

### Keywords
Natural history, salticid, taxonomy

## Introduction

Jumping spiders (Salticidae) constitute a relatively young family, which has rapidly radiated (Bodner and Maddison 2012; Hill and Edwards 2013). This is the largest family of spiders, with 5838 described species (WSC 2015). However, one major problem is that this diversity may be hyper-inflated due to lack of matching of single-sex known species (Edwards 2014). This problem, in part, may have contributed to some quantitative inconsistencies between salticid databases, as observed among those of Prószyński (2015), the WSC (2015), and Metzner (2015).

In Prószyński's database, accepted species status means that both sexes are adequately described and illustrated, while incomplete is due to the description and illustration of only one sex. A proposal for matching sexes was given by Edwards (2014) that consists in searching for an autapomorphy shared by both sexes as an intraspecific counterpart to an interspecific synapomorphy, considering also geographical and other data in an analysis (Edwards 2014). Use of this methodology is strengthened when the species in question belongs to a group that is already well-defined by morphology that agrees with its phylogenetic placement based on molecular data, e.g. Marpissinae (Maddison et al. 2014) [recently reclassified as Subtribe Marpissina (Maddison 2015)].

The marpissine genus *Balmaceda* Peckham & Peckham, 1894 is exclusive to the Americas. It currently includes eight valid species (WSC 2015; Metzner 2015), of which only the type species is known for both sexes, and five are known only for male or female (WSC 2015; the sixth is a nomen dubium). The latter is the case for *B. nigrosecta* Mello-Leitão, 1945. These spiders are similar to other marpissines as *Metacyrba* and *Platycryptus* (see Edwards 2006) in body and external genitalia forms. There is also some similarity to the euophryine *Corticattus* in the body form, but an examination of the genitalia can easily distinguish them (see Zhang and Maddison 2012).

In recent surveys of the salticid fauna from Misiones, in Northeastern Argentina (Rubio 2014), males and females of *B. nigrosecta* were observed and collected together. The coexistence of male and female in the same retreat observed in the field provided definitive evidence for conspecificity in our samples. In this paper, the female of *B. nigrosecta* is described for the first time and its somatic and genital morphology is illustrated. Some data on natural history are also presented.

## Methods

Field observations of living specimens were made in Misiones Province, Northeastern Argentina. Specimens were collected on walls of brick houses in Iguazú National Park and peri-urban habitats of Puerto Iguazú. This area corresponds to the Upper Parana Atlantic Forest Eco-region (Olson et al. 2001).

Morphological terms and description formats follow the main recent works about marpissines (Edwards 2006) and similar jumping spiders (Ruiz and Brescovit 2013). Female genitalia were cleared in clove oil to study the internal structures after digestion in a hot 10–20% KOH solution (Ramírez 2014). Temporary preparations were examined using a Leica DM500 compound microscope and a Leica M60 stereomicroscope. All measurements are in millimeters, and were obtained with an ocular micrometer following Ruiz and Brescovit (2013). Leg segments are measured for length, except the first two femora and tibiae which are measured length x width. Photographs in nature were taken with a Nikon D80 digital camera using a Micro-Nikkor 85 mm lens. Specimens examined are deposited at the arachnological collections of the Instituto de Biología Subtropical, Misiones (IBSI-Ara, G. Rubio).

Abbreviations used are updated, following Zhang and Maddison (2015):

**AG** accessory gland;
**AT** atrium;
**CD** copulatory duct;
**CO** copulatory opening;
**E** embolus;
**FD** fertilization duct;
**RTA** retrolateral tibial apophysis;
**S** spermatheca;
**SP** spermophore;
**T** tegulum.

## Results

### Taxonomy

**Family Salticidae Blackwall, 1841**
**Subfamily Salticinae Blackwall, 1841**
**Tribe Dendryphantini Menge, 1879**
**Subtribe Marpissina Simon, 1901**
**Genus *Balmaceda* Peckham & Peckham, 1894**

***Balmaceda nigrosecta* Mello-Leitão, 1945**
Figs 1–11

*Balmaceda nigrosecta* Mello-Leitão, 1945: 277.
*Metacyrba nigrosecta*, Galiano 1980: 35.
*Balmaceda nigrosecta*, Edwards 2006: 211, figs 123–126; Rubio 2014: 7, fig 11; World Spider Catalog 2015.

**Material examined.** Argentina: Misiones: 1 ♂ (holotype), Puerto Victoria, S26.33441°, W54.65540°, VI.1943, Zenzes leg. (MLP 16710; examined); 1 ♀, Iguazú National Park, Centro de Investigaciones Ecológicas Subtropicales, -25.67859°, -54.44927°, 20.IX.2014, G.D. Rubio leg. (IBSI-Ara 00198; tissue sample GDR 4126); 1 ♂ and 1 ♀, same locality, 17.X.2014, G.D. Rubio leg. (IBSI-Ara 00207); 2 ♀, same locality, 4.III.2015, G.D. Rubio leg. (IBSI-Ara 00291; tissue sample GDR 4143); 1 ♂, Puerto Iguazú, -25.59351°, -54.56968°, 20.XII.2014, J. Baigorria leg. (IBSI-Ara 00246).

**Diagnosis.** Specimens of *B. nigrosecta* resemble *B. picta* Peckham & Peckham, 1894 and *B. reducta* Chickering, 1946 in general body coloration (Chickering 1946; Edwards 2006: figs 115, 118, 123), and in general structure of epigyne, having an anterior atrium with narrow sclerotized rims of the copulatory openings on the posterior edge of the atrium (Figs 4, 5; arrow, rim of CO) (Chickering 1946: fig 47; Edwards 2006: fig 116), respectively. It can be distinguished from *B. picta* by having a larger

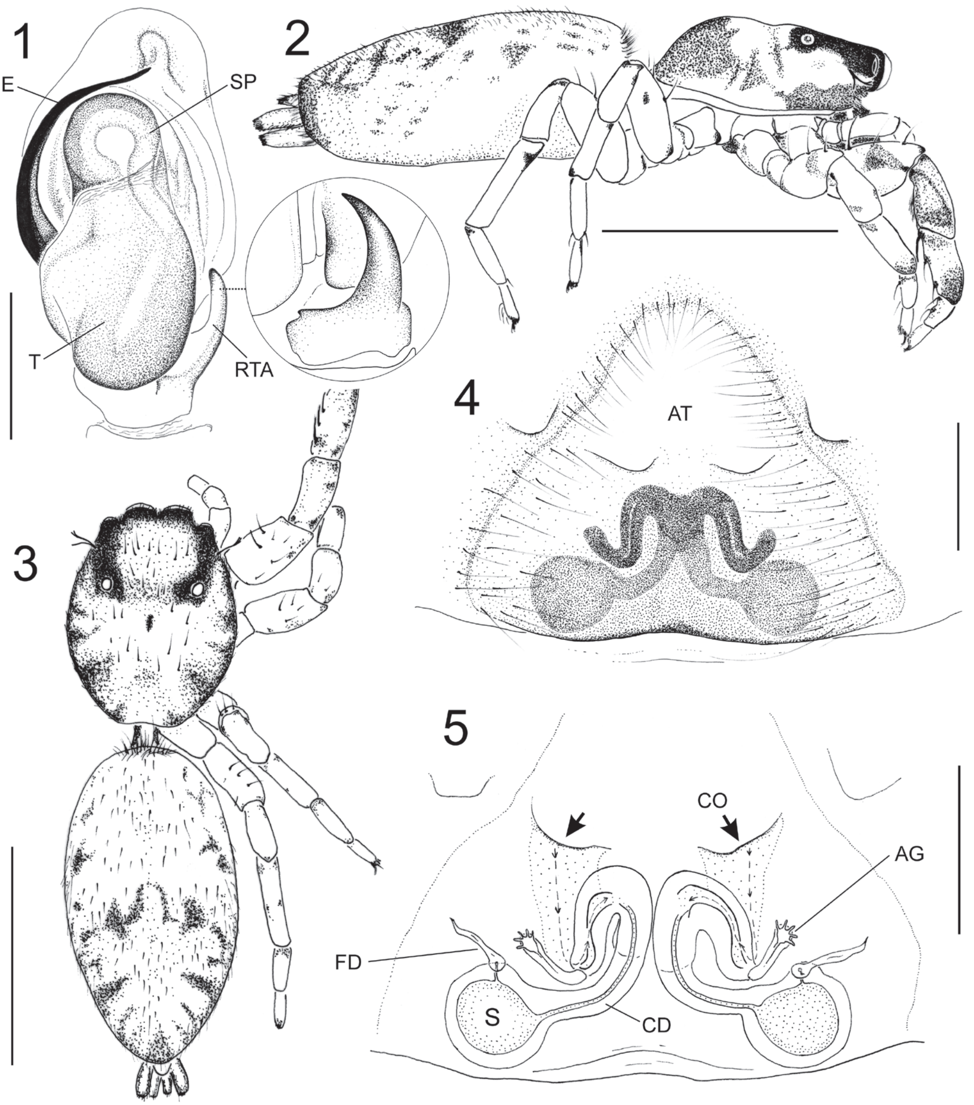


**Figure 1–5.** *Balmaceda nigrosecta* Mello-Leitão. **1** Male palp, ventral view (inset with close-up of RTA in retrolateral view) **2** female, lateral habitus **3** same, dorsal habitus **4** epigyne, ventral view **5** same, cleared. (AG–accessory gland; AT–atrium; CD–copulatory duct; CO–copulatory opening; E–embolus; FD–fertilization duct; RTA–retrolateral tibial apophysis; S–spermatheca; SP–spermophore; T–tegulum). Scale bars: 0.35 mm (**1**); 3 mm (**2**, **3**); 0.2 mm (**4**, **5**).

atrium, with the copulatory openings (CO) farther apart, anteriorly concave, and nearly transverse in orientation (*B. picta* has the COs nearly touching, anteriorly convex, and strongly oblique in orientation), and a thinner and curved male retrolateral tibial apophysis (Figs 1, 4, 5; compare with Edwards 2006: figs 116, 121, 122, 126). Also,

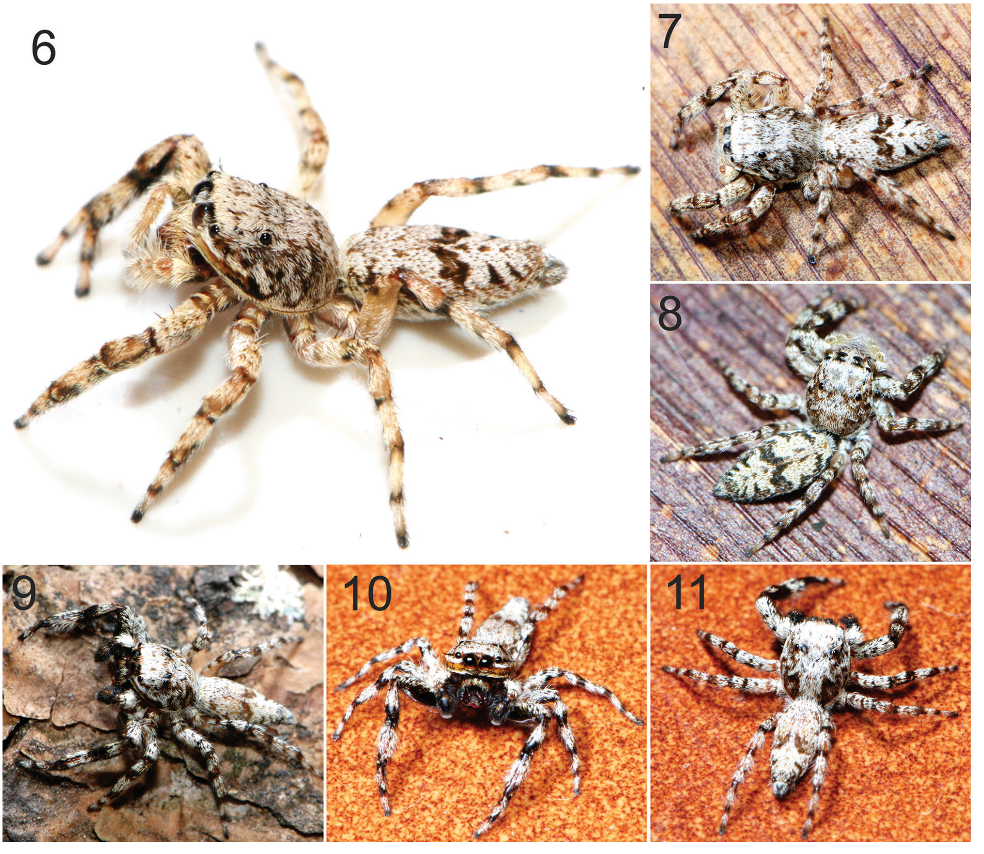


**Figure 6–11.** *Balmaceda nigrosecta* Mello-Leitão, habitus of living specimens from Iguazú National Park. Female (**6–8**); male (**9–11**).

the "W" shaped transverse mark across the middle of the abdomen is distinctive for both sexes, as only the lateral parts of this mark are evident and the medial connecting parts are absent for *B. picta*. It can be distinguished from *B. reducta* by having the copulatory ducts (CD) contiguous along the mid-line of the body in the middle of the duct (Fig 5; compare with Chickering 1946: fig 47).

**Description.** Female from Iguazú National Park (IBSI-Ara 00207) (Figs 2–8). Total length: 7.84. Carapace length: 3.10; width: 2.44; height: 1.00. Carapace low, reddish brown, darker toward the borders and in the cephalic region, covered with white scales and sparse black hairs (Figs 6–8). Length of the dorsal eye field: 1.30. Width of the anterior eye row: 1.65; posterior: 1.45. Clypeus very low (0.05 height), with white hairs. Chelicera dark orange, vertical, with two teeth on promargin and one bicuspid tooth on retromargin. Labium and endites brown, sternum lighter. Palp yellow. Leg I stout, especially tibia and femur. Legs 4123, light brown with scattered dark spots usually where legs articulate, covered with sparse black hairs. Prolateral ventral margin of leg I spotted with brown, mainly on femur, patella and proximal tibia. Femur I 1.68×0.76; II

1.45x0.60; III 1.32; IV 1.67. Patella I 1.15; II 0.92; III 0.80; IV 0.97. Tibia I 1.25x0.42; II 1.00x0.35; III 0.83; IV 1.45. Metatarsus I 0.82; II 0.80; III 0.92; IV 1.09. Tarsus I 0.42; II 0.42; III 0.50; IV 0.52. Leg macrosetae: femur I, II d 1-1-p1, p 0-1-2(d1+v1); III d 1-1-p1, r 0-1-2(d1+v1); IV d 1-1-1. Tibia I v 2-2-2, p 0-1; II v 2-2-2; III, IV v p1di; Metatarsus I, II v2-2; III, IV v 2di, r 1di, p 1di. Abdomen length: 4.20; width: 2.32. Abdomen oval, with sparse black hairs; coloration pale yellow with a brown irregular W-shaped mark in middle of dorsum (Figs 6–8); margins spotted with brown, and four short bands inclined posterolaterally (Figs 6–8); venter pale yellow. Epigyne (Figs 4, 5): epigynal plate large, with a broad anterior atrium (a wide, shallow concavity); atrium shaped like a broad inverted V, each branch of which intersects (in a position just anterior to median) a slightly oblique slit-like copulatory opening that has a narrow strongly sclerotized rim (Fig 5); copulatory ducts short, with a 180° bend backwards, connecting to a spherical spermatheca. An accessory gland (AG) occurs posterior to copulatory opening at posterior end of copulatory duct head (region of duct from CO to gland and first bend in duct). Fertilization duct anterior to spermatheca (Fig. 5). Spinnerets pale yellow. Variability: without significant variation in color pattern (n=4), otherwise total length: 6.19–7.84; carapace length: 2.65–3.10, width: 2.15–2.44, height: 0.87–1.12; abdomen length: 3.36–4.80, width: 1.70–2.70; epigynal plates may vary slightly in amount of sclerotization, and some abdomens are more pigmented than others.

Male (Holotype, MLP 16710): See Mello-Leitão (1945: 277) and Edwards (2006: 211, figs 123–126). Left palp as in figure 1, habitus as in figures 9–11.

**Comments.** From the illustrations in the original description it is clear that this species is very closely related to *B. reducta* (Chickering 1946: 64), and *B. nigrosecta* possibly is a senior synonym of *B. reducta*. Because the known distribution of *B. reducta* is limited (Panama) and far from Argentina, no synonymy will be made, as the genus is not yet adequately sampled in the region.

Because we note that the morphological data are quite similar among members of the genus, it is difficult to establish an intersexual autapomorphy (see Edwards 2014) for *B. nigrosecta*. Nevertheless, it appears that the irregular W-shaped mark in the middle of the abdominal dorsum is a species shared autapomorphy(Figs 6–11). Sex matching is also supported by geographic and phenological evidence, and by an instance of both sexes cohabiting in the same retreat, where an adult male and an adult female were found together.

**Natural history.** *Balmaceda nigrosecta* has sexual dimorphism as frequently occurs in other salticids, althoughstrong dimorphism is uncommon in marpissines. In this case the sexual dimorphism is weak; the males only show a slightly darker color in palps and first pair of legs. They live in many parts of the peri-urban area (Fig 12), even on light poles. They make a flat retreat or nest, about 15 mm long, always placed perpendicular to the ground, between 1 and 2 meters above ground (Figs 13–17). The entrance opening can be on either side. Spiders are positioned at the entrance, with the carapace leaning out (Figs 15, 16), usually looking down (as figure 16). In fifty-one observations, we saw them hunt in the same way: locate prey from the retreat or while actively searching (sometimes we observed them walking in the vicinity of the

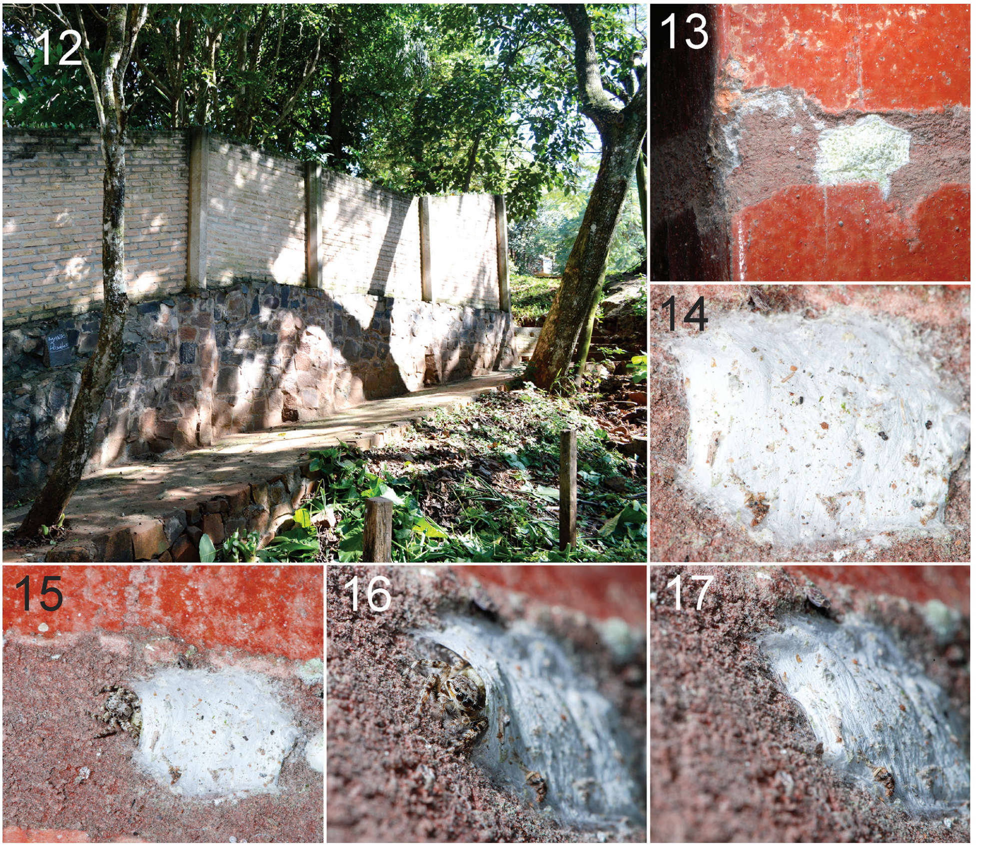


**Figure 12–17.** Habitat and natural retreat of *Balmaceda nigrosecta* Mello-Leitão. Puerto Iguazú (**12**), Iguazú National Park (**13–17**). Note the typical stalking position in **16**.

retreat in search of prey); when they detected something moving, they would accelerate towards it. Here one of two things would happen: if it was an unpalatable prey (e.g. an ant), spiders did not attack and returned quickly to the retreat; but if it was a potential prey, the spiders accelerated a definite distance, about 10 body lengths, and then lowered themselves close to the substrate, and continued approaching very slowly, like a cat stalking, then jumped extremely quickly over the prey. The catch was always observed to be successful, in one movement.

**Distribution.** Only known from northeast Argentina, in Misiones Province.

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# *Bambusimukaria*, a new bamboo-feeding leafhopper genus from China, with description of one new species (Hemiptera, Cicadellidae, Deltocephalinae, Mukariini)

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## Abstract

A new genus and species, *Bambusimukaria quinquepunctata* **gen. & sp. n.**, feeding on bamboo in Guizhou and Fujian, China, are described and illustrated. The characters of crown, frontoclypeus, forewing venations and male genitalia place the new genus in the tribe Mukariini.

## Keywords

Cicadomorpha, Oriental region, species diversity, taxonomy

## Introduction

The bamboo feeding leafhoppers from China were reviewed by Chen et al. (2012). Four of the new species described in this work, i.e., *Abrus xishuiensis* Yang & Chen, *Bambusimukaria quinquepunctatus* Yang, Chen & Li, *Bundera bambusana* Yang & Chen and *Paraonukia wangmoensis* Yang & Chen, were stated as species in press. Although, for all intents and purposes, these species were well described in this work, they do not fit the criteria of the Code (Art. 16.1) in one respect: it was not the authors' intention to formally describe them as new in that publication. Subsequently, the first of these species was named by Yang and Chen (2013) and the last two by Yang et al. (2013). It is the purpose of this paper to formally describe the fourth species *Bambusimukaria quinquepunctatus* and to also assign it to a new genus.

The tribe Mukariini was erected by Distant (1908), placed in the subfamily Nirvaninae (Evans 1947; Li and Chen 1999), and then raised to Mukariinae (Linnavuori 1979; Oman et al. 1990; Hayashi 1996). Recently, it was transferred to the subfamily Deltocephalinae based on molecular and morphological data (Zahniser and Dietrich 2010, 2013). The tribe contains the following genera: *Agrica* Strand, 1942 (three species); *Benglebra* Mahmood & Ahmad, 1969 (two species, reviewed by Khatri and Webb 2011); *Buloria* Distant, 1908 (one species); *Flatfronta* Chen & Li, 1997 (two species); *Mohunia* Distant, 1908 (six species, reviewed by Chen et al. 2007); *Mukaria* Distant, 1908 (13 species, reviewed by Yang and Chen 2011); *Neobassareus* Koçak, 1981 (nine species); *Neomohunia* Chen & Li, 2007 (one species); *Paramohunia* Chen & Li, 2007 (one species); *Pseudobalbillus* Jacobi, 1912 (18 species); *Pseudomohunia* Li, Chen & Zhang, 2007 (one speices); *Scaphotettix* Matsumura, 1914 (four species, reviewed by Dai et al. 2009); *Tiaobeinia* Chen & Li, 2008 (three species).

The following characters place the new genus in Mukariini: crown strongly sloping, frontoclypeus mostly flat, forewing venation obscure except near apex, with four apical cells and appendix well developed and aedeagus with paired shafts and two gonopores.

## Materials and methods

The study on bamboo leafhoppers in China was carried out from 2001 to 2011 for a minimum of ten weeks each year (June to October). All specimens were collected by sweep net in southern provinces of China and were counted and identified in the laboratory using a binocular microscope. A total of 8,000 leafhopper specimens from bamboo were examined and a total of 58 different genera and at least 123 species were identified, belonging to eight subfamilies (Chen et al. 2012).

In the present paper, terminology follows Li et al. (2011) except leg chaetotaxy, which follows Rakitov (1997). Dry specimens were used for the descriptions and illustrations. External morphology was observed under a stereoscopic microscope and characters were measured with an ocular micrometer. Measurements are given in millimeters; body length is measured from the apex of the head to the apex of the forewing in repose. The genital segments of the examined specimens were macerated in 10% KOH, washed in water and transferred to glycerin. Illustrations of the specimens were made with a Leica MZ 12.5 stereomicroscope. Photographs were taken with a Leica D-lux 3 digital camera. The digital images were then imported into Adobe Photoshop 8.0 for labeling and plate composition.

Type specimens of the new species here described are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (IEGU).

## Taxonomy

### Key to genera of Mukariini

| | | |
|---|---|---|
| 1 | Apex of head in profile thin and acuminate, ventral part of face flat and lying nearly horizontally (Figs 6, 8) | **2** |
| – | Apex of head in profile thick and truncate, ventral part of face tumid distally | **5** |
| 2 | Aedeagus with single shaft and 1 gonopore | **3** |
| – | Aedeagus with 2 shafts and 2 gonopores (Figs 21, 23) | **4** |
| 3 | Forewing with vein $M_{3+4}$ originating from the central anteapical cell; male pygofer with one process at inside of posterior margin; subgenital plate with a single row of macrosetae; connective V-shaped | ***Flatfronta*** |
| – | Forewing with vein $M_{3+4}$ originating from inner anteapical cell; male pygofer with two processes at posterior margin; subgenital plate with several rows of macrosetae; connective Y-shaped | ***Tiaobeinia*** |
| 4 | Hindwing with veins $R_{4+5}$ and $M_{1+2}$ separated basally (Fig. 9); male anal segment with large process ventrally (Figs 16–18) | ***Bambusimukaria*** |
| – | Hindwing with veins $R_{4+5}$ and $M_{1+2}$ confluent basally; male anal segment without process ventrally | ***Pseudobalbillus*** |
| 5 | Crown in dorsal view rather short, anterior margin broadly rounded | ***Buloria*** |
| – | Crown in dorsal view relatively long, anterior margin acutely rounded | **6** |
| 6 | Aedeagus with 2 shafts and 2 gonopores | **7** |
| – | Aedeagus with single shaft and 1 gonopore | **9** |
| 7 | Male pygofer side with process | **8** |
| – | Male pygofer side without process | ***Neobassareus*** |
| 8 | Body broad and dorsoventrally depressed, black, without longitudinal stripe dorsally; anterior margin of head with several carinae; male pygofer with process at posterior or ventral margin | ***Mukaria*** |
| – | Body normal, yellowish white, with dark longitudinal stripe dorsally; anterior margin of head without carina; male pygofer with process at inside of dorsal margin | ***Pseudomohunia*** |
| 9 | Valve and subgenital plates fused | ***Agrica*** |
| – | Valve and subgenital plates not fused | **10** |

10 Forewing with vein $M_{3+4}$ originating from central anteapical cell .............**11**
– Forewing with vein $M_{3+4}$ originating from inner anteapical cell.................**13**
11 Male pygofer with process at posterior margin ..............................***Mohunia***
– Male pygofer with process at ventral margin ............................................**12**
12 Male pygofer with a single process at inside of ventral margin ......***Benglebra***
– Male pygofer with paired processes at ventral margin...............***Scaphotettix***
13 Hindwing with veins $R_{4+5}$ and $M_{1+2}$ confluent basally; connective Y-shaped ..... ........................................................................................***Neomohunia***
– Hindwing with veins $R_{4+5}$ and $M_{1+2}$ separated basally; connective slender quadrate........................................................................... ***Paramohunia***

***Bambusimukaria*** **gen. n.**
http://zoobank.org/F6030468-65D6-48A2-A5B7-3B36477D9DB9
Figs 1–23, 26, 27

**Type species.** *Bambusimukaria quinquepunctata* sp. n., here designated.

**Diagnosis.** Crown with anterior and submarginal carinae; entire second segment of antenna visible from above. Frontoclypeus transversely impressed across base beneath prominent overhanging anterior edge of head. Forewing with four apical cells, venation obscure except near apex, vein $M_{3+4}$ originating from junction of inner and central anteapical cell. Hind wing with four closed apical cells. Ventral margin of male pygofer without process. Style with short articulating arm and broad outer basal arm. Connective Y-shaped, fused with aedeagus. Aedeagus with paired stout shafts diverging from base, gonopores subapical, large; basal apodeme short.

**Description. Head and thorax.** Crown (Figs 4, 7) shorter than pronotum, subconically anteriorly rounded, more than half as long as breadth between eyes, with anterior and submarginal carinae, posterior end of anterior carina strongly incurved before eyes; disk strongly sloping posteriorly, texture smooth; ocelli on crown, distant from eyes and close to anterior margin; entire second segment of antenna visible from above; eyes long, oblique, extending backward over anterior angles of pronotum; face (Fig. 5) including eyes as long as broad, frontoclypeus transversely impressed across base beneath prominent overhanging anterior edge of head, narrowed towards clypeus; clypellus narrowing apically; lorum broad. Pronotum (Figs 4, 7) elevated centrally, arched, anterior margin convexly rounded between eyes, posterior margin slightly concave, lateral margin short. Scutellum (Figs 4, 7) large, broad, basal margin longer than lateral margin, transverse depression slightly curving. Forewing (Figs 1–3, 9) elongate, considerably longer than abdomen, slightly widened posteriorly, with four apical cells, venation obscure except near apex, vein $M_{3+4}$ originating from junction of inner and central anteapical cell; appendix well developed. Hind wing (Fig. 10) with four closed apical cells. Profemur (Fig. 11) with 2 dorsoapical setae, row AM with 1 stout seta, and row AV with several fine setae. Protibia (Fig. 11) with 4 macrosetae in row AD and with 13 macrosetae approximately equal in length in row AV. Hind femur broadened distally and slightly bowed; apical setal formula 2+2+1. Hind tibia flattened and nearly straight, with PD setae very long, alternating in length and with 1 smaller setae between macrosetae; row AD with 14 macrosetae interspersed by 1 to 2 small stout setae; several supernumeral setae present between AD and AV rows. Metabasitarsomere with 3 platellae and 2 setae on apical transverse row, and one row of 6 stout setae at middle and one row of 4 stout setae at lateral margin.

**Male genitalia.** Male pygofer (Figs 15, 16) rather dorso-ventrally depressed, with macrosetae caudally; ventral margin without process. Valve (Fig. 19) broad, subtriangular. Subgenital plate (Fig. 19) very short, broad basally, with group of moderately long fine setae laterobasally and few short fine setae apically. Style (Fig. 20) with short articulating arm and broad outer basal arm. Connective (Fig. 21) Y-shaped, fused with aedeagus. Aedeagus (Figs 21–23) with paired stout shafts diverging from base, gonopores subapical, large; basal apodeme short, thumb-like in lateral view.

**Female genitalia.** Sternite VII (Fig. 12) with hind margin broadly concave. Pygofer with numerous macrosetae. Ovipositor protruding slightly beyond pygofer apex. First valvula (Fig. 13a, b) weakly curved; dorsal sculpturing pattern strigate, reaching dorsal margin; without distinctly delimited ventroapical sculpturing. Second valvula (Fig. 14a, b) broad, widest near mid-length, thereafter gradually tapered to acute apex; with broad dorsal sclerotized area, thereafter dorsal margin with numerous fine regular teeth after dorsal prominences.

**Host plant.** Bamboo (Figs 24–27).

**Distribution.** Southwest and south China.

**Etymology.** The genus name, which is feminine, is a combination of “bambus” (bamboo) and “*Mukaria*” (name of the type genus of Mukariini), meaning that members of this genus feeding exclusively on bamboo (Bambusoideae).

**Remarks.** The new genus can be distinguished from other genera of Mukariini by the very large anal tube process (see also above key to genera of Mukariini). Among other Chinese mukariin genera, the new genus is somewhat similar to *Flatfronta* Chen & Li, 1997 and *Tiaobeinia* Chen & Li, 2008 in the shape of head, and also similar to *Mukaria* Distant, 1908 in the shape of male genitalia. See also Table 1 for further comparisons.

### ***Bambusimukaria quinquepunctata* sp. n.**

http://zoobank.org/A5330454-C791-40F9-8BAC-9FCEFD88EFEF
Figs 1–23, 26, 27

*Bambusimukaria quinquepunctatus*, in press, Chen et al. (2012).

**Type material.** Holotype: ♂, **China:** Forest Park (26°35'N, 106°42') (1100 m), Guiyang, Guizhou, on bamboo (*P. bambusoides*), 11 Aug. 2006, X.-S. Chen and L. Yang;

paratypes: 4♂♂, 7♀♀, data same as holotype; 1♀, Dongtang (25°24'N, 107°52'), Maolan, Libo, Guizhou, on bamboo, 24 May 1998, X.-S. Chen; 10♀♀, Dayi (25°21'N, 106°06'), Wangmo, Guizhou, on bamboo (*P. bambusoides*), 28 July 1998, X.-S. Chen; 25♂♂, 6♀♀, Forest Park, Guiyang, Guizhou, on bamboo, 11 July 2006, Q.-Z. Song; 1♀, Weiyuan (26°01'N, 106°31'), Changshun, Guizhou, on bamboo, 11 July 2007, X.-S. Chen; 6♀♀, Daxianfeng (26°55'N, 116°59'), Datian, Sanming, Fujian, on bamboo, 14 May 2011, Z.-M. Chang and J.-K. Long; 5♀♀, Tianyanbao (26°39'N, 118°53'), Yongan, Fujian, on bamboo, 17 May 2011, Z.-M. Chang and W.-C. Yang. All types are deposited in IEGU except two males and two females deposited in BMNH where indicated.

**Diagnosis.** General color yellowish white to yellowish orange. Head and thorax with five black markings. Female sternite VII with two blackish brown markings. Anal (Xth) segment with a very large process at apical-ventral margin. Aedeagus with shafts diverging from base, each shaft narrower at base, broad to near apex, outer margin extended apically into a stout acute process inner margin with a stout subapical tooth-like process directed medially, dentate on dorsal suface, gonopores subapical on ventral surface.

**Description. Measurements.** Body length including forewing: male 5.30–5.40 mm (n = 30), female 5.50–5.60 mm (n = 36).

**Coloration.** General color yellowish white to yellowish orange (Figs 1–6, 26, 27). Eyes yellowish brown. Head and thorax (Figs 4, 7) with five black markings, one at apex of crown, two on anterior margin of pronotum and two on anterior margin of mesonotum. Fore tibia with one dark brown mark subapically. Female sternite VII with two blackish brown markings (Fig. 12).

**Head and thorax.** Crown (Figs 4, 7) with median length shorter than width between eyes (0.62:1). Face including eyes (Fig. 5) slightly shorter in middle line than broad at widest part (0.81:1). Pronotum (Figs 4, 7) wider than head including eyes (1.17:1), longer than vertex in middle line (1.48:1). Scutellum (Figs 4, 7) as long as pronotum in middle. Forewing (Fig. 9) 3.4 times longer in middle line than widest part. Hindwing (Fig. 10) 2.13 times longer in middle than widest part.

**Male genitalia.** Anal (Xth) segment (Figs 15–18) with a very large process at apical-ventral margin, directed cephalad, tapering distally to acute apex. Pygofer (Figs 15, 16) broad and rounded in lateral view, with many macrosetae. Valve (Fig. 19) with basal width 2 times longer than median length, posterior margin rounded. Subgenital plate (Fig. 19) very short, broad at base, tapering to acutely rounded apex. Style apophysis (Fig. 20) thumb-like, slightly sinuate, apex rounded. Connective stem (Figs 21, 22) slightly shorter than arms, fused with base of aedeagus. Aedeagus (Figs 21–23) in ventral view with shafts diverging from base, each shaft narrower at base, broad to near apex, outer margin extended apically into a stout acute process inner margin with a stout subapical tooth-like process directed medially, dentate on dorsal suface, gonopores subapical on ventral surface.

**Female genitalia.** Sternite VII (Fig. 12) with anterior margin angularly produced laterally, posterior margin strongly and broadly concaved. First and second valvulae

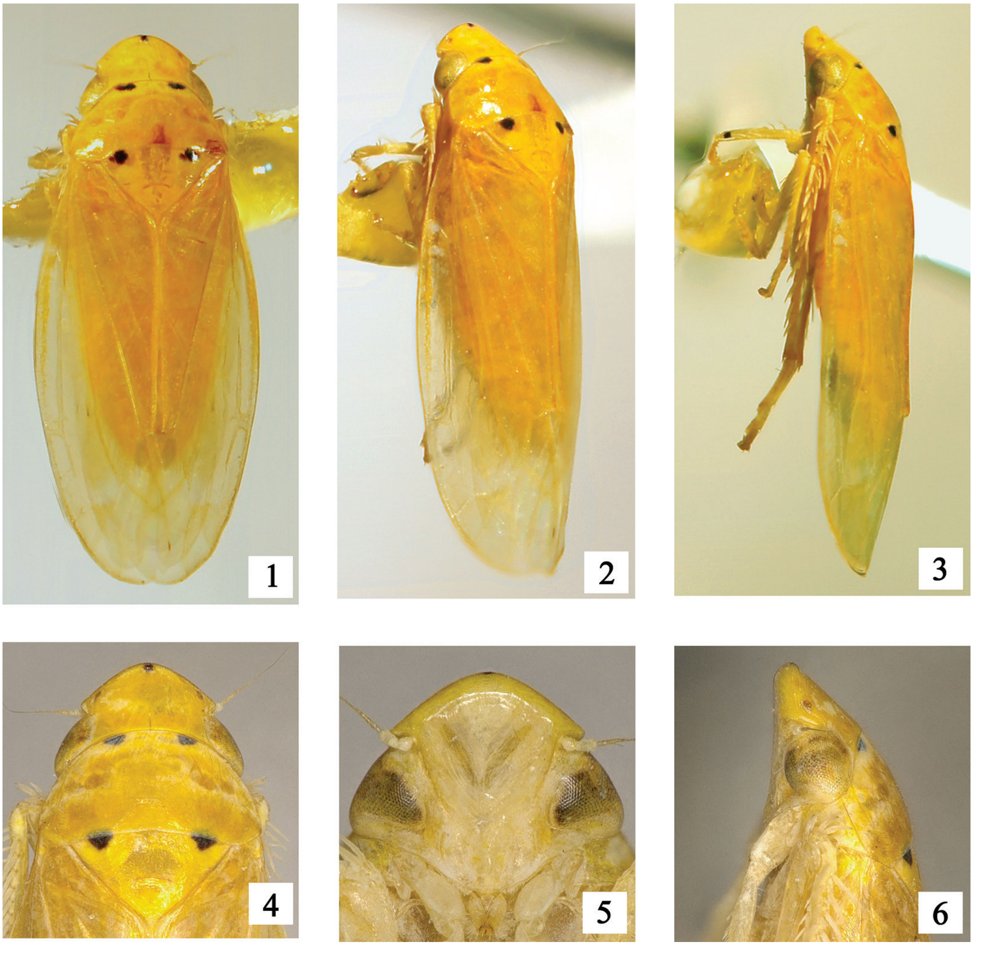
(Fig. 13a, b) as in generic description; second valvulae (Fig. 14a, b) bearing approximately 36 fine teeth on apical half behind dorsal prominence and basal curvature.

**Host plant.** Bamboo (*Phyllostachys bambusoides* f. *lacrimadeae* Keng *et* Wen) (Figs 24–27).

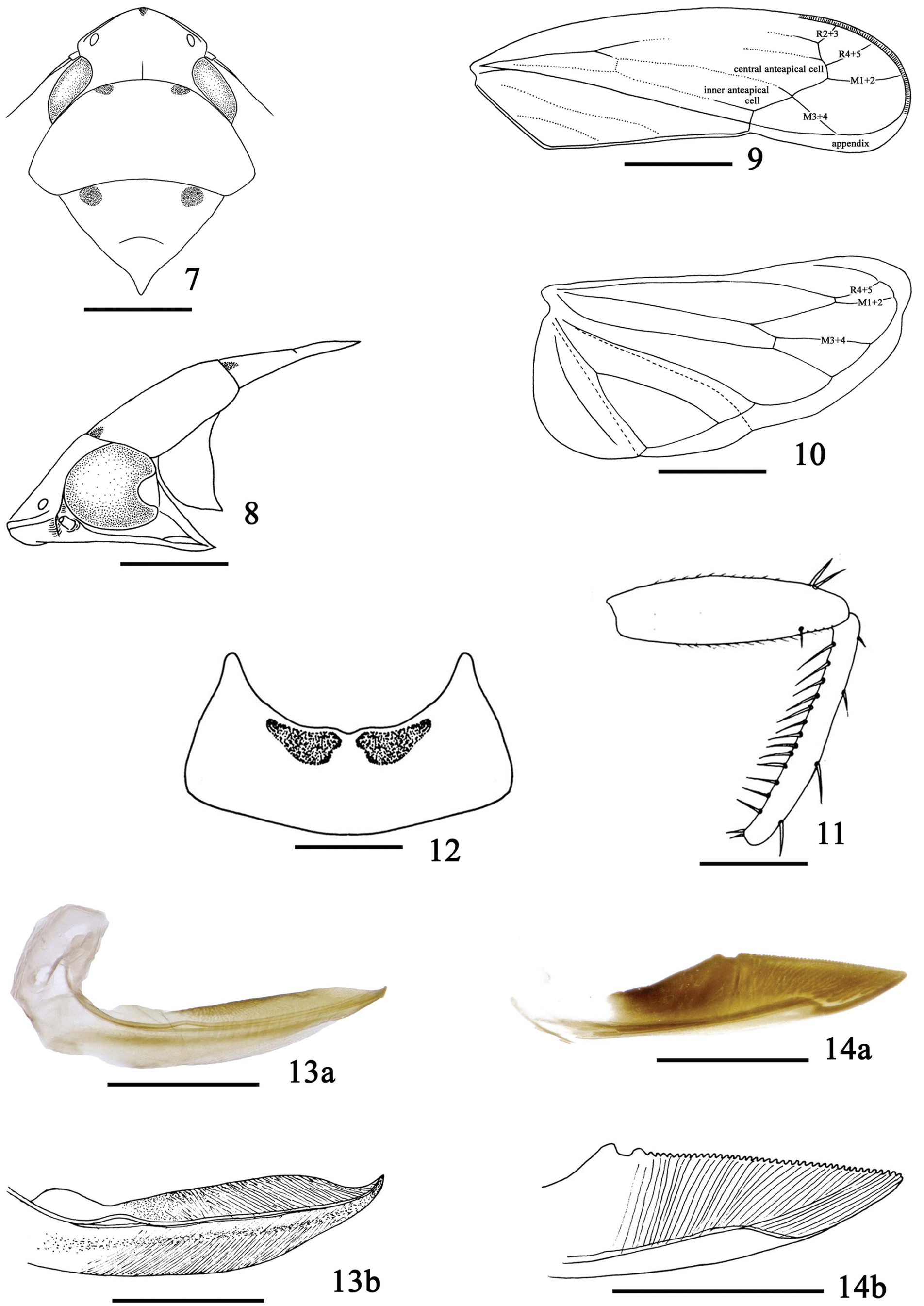
**Distribution.** Southwest and south China (Guizhou, Fujian).

**Etymology.** The name is a combination of the Latin words "quinque" (five) and "punctata" (spots), which refers to the dorsum of head and thorax with five small dark spots.

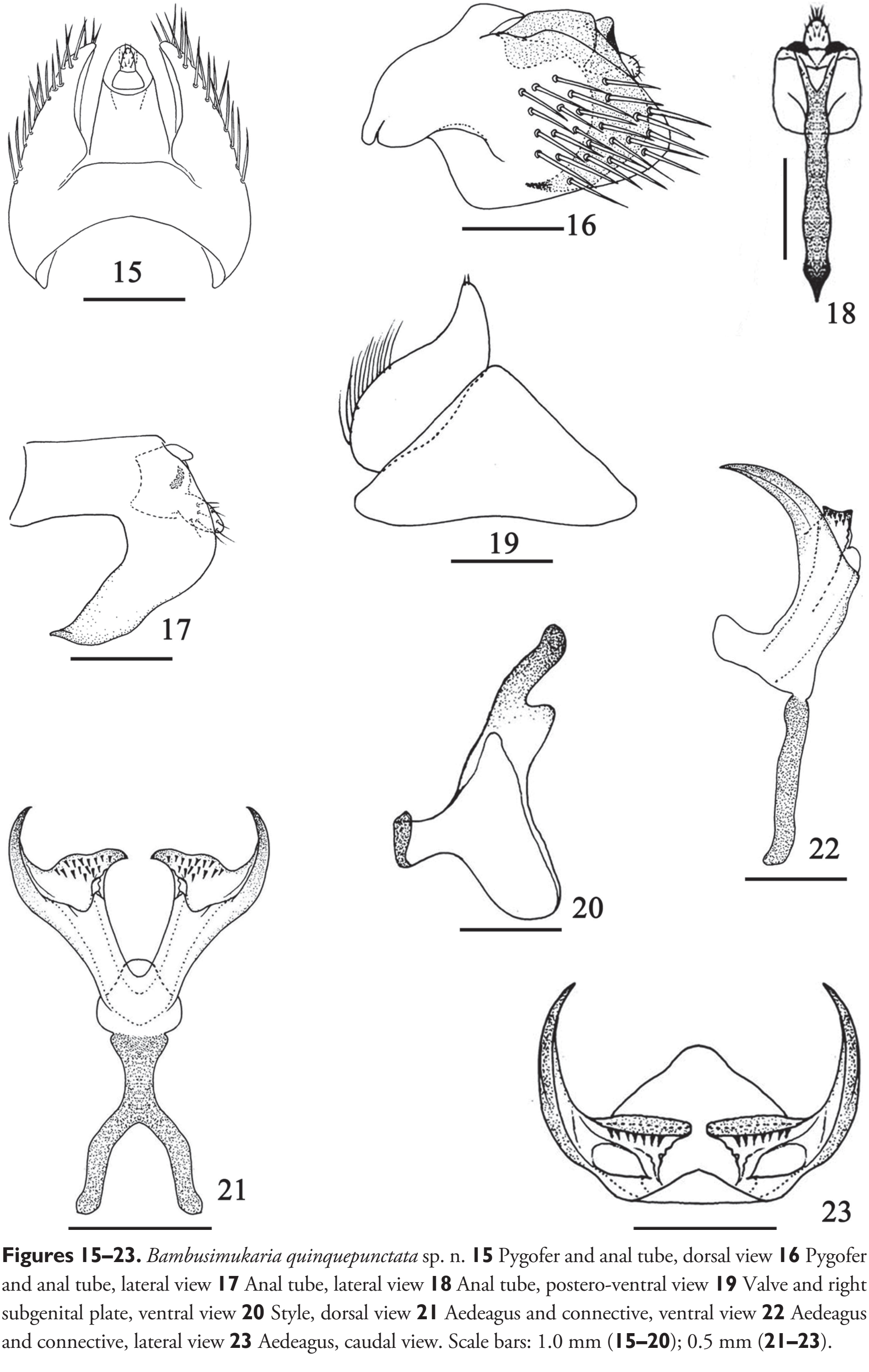
**Remarks.** The new species can be distinguished from other species of Mukariini by the very large anal tube process.



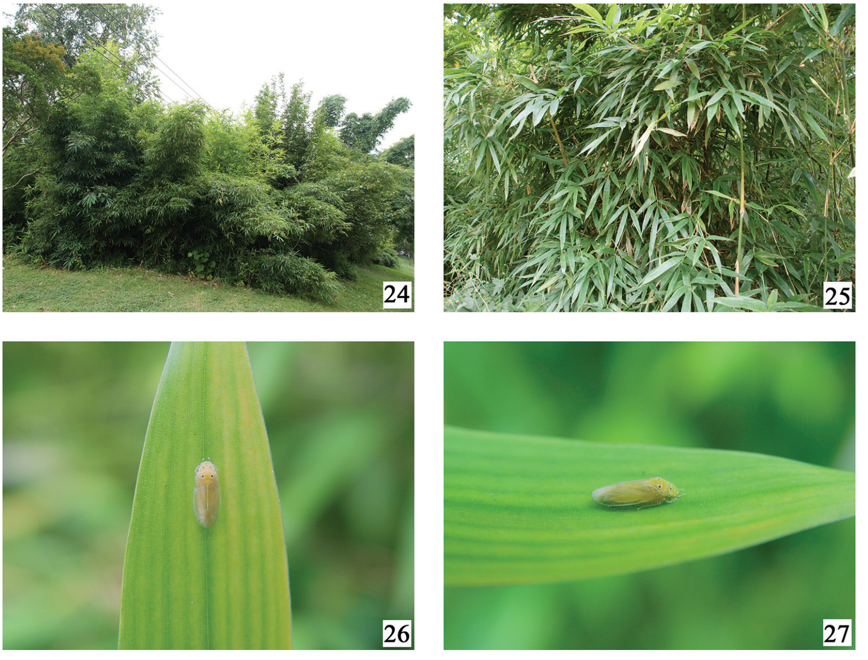

**Figures 1–6.** *Bambusimukaria quinquepunctata* sp. n. **1** Male habitus, dorsal view **2** Male habitus, dorsal and lateral view **3** Male habitus, lateral view **4** Head and thorax, dorsal view **5** Face **6** Head and thorax, lateral view.




**Figures 7–14.** *Bambusimukaria quinquepunctata* sp. n. **7** Head and thorax, dorsal view **8** Head and thorax, lateral view **9** Forewing **10** Hindwing **11** Fore femur and tibia, anterior surface **12** Female sternite VII, ventral view **13a** First valvula and valvifer, lateral view **13b** Apex of first valvula, lateral view **14a** Second valvula, lateral view **14b** Apex of second valvula, lateral view. Scale bars: 1.0 mm (**7–12**); 0.5 mm (**13–14**).




**Figures 15–23.** *Bambusimukaria quinquepunctata* sp. n. **15** Pygofer and anal tube, dorsal view **16** Pygofer and anal tube, lateral view **17** Anal tube, lateral view **18** Anal tube, postero-ventral view **19** Valve and right subgenital plate, ventral view **20** Style, dorsal view **21** Aedeagus and connective, ventral view **22** Aedeagus and connective, lateral view **23** Aedeagus, caudal view. Scale bars: 1.0 mm (**15–20**); 0.5 mm (**21–23**).



**Figures 24–27.** Host plant of *Bambusimukaria quinquepunctata* sp. n. **24** View of the area where the types of *B. quinquepunctata* were captured, in Guiyang Forest Park (Guizhou, China) with *Phyllostachys bambusoides* f. *lacrimadeae* Keng & Wen **25** View of the plant **26** *B. quinquepunctata* resting on a leaf of *P. bambusoides* f. *lacrimadeae*, dorsal view (Guiyang Forest Park, Guizhou) **27** same, lateral view. (11 Aug 2006, photography by X.-S. Chen)

**Table 1.** Morphological comparison of *Bambusimukaria* to similar genera, *Flatfronta*, *Tiaobeinia* and *Mukaria*.

| | *Bambusimukaria* | *Flatfronta* | *Tiaobeinia* | *Mukaria* |
|---|---|---|---|---|
| Body form | Depressed | Depressed | Depressed | Weakly depressed |
| No. of carinae on crown | Two | One | One | Two or three |
| Anterior margin of crown in dorsal view | Strongly incurved before eyes | Smoothly curved | Smoothly curved | Smoothly curved |
| Disk of crown | Strongly elevated posteriorly | Weakly elevated posteriorly | Weakly elevated posteriorly | Strongly elevated posteriorly |
| Frontoclypeus form | Mainly flat | Mainly flat | Mainly flat | Tumid anteriorly and depressed posteriorly |
| Forewing vein $M_{3+4}$ originating from | Inner anteapical cell | Central anteapical cell | Inner anteapical cell | Inner anteapical cell |
| Hindwing veins $R_{4+5}$ and $M_{1+2}$ | Separated basally | Confluent basally | Separated basally | Separated basally |
| Hind femur macrosetae | 2+2+1 | 2+2+1 | 2+2+1+1 | 2+2+1 |
| Pygofer process | Absent | Present | Present | Present or absent |
| Subgenital plate macrosetae | Absent | One row | Several rows | Absent |
| Connective form | Y-shaped | V-shaped | Y-shaped | U-shaped |
| Ventral process of anal segment | Present | Absent | Absent | Absent |
| Number of gonopores | Two | One | One | Two |

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# New species of subgenus *Tipula* (*Sivatipula*) from China, with redescription of *T.* (*S.*) *parvauricula* and a key to all known species of the Oriental Region (Diptera, Tipulidae, *Tipula*)

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## Abstract

Species of *Tipula* (*Sivatipula*) *biprocessa* **sp. n.** from Guangxi, China is described and illustrated as new in the subgenus *Tipula* (*Sivatipula*) Alexander, 1964. *T.* (*S.*) *parvauricula* Alexander, 1941 is redescribed and illustrated based on additional morphological characters. Semen pump of this subgenus is discussed. A key to all described species in this group is compiled.

## Keywords

China, crane flies, new species, semen pump, *Sivatipula*, *Tipula*, Tipulidae

## Introduction

*Tipula* (*Sivatipula*) Alexander, 1964 is a small subgenus with *Tipula mitocera* Alexander, 1927 from the eastern Himalayas, India as its type species. The other species in this subgenus include *T.* (*S.*) *pullimargo* Alexander, 1951 from Myanmar, *T.* (*S.*) *alhena* Alexander, 1953 from Thailand, *T.* (*S.*) *filicornis* Brunetti, 1918 and *T.* (*S.*) *bhishma* Alexander, 1964 from India, *T.* (*S.*) *lackschewitziana* Alexander, 1928, *T.* (*S.*) *suensoniana* Alexander, 1940 and *T.* (*S.*) *parvauricula* Alexander, 1941 from China (Oosterbroek 2015). All these species are restricted to the Oriental region. The Chinese fauna of *Tipula* (*Sivatipula*) is poorly represented with only three known species.

This subgenus is characterized by the following characters: male with antennae very long, slightly shorter, equal to or longer than body length, female with antennae relatively short, not beyond half length of body, flagellomere covered with six or seven long strong verticils; wing with squama naked, outer wing veins scattered with small, abundant macrotrichia, $R_{1+2}$ entire, Rs longer than m-cu; ninth tergite and sternite fused, median region of sternite extensive, more or less protrudent, forming amembranous extension; inner and outer gonostylus irregularly varied in shape. The species of subgenus *Tipula* (*Sivatipula*) had been placed previously in subgenus *Tipula* (*Acutipula*) Alexander, 1924, but treated subsequently as a distinct group based on the combined structural characters of antennae, hypopygium, and wing (Alexander 1964).

A previously unknown taxon of *Tipula* (*Sivatipula*) was noticed while sorting crane flies specimens collected from Leigongshan Mountain, Guizhou Province, and Cenwanglaoshan Mountain, Guangxi Zhuang Autonomous Region, China. In the present paper, the new species is described and illustrated, and a key is provided for separating all known species. *Tipula* (*S.*) *parvauricula* is redescribed based on newly available morphological characters with detailed illustrations. The original description of this species is insufficient and the illustrations are too simple to reveal necessary characters. The character of semen pump of subgenus *Tipula* (*Sivatipula*) is described for the first time. The current study also demonstrated the new distribution pattern for the subgenus *Tipula* (*Sivatipula*) in both Guangxi Zhuang Autonomous Region and Guizhou Province. Future collecting and investigation would undoubtedly increase the species numbers and range extension of this group in China.

## Material and methods

The genital segments of the specimens were soaked in 10% NaOH overnight and observed or drawn in glycerine using a Leica MZ125 (Leica, Germany) stereomicroscope. The genital segments were then preserved in glycerine in 0.20 ml centrifuge tubes. Photographs of partial body of male were taken by Canon 5D Mark II digital single lens reflex camera (Canon, Japan) with MP-E 65mm f/2.8 1-5X macro lens (Canon, Japan). All measurements are in millimeters (mm), made with the aid of a digital caliper. The terminology and methods of description and illustration follow those of Alexander and Byers (1981) and Frommer (1963). The type specimens of the new species are deposited in the animal specimen room, School of Life Sciences, Anqing Normal University, Anqing, Anhui Province, China.

The key was principally constructed from descriptions in the literature without examination of the type species of most of these species, and should be considered preliminary. The characters used in the key rely primarily on the structures of genitalia and the length of antenna of male specimens.

## Taxonomy

### *Tipula* (*Sivatipula*) *parvauricula* Alexander, 1941

Figs 1–11

*Tipula parvauricula* Alexander, 1941: 400 (original description), Pl. 1, fig. 14, Pl. 4, fig. 44.

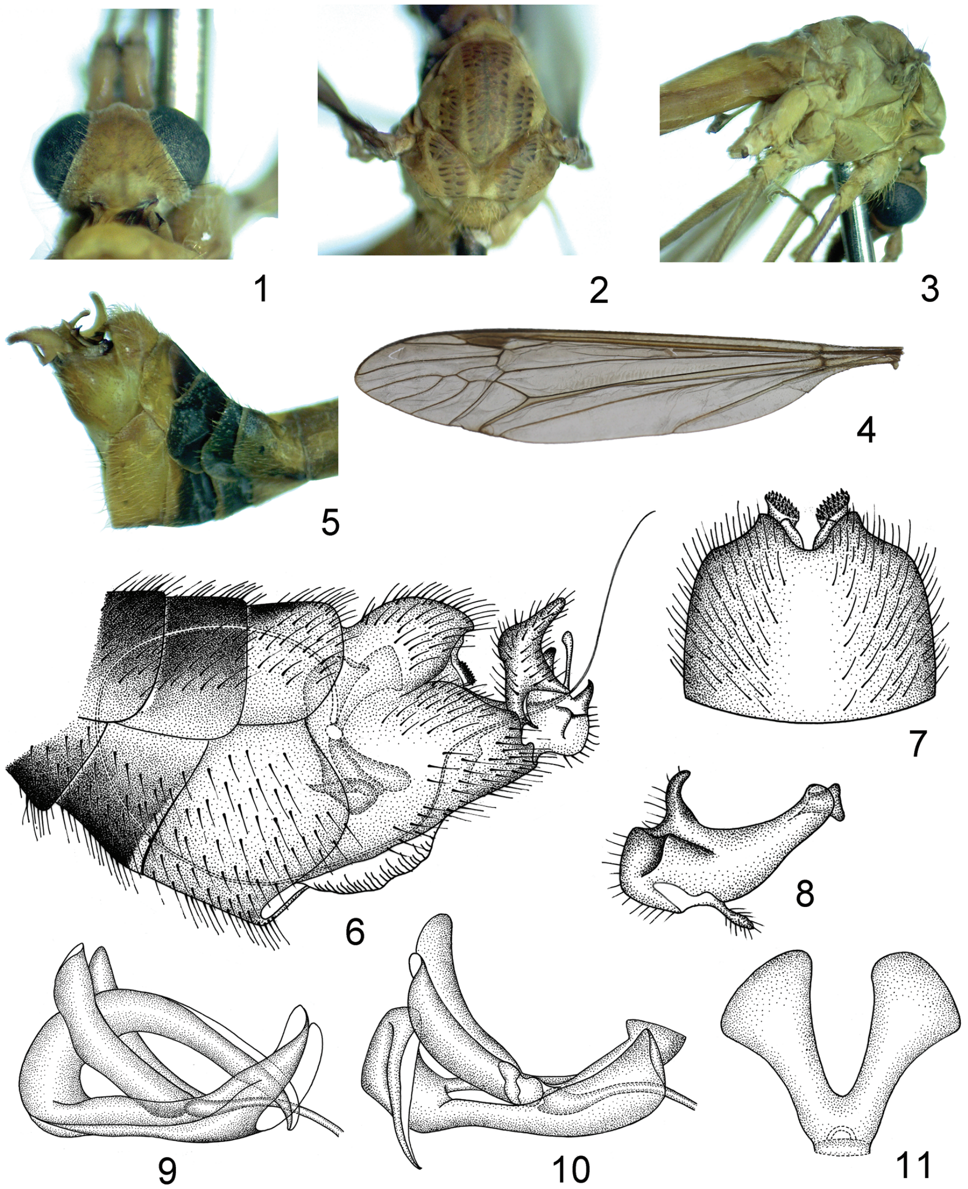
**Diagnosis.** Generally orange-yellow in coloration; antenna distinctly longer than body; prescutum orange-yellow with three light brown stripes, sometimes not clear; wings pale brown with a dark brown stigma; abdomen orange with segments six and seven black; hypopygium orange, tergite nine divided by a V-shaped notch, produced into a pair of ear-like processes, sometimes terminated into spinous point, ventral-lateral portions of tergite nine projected into two lobes, densely covered with black setae.

**Redescription.** Male. Length: *Body*: 14.0–18.0 mm (not including antenna, n = 5); *Wing*: 18.0–20.0 mm (n = 5); *Antenna*: 18.0–20.0 mm (n = 5).

*Head* orange. Rostrum orange with distinct orange nasus. Marking of vertex absent (Fig. 1). Eyes black (Fig. 1). Antenna: 12-segmented, distinctly longer than body; scape orange, expanded apically; pedicel orange, short; flagellomeres dark brown, each flagellomere cylindrical and subequal in length, with abundant black verticils, longest one longer than one third of flagellomeres length. Palpi light brown.

*Thorax* with pronotum entirely orange-yellow (Figs 2–3). Prescutum with three light brown stripes, sometimes not clear (Fig. 2). Scutum orange-yellow with two light brown stripes (Fig. 2). Scutellum and postnotum orange-yellow (Fig. 2). Pleura entirely bright yellow (Fig. 3). Legs slender, coxae, trochanters and femora orange-yellow, tibiae orange-yellow at basal half, changing to brown at apical half, tarsi brownish black. Halteres with stem yellow, knob darker. Wings pale brown, cell sc darker than ground color, stigma dark brown (Fig. 4). Sc relatively short, subequal to $R_3$ in length, petiole of cell m1 as long as or slightly longer than m-m, approximately one-half length of discal cell (Fig. 4).

*Abdomen* orange with segments six and seven black. Hypopygium orange, broad, compressed (Figs 5–6). Tergite nine separated by a V-shaped notch in ventral view; gradually narrowed to apex, produced into a pair of ear-like processes, sometimes terminated into spinous point in lateral view; ventral-lateral portions of tergite nine pro-




**Figures 1–11.** *T.* (*S.*) *parvauricula* **1** head, dorsal view **2** thorax, dorsal view **3** thorax, right lateral view **4** wing **5** hypopygium, right lateral view **6** perspective hypopygium, left lateral view **7** ninth tergite, dorsal view **8** inner gonostylus, left lateral view **9** and **10** semen pump, left lateral view **11** compressor apodeme, dorsal view.

jected into two lobes, densely covered with blackened setae (Figs 5–7). Sternite nine broader than tergite nine, median region of sternite nine protruded to a membranous extension (Fig. 6). Outer gonostylus flattened, widened medially, with a slender rod on ventral-lateral margin, directed dorsally (Fig. 6). Inner gonostylus broad basally, gradually narrowed to apex, rounded with a process truncated apically, ventral margin with a horn-shaped projection, dorsal margin with a finger-like process, basal region of inner gonostylus with a pyramidal process (Fig. 8).

*Semen pump* with compressor apodeme V-shaped, the arms expanded at apex (Fig. 11). Posterior immovable apodeme with only one arm, distinctly longer than compressor apodeme, gradually narrowed to apex and curved cephalad in lateral view (Fig. 9), sometimes abruptly bent to ventral margin (Fig. 10), the arm deeply grooved in dorsal view. Anterior immovable apodeme flattened, shorter than compressor apodeme, gradually narrowed to apex in lateral view (Figs 9–10). Aedeagus elongated, tubular, at least ten times longer than semen pump (Fig. 6).

**Material examined. CHINA:** Guangxi Zhuang Autonomous Region: 2 males, Dalongping, Cenwanglaoshan Mountain, 24°31'N, 106°17'E, 1300 m, 11 May 2015, Guo-Xi Xue leg.; Guizhou Province: 3 males, Leigongshan Mountain, 26°21'N, 108°13'E, 4 Jun. 2015, Guo-Xi Xue leg.

**Distribution.** China (Fujian, NW. Guangxi, SE. Guizhou).

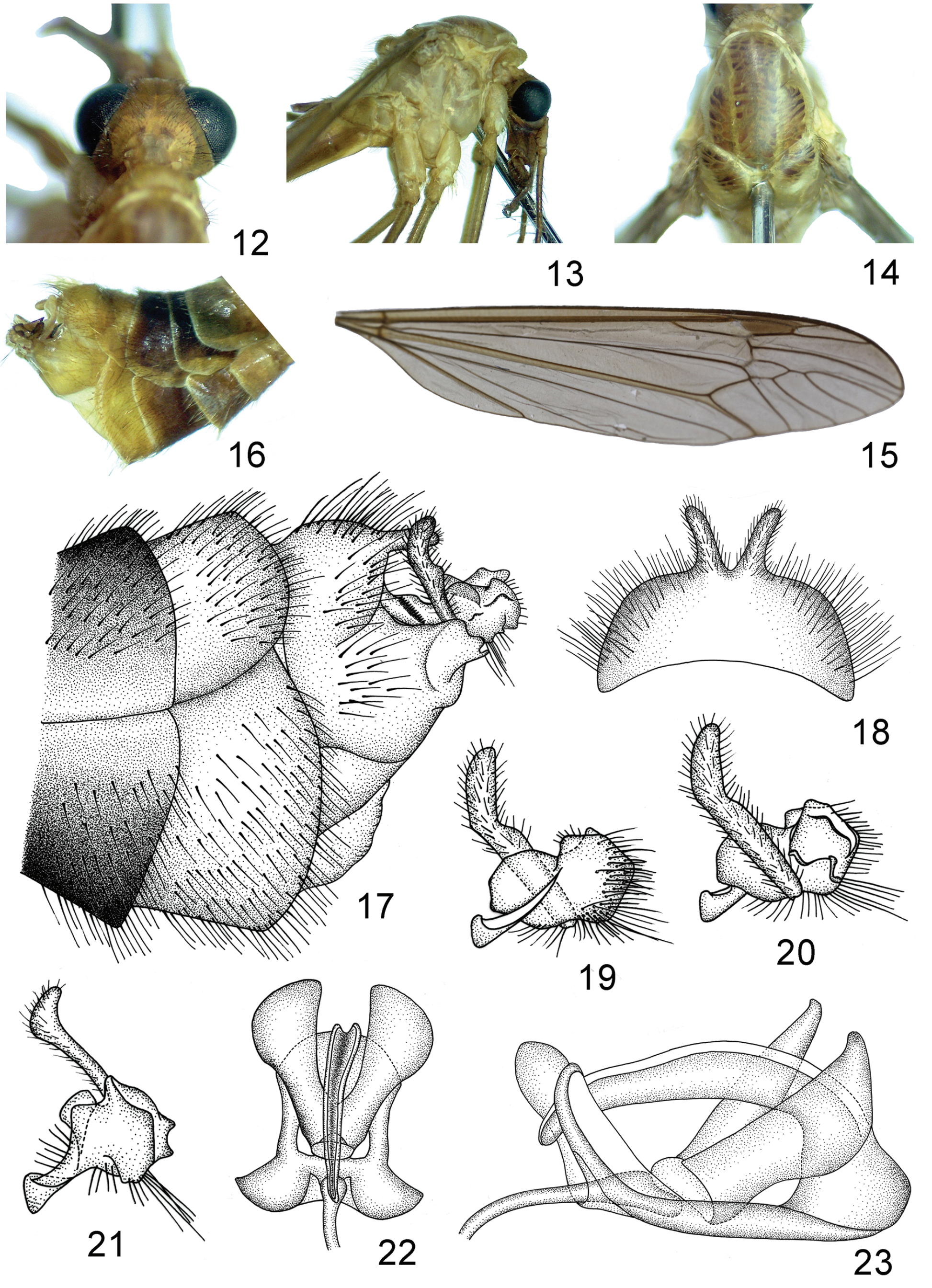
**Remarks.** In the original description of *T.* (*S.*) *parvauricula* (Alexander 1941), the prescutum is unmarked and the tergite nine is terminated into a pair of spinous points in lateral view. After observing five specimens, we noticed that the prescutum generally has three light brown stripes and the spinous point on tergite nine is not always present.

Three types of semen pumps were defined by Frommer (1963) based on morphological studies of the reproductive system of North American crane flies. Type III is the most common type characterized by the strongly bowed intromittent organ and by posterior immovable apodeme generally with two arms (Frommer 1963). According to the overall morphology, the semen pump of *T.* (*S.*) *parvauricula* should belong to Type III. However, its posterior immovable apodeme has only one arm, which differs from the results in previous works in Chinese species (*Ctenophora fumosa* Men, 2014 in Men and Huang 2014; *Tipula* (*Vestiplex*) *coxitalis* Alexander, 1935, *Tipula* (*Pterelachisus*) *biaciculifera* Alexander, 1937and *Tipula* (*Emodotipula*) *yaoluopingensis* Men, 2015 in Men 2015; *Tipula* (*Yamatotipula*) *nova* Walker, 1848 in Men et al. 2015a; *Nephrotoma liankangensis* Men, Xue & Yang and *N. pseudoliankangensis* Men, Xue & Yang in Men et al. 2015b). This may represent a special form of type III.

### ***Tipula* (*Sivatipula*) *biprocessa* sp. n.**

http://zoobank.org/0121BA77-9947-486A-91B8-15EAABEEFAB8
Figs 12–23

**Diagnosis.** Generally straw-yellow; prescutum straw-yellow with three light brown stripes; wings pale brown with a dark brown stigma; abdomen bright yellow on basal three segments, gradually changed to light brown on apical ones, segments six and seven suffused with black; hypopygium straw-yellow, tergite nine rounded at posterior




**Figures 12–23.** *T.* (*S.*) *biprocessa* sp. n. **12** head, dorsal view **13** thorax, right lateral view **14** thorax, dorsal view **15** wing **16** hypopygium, right lateral view **17** hypopygium, left lateral view **18** ninth tergite, dorsal view **19** inner gonostylus and outer gonostylus, left view **20** inner gonostylus and outer gonostylus, right view **21** inner gonostylus and outer gonostylus, dorsal view **22** semen pump, dorsal view **23** semen pump, right lateral view.

margin and equipped with two finger-like processes, ventral-lateral portions of tergite nine projected into two lobes, densely covered with black setae.

**Description.** Male. Length: *Body*: 14.0–15.0 mm (not including antenna, n = 2); *Wing*: 19.0–20.0 mm (n = 2); *Antenna*: 18.0–19.0 mm (n = 2).

*Head* straw-yellow (Figs 12–13). Rostrum light brown with a light brown nasus. Vertex without marking (Fig. 12). Eyes black (Fig. 12). Antenna: 12-segmented, distinctly longer than body; scape light yellow, expanded apically; pedicel light yellow, short; flagellomeres light brown, each flagellomere cylindrical, subequal in length, with abundant black verticils. Palpi light brown.

*Thorax* with pronotum entirely orange-yellow (Figs 13–14). Prescutum straw-yellow with three light brown stripes (Fig. 14). Scutum orange-yellow with two light brown stripes (Fig. 14). Scutellum and postnotum orange-yellow (Fig. 14). Pleura entirely bright yellow (Fig. 13). Legs slender, coxae and trochanters straw-yellow, femora straw-yellow with light brown tip, tibiae and tarsi light brown. Halteres with stem yellow, knob darker. Wings pale brown, cell sc darker than ground color, stigma dark brown (Fig. 15). Sc relatively short, subequal to $R_3$ in length, petiole of cell m1 slightly longer than m-m, approximately one-half length of discal cell (Fig. 15).

*Abdomen* bright yellow on basal three segments, gradually changed to light brown on apical ones, segments six and seven suffused with black (Fig. 16). Hypopygium straw-yellow (Fig. 16). Hypopygium broad, compressed (Figs 16–17). Tergite nine rounded at posterior margin with two finger-like processes, lateral sides of tergite nine with numerous long hairy setae, longest one longer than finger-like process (Fig. 18). Sternite nine broader than tergite nine, median region of sternite nine protruded to a membranous extension (Fig. 17). Outer gonostylus narrow, flattened, apical two-fifths curved caudad (Figs 19–21). Inner gonostylus flattened, narrowed medially, a slender lobe generated from the median region of inner gonostylus (Figs 19–21).

*Semen pump* with compressor apodeme V-shaped, the arms expanded at apex, distinctly broader than that of *T.* (*S.*) *parvauricula* (Fig. 22). Posterior immovable apodeme with one arm, distinctly longer than compressor apodeme, gradually narrowed to apex and curved cephalad in lateral view, the arm deeply grooved in dorsal view, basal region distinctly wider than that of *T.* (*S.*) *parvauricula* (Figs 22–23). Anterior immovable apodeme flattened, gradually narrowed to apex in lateral view (Fig. 22). Aedeagus elongated, tubular, at least ten times longer than semen pump.

**Material examined. Holotype** male. **CHINA:** Guangxi Zhuang Autonomous Region, Dalongping, Cenwanglaoshan Mountain, 24°31'N, 106°17'E, 1300 m, 7 May 2015, Guo-Xi Xue leg. **Paratype.** 1 male, same data as holotype.

**Remarks.** We compared the new species with all known species based on published descriptions and illustrations, and found that it is mostly similar to *T.* (*S.*) *parvauricula* by the color of body, the structures of antenna and hypopygium. It can be easily distinguished from the latter by the shape of tergite nine which is rounded at posterior margin with two distinct finger-like processes. The latter species has its tergite nine separated by a V-shaped notch with two short truncated processes and produced into a pair of ear-like processed ventrally. There is also a noticeable difference in the

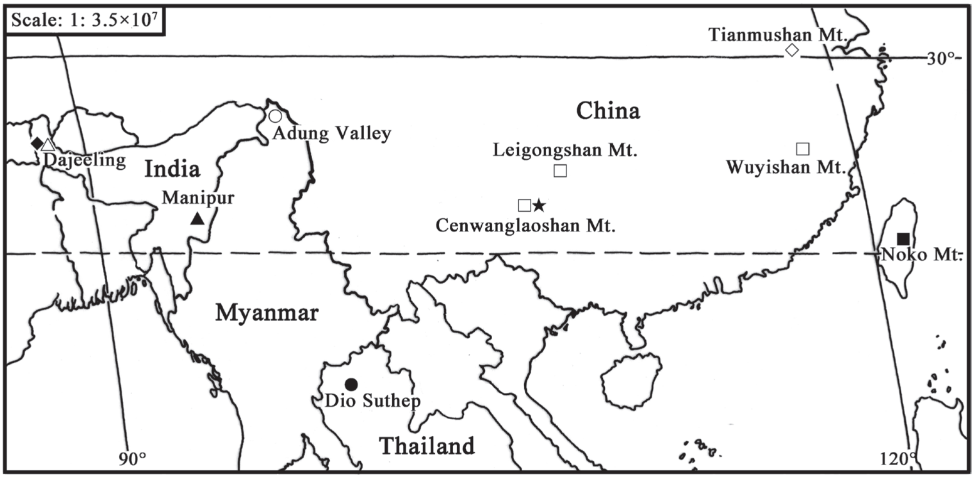



**Figure 24.** Geographic distribution of *Tipula* (*Sivatipula*) species: *T.* (*S.*) *alhena* (●), *T.* (*S.*) *bhishma* (▲), *T.* (*S.*) *filicornis* (♦), *T.* (*S.*) *lackschewitziana* (■), *T.* (*S.*) *mitocera* (△), *T.* (*S.*) *pullimargo* (○), *T.* (*S.*) *suensoniana* (◊), *T.* (*S.*) *parvauricula* (□), *T.* (*S.*) *biprocessa* sp. n. (★).

shape of the outer gonostylus which is simple and narrowed in the new species, but flattened and widened medially with a slender rod on ventral-lateral margin in that of *T.* (*S.*) *parvauricula*. Distinct interspecific difference is also found in the shape of inner gonostylus as Figures 8, 19 and 20.

**Etymology.** The specific epithet is a noun derived from the Latin '*processa*' with Latin prefix '*bi*', referring to the presence of two finger-shaped processes at posterior margin of tergite nine.

**Distribution.** China (NW. Guangxi).

## Key to species of subgenus *Tipula* (*Sivatipula*)

| | |
|---|---|
| 1 | Hind margin of ninth tergite rounded apically with a pair of finger-like processes (see Figs 16–18) .......... ***T.* (*S.*) *biprocessa* sp. n.** (China: Guangxi, Cenwanglaoshan Mountain. Fig. 24) |
| – | Hind margin of ninth tergite without such process .......... **2** |
| 2 | Outer gonostylus with an appressed pubescence on outer surface of apical arm (see Alexander 1928: 459, Pl. 2, fig. 5) .......... ***T.* (*S.*) *lackschewitziana* Alexander, 1928** (China: Taiwan, Noko Moutain. Fig. 24) |
| – | Outer gonostylus without such pubescence .......... **3** |
| 3 | Ninth tergite laterally bearing two spinous projections, lower to them submedially with two short spiculose projections (see Joseph, 1974: 277, figs 137–142) .......... ***T.* (*S.*) *filicornis* Brunetti, 1918** (India: West Bengal, Dajeeling. Fig. 24) |
| – | Ninth tergite not as above .......... **4** |
| 4 | Antenna slightly shorter than body .......... **5** |

– Antenna equal to or longer than body........................................................**6**

5 Outer gonostylus club-shaped (see Alexander, 1953: 348, fig. 12d) ............... ***T.* (*S.*) *pullimargo* Alexander, 1951** (Myanmar: Adung Valley. Fig. 24)

– Outer gonostylus long-attenuate (see Alexander 1940: 110, fig. 11) ............ .................................................................................... ***T.* (*S.*) *suensoniana* Alexander, 1940** (China: Zhejiang, Tianmushan Mountain. Fig. 24)

6 Ninth tergite produced into a rounded apex, with numerous black setae (see Alexander 1971: 81, fig. 6) ..................................................***T.* (*S.*) *alhena* Alexander, 1953** (Thailand: Chiengmai, Dio Suthep. Fig. 24)

– Ninth tergite not produced into a rounded apex.......................................**7**

7 Ninth tergite with powerful lateral arms that are tipped with abundant blackened pegs (see Alexnader 1964: 105, Pl. 4, fig. 40) ..................................... ...........***T.* (*S.*) *bhishma* Alexander, 1964** (India: Assam, Manipur. Fig. 24)

– Ninth tergite without such pegs..................................................................**8**

8 Median lobe of ninth sternite bearing terminal brush-like setae (see Alexander 1927: 182, fig. 3)........................................................................................ ... ***T.* (*S.*) *mitocera* Alexander, 1927** (India: West Bengal, Dajeeling. Fig. 24)

– Median lobe of ninth sternite without such brush-like setae (see Alexander 1941: 401, Pl. 4, fig. 44; Fig. 6) .....................................................................***T.* (*S.*) *parvauricula* Alexander, 1941** (China: Fujian, Wuyishan Mountain; Guangxi, Cenwanglaoshan Mountain; Guizhou, Leigongshan Mountain. Fig. 24)

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# Uncovering the diversity in the *Amazophrynella minuta* complex: integrative taxonomy reveals a new species of *Amazophrynella* (Anura, Bufonidae) from southern Peru

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## Abstract

A new species of the genus *Amazophrynella* (Anura, Bufonidae) is described from the departments of Madre de Dios, Cusco and Junin in Peru. An integrative taxonomy approach is used. A morphological diagnosis, morphometrics comparisons, description of the advertisement call, and the phylogenetic relationships of the new species are provided. *Amazophrynella javierbustamantei* **sp. n.** differs from other species of *Amazophrynella* by: intermediate body-size (snout-vent length 14.9 mm in males, n = 26 and 19.6 mm in females, n = 20), tuberculate skin texture of body, greatest hand length of the *Amazophrynella* spp. (3.6 mm in males, n = 26 and 4.6 mm in females, n = 20), venter coloration yellowish, tiny rounded black points covering the venter, and thirteen molecular autapomorphies in the 16S RNA gene. Its distribution varies from 215 to 708 m a.s.l. This discovery highlights the importance of the remnant forest in preserving the biodiversity in Peru, and increase in seven the species formally described in the genus *Amazophrynella*.

**Resumen**

Describimos una nueva especie del género *Amazophrynella* (Anura, Bufonidae) del Perú de los Departamentos de Madre de Dios, Cusco y Junin de Peru. Utilizamos un método de taxonomía integrativa. Obtuvimos la diagnosis morfológica, comparaciones morfométricas, descripción del canto de reproducción y las relaciones filogenéticas de la nueva especie. *A. javierbustamantei* **sp. n.** difiere de las otras *Amazophrynella* spp. por poseer tamaño medio (Hocico-cloaca en machos 16.9 mm, n = 26 y en hembras 19.6 mm, n = 20); textura de la piel tuberculada; tamaños de las manos mayores (3.6 mm en machos, n = 26 y 4.6 mm en hembras, n = 20); coloración ventral amarillento-pálida, pequeños puntos redondos de color negro en el vientre y por trece autopomorfias moleculares en el gen 16S RNA. Su distribución varía desde 215 m hasta 708 m a.s.n.m. Este descubrimiento resalta la importancia de los remanentes de la selva Peruana en términos de conservación, e incrementa en siete las especies formalmente descritas en del género *Amazophrynella*.

**Resumo**

Descrevemos uma nova espécie do gênero *Amazophrynella* (Anura, Bufonidae) dos departamentos de Madre de Dios, Cusco e Junin do Peru. Utilizamos um método de taxonomia integrativa. Apresentamos a diagnose morfológica, comparações morfométricas, descrevemos o canto de anúncio e geramos uma hipótese filogenética da nova espécie. *Amazophrynella javierbustamantei* **sp. n.** difere das outras *Amazophrynella* spp. por possuir tamanho médio (Comprimento rostro-cloacal 16.9 mm em machos, n = 26 e 19.6 mm em fêmeas, n=20); textura da pele tuberculada; tamanhos das mãos maiores (3.6 mm em machos, n = 26 e 4.6 mm em fêmeas, n = 20); coloração ventral amarelo-clara, coberta por pequenos pontos redondos pretos e por treze autapomorfias moleculares no gene 16S RNA. Sua distribuição varia entre os 215 m até os 708 m a.n.m. Nossa descoberta aumenta a importância dos remanescentes da floresta Peruana em termos de conservação e incrementa em sete as espécies formalmente descritas no gênero *Amazophrynella*.

**Keywords**

Amphibian, Tree Toad, conservation, Southern Peru, integrative taxonomy

**Palabras claves**

Anfibios, Sapo del árbol, conservación, Sur del Perú, taxonomía integrativa

**Palavras chaves**

Anfíbios, Sapo do arvore, conservação, Sul do Peru, taxonomia integrativa

## Introduction

Until 2012, two species of *Amazophrynella* were placed in the genus *Dendrophryniscus* Jimenez de la Espada, 1868. Fouquet et al. (2012a) recognized that species of *Dendrophryniscus* from the Amazon and Atlantic rainforests represented morphologically and genetically deeply divergent lineages, and thus the authors proposed a new genus: *Amazophrynella* Fouquet, Recorder, Texeira, Cassimiro, Amaro, Camacho, Damaceno, Carnaval, Moritz & Rodrigues, 2012 for the Amazonian species *A. minuta* and *A. bokermanni*.

In the following years, an additional four new species of the genus were described: *A. vote* Ávila, Carvalho, Gordo, Ribeiro & Morais, 2012 and *A. manaos* Rojas, Carvalho, Gordo, Ávila, Farias & Hrbek, 2014 based on morphology; *A. amazonicola* and *A. matses* Rojas, Carvalho, Gordo, Ávila, Farias & Hrbek, 2015, based on morphology and genetic data (Ávila et al. 2012; Rojas et al. 2014, 2015). Species of the genus are distributed in nine South American countries: Bolivia, Peru, Ecuador, Colombia, Venezuela, Guiana, French Guiana Brazil, and presumably in Suriname (Frost et al. 2015).

Using a phylogenetic analysis based on mitochondrial and nuclear genes (Fouquet et al. 2007, 2012a), the existence of three independent evolutionary lineages was discovered within the nominal species *A. minuta* from Ecuador and French Guianas; subsequently, other independent evolutionary lineages were discovered from Brazil and Peru (Rojas et al. 2014, 2015). The difficulties in delimiting species within the *A. minuta* species complex resides in the relatively generalized diagnosis (see Melin 1941) and the poor geographic sampling. For these reasons, historically, the name *A. minuta* has been used for individuals distributed throughout the Amazonian biome (e.g. Duellman 1978; Zimmerman and Rodrigues 1990; Magnusson and Hero 1991; Rodrigues and Duellman 1993; Duellman and Mendelson 1995; Fouquet et al. 2012a). Thus, taxonomy and systematics of populations that are currently part of the *A. minuta* complex remains largely unresolved (Rojas et al. 2014), in turn limiting the knowledge of the true taxonomic diversity of the genus (Ávila et al. 2012; Rojas et al. 2014, 2015).

Given this scenario, herein is described an additional new species of *Amazophrynella* from the departments of Madre de Dios, Cusco and Junin, Peru, founded on the principles of integrative taxonomy. Morphological, morphometric, bioacoustic and phylogenetic relationships are provided as evidence for the existence of the new taxon.

## Material and methods

### Morphology

Forty eight specimens previously identified as *Amazophrynella minuta* (Melin, 1941), deposited at the Museo de Historia Natural del Cusco, Universidad Nacional de San Antonio Abad del Cusco (MHNC) and Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MHNSM) were analyzed. This material was compared with twenty preserved specimens of *A. minuta* from the type locality (Taracuá mission, on the right bank of the Uaupés River, municipality of São Gabriel da Cachoeira, Brazil), deposited in the Collection of Amphibians and Reptiles of the Instituto Nacional de Pesquisas da Amazônia–INPA, Brazil (INPA-H). Further comparisons were made with three syntypes deposited at the Naturhistoriska Museet, Göteborg, Sweden (NHMG), and the original description of the species (Melin 1941).

Additionally five preserved specimens of *Amazophrynella bokermanni* (Izecksohn, 1993) from near the type locality (Juruti, 30 Km from type locality), the holotype and paratypes of *A. manaos* deposited in the Collection of Amphibians and Reptiles of the Instituto Nacional de Pesquisas da Amazônia–INPA, Manaus, Amazonas, Brazil (INPA-H), the holotype of *Amazophrynella vote*, deposited in the Coleção Zoológica de Vertebrados of the Universidade Federal de Mato Grosso–UFMT, Cuiabá, Mato Grosso, Brazil (UFMT-A), seventeen paratypes deposited in the Collection of Amphibians and Reptiles of the Instituto Nacional de Pesquisas da Amazônia–INPA, Manaus, Amazonas, Brazil (INPA-H), and the holotype and paratypes of *A. amazonicola* and *A. matses*, deposited at the Museo de Zoologia de la Universidad Nacional de la Amazonia Peruana (MZUNAP) were analyzed (see Appendix 1 listing all the revised specimens).

Morphological character analyses were carried out according to Cruz and Fussinato (2008) and Fouquet et al. (2012a). Sex was determined by gonad analysis.

Measurements were carried out with a digital caliper following Kok and Kalamandeen (2008) and Duellman (1978). SVL (snout-vent length) from the tip of the snout to the posterior edge of the cloaca; HL (head length) from the posterior edge of the jaw to the tip of the snout; HW (head width), the greatest width of the head, usually at the level of the posterior edges of the tympanum; ED (eye diameter); IND (internarinal distance), the distance between the edges of the nares; SL (snout length) from the anterior edge of the eye to the tip of the snout; HAL (hand length) from the proximal edge of the palmar tubercle to the tip of Finger III; UAL (upper arm length) from the edge of the body insertion to the tip of the elbow; THL (thigh length) from the vent to the posterior edge of the knee; TL (tibia length) from the outer edge of the knee to the tip of the heel; TAL (tarsal length) from the heel to the proximal edge of the inner metatarsal tubercle; FL (foot length) from the proximal edge of the inner metatarsal tubercle to the tip of Toe IV. Diagnosis of characters follow Chaparro et al. 2015.

*Statistical analysis*. We used a total of 80 adult males of the *Amazophrynella minuta* species complex (numbers of individuals and populations of origin in parentheses): *A. minuta* sensu stricto (n = 23, from Taracuá), *A. amazonicola* (n = 15, from Puerto Almendras and Fazenda Zamora); *A. matses* (n = 13, from Nuevo Salvador) and the new species of *Amazophrynella* (n = 29, from Tambopata, Nuevo Arequipa, Candamo and Inambari).

All morphometric measures were log10 transformed to conform to requirements of normality (Hayek et al. 2001). The effect of size was removed from all variables by regressing them against SVL and using the residuals of each variable in a Principal Component Analysis (PCA). Significance of morphometric differences was tested with Multivariate Analysis of Variance (MANOVA) with the two first principal components being treated as dependent variables and species as independent variables. The first two principal components were used since they explained the majority of observed variation in shape. A Discriminant Function Analysis (DFA) was performed to test classification of individuals in predicted groups. All the statistical analysis were performed in R (R Development Core Team 2011) adopting a 5% significance cut-off. PCA was used to detect groups representing putative cryptic species and DFA was subsequently applied to identify the set of characters that best diagnose those groups (Padial and De la Riva 2009). Additionally we noted large size in the HAL of the new species of *Amazophrynella*, and we used an Analysis of Variance (ANOVA) of the original data (from *A. minuta*, *A. matses*, *A. amazonicola* and the new species) to statistically support this hypothesis.

## Molecular data

*Laboratory procedure.* Total DNA was extracted from muscle tissue using standard phenol/chloroform extraction (Sambrook et al. 1989). A 480 bp fragment of the 16S rDNA was PCR amplified using the 16Sar and 16Sbr primers (Palumbi 1996). Amplification was carried out under the following conditions: 60 s hot start at 92 °C followed by 35 cycles of 92 °C (60 sec), 50 °C (50 sec) and 72 °C (1.5 min). Final volume of the PCR reaction was 12 µl and contained 4.4 µL $ddH_2O$, 1.5 µL of 25 mM $MgCl_2$, 1.25 µL of 10 mM dNTPs (2.5mM each dNTP), 1.25 µL of 10x buffer (75 mM Tris HCl, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), 1 µL of each 2 µM primer, 0.3 µL of 5 U/µL DNA Taq Polymerase (Biotools, Spain) and 1 µL of DNA (about 30 ng/µL). Sequencing reactions were carried out according to the manufacturer's recommendation for the ABI BigDye Terminator cycle sequencing mix, using 16Sa primer and an annealing temperature of 50 °C. Sequencing reactions were precipitated using standard EDTA/EtOH protocol, and resolved in an ABI 3130xl automatic sequencer.

*Phylogenetic analysis.* We obtained 16S rDNA sequence data from two specimens of the new species (Accession numbers: KR905184, KR905185), two paratypes of *A. vote* (Accession numbers: KF433970, KF433971), two specimens of *A. bokermanni* (Accession numbers: KF433975, KF433976), two topotypic specimens of *A. minuta* (Accession numbers: KF792834, KF792836), two paratopotypes of *A. matses* (Accession number: KP681688, KP681689), the holotype and one paratopotype of *A. amazonicola* (Accession number: KP681868, KP681669) and two paratypes of *A. manaos* (Accession number: KF433954, KF433957) deposited in the tissue collection of the Laboratório de Evolução e Genética Animal of the Universidade Federal do Amazonas (CTGA-ICB/UFAM). The dataset also included two sequences of *A.* sp. aff. *minuta* (Accession number: AY326000, DQ158420) from Darst and Canatella (2004), Pramuk (2006) and two sequences of *A.* sp. aff. *manaos* (Accession number: EU201057, JN867570) from Fouquet et al. (2007). As outgroups we used species of the sister taxon *Dendrophryniscus* (see Table 2 for samples information).

Sequences were aligned using the Clustal W algorithm (Thompson et al. 1996) implemented in BioEdit (Hall 1999) and alignment was adjusted as necessary against the secondary structure of the 16S rDNA. The existence of lineages in a phylogenetic tree-based context (Baum and Donoghue 1995) was performed using Maximum Likelihood analysis (Felsenstein 1981) in the program Treefinder (Jobb 2008) using the GTR+I+G model of substitution, selected via Akaike information criterion as implemented in Modeltest 3.7 (Posada 2006). Phylogenetic support was assessed via 10 000 non-parametric bootstrap (Felsenstein 1985). Additionally uncorrected pairwise genetic distances between linages identified by phylogenetic inference of *Amazophrynella* were calculated in MEGA 5.05 (Tamura et al. 2007).

*Molecular species delimitation.* Evolutionary lineages are diagnosed by discontinuities in character variation among lineages, and correspond to phylogenetic species. The existence of lineages is therefore a necessary and sufficient prerequisite for inferring the existence of a species under the different conceptualizations of the Phylogenetic Species Concept (PSC) (Cracraft 1983; Baum and Donoghue 1995; De Queiroz 2007). The existence of lineages in a non-tree-based context (Cracraft 1983) was inferred using Population Aggregation Analysis performed at the level of an individual (Davis and Nixon 1992; Rach et al. 2008) using the dataset with the *Amazophrynella minuta* species complex: *A. matses*, *A. minuta*, *A. amazonicola* and the new species. The analyses were performed in the program R (R Development Core Team 2011).

### Bioacoustics

We analyzed one advertisement call obtained from the CD of Frogs of Tambopata, Peru (Macauly Library of Natural Songs and Cornell Laboratory of Ornithology) by the authors Cocroft et al. (2001) from the Natural Reserve of Tambopata, a locality of occurrence of the new species. The call was edit with the software Audacity 1.2.2 for Windows (Free Software Foundation Inc. 1991). The spectral and temporal parameters of the recording were analyzed in the software Raven Pro. 1.3 for Windows (Cornell Laboratory of Ornithology). The advertisement call was obtained from one male in a temperature 25 °C (Crocoft et al. 2001). We measured the following quantitative parameters: call duration (seconds); pulses per call; length of silence between calls (seconds); dominant frequency (kHz); fundamental frequency (kHz) and time to peak at maximum frequency (seconds).

## Results

### Phylogenetic analysis and systematics

In the resulting phylogeny, the six nominal species of *Amazophrynella* were recognized as monophyletic (Fig. 1). In the genus we can distinguish two monophyletic groups: One clade (bootstrap support = 100) formed by the species: *A. manaos*, *A. bokermanni* and *A. vote* and another represented by the species of the *A. minuta* "species complex" (bootstrap support = 98): *A. minuta*, *A. amazonicola*, *A. matses* and the new species described herein.

In the first clade the *Amazophrynella* species: *A. manaos* is sister taxon of the possible new specie from the Guiana Shield: *A.* sp. aff. *manaos* (bootstrap support= 91), and both are sister to *A. bokermanni* (bootstrap support= 98). *Amazophrynella vote* is sister of *A. bokermanni* + (*A. manaos* + *A.* sp. aff. *manaos*) with a bootstrap support of 81.

The second clade corresponding to the *A. minuta* "species complex", *A. amazonicola* is sister of *A. minuta* + *A.* sp. aff. *minuta* from western Amazonia (bootstrap support= 99). Our analysis further highlighted the occurrence of a new monophyletic lineage (*A. javierbustamantei* sp. n.) showing sister relationship with *A. matses* (bootstrap support = 96), both being in turn sister group of *A. amazonicola* + (*A. minuta* + A. sp. aff. *minuta*) with a bootstrap support of 99.

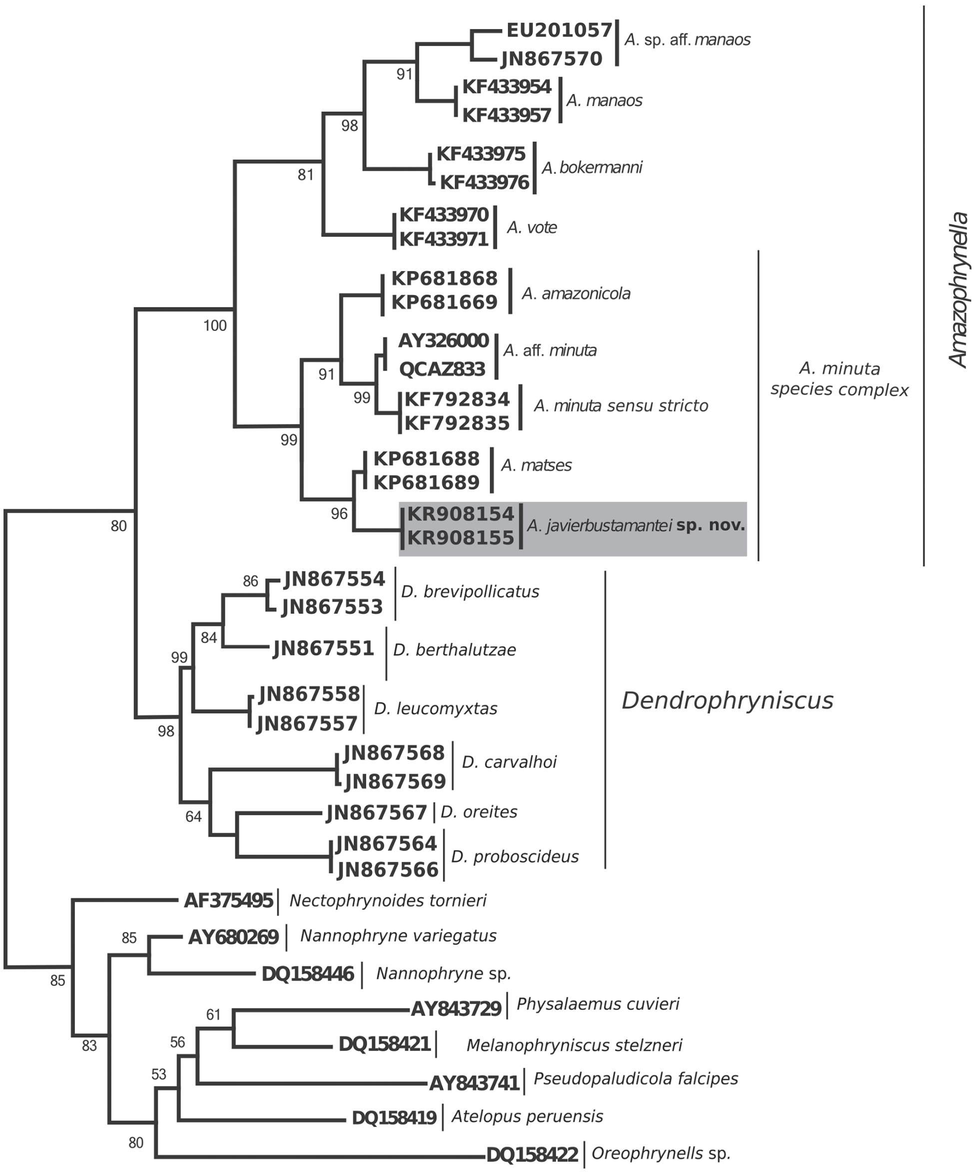


**Figure 1.** Maximum Likelihood tree of the *Amazophrynella* species based on the GTR+I+G model, analyzing 480 bp of 16S rDNA. Numbers below branches represent bootstrap support for 10 000 pseudoreplications.

Smallest uncorrected 16S rDNA p-distances estimated between phylogenetic linages was observed between *A. minuta* and *A.* sp. aff. *minuta* (= 3%). Greatest interspecific distance (= 14%) was observed between *Amazophrynella javierbustamantei* sp. n. and *A. bokermanni* and was comparable to divergence observed between *A. manaos* and *A. minuta*. Within the "*A. minuta*" species complex, the new species shows a high degree of genetic divergence from *A. minuta* (= 7%), *A. amazonicola* (= 9%) and minor genetic distance with their sister taxon *A. matses* (= 3%) (see all pairwise genetic distance values summarized in Table 3). According to the Population Aggregation Analysis, the newly identified lineage was also diagnosable by thirteen molecular autapomorphic characters (Table 4) leading us to the conclusion that this lineage corresponds to a new species.

## Morphometric analysis

Comparative analysis of quantitative morphological data allowed us to distinguish *Amazophrynella* sp. n. from the other members of the *A. minuta* "species complex". The first two principal components extracted by the PCA account for 48.56% of the variation found in the dataset. The first component (PC1) explained 24.93% of total variation. In the first principal component axis, *A. amazonicola* is distinguished from the other species due to its larger size (SVL = 14.9 ± 0.7 mm, see Table 1), sharing relative size with *A. minuta* sensu stricto (SVL = 13.5 ± 0.6 mm, see Table 1), the species *A. matses* is distinguish by having the smallest size of the genus (SVL range= 12.1 ±0.6 mm, see Table 1), and shares this characteristic with *Amazophrynella* sp. n. (SVL = 14.9 ± 0.9 mm, see Table 1) (Fig. 2). The second component explains 23.63% of the variation. This axis represents a shape variation vector; in this axis *Amazophrynella javierbustamantei* sp. n. is well distinguished from the three formally described species, sharing more similarity with *A. matses* (Table 5).

All the species of the group are significantly different in shape (MANOVA, $F_{24.3}$, *Pillae´s trace* < 0.001). The discriminate function analysis (DFA) found specimens correctly classified in 56.6% of cases and a moderate prior probabilities of groups (*A. minuta* = 28.75%, *A. amazonicola* = 18.75%, *A. matses* = 16.25% and *A. javierbustamantei* sp. n. = 36.25%). The variables that contributed most to the classification were HAL, SVL and TAL (Table 6). The differences in HAL were significant (ANOVA, $F_{45.27}$, $P < 0.001$) among all the species of *A. minuta* "species complex" (see Fig. 1), and reveals *Amazophrynella javierbustamantei* sp. n. as the species with the largest HAL (Fig. 3).

## Morphological description

***Amazophrynella javierbustamantei* sp. n.**
http://zoobank.org/A946B949-1D1F-4FF5-B722-0B33435EE610

**Holotype** (Fig. 4). MHNC 8331 (Genbank *16S rRNA*: KR905184). Adult male, collected at Quebrada Guacamayo (12°54'24.5"S; 69°59'32.7"W, 215 m a.s.l.) km 105 of the highway Puerto Maldonado–Cusco City, District Inambari, Province Tambopata, Department Madre de Dios, Peru, on 27 October 2009 by Juan C. Chaparro and Oscar Quispe.

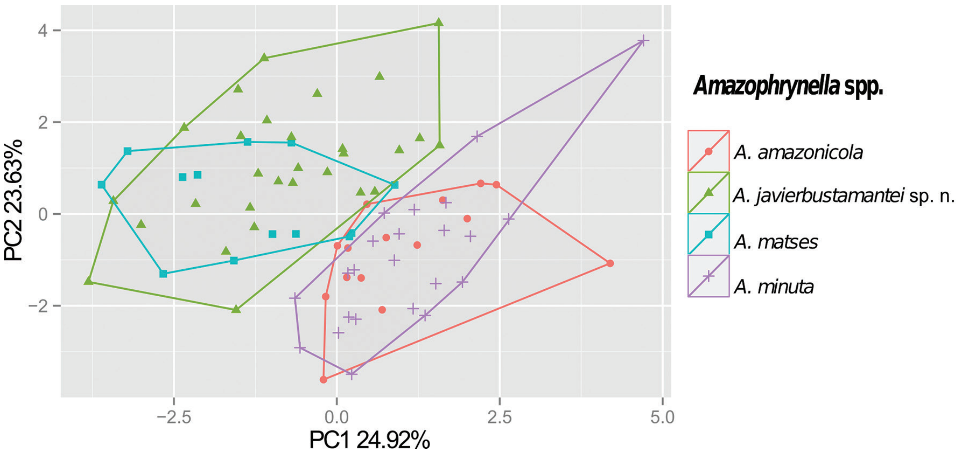


**Figure 2.** Principal Component Analysis (PCA) from: *Amazophrynella minuta* species complex. See Table 5 for character loadings on each component.

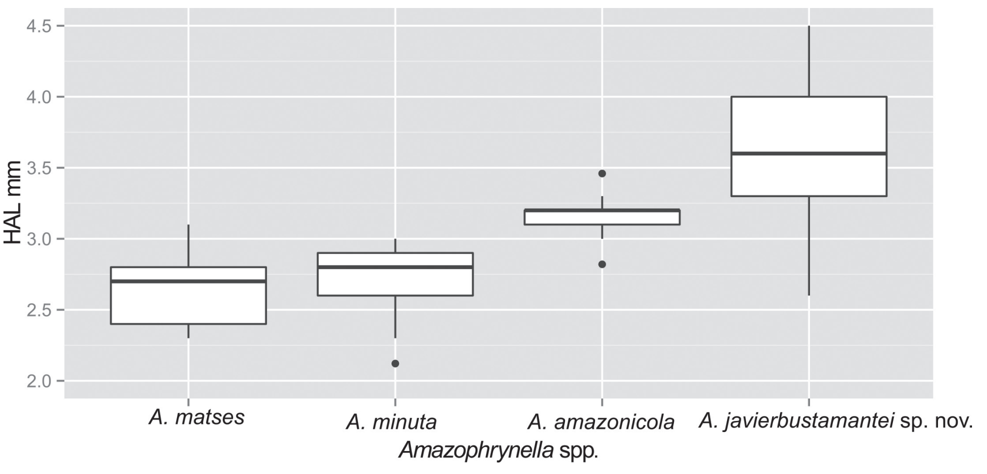


**Figure 3.** Measurement comparison of the Hand Length (HAL) between species of *Amazophrynella minuta* complex.

**Paratypes** (Fig. 5)**.** Twenty-two specimens (males= 09, females= 13). MHNC 8363, MHNC 8245, MHNC 8238, adult males, MHNC 8316, MHNC 8484, MHNC 8362, MHNC 8354, adult females, collected with the holotype (12°28'25"S, 69°12'36"W, 205 m a.s.l.). MHNC 11001, adult male, MHNC 11002, MHNC 11003, MHNC 11004, adult females collected by E. Aguilar on 17 May 2009, from La Pampa km 107 highway Puerto Maldonado–Cusco City, Department Madre de Dios (12°40'14.14"S, 72°27'30"W, 250 m a.s.l.). MHNSM 17993, adult male collected by A. Angulo in 1999; from Province Manu, locality of Inambari, Department Madre de Dios (13°02'29.28"S, 70°22'46.65"W, 306 m a.s.l.). MHNSM

**Table 1.** Measurements (mm) of adult male specimens (including the holotype) in the type series *Amazophrynella* spp. Mean ± standard deviation, with ranges in parentheses. Abbreviations are defined in material and methods.

| Variable | ***A. minuta*** **sensu stricto** (n = 15) | ***A. manaos*** (n = 29) | ***A. bokermanni*** (n = 5) | ***A. vote*** (n = 14) | ***A. amazonicola*** (n = 15) | ***A. matses*** (n = 13) | ***A. javierbustamantei*** **sp. n.** (n = 26) |
|---|---|---|---|---|---|---|---|
| **SVL** | 13.5 ± 0.6 (12.5–14.2) | 14.2 ± 0.7 (12.3–15.0) | 16.8 ± 1.4 (14.6–18.2) | 13.1 ± 0.7 (12.0–14.1) | 14.5 ± 0.7 (13.3–15.4) | 12.1±0.6 (11.5–13.5) | 14.9 ± 0.9 (12.7–16.4) |
| **HW** | 4.2 ± 0.2 (4.0–4.3) | 4.2 ± 0.3 (3.7–4.7) | 3.2 ± 0.3 (2.5–3.3) | 4.0 ± 0.7 (3.3–4.4) | 4.4 ± 0.3 (4.2–4.6) | 3.6 ±0.2 (3.1–3.8) | 4.2 ± 0.2 (3.5–4.7) |
| **HL** | 4.9 ± 0.2 (4.8–5.3) | 5.3 ± 0.3 (4.7–5.6) | 3.4 ± 0.4 (2.8–3.8) | 4.6 ± 0.3 (4.0–5.2) | 5.2 ± 0.3 (5.0–6.2) | 4.3 ± 0.3 (3.9–4.8) | 5.1 ±0.3 (4.4–5.6) |
| **SL** | 2.3 ± 0.1 (2.2–2.5) | 2.7 ± 0.2 (2.3–2.7) | 3.0 ± 0.4 (2.2–3.1) | 2.1 ± 0.2 (1.9–2.6) | 2.4 ± 0.2 (2.2–2.5) | 2.0 ± 0.3 (1.6–2.3) | 2.2 ± 0.2 (1.7–2.6) |
| **ED** | 1.4 ± 0.1 (1.3–1.5) | 1.3 ± 0.1 (1.2–1.6) | 1.8 ± 0.2 (1.5–2.0) | 1.3 ± 0.1 (1.2–1.5) | 1.2 ± 0.1 (0.9–1.2) | 1.1 ± 0.1 (0.9–1.2) | 1.3 ± 0.1 (1.0–1.6) |
| **IND** | 1.2 ± 0.1 (1–1.3) | 1.1 ± 0.1 (1.0–1.4) | 1.4 ± 0.2 (1.0–1.5) | 1.1 ± 0.1 (1.0 –1.3) | 1.2 ± 0.1 (1.0–1.3) | 1.0 ± 0.1 (0.8–1.2) | 0.9 ± 0.1 (0.8–1.2) |
| **UAL** | 3.8 ± 0.2 (3.2–4.1) | 3.6 ± 0.4 (2.9–4.1) | 5.5 ± 0.6 (5.0–5.6) | 3.9 ± 0.5 (2.8–3.9) | 4.5 ± 0.3 (4.2–5.3) | 3.5 ± 0.4 (2.9–4.2) | 4.5 ± 0.4 (3.8–5.7) |
| **HAL** | 2.8 ± 0.2 (2.6–3.0) | 2.8 ± 0.6 (1.9–2.9) | 3.4 ± 0.6 (2.8–4.2) | 2.7 ± 0.3 (2.3–3.2) | 3.2 ± 0.2 (2.8–3.3) | 2.7 ± 0.2 (2.3–3.1) | 3.6 ± 0.4 (2.5–4.5) |
| **THL** | 6.8 ± 0.2 (6.4–7.2) | 6.7 ± 0.3 (2.3–3.1) | 8.7 ±1.4 (7.2–8.9) | 6.5 ± 0.7 (5.4–7.2) | 7.7 ± 0.6 (6.3–8.0) | 6.2 ± 0.4 (5.1–6.3) | 7.6 ± 0.7 (6.2–9.2) |
| **TAL** | 6.7 ± 0.3 (6.3–7.1) | 6.9 ±0.6 (4.2–7.3) | 8.3 ± 1.0 (6.7–9.2) | 5.7 ± 0.7 (4.8–7.0) | 7.2 ± 0.6 (6.1–7.9) | 5.8 ± 0.3 (5.1–6.3) | 7.6 ± 0.7 (6.2–8.8) |
| **TL** | 4.1 ± 0.2 (3.8–4.6) | 4.6 ± 0.4 (4.3–6.3) | 5.4 ± 1.4 (2.9–6.2) | 3.8 ± 1.0 (4.2–7.0) | 4.2 ± 0.6 (6.3–8.0) | 3.8 ± 0.2 (3.6–4.3) | 4.7 ± 0.8 (3.9–8.7) |
| **FL** | 4.8 ± 0.4 (4.2–5.2) | 5.2 ± 0.5 (4.7–6.1) | 6.3 ± 1.3 (3.9–7.6) | 4.4 ± 0.6 (3.2–5.4) | 5.1 ± 0.4 ( 4.7–6.0) | 4.3 ± 0.4 (5.5–3.0) | 5.7 ± 0.6 (4.5–7.2) |

**Table 2.** Individuals of the genus *Amazophrynella* (A) and *Dendrophryniscus* (D) used in the molecular analyses. Information includes samples, collecting locality, GenBank accession number for the 16S rDNA fragment, voucher number and specimen status.

| Sample | Locality | Accession Number | Voucher number | Specimen status |
|---|---|---|---|---|
| *A. javierbustamantei* | Quebrada Guacamayo, Peru | KR905184 | MHNC 8331 | Holotype |
| *A. javierbustamantei* | Quebrada Guacamayo, Peru | KR905185 | MHNC 8363 | Paratype |
| *A. matses* | Nuevo Salvador, Peru | KF681688 | MZUNAP 928 | Paratopotype |
| *A. matses* | Nuevo Salvador, Peru | KF681689 | MZUNAP 941 | Paratopotype |
| *A. minuta* sensu stricto | Taracuá, Brazil | KF792834 | INPA-H 32729 | Topotype |
| *A. minuta* sensu stricto | Taracuá, Brazil | KF792835 | INPA-H 32730 | Topotype |
| *A. amazonicola* | Puerto Almendras, Peru | KF681868 | MZUNAP 901 | Holotype |
| *A. amazonicola* | Puerto Almendras, Peru | KF681669 | MZUNAP 915 | Paratopotype |
| *A. vote* | Parque Nacional Nascentes do Lago Jari, Brazil | KF433970 | INPA-H 28720 | Paratype |
| *A. vote* | Parque Nacional Nascentes do Lago Jari, Brazil | KF433971 | INPA-H 28722 | Paratype |
| *A. bokermanni* | Juruti, Pará, Brazil | KF433975 | INPA-H 31864 | |
| *A. bokermanni* | Juriti, Pará, Brazil | KF433976 | INPA-H 31861 | |
| *A. manaos* | Mineração taboca, Brazil | KF433954 | INPA-H 29566 | Paratype |
| *A. manaos* | Mineração taboca, Brazil | KF433957 | INPA-H 29567 | Paratype |
| *A.* sp. aff. *manaos* | Mitaraka, French Guiana | JN867570 | 296MC | |
| *A.* sp. aff. *manaos* | Mitaraka, French Guiana | EU201057 | 3035T | |
| *A.* sp. aff. *minuta* | Rio Lagarto Cocha, Peru | AY326000 | USNM 520905 | |
| *A.* sp. aff. *minuta* | Equador | DQ158262 | QCAZ833 | |
| *D. proboscideus* | Mata Escura, Brazil | JN867566 | MTR17173 | |
| *D. proboscideus* | Mata Escura, Brazil | JN867564 | MTR17171 | |
| *D. oreites* | Serra das lontras, Brazil | JN867567 | MTR16368 | |
| *D. carvalhoi* | Parna Caparão, Brazil | JN867568 | MTR15755 | |
| *D. carvalhoi* | Parna Caparão, Brazil | JN867569 | MTR15757 | |
| *D. leucomyxtas* | Ilha grande, Brazil | JN867558 | MTR15547 | |
| *D. leucomyxtas* | Ilha grande, Brazil | JN867557 | MTR15548 | |
| *D. berthalutzae* | Treviso, Brazil | JN867551 | CFBH10322 | |
| *D. brevipollicatus* | Estação Biológica de Boracia, Brazil | JN867554 | AF1541 | |
| *D. brevipollicatus* | Estação Biológica de Boracia, Brazil | JN867553 | AF1175 | |

**Table 3.** Uncorrected *p*-distances between *Amazophrynella* (A), species and the sister genus *Dendrophryniscus* (D). Molecular distances are based on the 480-bp fragment of 16S rDNA. We included *A. minuta* sensu stricto from its type locality and two candidate species, *Amazophrynella* sp. aff. *manaos* and *A.* sp. aff. *minuta* mentioned in Fouquet et al. (2012a).

| 16S rDNA | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 *A. amazonicola* | | | | | | | | | |
| 2 *A. matses* | 0.08 | | | | | | | | |
| 3 *A.* sp. aff. *minuta* | 0.06 | 0.07 | | | | | | | |
| 4 *A. minuta* | 0.05 | 0.08 | 0.03 | | | | | | |
| 5 *A. javierbustamantei* sp. n. | 0.09 | 0.03 | 0.06 | 0.07 | | | | | |
| 6 *A. vote* | 0.12 | 0.12 | 0.12 | 0.12 | 0.13 | | | | |
| 7 *A. bokermanni* | 0.12 | 0.12 | 0.11 | 0.11 | 0.13 | 0.10 | | | |
| 8 *A. manaos* | 0.12 | 0.12 | 0.12 | 0.14 | 0.12 | 0.10 | 0.08 | | |
| 9 *A.* sp. aff. *manaos* | 0.12 | 0.11 | 0.12 | 0.13 | 0.12 | 0.10 | 0.07 | 0.04 | |
| 10 *D. leucomystax* | 0.19 | 0.21 | 0.17 | 0.18 | 0.20 | 0.22 | 0.18 | 0.20 | 0.20 |

**Table 4.** Species level diagnostic characters observed in the 16S rDNA gene of *Amazophrynella javierbustamantei* sp. n. and other species of genus *Amazophrynella*. First line indicates position of the character within the 16S rDNA gene; (-) indicates a deletion.

| Species | 213 | 232 | 271 | 276 | 470 | 471 | 473 | 474 | 476 | 477 | 478 | 479 | 480 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. manaos* | A | C | A | C | A | T | G | T | C | A | A | A | A |
| *A. vote* | A | T | A | C | C | C | C | T | T | A | A | A | G |
| *A. minuta* | C | T | A | A | C | C | C | T | T | A | A | A | G |
| *A. bokermanni* | A | T | A | C | A | T | G | T | C | A | A | A | A |
| *A. amazonicola* | C | C | A | C | C | C | C | T | T | A | A | T | G |
| *A. javierbustamantei* sp. n. | T | G | G | T | T | G | T | G | A | G | C | C | - |
| *A. matses* | C | T | A | C | C | C | C | T | T | A | A | T | T |

**Table 5.** Character loadings, eigenvalues, and percentage of explained variance for Principal Components (PC) 1–2. The analysis was based on eleven morphometric variables of adult males: *Amazophrynella minuta* complex (*A. minuta* sensu stricto; *A. amazonicola*; *A. matses* and *A. javierbustamantei* sp. n.).

| Variables | PC1 | PC2 |
|---|---|---|
| HW | 0.462 | -0.146 |
| HL | 0.455 | -0.104 |
| SL | 0.374 | -0.244 |
| ED | 0.261 | 0.052 |
| IND | 0.369 | -0.271 |
| UAL | 0.139 | 0.258 |
| HAL | -0.032 | 0.484 |
| THL | 0.311 | -0.295 |
| TAL | 0.314 | 0.350 |
| TL | 0.116 | 0.364 |
| FL | 0.063 | 0.433 |
| % of variation | 24.93 | 23.63 |
| % | 24.93 | 48.56 |

**Table 6.** Character loadings of explained variance for Discriminant Function Analysis (DFA). The analysis was based on twelve morphometric variables of adult males of the *Amazophrynella minuta* complex (*A. minuta* sensu stricto; *A. amazonicola*; *A. matses* and *A. javierbustamantei* sp. n.).

| Variables | Discriminant Function |
|---|---|
| SVL | 6.343 |
| HW | -7.628 |
| HL | 0.146 |
| SL | -5.479 |
| ED | -1.175 |
| IND | -6.015 |
| UAL | 1.313 |
| HAL | 5.744 |
| THL | -3.871 |
| TAL | 13.944 |
| TL | -1.250 |
| FL | 1.016 |

25651, adult female, collected by D. Rodriguez on April 2007, from Province La Convención, locality of Camana, Department Cusco (12°05'9.25”S, 73°03'2.61”W, 680 m a.s.l.). MHNC 9939, MHNC 9940, adult females, collected by J. Delgado on 17 January 2010 from Province La Convención, locality of Mapi, Department Cusco (11°31'19.17”S, 73°28'29.83”W, 708 m a.s.l.). MHNC 9387, adult male, collected by G. Estrada on 21 January 2010, from locality of Tambo Poyeni near Quebrada Mayapo, Department Junin (11°19'29.9”S, 73°32'16.7"W, 388 m a.s.l.). MHNC 9754, MHNC 9756, adult males, MHNC 9626 , MHNC 9679, MHNC 9680, MHNC 9757, adult females, collected by A. Pari on January 2010, from locality of Tsoroja, Department Junin (11°18'56.06”S, 73°32'32.11”W, 399 m a.s.l. and 11°23'14.50”S , 73°29'43.00”W, 450 m a.s.l.).

**Diagnosis.** The new species is part of *Amazophrynella* based on molecular phylogenetic relationships (Fig. 1) and morphological synapomorphies (Fouquet et al. 2012a).

*Amazophrynella javierbustamantei* sp. n. is characterized by: (1) skin on dorsum tuberculate, with many subconical tubercles disperse on arms, legs, head and body; ventral skin coarsely areolate, throat and chest aerolate; (2) tympanic membrane and tympanic annulus not apparent through the skin; (3) snout long, subacuminated, protruding in lateral views; (4) upper eyelid with smaller tubercles, cranial crests absent; (5) dentigerous process of vomers absent; (6) vocal sac, vocal slits and nuptial pads absent; (7) finger I shorter than finger II, tips of digits rounded; (8) fingers lacking lateral fringes; (9) ulnar tubercles present; (10) heel bearing eight or more small low tubercles, tarsus with small tubercles and lack of folds; (11) plantar surfaces of feet bearing one metatarsal tubercle, the inner 2.5x larger than the outer, outer subconical; supernumerary plantar tubercles round and low; (12) toes lacking lateral fringes; webbing basal; toe III equal than toe V, tips of digits rounded; (13) dorsally is dark brown to light brown, and gray to black in some, ventrally, cream with yellow to orange marks, with

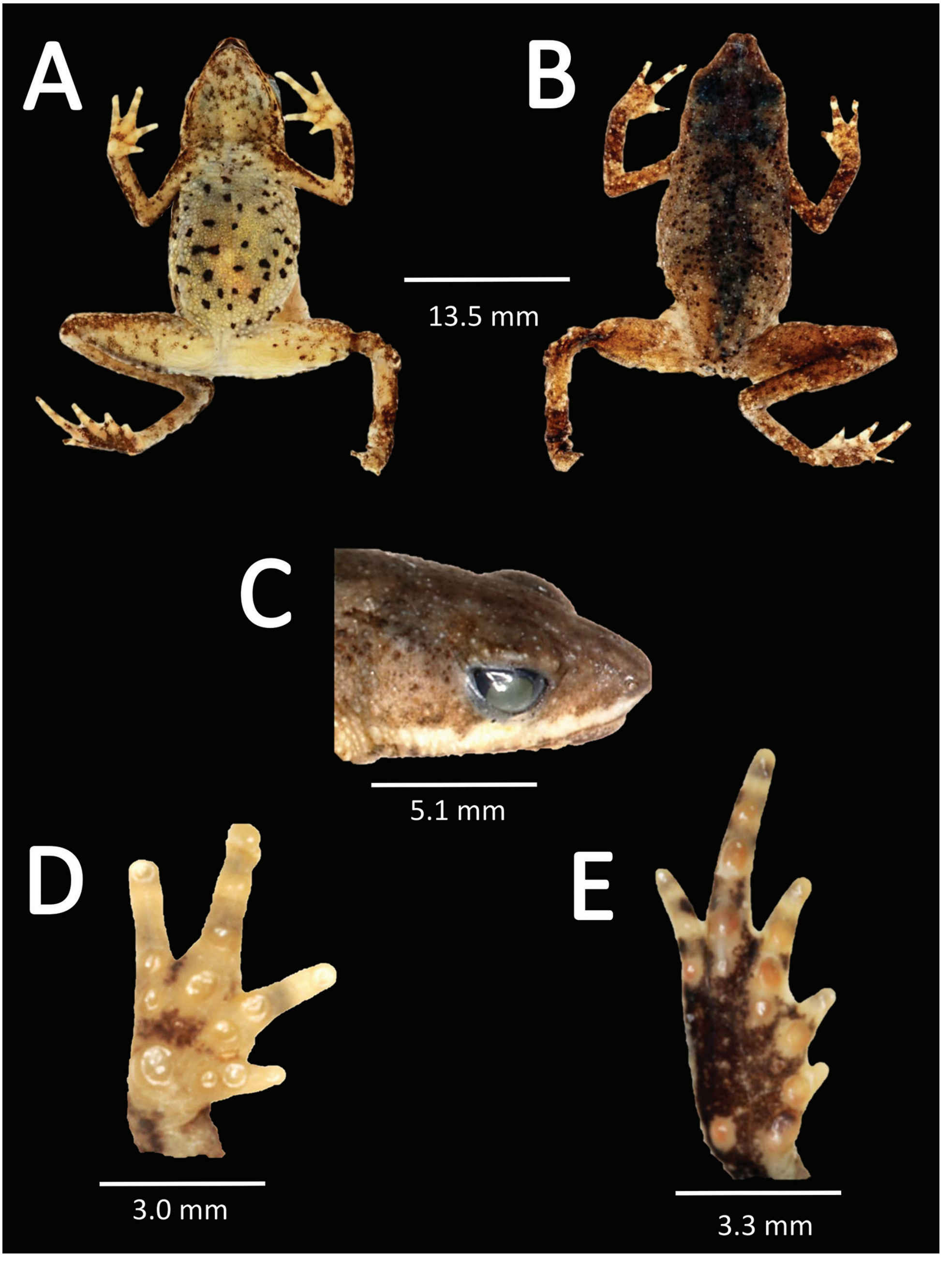


**Figure 4.** Holotype of *Amazophrynella javierbustamantei* sp. n. (MHNC 8331); **A** dorsal view **B** ventral view **C** dorsolateral view **D** right hand **E** right foot.

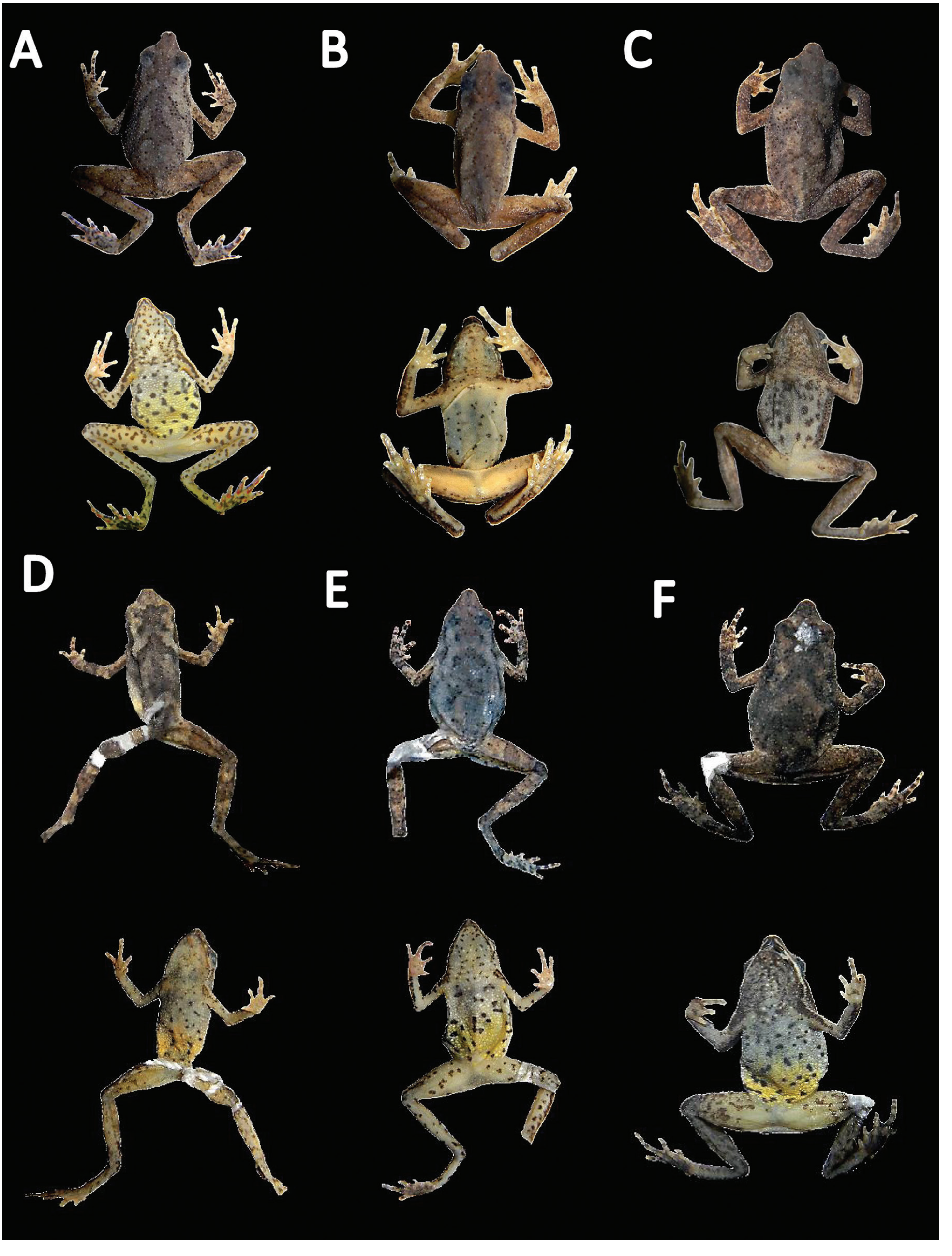


**Figure 5.** Dorsal and ventral view of some Paratypes of *Amazophrynella javierbustamantei* sp. n. Adult males (MHNC 8245: SVL 13.6 mm; MHNSM 31255: SVL 15.9 mm; MHNSM: 17993 SVL 14.2 mm; Adult females (MHNC 11002: SVL 17.2 mm, MHNC 9739: SVL 21.5, MHNC 8362: SVL 18.0 mm).

black to dark brown spots; (14) SVL 16.39–22.25 mm in females, 12.79–16.42mm in males; (15) hand length is the greatest of all species of *Amazophrynella*: 3.6 mm in males (n= 26) and 4.6 mm in females (n=20), see Fig. 3; (16) thirteen molecular autapomorphies in the 16S rDNA gene.

**Comparison with other species.** *Amazophrynella javierbustamantei* sp. n. (Figs 4, 5, 6) differs in the following character states (states of other species in parentheses). From *A. minuta* (Fig. 6A) by having body skin texture tuberculate (roughly granular); relative abundance of spiny granules on the forelimbs (prickly warty skin on axillary region of the forelimbs); absence of large warts on dorsum (presence of large warts); throat and chest cream-grayish (light brown); posterior side of belly color pale orange yellowish with tiny rounded black or dark brown spots (throat and the whole belly intensely orange yellowish); tiny rounded black spots covering the belly (irregular black ocelli or blotches); metatarsal tubercle rounded (oval). From *A. bokermanni* (Fig. 6B) relative size of fingers, with finger I shorter than II (I>II); snout vent length smaller in males (15.8 mm) and females (22.25 mm) (*A. bokermanni* with maximum 22 mm SVL in males and 28 mm SVL in females, see Izecksohn 1993); smaller snout in males, with 2.2 mm SL, n = 26 (2.7 mm SL, n = 5; see Table 1); posterior side of belly color pale orange yellowish with tiny rounded black or dark brown spots (white coloration with small black dots). From to *A. vote* (Fig. 6C) snout subacuminated in dorsal view (rounded); posterior side of belly color pale orange yellowish with tiny rounded black or dark brown spots (ventral color pattern reddish brown, with presence of small white dots). From *A. manaos* (Fig. 6D) snout subacuminated (snout truncate); dorsal skin finely granular (dorsal surfaces granular); throat and chest grayish (dark coloration); posterior side of belly color pale orange yellowish with tiny rounded black spots (venter cream with black spots or stripes). From to *A. matses* (Fig. 6E) snout subacuminated (snout slightly truncate), edges of nasal protrusion not dilated (dilated in ventral view); shape of palmar tubercle rounded (palmar tubercles elliptical); finger tips unexpanded (expanded), rounded tiny black spots covering the belly (medium-sized black ocelli or streaks); coloration of the belly pale yellow (belly completely yellow). From *A. amazonicola* (Fig. 6F) by the absence of small triangular protrusion on the tip of the snout in both dorsal and ventral views (presence); body surface granular (finely granular), dorsum uncovered with medium-sized granules scattered irregularly (covered with medium-sized granules scattered irregularly); posterior side of belly color pale orange yellowish with tiny rounded black or dark brown spots (orange yellowish with dark red and brown blotches).

**Description of the holotype.** Body slender, head triangular, slightly longer than wide; head length 35.5% of SVL, head width 30.9% of SVL. Snout long, subacuminate in dorsal view, protruding in lateral view; *canthus rostralis* straight and loreal region vertical; without papilla; snout length 39.0% of head length; tympanic membrane and tympanic annulus not apparent through the skin, skin of the tympanic area covered by round sub-conical warts; vocal sac externally not visible, vocal slits absent; eyes prominent 23.8% of head length; upper eyelid covered with small tubercles; those close to the external margin aligned in a more or less distinct row; nostril closer to snout than

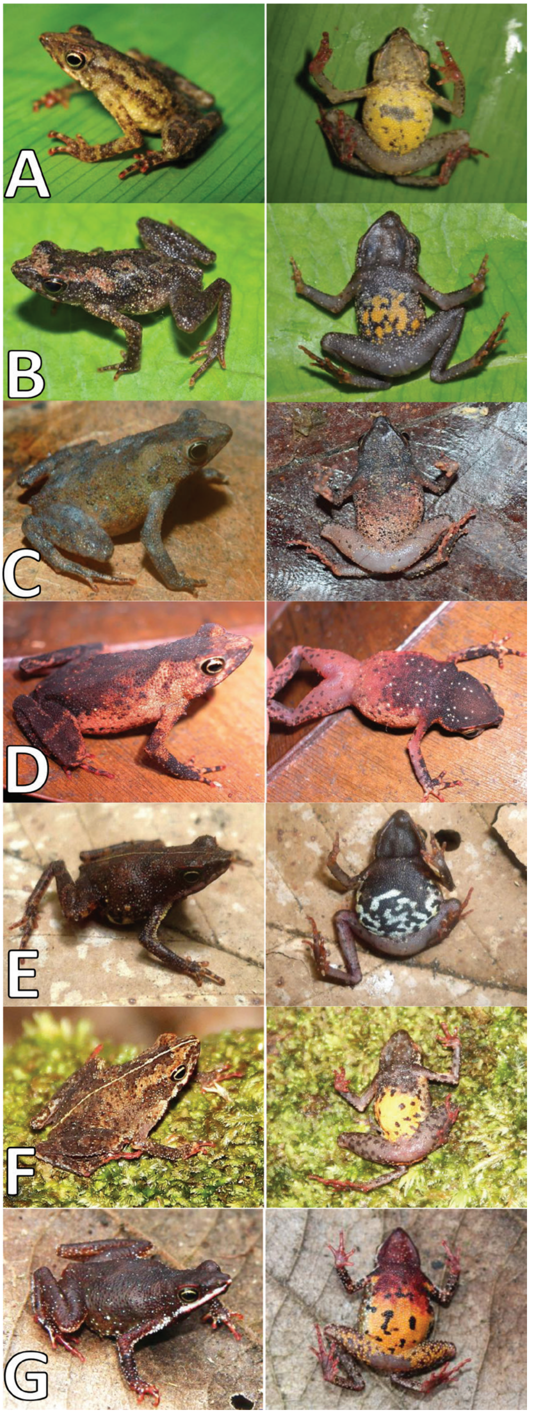


**Figure 6.** Dorsal and ventral morphological comparison between the *Amazophrynella* spp. (Unvoucher specimens): **A** *A. javierbustamantei* sp. n. **B** *A. minuta* **C** *A. bokermanni* **D** *A. vote* **E** *A. manaos* **F** *A. matses* **G** *A. amazonicola*.

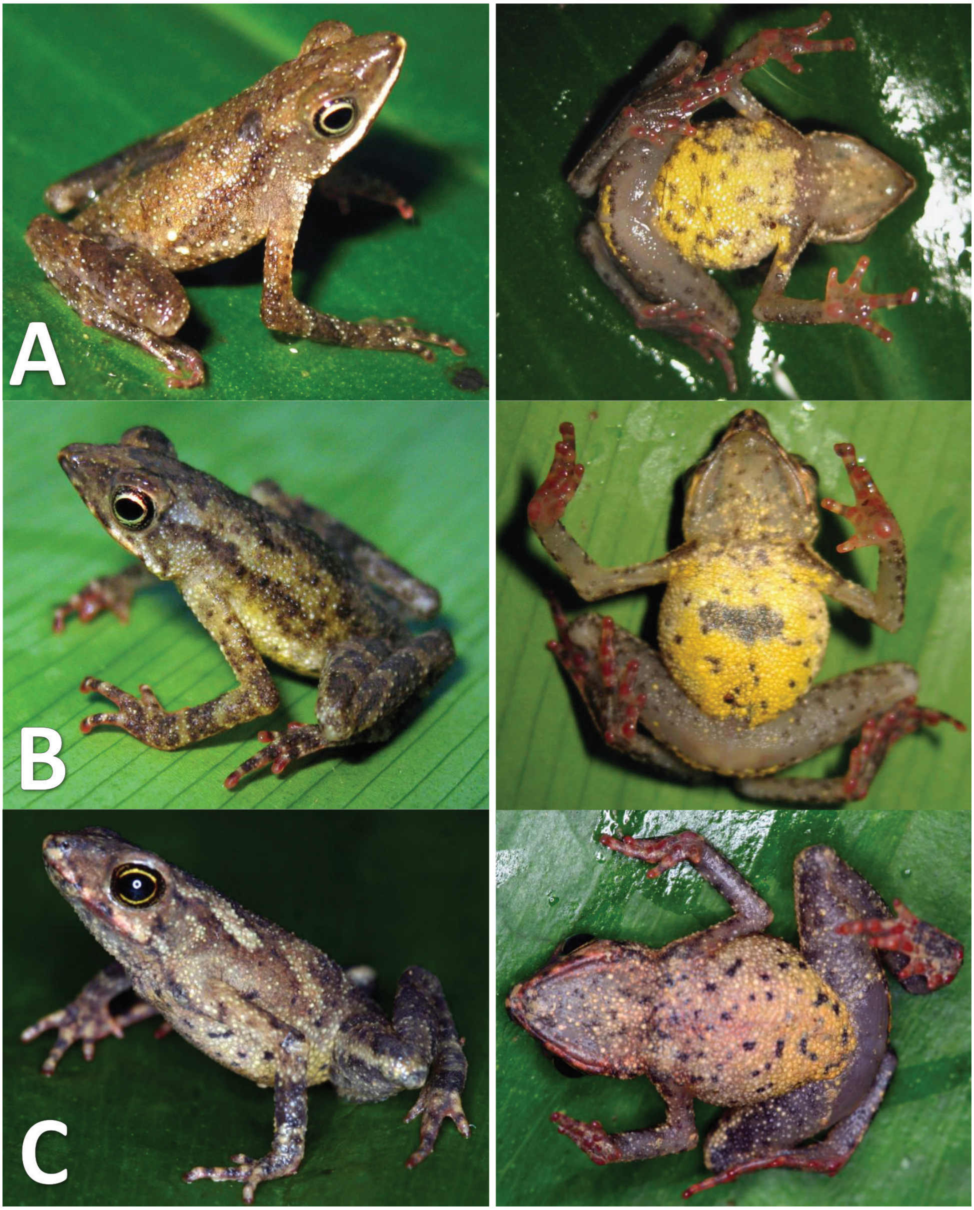


**Figure 7.** Dorsal and ventral variation of *Amazophrynella javierbustamantei* sp. n. (Unvoucher specimens): **A–C** Nueva Arequipa, Madre de Dios Department **B** Basin of Bajo Urubamba, Cusco Department.

to eyes; internarial distance smaller than eye diameter; presence of a line of small spiny granules from the outer edge of the mouth to upper arm, choanas small and circular.

Dorsal skin finely tuberculate with several large tubercles scattered sub-conical tubercles on upper arm; texture of ventral skin granular, covered by rounded granules. Dorsolateral surfaces, granular, with presence of large rounded tubercles. Forelimbs slender, upper arm length 29.6% of SVL; edges of lower arm and upper arm finely tuberculate with several large sub-conical and spiny granules; hand length 76.5% of upper arm length; fingers slender, tips not expanded; relative length of fingers I<II<IV<III; supernumerary tubercles and accessory palmar tubercles present, palmar large and rounded, supernumerary tubercles low, small rounded; subarticular tubercles rounded and small, one tubercle on fingers I, II and IV and two on finger III; fingers I and II basally webbed; indistinct nuptial pad. Hind limbs slender; ventral skin from thigh to tarsus covered by spiny tubercles, foot length 66% of thigh length; relative length of toes I<II<V<III<IV: inner metatarsal tubercle oval, 2.5× larger than outer; outer metatarsal tubercles small, rounded; subarticular tubercles present, rounded, present one on fingers I, II, and two on fingers III, V and three on finger IV; and tip of toes not expanded.

**Measurements of the holotype** (in millimeters)**.** SVL 15.1; HW 4.6; HL 5.3; SL 2.1; ED 1.2; IND 1.0; UAL 4.4; HAL 3.4; THL 8.1; TAL 8.1; TL 4.5; FL 5.3.

**Coloration of the holotype.** In life: dorsum of the holotype mostly light brown with dark brown in the dorsum; dorsolaterally creamish-brown with scattered black blotches; dorsal surfaces of hands and feet creamish-brown, and gray on arms and legs; belly creamish-gray with black dots, and the throat gray; fingers, toes and plantar surfaces reddish-black; groin with orange marks; iris with a bronze ring; cloaca with orange flap, black pupil and bronze iris. In alcohol: dorsum brownish-grey; venter cream with black and brown dots; orange surfaces turned cream, with a white longitudinal stripe on upper jaw extending from nostril to forearm.

**Variation.** The new species is phenotypically variable. In some individuals (e.g. MHNC 8245 and MHNC 11002, see Fig. 5) patterning on the dorsum varies, with these specimens presenting brown chevrons extending from the head to the vent. Some individuals showed a white line extending from the tip of the nose to the upper arm. Another specimen (MHNC 9739, see Fig. 5) presented a yellow pale coloration in the axillary region (in ventral view). In some individuals, the coloration of the throat extended onto the chest (e.g. MHNC 11002, MHNC 9739 and MHNC 8245, see Fig. 5). The pale yellow coloration of the belly surface may extend from thighs to the chest or just to the middle of the belly (e.g. MHNC 8362, see Fig. 5 and Fig. 7B). In some individuals, the thighs are abundantly covered by rounded tiny spots extending to the shank (Fig. 7B). In preserved specimens the dorsum becomes light brown and the belly coloration vary from white to yellow pale (e.g. MHNSM 31255 and MHNSM 17993, see Fig. 5). The color of the finger becomes pale red and in other individuals the red coloration of the fingers became brown or orange (Fig. 5).

**Bioacoustics.** The following values are presented as: min-max (average ± SD, number of notes). The call is a trill type call issued during continuous and regular intervals (Fig. 8). Each note had a duration of between 0.03 to 0.08 seconds (0.05 ± 0.01 seconds, n = 20). The number of pulses varied between 8 to 18 pulses per note (10.4 ± 2.6 pulses/note, n = 20). The silence between notes varied from 0.4 to 1.6 seconds (0.8 ± 0.3 seconds, n = 20). The dominant frequency varied from 3962.1 to 3789.8 kHz (3927.6 ± 70.7 kHz, n = 20), and coincides with the fundamental frequency. Time to peak amplitude was around 0.014 to 0.04 seconds (0.02 ± 0.01 seconds, n = 20).

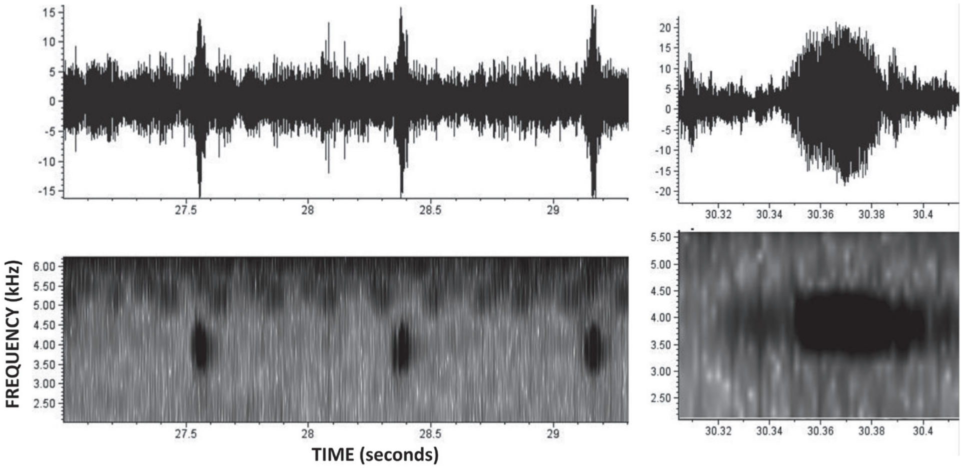


**Figure 8.** Advisement call of *Amazophrynella javierbustamantei* sp. n. from the Tambopata National Reserve, Madre de Dios, Peru (207 meters a.s.l.) (Macauly Library of Natural Songs and Cornell Laboratory of Ornithology) by the authors Crocoft, Morales and Mc Diarmid (2007). **A** Oscilogram and spectrogram by one note **B** Oscilogram and spectrogram of notes from the advisement Call.

**Distribution, ecology and conservation.** *Amazophrynella javierbustamantei* sp. n. is known from the Department of Cusco, in the lower Urubamba river basin and Department of Madre de Dios (Inambari, Candamo and Nueva Arequipa) in Peru (Fig. 9). Its distribution can vary from 215 m a.s.l. to 708 m a.s.l. Additional specimens were recorded at Los Amigos Biological Station , Tapir Lodge, and Explorers Inn, in Tambopata National Reserve. Individuals were active during the day, jumping on leaf litter, at night they were sleeping on leaves around 30 cm above ground. This species breeds close to the edges of permanent oxbow lakes, males call during the day while perched above streams in tangles (Cocroft et al. 2001). Three of the localities, km 105, 107 and 117 of the highway Puerto Maldonado–Cusco, Department Madre de Dios, show evidence of serious environmental impacts due to illegal gold mining activities, with forest and soil removed, and environmental pollution via organic and inorganic chemicals and heavy metal (specially mercury) poisoning. In addition, the new species is distributed inside of territories where oil companies are operating. On the other hand, the species is present in two protected areas, the Tambopata Natural Reserve and Machiguenga Communal Reserve. The conservation status of this species remains unknow, but was listed in 2008 as Least Concern on the IUCN red list (2015), because it was confused with *Amazophrynella minuta*, and because *Amazophrynella minuta* s.l. had wide distribution at that time, apparent tolerance of a certain degree of habitat modification, presumed large population, and because it is unlikely to be declining, and thus did not qualify for listing in a more threatened category. With recent studies the genus, the species complex of *Amazophrynella minuta*, was split in five species, three of them are now formally described for Peru (*Amazophrynella matses*, *A. amazonicola* and *A. javierbustamantei* sp. n.). The recognition of these new species will require the

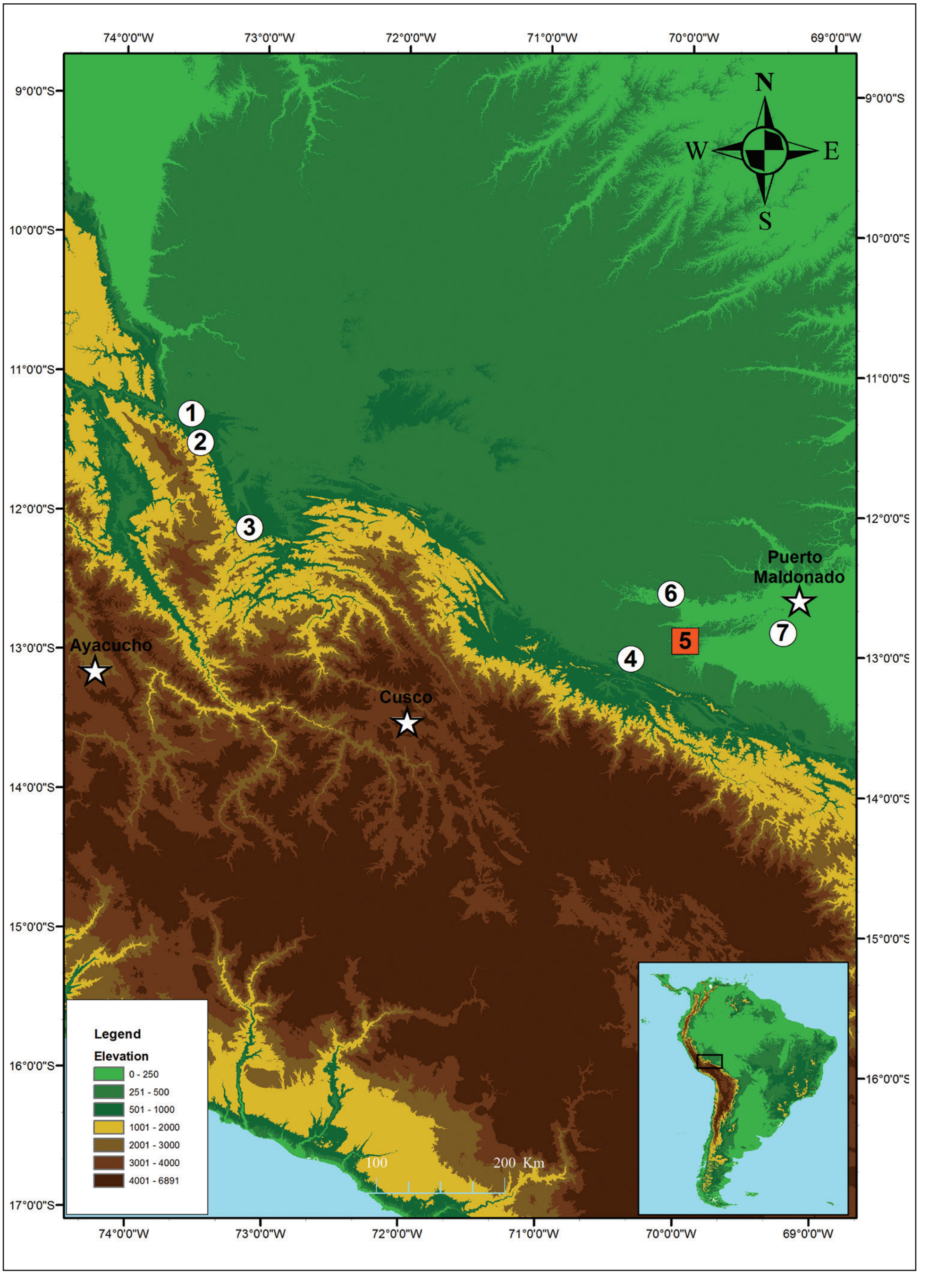


**Figure 9.** Distribution map of *Amazophrynella javierbustamantei* sp. n. in Peru. Holotype locality in square orange, **5** Guacamayo Creek, Department Madre de Dios. Paratypes localities in white circles **1** Tsoroja, Department Junin **2** Mapi, Department Cusco **3** Camana, Department Cusco **4** Inambari, Department Madre de Dios **6** Los Amigos Biological Station, Department Madre de Dios **7** Explorer's Inn, Department Madre de Dios.

reevaluation of the conservation status of these species. It should also act as an impetus for additional field and laboratory studies of Peruvian amphibians, in order to understand the real conservation status of this fauna.

**Etymology.** The species is named after Dr. Javier Bustamante, a Peruvian residing in United States, to whom we dedicate this species in recognition of his friendship and support of herpetological taxonomy and systematics research and amphibian conservation in Peru.

## Discussion

Taxonomic reviews of Amazonian amphibians suggests that morphological characters are too conservative to permit delimiting species since closely related species share similar morphologies, and amphibians in general are morphologically conservative (e.g., Elmer et al. 2007; Fouquet et al. 2007c; Funk et al. 2011; Padial et al. 2009). Thus, the use of integrative techniques in taxonomy is revolutionizing the identification and delimitation of species based on independent lines of evolutionary evidence (Dayrat 2005; Padial and De la Riva 2009). The use of an integrative approach not only allows for the discovery and delimitation of new species, it also helps us to understand the mechanism of species formation. Thus, integrative taxonomy allows us to have a better understanding of the true scope of anuran diversity in the Amazon, and it allows us to better understand the processes that generated this biodiversity.

The taxonomic ambiguity surrounding the name *A. minuta* and to a lesser extent *A. bokermanni* resulted in a severe underestimation of the taxonomic diversity of this genus. Since the descriptions of *A. minuta* in 1941 and *A. bokermanni* in 1993, the taxonomy of the genus has not been revised, leading to misdiagnoses of other species as either *A. minuta* or *A. bokermanni* due to the relatively generalized descriptions of these taxa. Three publications since 2012 (Ávila et al. 2012; Rojas et al. 2014, 2015) described four new species, increasing the taxonomic diversity of the genus by 200%. All four species were previously classified as populations of a single species with a large distribution (*A. minuta* sensu lato). Although striking, the severe underestimation of taxonomic diversity observed in *Amazophrynella* and the existence of multiple lineages in *Amazophrynella minuta* is nothing particular to this group. Examples of other Amazonian species complexes include *Rhinella margaritifera* and *Scinax ruber*, *Pristimantis ockendeni*, *Pristimantis fenestratus*, *Engystomops petersi*, *Hybsiboas fasciatus*, *Dendropsophus minutus* and *Osteocephalus taurinus* (Fouquet et al. 2007; Elmer and Canatella 2008; Padial et al. 2009; Funk et al. 2011; Caminer and Ron 2014; Gehara et al. 2014, Jungfer et al. 2013).

The descriptions by Rojas et al. (2014, 2015) were based, in part, on diagnostic characters observed in the 16S rDNA. This gene is widely used as a DNA barcode for amphibians, for reliable species identification (Vences et al. 2005, Fouquet et al. 2007), for evaluating monophyly of species and for discovering divergent lineages (Padial et al. 2009, Crawford et al. 2010; Padial et al. 2010 and Padial et al. 2012). Based on 16S rDNA analyses, we also have evidence that *A. bokermanni* and *A. vote* represent species complexes (RRRZ, personal observation). This observation is in addition to the existence of the two candidate species of *Amazophrynella* already observed in previous analyses: one from the Guiana Shield (*A.* sp. aff. *manaos*), sister taxon of *A. manaos*, and another from Ecuador (*A.* sp. aff. *minuta*), sister taxon of *A. minuta* sensu stricto (Fig. 1). Although the taxonomic status of these candidate species will need to be confirmed using morphological and bioacoustics data, it is clear that even with the recent descriptions, the taxonomic diversity of the genus remains underestimated.

While part of our evidence for the existence of the new species as well as those described previously by Rojas et al. (2014, 2015) comes from the use of molecular data, the descriptions make use of other data types and non-molecular diagnoses. Thus these undiscovered lineages were not truly cryptic (morphologically cryptic), but rather the result of poor taxonomic knowledge of the group. In this respect, the genus *Amazophrynella* again is not the exception, but rather the norm.

The new species *A. javierbustamantei* sp. n. is clearly differentiated in multivariate morphometric space from the other members of the *Amazophrynella minuta* "species group" (*A. minuta*, *A. amazonicola* and *A. matses*). Together with the description of *Amazophrynella javierbustamantei* sp. n. we also provide advertisement call. *Amazophrynella javierbustamantei* sp. n. is only the second species of the genus for which an advertisement call is known and recorded (see Duellman 1978). Acoustics can provide evidence of potentially new species with behavioral or premating isolating mechanisms (e.g. De la Riva et al. 1997; Gerhardt 1998; Simões et al. 2008, Padial and De la Riva 2009; Padial et al. 2012), thus providing evidence of evolutionary mechanisms that contributed to the species diversity of the genus *Amazophrynella*.

The threats to the biological conservation of *A. javierbustamantei* sp. n. are evident, with uncontrolled exploration for gold, illegal mining and the destruction of habitat in the Departments of Madre de Dios and Cusco, probably causing a significant reduction in the population sizes of the species and fragmenting its distribution. For these reasons is necessary to analyze the current population status and trends of this and another amphibian species in this Department of southern Peru.

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## Appendix 1

### Specimens examined

*Amazophrynella minuta*—BRAZIL: Taracuá, Uaupés River: INPA-H 32725, INPA-H 32723, INPA-H 32729, INPA-H32730, INPA-H32736, INPA-H32731 (females) and INPA-H 32724, INPA-H32728, INPA-H 32733, INPA-H 32735, INPA-H 32722, INPA-H 32738, INPA-H 32737, INPA-H 32739. INPA-H 32720, INPA-H 32732, INPA-H 32726, INPA-H 32730, INPA-H 32740, INPA-H 32734, INPA-H 32721 (males).

*Amazophrynella bokermanni*—BRAZIL: Juriti, Pará: INPA-H 31861, INPA-H 31864, INPA-H 31863, INPA-H 31862, INPA-H 31865, municipality of Juriti, Pará State, Brazil (50 km from type locality).

*Amazophrynella vote*—BRAZIL: Fazenda São Nicolau, Cotriguaçu, Mato Grosso (Holotype: UFMT-A 11138); Madeira River, Manicoré (Paratypes: INPA-H 12256, 12331, 12255, 12342, 12343, 12366, 12267); Aripuanã River, Novo Aripuanã (Paratype: INPA-H 12326); Parque Estadual do Guariba: Manicoré (Paratypes: INPA-H 21558); Parque Nacional Nascentes do Lago Jari, Tapauá: Amazonas (Paratypes: INPA-H 27412, 27417-27419, 27421-27423, 27425-27426).

*Amazophrynella manaos*—BRAZIL: Campus da Universidade Federal do Amazonas, Amazonas (Holotype: INPA-H 31866, paratypes: INPA-H 6983, INPA-H 6984, INPA-H 6987, INPA-H 7797); Presidente Figueiredo, Amazonas (Paratypes: INPA-H 29568, INPA-H 29569, INPA-H 29571, INPA-H 29570, INPA -H 29572, INPA-H 20986; INPA-H 21217, INPA-H 30577, INPA-H 30575, INPA-H 30573, INPA-H 30572, INPA-H 30576); Reserva Florestal Adolpho Ducke, Amazonas (INPA-H 21028, INPA-H 21170, INPA-H 21060, INPA -H 31866, INPA-H 21007, INPA-H 21008, INPA-H 21009, INPA-H 21010, INPA-H 21011, INPA-H 21012, INPA-H 21013).

*Amazophrynella amazonicola*—PERU: Puerto Almendra San Juan Bautista, Loreto (Holotype: MZUNAP 901, paratopotypes: MZUNAP 906; MZUNAP 915; MZUNAP

110; MZUNAP 907, MZUNAP 917; MZUNAP 889; MZUNAP 910; MZUNAP 911; MZUNAP 916; MZUNAP 913; MZUNAP 914; paratypes: MZUNAP 906; MZUNAP 915; MZUNAP 110; MZUNAP 907, MZUNAP 917; MZUNAP 889; MZUNAP 910; MZUNAP 911; MZUNAP 916; MZUNAP 913; MZUNAP 914); 58 km of Iquitos–Nauta highway on Fundo Zamora, San Juan Bautista, Loreto (Paratypes: MZUNAP 908, MZUNAP 924, MZUNAP 886, MZUNAP 900, MZUNAP 888, MZUNAP 919, MZUNAP 902, MZUNAP 887, MZUNAP 905, MZUNAP 920); Nauta, Maynas (Paratypes: MZUNAP 918, MZUNAP 909); Fundo UNAP, Maynas, Loreto (Paratype: MZUNAP 242)

*Amazophrynella matses*—PERU: Nuevo Salvador, Requena, Loreto (Holotype: MZUNAP 921, paratopotypes: MZUNAP 934, MZUNAP 955 MZUNAP 940, MZUNAP 948 MZUNAP 943, MZUNAP 952, MZUNAP 953, MZUNAP 958, MZUNAP 922, MZUNAP 923, MZUNAP 925, MZUNAP 926, MZUNAP 927, MZUNAP 944, MZUNAP 938, MZUNAP 936); Jenaro Herrera, Requena, Loreto (Paratypes: MZUNAP 928, MZUNAP 929, MZUNAP 930, MZUNAP 931, MZUNAP 933, MZUNAP 955, MZUNAP 935, MZUNAP 950, MZUNAP 937, MZUNAP 939, MZUNAP 941, MZUNAP 942, MZUNAP 946, MZUNAP 947, MZUNAP 949).

*Amazophrynella javierbustamantei* sp. n. —PERU: Quebrada Guacamayo, Tambopata, Madre de Dios (Holotype: MHNC 8331; MHNC 8363, MHNC 8245, MHNC 8238, MHNC 8316, MHNC 8484, MHNC 8362); La Pampa, Tambopata, Madre de Dios (MHNC 11101, MHNC 11102, MHNC 11103, MHNC 11104); Inambari, Manu, Madre de Dios (MHNSM 17993); Nuevo Arequipa, Tambopata, Madre de Dios (MHNC 8363, MHNC 8245, MHNC 8331, MHNC 8238, MHNC 8354, MHNC 8316, MHNC 8484, MHNC 8362); Rio Tambopata, Tambopata, Madre de Dios (MHNSM 9635, MHNSM 9641, MHNSM 9647, MHNSM 9642, MHNSM 9648, MHNSM 9633, MHNSM 9644, MHNSM 9646, MHNSM 9657, MHNSM 9640); Camana, La Convencion, Madre de Dios (MHNSM 25651); Mapi, La Convencion, Madre de Dios (MHNC 9939, MHNC 9940); Tambo Poyeni, Junin (MHNC 9387); Tsoroja, Junin (MHNC 9754, MHNC 9756, MHNC 9626, MHNC 9679, MHNC 9680, MHNC 9757). Rio Urubamba, Urubamba, Cusco (MHNC 9939, MHNC 9626, MHNC 9686, MHNC 9679, MHNC 9940, MHNC 9757, MHNC 9387, MHNC 9754, MHNC 9756).

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# A checklist of helminth parasites of Elasmobranchii in Mexico

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## Abstract

A comprehensive and updated summary of the literature and unpublished records contained in scientific collections on the helminth parasites of the elasmobranchs from Mexico is herein presented for the first time. At present, the helminth fauna associated with Elasmobranchii recorded in Mexico is composed of 132 (110 named species and 22 not assigned to species), which belong to 70 genera included in 27 families (plus 4 *incertae sedis* families of cestodes). These data represent 7.2% of the worldwide species richness. Platyhelminthes is the most widely represented, with 128 taxa: 94 of cestodes, 22 of monogeneans and 12 of trematodes; Nematoda and Annelida: Hirudinea are represented by only 2 taxa each. These records come from 54 localities, pertaining to 15 states; Baja California Sur (17 sampled localities) and Baja California (10), are the states with the highest species richness: 72 and 54 species, respectively. Up to now, 48 elasmobranch species have been recorded as hosts of helminths in Mexico; so, approximately 82% of sharks and 67% of rays distributed in Mexican waters lack helminthological studies. The present list provides the host, distribution (with geographical coordinates), site of infection, accession number in scientific collections, and references for the parasites. A host-parasite list is also provided.

## Keywords

Platyhelminthes, Nematoda, Hirudinea, Sharks, Rays, Richness, Selachii, Batoidea

## Introduction

According to Eschmeyer and Fong (2015), 1338 species of elasmobranchs have been described worldwide (768 rays and 570 sharks). However, Naylor et al. (2012), based on the fact that since 2005 more than 130 new species have been described, considered that more species remain to be discovered. According to these authors, this increase is a result of reassessment of geographic variation; some of the increase represents recognition of subtle morphological variants among congeneric forms that nevertheless exhibit substantial molecular sequence divergence (cryptic species). In Mexico, this group is represented by 204 known species (95 rays and 109 sharks) (Del Moral-Flores and Pérez-Ponce de León 2013) (Table 1); this richness constitutes 15% of the living species in the world. Nonetheless, most of the species recorded in Mexican waters also have been found in international waters, and many of them are cosmopolitan (Espinoza-Pérez et al. 2004).

Elasmobranchs (sharks, skates and rays) are host to a great variety of parasites in nature, particularly helminths. Up to now, more than 1500 helminth species have been recorded in association with these hosts worldwide; cestodes represent the most diverse group, with approximately 1133 species, followed by monogeneans with 226, nematodes with 83, digeneans with 50-60, leeches with 23, and aspidogastreans with 2 (Caira et al. 2012). In addition, 4 species of acanthocephalans have been found only in elasmobranchs (Weaver and Smales 2014). In Mexico, the first record of a helminth parasitizing an elasmobranch was made by Caballero y Caballero (1945), who described the digenean *Staphylorchis pacifica* (=*Petalodistomum pacificum*) from the body cavity of an undetermined shark in the Pacific slope of this country. Since then, a great amount of information has been generated, most of it in the last 2 decades, particularly in the Gulf of California. The main goal of this checklist is to compile and discuss all these data and to establish some patterns of richness, geographical distribution and host spectrum.

## Methods

This checklist contains information updated until December, 2015, and comes from two different sources: 1) retrospective bibliographical search, using different databases such as CAB Abstracts, Biological Abstracts, and Zoological Record; 2) Search in databases of national [Colección Nacional de Helmintos (CNHE), Instituto de Biología, UNAM, Mexico City, Mexico] and international [Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska-Lincoln, USA; National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C., USA, formerly United States National Parasite Collection (USNPC), Beltsville, Maryland, USA] parasite collections.

The checklist is divided into two sections; the first includes a parasite-host list, presented in phylogenetic order, starting with the phylum Platyhelminthes (Trematoda,

**Table 1.** Species of Elasmobranchii reported from Mexico and richness of associated helminths (modified from Del Moral-Flores and Pérez-Ponce de León 2013).

| Subclass | Order | Family | Genera | Species | Sampled species | Helminth recorded |
|---|---|---|---|---|---|---|
| **Selachii** | Hexanchiformes | Chlamydoselachidae | 1 | 1 | 0 | 0 |
| | | Hexanchidae | 3 | 4 | 1 | 1 |
| | | Echinorhinidae | 1 | 1 | 0 | 0 |
| | | Squalidae | 2 | 4 | 0 | 0 |
| | | Centrophoridae | 1 | 4 | 0 | 0 |
| | Squaliformes | Etmopteridae | 2 | 8 | 0 | 0 |
| | | Somniosidae | 3 | 3 | 0 | 0 |
| | | Oxynotidae | 1 | 1 | 0 | 0 |
| | | Dalatiidae | 4 | 5 | 0 | 0 |
| | Squatiniformes | Squatinidae | 1 | 4 | 1 | 2 |
| | Heterodontiformes | Heterodontidae | 1 | 2 | 2 | 7 |
| | | Ginglymostomatidae | 1 | 1 | 1 | 3 |
| | Orectolobiformes | Rhicodontidae | 1 | 1 | 0 | 0 |
| | | Odontaspididae | 2 | 3 | 0 | 0 |
| | | Pseudocarcharhiidae | 1 | 1 | 0 | 0 |
| | | Megachasmidae | 1 | 1 | 0 | 0 |
| | Lamniformes | Alopidae | 1 | 4 | 2 | 6 |
| | | Cetorhinidae | 1 | 1 | 0 | 0 |
| | | Lamnidae | 3 | 4 | 0 | 0 |
| | | Scyliorhinidae | 6 | 15 | 0 | 0 |
| | | Triakidae | 3 | 11 | 5 | 9 |
| | Carcharhiniformes | Carcharhinidae | 7 | 25 | 5 | 19 |
| | | Sphyrnidae | 1 | 6 | 3 | 5 |
| TOTALS* | 7 | 23 | 48 | 109 | 20 | 52 |
| **Batoidea** | Torpediniformes | Torpedinidae | 1 | 2 | 0 | 0 |
| | | Narcinidae | 2 | 4 | 2 | 5 |
| | Pristiformes | Pristidae | 1 | 3 | 0 | 0 |
| | | Rhinobatidae | 2 | 10 | 5 | 10 |
| | Rhinobatiformes | Platyrhinidae | 1 | 1 | 0 | 0 |
| | | Arhynchobatidae | 2 | 4 | 0 | 0 |
| | Rajiformes | Rajidae | 9 | 29 | 2 | 2 |
| | | Anacanthobatidae | 2 | 4 | 1 | 1 |
| | | Urotrygonidae | 2 | 10 | 5 | 27 |
| | | Dasyatidae | 3 | 9 | 5 | 26 |
| | | Gymnuridae | 1 | 4 | 0 | 0 |
| | Myliobatiformes | Myliobatidae | 3 | 8 | 8 | 38 |
| | | Rhinopteridae | 1 | 2 | 0 | 0 |
| | | Mobulidae | 2 | 6 | 0 | 0 |
| TOTALS* | 5 | 14 | 32 | 95 | 28 | 108 |

* The totals in the table are greater than in the text because some species infect 2 or more host species (sharks and/or rays).

Monogenoidea and Cestoda), and followed by the phyla Nematoda and Annelida (Hirudinea). Each phylum contains families, genera, and species in alphabetical order. The nomenclature and classification for each metazoan group is based on the following references: Trematoda (Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008), Monogenoidea (Boeger and Kritsky 1993), Cestoda (Caira et al. 2014b), Nematoda (Anderson et al. 1974–1983; Gibbons 2010), and Hirudinea (Sawyer 1986; Davies 1991). The information for each helminth species includes species name, authority, and site of infection. We use "NA" when some data are not available in the original source. Next, we present species distributions, referring to states of the Mexican Republic (in caps) where the record was made as well as the specific locality name, followed by the species of host and the bibliographic references related to records. For specimens deposited in a collection, acronyms are as follows:

**BMNH** The British Museum (Natural History) Collection at the Natural History Museum, London, UK.

**CNHE** Colección Nacional de Helmintos, Instituto de Biología, UNAM, Mexico City, México.

**CPMHN-UABCS** Colección Parasitológica del Museo de Historia Natural de la Universidad Autónoma de Baja California Sur, La Paz, Baja California Sur, Mexico.

**ECOPA** El Colegio de la Frontera Sur, Chetumal, Quintana Roo, Mexico.

**HWML** Harold W. Manter Laboratory of Parasitology, University of Nebraska-Lincoln, Nebraska, United States.

**IPCAS** Institute of Parasitology, Academy of Sciences of the Czech Republic, Česke Budějovice, Czech Republic.

**LRP** Lawrence R. Penner Collection, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT, USA.

**MNHG-INV** or **PLAT** Museum of Natural History, Geneva, Switzerland.

**SBMNH** Santa Barbara Museum of Natural History, Santa Barbara, California, United States.

**TINRO** Pacific Fisheries Research Center, Vladivostok, Russian Federation.

**UCLA** Helminthological Collection, Zoology Department, University of California at Los Angeles.

**USNPC** Accession numbers used in this work correspond to those given by United States National Parasite Collection, Beltsville, Maryland, USA, which was recently transferred to the National Museum of Natural History (USMN), Smithsonian Institution, Washington, D.C., USA.

The name of the type locality (TL), type host (TH), and original reference (OR) of the new species described from elasmobranchs recorded in Mexico are indicated with abbreviations of these words in superscript.

The host-parasite list is ordered alphabetically by families of elasmobranchs; each family includes the scientific name of the host and the authority name. Then, the scientific names of the species of helminths are listed in alphabetical order, indicating in parentheses the parasite group to which they belong. The scientific names of elasmobranchs were updated following Froese and Pauly (2014); higher levels of classification follow Del Moral-Flores and Pérez-Ponce de León (2013).

## Results

To date, 48 species of elasmobranchs (20 sharks and 28 rays) have been recorded as host of 132 taxa of helminths (110 named species and 22 not assigned to species); these parasite species belong to 70 genera included in 27 families (plus 4 families of cestodes that are *incertae sedis*). Platyhelminthes is represented by 128 taxa: 94 taxa of cestodes, 22 taxa of monogeneans and 12 taxa of trematodes; for both Nematoda and Annelida (Hirudinea) only 2 species have been recorded. The 54 sampled sites for helminths are located in 15 states; Baja California Sur (17 localities) and Baja California (10), are the states with the highest species richness (72 and 54, respectively) (Fig. 1). Up to now, no helminths parasitizing elasmobranchs from Mexican waters have been

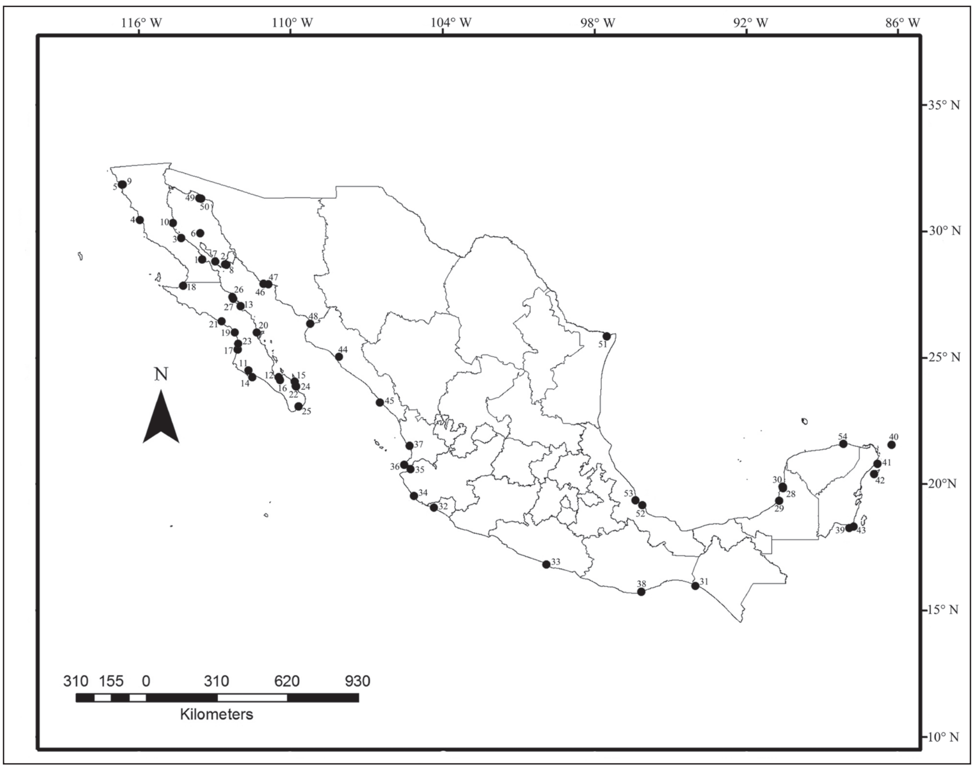


**Figure 1.** Map of Mexico showing the localities that have been sampled for elasmobranchs as hosts of helminth species.

reported from the states of Michoacán and Tabasco; for Chiapas, Colima, Tamaulipas and Yucatán, only one record each has been reported. Below, we present the checklist of helminth parasites recorded in elasmobranch species caught in Mexico, which summarizes the current knowledge on this group in the country.

## Parasite-host list

### Trematoda Rudolphi, 1808
### Acanthocolpidae Lühe, 1906

#### *Pleorchis magniporus* Arai, 1962

**Site of infection.** Intestine.
**Locality.** BAJA CALIFORNIA SUR: Bahía Magdalena[TL]: *Urolophus maculatus*[TH] (see Arai 1962[OR]).
**Specimens in collections.** UCLA.

### Azygiidae Lühe, 1909

#### *Otodistomum veliporum* (Creplin, 1837) Stafford, 1904

**Site of infection.** Body cavity, stomach.
**Locality.** BAJA CALIFORNIA SUR: Santa Rosalía: *Heterodontus francisci*, *Heterodontus mexicanus*, *Mustelus henlei*, *Squatina californica* (see Curran and Overstreet 2000).
**Specimens in collections.** CNHE (3852).

### Bucephalidae Poche, 1907

#### *Prosorhynchus truncatus* Verma, 1936

**Site of infection.** Intestine.
**Locality.** BAJA CALIFORNIA SUR: El Comitán: *Dasyatis brevis* (see Villarreal-Lizárraga 1995).
**Specimens in collections.** CPMHN-UABCS (20).

### Gorgoderidae Looss, 1899

#### *Anaporrhutum euzeti* Curran, Blend & Overstreet, 2003

**Site of infection.** Pericardial and body cavities.

**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Dasyatis brevis* (see Curran et al. 2003). BAJA CALIFORNIA SUR: Loreto[TL]: *Myliobatis longirostris*[TH] (see Curran et al. 2003[OR]). NA: Golfo de California: *Dasyatis longa*, *Diplobatis ommata*, *Mobula munkiana*, *Myliobatis californica*, *Narcine entemedor*, *Rhinobatos productus*, *Urolophus halleri*, *Urolophus maculatus*, *Zapteryx exasperata* (see Curran et al. 2013)
**Specimens in collections.** CNHE (4499); HWML (16702); SBMNH (345780).

### *Nagmia cisloi* Curran, Blend & Overstreet, 2009

**Site of infection.** Body cavity.
**Locality.** BAJA CALIFORNIA SUR: Bahía de La Paz[TL]: *Mobula thurstoni*[TH] (see Curran et al. 2009[OR]).
**Specimens in collections.** CNHE (6198).

### *Nagmia rodmani* Curran, Blend & Overstreet, 2009

**Site of infection.** Body cavity.
**Locality.** BAJA CALIFORNIA SUR: Loreto [TL]: *Narcine entemedor* [TH] (see Curran et al. 2009 [OR]).
**Specimens in collections.** CNHE (6199); HWML (48889); SBMNH (423115).

### *Probolitrema richiardii* (López, 1888) Looss, 1902

**Site of infection.** Body cavity.
**Locality.** BAJA CALIFORNIA: Isla San Esteban: *Urobatis* sp. (see Curran et al. 2009). BAJA CALIFORNIA SUR: Bahía de Santa Inés: *Dasyatis brevis*, *Mustelus lunulatus*, *Urolophus maculatus* (see Markell 1956). NA: Golfo de California: *Dasyatis brevis*, *Dasyatis longa*, *Myliobatis californica*, *Myliobatis longirostris*, *Rhinobatos leucorhynchus* (see Curran et al. 2009)
**Specimens in collections.** CNHE (6200); HWML (48890); SBMNH (423116); USNPC (49354).
**Notes.** The specimens of Bahía de Santa Inés were identified as *Probolitrema mexicana*, but this species is a synonym of *P. richiardi* according to Curran et al. (2009).

### *Staphylorchis pacifica* (Caballero y Caballero, 1945) Campbell, 2008

**Site of infection.** Body cavity.
**Locality.** COLIMA: Manzanillo [TL]: “Tiburón no determinado[TH]” (see Caballero y Caballero 1945[OR]). JALISCO: Puerto Vallarta: “Elasmobranchii” (CNHE); NA-

YARIT: Punta Mita: "Tiburón no determinado" (see Bravo-Hollis 1954); San Blás: *Carcharhunus limbatus* (see Lamothe-Argumedo 1969). SINALOA: Mazatlán: *Galeorhinus galeus* (see Winter 1959).

**Specimens in collections.** CNHE (921, 1069, 1111, 1585, 3246).

**Notes.** The original description of this species was made under the name *Petalodistomum pacificum* (Caballero y Caballero 1945); later, this species was transferred to *Nagmia* by Markell (1953). This act was accepted by Sogandares-Bernal (1959) and Curran et al. (2009) but rejected by Caballero y Caballero et al. (1956) and Winter (1959). Lamothe-Argumedo (1969) erected *Winteria* to accommodate this species, but this genus was considered a synonym of *Nagmia* (Curran et al. 2009). Pigulevski (1953) divided the genus *Petalodistomum* in 2 subgenera, including the species of *Petalodistomum* described by Caballero y Caballero (1945) in *Petalodistomum* (*Petalodistomum*). Currently, this trematode species is accepted as *Staphylorchis pacifica* (see Campbell 2008).

## Opecoelidae Ozaki, 1925

### *Helicometrina nimia* Linton, 1910

**Site of infection.** Stomach.

**Locality.** BAJA CALIFORNIA SUR: Las Barrancas: *Prionace glauca* (see Méndez 2005).

**No specimens in collections.**

## Ptychogonimidae Dollfus, 1937

### *Ptychogonimus megastomum* (Rudolphi, 1819) Lühe, 1900

**Site of infection.** Stomach.

**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Mustelus californicus* (see Curran and Overstreet 2000); Puertecitos: *Mustelus lunulatus* (see Curran and Overstreet 2000).

**Specimens in collections.** CNHE (3853-4).

## Syncoeliidae Loos, 1899

### *Paronatrema vaginicola* Dollfus, 1937

**Site of infection.** Buccal cavity, cloaca, gills.

**Locality.** BAJA CALIFORNIA SUR: Boca de Álamo: *Alopias pelagicus*, *Prionace glauca* (see Curran and Overstreet 2000); Puntarena, San Isidro: *Prionace glauca* (see Cur-

ran and Overstreet 2000). SINALOA: Bahía Santa María: *Alopias pelagicus* (see Curran and Overstreet 2000).
**Specimens in collections.** CNHE (3855); HWML (15263, 15265).

### *Syncoelium vermilionensis* Curran & Overstreet, 2000

**Site of infection.** Gills.
**Locality.** BAJA CALIFORNIA SUR: Puntarena: *Mobula japanica* (see Curran and Overstreet 2000); Santa María[TL]: *Mobula thurstoni*[TH] (see Curran and Overstreet 2000[OR]).
**Specimens in collections.** CNHE (3850); HWML (15261).

## Monogenoidea Bychowsky, 1937
## Capsalidae Baird, 1853

### *Benedeniella posterocolpa* (Hargis, 1955) Yamaguti, 1963

**Site of infection.** Skin.
**Locality.** CAMPECHE: Estuario Champotón: *Rhinoptera bonasus* (see Pulido-Flores and Monks 2005).
**Specimens in collections.** CNHE (4370).

### *Listrocephalos guberleti* (Caballero y Caballero & Bravo-Hollis, 1962) Bullard, Payne & Braswell, 2004

**Site of infection.** Gills, skin.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Urolophus halleri* (see Bullard et al. 2004); Isla San Esteban: *Urobatis concentricus*, *Urolophus maculatus*, *Urobatis* sp. (see Bullard et al. 2004). SONORA: Bahía de Guaymas[TL]: *Urolophus halleri*[TH] (see Caballero y Caballero and Bravo-Hollis 1962[OR]).
**Specimens in collections.** CNHE (34-5); USNPC (94826-8).
**Notes.** This species was described as *Entobdella guberleti* (Caballero y Caballero and Bravo-Hollis 1962) and transferred to *Listrocephalos* by Bullard et al. (2004).

### *Listrocephalos kearni* Bullard, Payne & Braswell, 2004

**Site of infection.** Skin.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Dasyatis brevis*[TH] (see Bullard et al. 2004[OR]). BAJA CALIFORNIA SUR: Santa Rosalía: *Dasyatis brevis* (see Bullard et al. 2004).
**Specimens in collections.** CNHE (5021-2); USNPC (94829-34).

### *Listrocephalos whittingtoni* Bullard, Payne & Braswell, 2004

**Site of infection.** Skin.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Dasyatis longa*[TH] (see Bullard et al. 2004[OR]). BAJA CALIFORNIA SUR: Bahía de La Paz: *Dasyatis longa* (see Bullard et al. 2004).
**Specimens in collections.** CNHE (5023-4); USNPC (94835-9).

## Hexabothriidae Price, 1942

### *Dasyonchocotyle dasyatis* (Yamaguti, 1968) Boeger & Kritsky, 1989

**Site of infection.** Gills.
**Locality.** SINALOA: Mazatlán: *Dasyatis longa* (see Escorcia-Ignacio et al. 2015).
**Specimens in collections.** CNHE (9361).

## Loimoidae Price, 1936

### *Loimos winteri* Caballero y Caballero & Bravo-Hollis, 1961

**Site of infection.** Gills.
**Locality.** SONORA: Bahía de Guaymas[TL]: *Carcharhinus brachyurus*[TH] (see Caballero y Caballero and Bravo-Hollis 1961[OR]).
**Specimens in collections.** CNHE (86-7).

### *Loimosina parawilsoni* Bravo-Hollis, 1970

**Site of infection.** Gills.
**Locality.** SINALOA: Mazatlán[TL]: *Sphyrna lewini*[TH] (see Bravo-Hollis 1970[OR]).
**Specimens in collections.** CNHE (153-4).

## Monocotylidae Taschenberg, 1879

### *Anoplocotyloides papillatus* (Doran, 1953) Young, 1967

**Site of infection.** Gills.
**Locality.** SINALOA: Mazatlán: *Rhinobatos glaucostigma* (see Bravo-Hollis 1969).
**Specimens in collection.** CNHE (178).
**Notes.** Based on the morphology of the posterior hooks of the haptor, Neifar et al. (2002) considered that this material is composed of 2 different monocotylideans.

***Calicotyle californiensis* Bullard & Overstreet, 2000**

**Site of infection.** Body cavity.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Mustelus californicus*[TH] (see Bullard and Overstreet 2000[OR]).
**Specimens in collections.** CNHE (3907).

***Calicotyle kroyeri* Diesing, 1850**

**Site of infection.** Cloaca, rectum.
**Locality.** CAMPECHE: Bancos de Campeche: *Anacanthobatis folirostris*, *Dipturus olseni* (see Chisholm et al. 1997).
**No specimens in collections.**

***Calicotyle urobati* Bullard & Overstreet, 2000**

**Site of infection.** Cloaca, rectum.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Urolophus halleri*[TH], *Urolophus maculatus* (see Bullard and Overstreet 2000[OR]); Bahía de San Francisquito: *Urolophus halleri* (see Bullard and Overstreet 2000); Puertecitos: *Urolophus maculatus* (see Bullard and Overstreet 2000). BAJA CALIFORNIA SUR: Santa Rosalía: *Urolophus halleri*, *Urolophus maculatus* (see Bullard and Overstreet 2000).
**Specimens in collections.** CNHE (3908-9); HWML (15365-6); USNPC (89777-8).

**Dasybatotreminae gen. sp.**

**Site of infection.** Gills.
**Locality.** GUERRERO: Acapulco: *Rhinoptera steindachneri* (see Carbajal-Violante 2012).
**Specimens in collections.** CNHE (8287-8).

***Decacotyle floridana* (Pratt, 1910) Chisholm & Whittington, 1998**

**Site of infection.** Gills.
**Locality.** CAMPECHE: Ciudad del Carmen: *Aetobatus narinari* (CNHE); Estuario Champotón: *Aetobatus narinari* (see Pulido-Flores and Monks 2005). QUINTANA ROO: Holbox: *Aetobatus narinari* (see Pulido-Flores and Monks 2005).
**Specimens in collections.** CNHE (327, 4368).

**Notes.** Specimens from Ciudad del Carmen were identified as *Heterocotyle aetobatis* Hargis, but this species was considered a synonym of *Decacotyle floridana* by Chisholm and Whittington (1998).

### *Denarycotyle gardneri* Pulido-Flores, Monks & Violante-González, 2015

**Site of infection.** Gills.
**Locality.** GUERRERO: Acapulco[TL]: *Rhinoptera steindachneri*[TH] (Pulido-Flores et al. 2015[OR]).
**Specimens in collections.** CNHE (9558-9); HWML (75364-7).

### *Dendromonocotyle cortesi* Bravo-Hollis, 1969

**Site of infection.** Skin.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL], Isla Rasa: "Mantarraya gris[TH]" (see Bravo-Hollis 1969[OR]).
**Specimens in collections.** CNHE (149-50).
**Notes.** Valid species according to Chisholm et al. (2004).

### *Dendromonocotyle octodiscus* Hargis, 1955

**Site of infection.** Skin.
**Locality.** GOLFO DE MEXICO (Mexico): *Dasyatis americana*, *Urobatis jamaicensis* (see Fehlauer-Ale and Littlewood 2011). QUINTANA ROO: Blanquizal, Holbox: *Dasyatis americana* (see Pulido-Flores and Monks 2005); El Paso de los Cedros (Cozumel), Ixmapuit (Isla Contoy), Xcalak: *Urobatis jamaicensis* (see Pulido-Flores and Monks 2005). YUCATÁN: Ría Lagartos: *Urobatis jamaicensis* (see Pulido-Flores and Monks 2005).
**Specimens in collections.** CNHE (4362-3, 4366-7); ECOPA (001); USNPC (90353).
**Notes.** Valid species accordig to Chisholm et al. (2004).

### *Euzetia lamothei* Pulido-Flores & Monks, 2008

**Site of infection.** Gills.
**Locality.** CAMPECHE: Ciudad del Carmen[TL]: *Rhinoptera bonasus*[TH] (see Pulido-Flores and Monks 2008[OR]). QUINTANA ROO: Isla Contoy: *Rhinoptera bonasus* (see Pulido-Flores and Monks 2008).
**Specimens in collections.** CNHE (6067-8); HWML (48817); CHE (P00056).

### *Heterocotyle* sp.

**Site of infection.** Gills.
**Locality.** GUERRERO: Acapulco: *Rhinoptera steindachneri* (see Carbajal-Violante 2012).
**No specimens in collections.**

### Monocotylidae gen. sp.

**Site of infection.** Gills.
**Locality.** BAJA CALIFORNIA SUR: Bahía Almejas: *Rhinoptera steindachneri* (see Gómez del Prado-Rosas and Euzet 1997).
**No specimens in collections.**
**Notes.** This material was recorded as *Quadritestis almehensis* n. gen., n. sp., but its description was not published, so that name is a *nomen nudum*.

### *Spinuris lophosoma* Doran, 1953

**Site of infection.** Gills.
**Locality.** BAJA CALIFORNIA SUR: Bahía Almejas: *Rhinobatos productus* (see Gómez del Prado-Rosas and Euzet 1999).
**No specimens in collections.**

### *Spinuris mexicana* Bravo-Hollis, 1969

**Site of infection.** Gills.
**Locality.** SINALOA: Mazatlán[TL]: *Rhinobatos glaucostigma*[TH] (see Bravo-Hollis 1969[OR]).
**Specimens in collections.** CNHE (151-2).

### *Spinuris zapterygis* Gómez del Prado-Rosas & Euzet, 1999

**Site of infection.** Gills.
**Locality.** BAJA CALIFORNIA SUR: Bahía Almejas[TL]: *Zapteryx exasperata*[TH] (see Gómez del Prado-Rosas and Euzet 1999 [OR]).
**Specimens in collections.** BM(NH) (1997.1.28.1); CNHE (2975-6); CPMHN-UABCS (54); MNHN (547HF Tk80); USNPC (87037).

**Cestoda Rudolphi, 1808**
**Anthocephaliidae Ruhnke, Caira & Cox, 2015**

### *Anthocephalum currani* Ruhnke & Seaman, 2009

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles, Puertecitos: *Dasyatis brevis* (see Ruhnke and Seaman 2009); Bahía de Los Ángeles: *Dasyatis dipterura* (see Ruhnke et al. 2015). BAJA CALIFORNIA SUR: Puntarena[TL]: *Dasyatis brevis*[TH] (see Ruhnke and Seaman 2009[OR]).
**Specimens in collections.** CNHE (6234-5); USNPC (100993).
**Notes.** This species was identified as *Anthocephalum* n. sp. 2. in Olson et al.'s (1999) phylogenetic analysis.

### *Anthocephalum duszynskii* Ruhnke, 1994

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco (Bahía Cholla)[TL]: *Urolophus halleri*[TH] (see Ruhnke 1994[OR]).
**Specimens in collections.** HWML (37095); USNPC (83437).

### *Anthocephalum lukei* Ruhnke & Seaman, 2009

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles, Puertecitos[TL]: *Dasyatis longa*[TH] (see Ruhnke and Seaman 2009[OR]). BAJA CALIFORNIA SUR: Bahía de La Paz: *Dasyatis longa* (see Ruhnke and Seaman 2009).
**Specimens in collections.** CNHE (6232-3); USNPC (100995).
**Notes.** This species was identified as *Anthocephalum* n. sp. 1. in the Olson et al.'s (1999) phylogenetic analysis.

### *Anthocephalum michaeli* Ruhnke & Seaman, 2009

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Dasyatis longa* (see Ruhnke and Seaman 2009); Isla San Esteban: *Urolophus maculatus* (see Caira et al. 2001). BAJA CALIFORNIA SUR: Bahía de La Paz, Loreto[TL], Puntarena, San José del Cabo: *Dasyatis longa*[TH] (see Ruhnke and Seaman 2009[OR]).
**Specimens in collections.** CNHE (6230-1); LRP (4232); USNPC (100998-9, 101000).

**Notes.** Specimens from Isla San Esteban, identified as *A. duszynskii* by Caira et al. (2001), were re-identified as *A. michaeli* by Ruhnke and Seaman (2009).

**Cathetocephalidae Dailey & Overstreet, 1973**

***Cathetocephalus resendezi* Caira, Mega & Ruhnke, 2005**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Carcharhinus leucas*[TH] (see Caira et al. 2005[OR]).
**Specimens in collections.** CNHE (5300).

***Cathetocephalus thatcheri* Dailey & Overstreet, 1973**

**Site of infection.** Spiral valve.
**Locality.** VERACRUZ: Playa Chachalacas: *Carcharhinus leucas* (see Méndez and Dorantes 2013).
**Specimens in collections.** CNHE (6860).

**Echeneibothriidae de Beauchamp, 1905**

***Echeneibothrium* sp.**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Santa Rosalía: *Myliobatis californicus* (see Caira et al. 1999), *Raja velezi* (see Healy et al. 2009).
**Specimens in collections.** LRP (4217).
**Notes.** This material was recorded as *Discobothrium* sp., but according to Euzet (1994), this genus is a synomym of *Echeneibothrium.*

**Echinobothriidae Perrier, 1897**

***Echinobothrium fautleyae* Tyler & Caira, 1999**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Rhinoptera steindachneri*[TH] (see Tyler and Caira 1999 [OR]); Puertecitos: *Myliobates californica*, *Rhinoptera steindachneri* (see Tyler and Caira 1999). BAJA CALIFORNIA SUR: Loreto: *Rhinoptera steindachneri* (see Tyler and Caira 1999); Puntarena: *Rhinoptera steindachneri*

(see Tyler and Caira 1999); Santa Rosalía: *Myliobates californica*, *Rhinoptera steindachneri* (see Tyler and Caira 1999).
**Specimens in collections.** CNHE (3340-1); HWML (33910-11); USNPC (88217-19).

### *Echinobothrium hoffmanorum* Tyler, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de San Francisquito: *Urolophus halleri*, *Urolophus maculatus* (see Tyler 2001); Isla San Esteban[TL]: *Urolophus maculatus*[TH] (see Tyler 2001[OR]). BAJA CALIFORNIA SUR: Puntarena: *Urobatis concentricus* (see Tyler 2001).
**Specimens in collections.** CNHE (3916-9).

### *Echinobothrium mexicanum* Tyler & Caira, 1999

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Myliobatis longirostris*[TH], *Myliobatis californica* (see Tyler and Caira 1999[OR]); Puertecitos: *Myliobatis californica* (see Tyler and Caira 1999). BAJA CALIFORNIA SUR: Loreto: *Myliobatis longirostris*, *Myliobatis californica* (see Tyler and Caira 1999); Santa Rosalía: *Myliobatis longirostris* (see Tyler and Caira 1999).
**Specimens in collections.** CNHE (3343-5); HWML (33912-14); USNPC (88220-21).

### *Echinobothrium rayallemangi* Tyler, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Rhinobatos leucorhynchus*[TH] (see Tyler 2001[OR]). BAJA CALIFORNIA SUR: Santa Rosalía: *Rhinobatos leucorhynchus* (see Tyler 2001).
**Specimens in collections.** CNHE (3920-22).

## Escherbothriidae Ruhnke, Caira & Cox, 2015

### *Escherbothrium molinae* Berman & Brooks, 1994

**Site of infection.** Spiral valve.
**Locality.** GUERRERO: Bahía de Acapulco: *Urotrygon* sp. (see Zaragoza-Tapia et al. 2013).
**Specimens in collections.** CNHE (8513-4); HWML (49850-3).

### Eutetrarhynchidae Guiart, 1927

#### *Dollfusiella litocephalus* (Heinz & Dailey, 1974) Beveridge, Neifar & Euzet, 2004

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de San Quintín: *Triakis semifasciata* (see Heinz and Dailey 1974).
**Specimens in collections.** USNPC (072672).
**Notes.** The original denomination of this species was *Eutetrarhynchus litocephalus*, but it was transferred to the genus *Dollfusiella* by Beveridge et al. (2004).

#### *Dollfusiella cortezensis* (Friggens & Duszynski, 2005) Schaeffner, 2014

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco[TL]: *Urolophus halleri*[TH] (see Friggens and Duszynski 2005[OR]).
**Specimens in collections.** USNPC (92215).
**Notes. Notes.** Published as *Eutetrarhynchus* sp. in Friggens and Brown (2005). The original denomination of this species was *Eutetrarhynchus cortezensis*, but it was transferred to the genus *Dollfusiella* by Schaeffner (2014).

#### *Fellicocestus mobulae* Campbell & Beveridge, 2006

**Site of infection.** Gall bladder.
**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz: *Mobula* sp. (see Campbell and Beveridge 2006a); Puntarena[TL]: *Mobula japanica*[TH] (see Campbell and Beveridge 2006a[OR]).
**Specimens in collections.** CNHE (5452); USNPC (97899, 9700).

#### Eutetrarhynchidae gen. sp.

**Site of infection.** Spiral valve.
**Locality.** VERACRUZ: Playa de Chachalacas: *Carcharhinus leucas* (see Mendez and Dorantes 2013).
**Specimens in collections.** CNHE (6169).

#### *Hemionchos maior* Campbell & Beveridge, 2006

**Site of infection.** Spiral valve.

**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz[TL]: *Mobula japanica*[TH] (see Campbell and Beveridge 2006a[OR]).
**Specimens in collections.** CNHE (5466-7).

### *Hemionchos mobulae* Campbell & Beveridge, 2006

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz[TL], Puntarena: *Mobula japanica*[TH] (see Campbell and Beveridge 2006a[OR]): Loreto, Santa Rosalía: *Mobula munkiana* (see Campbell and Beveridge 2006a).
**Specimens in collections.** CNHE (5465-6); LRP (3961); USNPC (97908-9).

### *Hemionchos striatus* Campbell & Beveridge, 2006

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz[TL]: *Mobula japanica*, *Mobula thurstoni*[TH] (see Campbell and Beveridge 2006a[OR]); Loreto: *Mobula thurstoni*, *Myliobatis californica* (see Campbell and Beveridge 2006a).
**Specimens in collections.** CNHE (5460); LRP (3948); USNPC (97904-6).

### *Mecistobothrium myliobati* Heinz & Dailey, 1974

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco: *Urolophus halleri* (see Friggens and Brown 2005).
**No specimens in collections.**

### *Mobulocestus lepidoscolex* Campbell & Beveridge, 2006

**Site of infection.** Nephridial system.
**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz[TL]: *Mobula thurstoni*[TH] (see Campbell and Beveridge 2006a[OR]).
**Specimens in collections.** CNHE (5458); USNPC (97902).

### *Mobulocestus mollis* Campbell & Beveridge, 2006

**Site of infection.** Cloaca.
**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz[TL]: *Mobula thurstoni*[TH] (see Campbell and Beveridge 2006a[OR]).
**Specimens in collections.** CNHE (5456).

***Mobulocestus nephriditis* Campbell & Beveridge, 2006**

**Site of infection.** Nephridial system.
**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz[TL]: *Mobula thurstoni*[TH] (see Campbell and Beveridge 2006a[OR]).
**Specimens in collections.** CNHE (5454); USNPC (97901).

***Oncomegas paulinae* Toth, Campbell & Schmidt, 1992**

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco[TL]: *Urolophus halleri*[TH] (see Toth et al. 1992[OR]).
**Specimens in collections.** BMNH (1991.10.30.1-3); USNPC (082186).

***Parachristianella dimegacantha* Krause, 1959**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Bahía de la Paz: *Dasyatis longa* (see Campbell and Beveridge 2007); Loreto: *Urotrygon simulatrix* (see Campbell and Beveridge 2007); San José del Cabo: *Sphyrna zygaena* (see Campbell and Beveridge 2007).
**Specimens in collections.** USNPC (97925-7).

***Parachristianella parva* Campbell & Beveridge, 2007**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Urolophus maculatus*[TH] (see Campbell and Beveridge 2007[OR]).
**Specimens in collections.** CNHE (5472).

***Parachristianella trygoni* Dollfus, 1946**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Dasyatis brevis*[TH] (see Campbell and Beveridge 2007[OR]). BAJA CALIFORNIA SUR: Loreto: *Mobula munkiana* (see Campbell and Beveridge 2007).
**Specimens in collections.** USNPC (97923-4).

### *Prochristianella minima* Hainz & Daily, 1974

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco: *Urolophus halleri* (see Friggens and Brown 2005).
**Specimens in collections.** USNPC (92211, 92216).

### *Prochristianella multidum* Friggens & Duzynski, 2005

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco[TL]: *Urolophus halleri*[TH] (see Friggens and Duszynski 2005[OR]).
**Specimens in collections.** USNPC (92218-9).

### *Pseudochristianella elegantissima* Campbell & Beveridge, 2006

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos: *Dasyatis brevis* (see Campbell and Beveridge 2006b). BAJA CALIFORNIA SUR: Bahía de la Paz[TL]: *Dasyatis brevis*[TH] (see Campbell and Beveridge 2006b[OR]); San José del Cabo: *Dasyatis longa* (see Campbell and Beveridge 2006b)
**Specimens in collections.** CNHE (5468); USNPC (97915-6).

### *Pseudochristianella nudiscula* Campbell & Beveridge, 2006

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Myliobatis longirostris*, *Rhinobatos productus* (see Campbell and Beveridge 2006b). BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Zapteryx exasperata*, *Rhinobatos productus*[TH] (see Campbell and Beveridge 2006b[OR]); San José del Cabo: *Dasyatis longa* (see Campbell and Beveridge 2006b).
**Specimens in collections.** CNHE (5470); USNPC (97917, 97921-2).

## Lacistorhynchidae Guiart, 1927

### *Callitetrarhynchus gracilis* (Rudolphi, 1819)

**Site of infection.** Spiral valve.
**Locality.** VERACRUZ: Playa Chachalacas: *Carcharhinus leucas* (see Méndez and Dorantes 2013).
**Specimens in collections.** CNHE (6867).

***Floriceps caballeroi* Cruz-Reyes, 1977**

**Site of infection.** Spiral valve.
**Locality.** SONORA: Laguna de Agiabampo[TL]: *Negaprion brevirostris*[TH] (see Cruz-Reyes 1977[OR]).
**Specimens in collections.** CNHE (479-80).
**Notes.** According to Palm (2004), this material is a synonym of *F. saccatus*. However, the poor condition of the type material re-examined during the present study precludes any conclusion about the taxonomic status of this species.

***Floriceps saccatus* Cuvier, 1817**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: NA: *Notorhynchus cepedianus* (see Heinz and Dailey 1974).
**No specimens in collections.**

**Litobothriidae Dailey, 1969**

***Litobothrium aenigmaticum* Caira, Jensen, Waeschenbach & Littlewood, 2014**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Santa María[TL], Santa Rosalía: *Alopias pelagicus*[TH] (see Caira et al. 2014a[OR]).
**Specimens in collections.** CNHE (8941-4).

***Litobothrium amplifica* (Kurochkin & Slankis, 1973) Euzet, 1994**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Alopias pelagicus* (see Olson and Caira 2001). BAJA CALIFORNIA SUR: Santa Rosalía: *Alopias pelagicus* (see Olson and Caira 2001). OAXACA: Golfo de Tehuantepec[TL]: *Alopias superciliosus*[TH] (see Kurochkin and Slankis 1973[OR]).
**Specimens in collections.** BMNH (2000.3.7.8.10); CNHE (4051); TINRO (72020).
**Notes.** This species was described as a member of the genus *Renyxa*, but Euzet (1994) considered *Renyxa* as a synonym of *Litobothrium*.

***Litobothrium daileyi* Kurochkin & Slankis, 1973**

**Site of infection.** Spiral valve.

**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Alopias pelagicus* (see Olson and Caira 2001). BAJA CALIFORNIA SUR: Santa Rosalía: *Alopias pelagicus* (see Olson and Caira 2001). OAXACA: Golfo de Tehuantepec[TL]: *Alopias superciliosus*[TH] (see Kurochkin and Slankis 1973[OR]).
**Specimens in collections.** CNHE (4050); TINRO (72012).

### *Litobothrium janovyi* Olson & Caira, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Alopias superciliosus*[TH] (see Olson and Caira 2001[OR]).
**Specimens in collections.** CNHE (4052-3).

### *Litobothrium nickoli* Olson & Caira, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Alopias pelagicus*[TH] (see Olson and Caira 2001[OR]). BAJA CALIFORNIA SUR: Santa Rosalía: *Alopias pelagicus* (see Caira et al. 2014a).
**Specimens in collections.** CNHE (4054-55); LRP (8321).

## Lecanicephalidea *incertae sedis* (Family)

### *Aberrapex senticosus* Jensen, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Myliobatis californica*[TH] (see Jensen 2001[OR]).
**Specimens in collections.** CNHE (4188); HWML (16374); USNPC (91208).
**Notes.** This species appears as *Discobothrium* n. sp. in Caira et al. (1999) and Caira et al. (2001).

### *Healyum harenamica* Jensen, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Punta Arena[TL]: *Mobula japanica*[TH] (see Jensen 2001[OR]).
**Specimens in collections.** CNHE (4186); HWML (16376); USNPC (91212).

***Healyum pulvis* Jensen, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Punta Arena[TL]: *Mobula japanica*[TH] (see Jensen 2001[OR]).
**Specimens in collections.** CNHE (4184); HWML (16377); USNPC (91213).
**Notes.** This taxon appears as New genus 3 n. sp., in the phylogenetic analysis done by Caira et al. (2001).

***Paraberrapex manifestus* Jensen, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Squatina californica*[TH] (see Jensen 2001[OR]).
**Specimens in collections.** CNHE (4179); HWML (16375); USNPC (91209).
**Notes.** *Paraberrapex manifestus* was referred to as New genus 2 n. sp. in the phylogenetic analysis done by Caira et al. (2001).

***Quadcuspibothrium francisi* Jensen, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Punta Arena[TL]: *Mobula japanica*[TH] (see Jensen 2001[OR]).
**Specimens in collections.** CNHE (4182); HWML (16378); USNPC (91214).
**Notes.** This species was referred to as New genus 4 n. sp. in the phylogenetic analysis done by Caira et al. (2001).

**Tetragonocephalidae Yamaguti, 1959**

***Tylocephalum* sp.**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Rhinoptera steindachneri* (see Caira et al. 1999). GUERRERO: Bahía de Acapulco: *Rhinoptera steindachneri* (see Carbajal-Violante 2012).
**Specimens in collections.** CNHE (8295-8296).

**Onchoproteocephalidea *incertae sedis* (Family)**

***Acanthobothrium bajaensis* Appy & Dailey, 1973**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de San Quintín[TL]: *Heterodontus francisci*[TH] (see Appy and Dailey 1973[OR]).
**Specimens in collections.** USNPC (72567-8).

***Acanthobothrium bullardi* Goshroy & Caira, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL], Puertecitos: *Dasyatis brevis*[TH] (see Goshroy and Caira 2001[OR]). BAJA CALIFORNIA SUR: Santa Rosalía: *Dasyatis brevis* (see Goshroy and Caira 2001).
**Specimens in collections.** CNHE (4045-6); LRP (2060–2062); USNPC (90466-8).

***Acanthobothrium cleofanus* Monks, Brooks & Pérez-Ponce de León, 1996**

**Site of infection.** Spiral valve.
**Locality.** JALISCO: Bahía de Chamela[TL]: *Dasyatis longa*[TH] (see Monks et al. 1996[OR]).
**Specimens in collections.** CNHE (2670-1); HWML; MNHG INV.

***Acanthobothrium dasi* Goshroy & Caira, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos[TL]: *Dasyatis brevis*[TH] (see Goshroy and Caira 2001[OR]).
**Specimens in collections.** CNHE (4043-4); HWML (15549-51); LRP (2051-4); USNPC (90463-5).

***Acanthobothrium dollyae* Caira & Burge, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL], Isla San Esteban: *Diplobatis ommata*[TH] (see Caira and Burge 2001[OR]). BAJA CALIFORNIA SUR: Punta Arena: *Diplobatis ommata* (see Caira and Burge 2001).
**Specimens in collections.** CNHE (4169-70); LRP (2097-2101); USNPC (90837-9).

***Acanthobothrium maryanskii*** **Caira & Burge, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Loreto[TL], Punta Arena: *Diplobatis ommata*[TH] (see Caira and Burge 2001[OR]).
**Specimens in collections.** CNHE (4171-2); LRP (2012-3); USNPC (90840-1).

***Acanthobothrium olseni*** **Dailey & Mudry, 1968**

**Site of infection.** Spiral valve.
**Locality.** Sonora: Puerto Peñasco: *Urolophus halleri* (see Friggens and Brown 2005).
**No specimens in collections.**

***Acanthobothrium parviuncinatum*** **Young, 1954**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos: *Urolophus halleri* (see Caira et al. 1999). SONORA: Puerto Peñasco: *Urolphus halleri* (see Friggens and Brown 2005).
**Specimens in collections.** CNHE (4171-2); LRP (2012-3); USNPC (90840-1).

***Acanthobothrium puertecitense*** **Caira & Zahner, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos: *Heterodontus francisci* (see Caira and Zahner 2001).
**Specimens in collections.** CNHE (4175-6); LRP (2105-6); USNPC (90843).
**Notes.** Caira et al. (2001) recorded this species as *Acanthobothrium* n. sp. 1.

***Acanthobothrium rajivi*** **Goshroy & Caira, 2001**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos[TL]: *Dasyatis brevis*[TH] (see Goshroy and Caira 2001[OR]).
**Specimens in collections.** CNHE (4043-4); HWML (15552); LRP (2055-6); USNPC (90461).

### *Acanthobothrium royi* Caira & Burge, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Loreto, Punta Arena[TL]: *Diplobatis ommata*[TH] (see Caira and Burge 2001[OR]).
**Specimens in collections.** CNHE (4173-4); LRP (2014); USNPC (90842).

### *Acanthobothrium santarosaliense* Caira & Zahner, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Heterodontus francisci*[TH] (see Caira and Zahner 2001[OR]).
**Specimens in collections.** CNHE (4177-78); LRP (2107); USNPC (90844).

### *Acanthobothrium soberoni* Goshroy & Caira, 2001

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles, Puertecitos[TL]: *Dasyatis brevis*[TH] (see Goshroy and Caira 2001[OR]). BAJA CALIFORNIA SUR: Loreto: *Dasyatis brevis* (see Goshroy and Caira 2001).
**Specimens in collections.** CNHE (4040-1); HWML (15548); LRP (2057-9); USNPC (90462).

### *Acanthobothrium* sp.

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Urolophus halleri* (see Caira et al. 2001), Puertecitos: *Heterodontus francisci* (see Caira et al. 2001). BAJA CALIFORNIA SUR: Santa Rosalía: *Urolophus maculatus* (see Caira et al. 2001). NA: NA: *Dasyatis longa* (see Healy et al. 2009). SONORA: Puerto Peñasco: *Urolophus halleri* (see Friggens and Brown 2005).
**No specimens in collections.**

**No specimens in collections**

### *Acanthobothroides* sp.

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Dasyatis brevis* (see Goshroy and Caira 2001). BAJA CALIFORNIA SUR: San José del Cabo: *Dasyatis brevis* (see Goshroy and Caira 2001).

**Specimens in collections.** CNHE (4048); USNPC (90439).
**Notes.** This material probably represents a new species as it differs from both *A. thorsoni* and *A. pacificus* (see Goshroy and Caira 2001).

### *Onchobothrium* sp.

**Site of infection.** Intestine.
**Locality.** BAJA CALIFORNIA: Ensenada: *Urolophus halleri* (HWML).
**Specimens in collections.** HWML (31324).

### *Phoreibothrium* sp.

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Carcharhinus leucas* (see Caira et al. 2001). NA: Golfo de México (Mexico): *Sphyrna mokarran* (see Caira et al. 2001). VERACRUZ: Playa Chachalacas: *Carcharhinus leucas* (see Mendez and Dorantes 2013).
**Specimens in collections.** CNHE (6866).

### *Platybothrium angelbahiense* Healy, 2003

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles[TL]: *Carcharhinus leucas*[TH] (see Healy 2003[OR]).
**Specimens in collections.** CNHE (4727-9); LRP (3213-3215); USNPC (92236).

### *Platybothrium auriculatum* Yamaguti, 1952

**Site of infection.** Intestine, spiral valve, stomach.
**Locality.** BAJA CALIFORNIA SUR: Bahía de La Paz, San Isidro: *Prionace glauca* (see Healy 2003); Las Barrancas, Punta Abreojos, Punta Belcher: *Prionace glauca* (see Méndez 2005).
**Specimens in collections.** CNHE (4730).

### *Platybothrium* sp.

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Carcharhinus leucas* (see Caira et al. 1999).

**Specimens in collections.** CSMNH.

**Notes.** Caira et al. (1999) identified this material as *Platybothrium cervinum*, but according to Healy (2003) these specimens represent an undescribed species.

### *Platybothrium tantulum* Healy, 2003

**Site of infection.** Spiral valve.

**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Sphyrna lewini*, *Sphyrna cygaena* (see Healy 2003). BAJA CALIFORNIA SUR: San José del Cabo: *Sphyrna lewini* (see Healy 2003).

**Specimens in collections.** CNHE (4731-3).

### *Prosobothrium armigerum* Cohn, 1902

**Site of infection.** Intestine, stomach.

**Locality.** BAJA CALIFORNIA SUR: Punta Abreojos, Punta Belcher: *Prionace glauca* (see Méndez 2005).

**No specimens in collections.**

**No specimens in collections**

## Otobothriidae Dollfus, 1942

### *Otobothrium* sp.

**Site of infection.** Spiral valve.

**Locality.** BAJA CALIFORNIA SUR: San José del Cabo: *Sphyrna zygaena* (see Schaeffner and Beveridge 2013). VERACRUZ: Playa Chachalacas: *Carcharhinus leucas* (see Méndez and Dorantes 2005).

**Specimens in collections.** CNHE (6863-3).

## Phyllobothriidae Braun, 1900

### *Orygmatobothrium* sp.

**Site of infection.** Spiral valve.

**Locality.** BAJA CALIFORNIA: Puertecitos: *Mustelus henlei* (see Caira et al. 1999).

**No specimens in collections.**

***Paraorygmatobothrium* sp.**

**Site of infection.** Spiral valve.
**Locality.** VERACRUZ: Playa Chachalacas: *Carcharhinus leucas* (see Méndez and Dorantes 2013).
**Specimens in collections.** CNHE (6864-5).
**Notes.** This material was recorded as *Paraorygmatobothrium* sp. 1 and sp. 2.

***Phyllobothrium hallericola* Church & Schmidt, 1990**

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco[TL]: *Urolophus halleri*[TH] (see Church and Schmidt 1990[OR]).
**Specimens in collections.** USNPC (81051-2).
**Notes.** The accession number published by Church and Schmidt (1990) is wrong.

***Phyllobothrium* sp.**

**Site of infection.** Intestine, spiral valve, stomach.
**Locality.** BAJA CALIFORNIA SUR: Las Barrancas, Punta Abreojos, Punta Belcher: *Prionace glauca* (see Méndez 2005). GUERRERO: Bahía de Acapulco: *Rhinoptera steindachneri* (see Carbajal-Violante 2012). SONORA: Puerto Peñasco: *Urolophus halleri* (see Friggens and Brown 2005, Church and Schmidt 1990).
**Specimens in collections.** CNHE (8291-2); USNPC (81053).
**Notes.** The accession number published by Church and Schmidt (1990) is wrong.

**Pterobothriidae Pintner, 1931**

***Pterobothrioides carvajali* Campbell & Beveridge, 1997**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos[TL]: *Dasyatis longa*[TH] (see Campbell and Beveridge 1997[OR]).
**Specimens in collections.** CNHE (3142); USNPC (85472).

**Rhinebothriidea *incertae sedis* (Family)**

***Serendip danbrooksi* Monks, Zaragoza-Tapia, Pulido-Flores & Violante-González, 2015**

**Site of infection.** Spiral valve.
**Locality.** GUERRERO: Bahía de Acapulco$^{TL}$: *Rhinoptera steindachneri*$^{TH}$ (Monks et al. 2015$^{OR}$). SINALOA: Mazatlán: *Rhinoptera steindachneri* (Monks et al. 2015).
**Specimens in collections.** CNHE (8293-4; 9725-7); HWML (75091-104); MNGH-PLAT (90513-5).
**Notes.** This species appears as *Serendip* sp. in Carbajal-Violante (2012). According to Ruhnke et al. (2015) the genus *Serendip* is clearly a candidate for membership in the Rhinebothriidea; a molecular analysis will be necessary to assign it to family level.

**Rhinebothriidae Euzet, 1953**

***Glyphobothrium zwerneri* Williams & Campbell, 1977**

**Site of infection.** Spiral valve.
**Locality.** CAMPECHE: Ciudad del Carmen: *Rhinoptera bonasus* (see Pulido-Flores and Monks 2014).
**Specimens in collections.** CNHE (8838).
**Notes.** The inclusion of this cestode species in Rhinebothriidae follows Appeltans et al. (2012).

***Rhinebothrium chollaensis* Friggens & Duszynski, 2005**

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco (Bahía Cholla)$^{TL}$: *Urolophus halleri*$^{TH}$ (see Friggens and Duszynski 2005$^{OR}$).
**Specimens in collections.** USNPC (92213-4).
**Notes.** Published as *Rhinebothrium* sp. in Friggens and Brown (2005).

***Rhinebothrium gravidum* Friggens & Duszynski, 2005**

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco$^{TL}$: *Urolophus halleri*$^{TH}$ (see Friggens and Duszynski 2005$^{OR}$).
**Specimens in collections.** USNPC (92212).
**Notes.** Published as *Rhinebothrium* sp. in Friggens and Brown (2005).

***Rhinebothrium maccallumi* Linton, 1924**

**Site of infection.** Spiral valve.
**Locality.** NA: NA: *Dasyatis americana* (see Healy et al. 2009).
**No specimens in collections.**
**Notes.** Species identification requires verification according to Healy et al. (2009).

***Rhinebothrium* sp.**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos: *Dasyatis brevis* (see Healy et al. 2009). BAJA CALIFORNIA SUR: Loreto: *Urolophus maculatus* (see Caira et al. 1999); San José del Cabo: *Dasyatis longa* (see Healy et al. 2009).
**Specimens in collections.** LRP (3893, 3896).
**Notes.** The records of Healy et al. (2009) of *D. brevis* and *D. longa* were made as *Rhinebothrium* sp.5 and *Rhinebothrium* sp.6, respectively.

***Rhinebothrium urobatidium* (Young, 1955) Appy & Dailey, 1977**

**Site of infection.** Spiral valve.
**Locality.** SONORA: Puerto Peñasco: *Urolophus halleri* (see Friggens and Brown 2005).
**Specimens in collections.** USNPC (92202-5).

***Scalithrium* sp.**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: San José del Cabo: *Dasyatis longa* (see Healy et al. 2009).
**Specimens in collections.** LRP (3895).
**Notes.** This record appears as *Scalithrium* n. sp. in Healy et al. (2009).

**Rhinoptericolidae Carvajal & Campbell, 1975**

***Rhinoptericola megacantha* Carvajal & Campbell, 1975**

**Site of infection.** Spiral valve.
**Locality.** GUERRERO: Bahía de Acapulco: *Rhinoptera steindachneri* (see Carbajal-Violante 2012).
**Specimens in collections.** CNHE (8297-8).

***Rhinoptericola* sp.**

**Site of infection.** Stomach.
**Locality.** GUERRERO: Bahía de Acapulco: *Rhinoptera steindachneri* (see Carbajal-Violante 2012).
**Specimens in collections.** CNHE (8299-8300).

**Tentaculariidae Poche, 1926**

***Nybelinia anthicosum* Heinz & Dailey, 1974**

**Site of infection.** Spiral valve, stomach.
**Locality.** BAJA CALIFORNIA: Playa María: *Heterodontus francisci* (see Heinz and Dailey 1974). SONORA: Bahía de San Carlos: *Heterodontus francisci* (see Heinz and Dailey 1974).
**Specimens in collections.** USNPC (72675).

***Nybelinia* sp.**

**Site of infection.** Stomach.
**Locality.** BAJA CALIFORNIA SUR: Las Barrancas: *Prionace glauca* (see Méndez 2005).
**No specimens in collections.**

**"Tetraphyllidea" *incertae sedis* (Family)**

***Anthobothrium* sp.**

**Site of infection.** Intestine, stomach.
**Locality.** BAJA CALIFORNIA SUR: Punta Abreojos, Punta Belcher, Las Barrancas: *Prionace glauca* (see Méndez 2005).
**No specimens in collections.**

***Caulobothrium opisthorchis* Riser, 1955**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Myliobatis californicus* (see Healy et al. 2009).
**Specimens in collections.** LRP (3910).

***Caulobothrium* sp.**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Myliobatis californicus* (see Healy et al. 2009).
**Specimens in collections.** LRP (3012).
**Notes.** According to Healy et al. (2009) this material represents an undescribed species; recorded as *Caulobothrium* n. sp. 1 in Caira et al. (1999) and Caira et al. (2001).

***Duplicibothrium cairae* Ruhnke, Curran & Holbert, 2000**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de los Ángeles, Puertecitos: *Rhinoptera steindachneri* (see Ruhnke et al. 2000). BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Rhinoptera steindachneri*[TH] (see Ruhnke et al. 2000[OR]).
**Specimens in collections.** CNHE (3846-7); HWML (15275,15276); USNPC (89726-7).
**Notes.** This species was reported as *Duplicibothrium* n. sp. 1 in the phylogenetic analysis done by Olson et al. (1999).

***Duplicibothrium paulum* Ruhnke, Curran & Holbert, 2000**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de los Ángeles, Puertecitos[TL]: *Rhinoptera steindachneri*[TH] (see Ruhnke et al. 2000[OR]).
**Specimens in collections.** CNHE (3848); HWML (15277, 15278); USNPC (89728-9).
**Notes.** This species was reported as *Duplicibothrium* n. sp. 2 in the phylogenetic analysis done by Olson et al. (1999).

***Pedibothrium brevispine* Linton, 1909**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA SUR: San José del Cabo: *Ginglymostoma cirratum* (see Caira and Euzet 2001).
**Specimens in collections.** CNHE (4191).

***Pedibothrium manteri* Caira, 1992**

**Site of infection.** Spiral valve.

**Locality.** BAJA CALIFORNIA SUR: San José del Cabo: *Ginglymostoma cirratum* (see Caira and Euzet 2001).
**Specimens in collections.** CNHE (4190).

### *Symcallio evani* (Caira, 1985) Bernot, Caira & Pickering, 2015

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía de Los Ángeles: *Mustelus lunulatus* (see Nasin et al 1997); Puertecitos[TL]: *Mustelus lunulatus*[TH] (see Caira 1985[OR]). BAJA CALIFORNIA SUR: San José del Cabo, Santa Rosalía: *Mustelus lunulatus* (see Nasin et al. 1997).
**Specimens in collections.** CNHE (3071); USNPC (78600, 87127).
**Notes.** This species was described as *Calliobothrium evani* and recently transferred to *Symcallio* by Bernot et al. (2015). Type host of *S. evani* was determined by Caira (1985) as "unidentified shark of the family Carcharhinidae"; its accurate specific identity was established by Nasin et al. (1997).

### *Symcallio riseri* (Nasin, Caira & Euzet, 1997) Bernot, Caira & Pickering, 2015

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Puertecitos: *Mustelus henlei* (see Caira 1985, Nasin et al. 1997). BAJA CALIFORNIA SUR: Santa Rosalía[TL]: *Mustelus henlei*[TH] (see Nasin et al. 1997[OR]).
**Specimens in collections.** CNHE (3068-70); HWML (22537).
**Notes.** Specimens from Puertecitos were identified by Caira (1985) as *Calliobothrium lintoni* Euzet, 1954 and re-identified by Nasin et al. (1997) as *C. riseri*. This species was recently transferred to *Symcallio* by Bernot et al. (2015).

## Nematoda Cobb, 1932
## Gnathostomatidae Lane, 1923

### *Echinocephalus jenzeni* Hoberg, Brooks, Molina & Erbe, 1998

**Site of infection.** Spiral valve.
**Locality.** CHIAPAS: Laguna Mar Muerto: *Himantura pacifica* (see Hoberg et al. 1998).
**Specimens in collections.** CNHE (2642).

***Echinocephalus pseudouncinatus* Millemann, 1951**

**Site of infection.** Spiral valve.
**Locality.** BAJA CALIFORNIA: Bahía San Francisquito, Isla Ángel de la Guarda (Puerto Refugio): *Heterodontus francisci* (see Milleman 1963); Bahía San Felipe: *Myliobatis californica* (see Milleman 1963). BAJA CALIFORNIA SUR: Laguna Ojo de Liebre (Guerrero Negro), Punta Abreojos: *Heterodontus francisci* (see Gómez del Prado-Rosas 1984).
**Specimens in collections.** CNHE (2289); USNPC (57448, 57450-2).

**Annelida Lamarck 1809**
**Hirudinea Lamarck, 1818**
**Piscicolidae Johnston, 1865**

**Piscicolidae gen. sp.**

**Site of infection.** Skin.
**Locality.** BAJA CALIFORNIA: Isla San Esteban: *Zapteryx exasperata* (CNHE).
**Specimens in collections.** CNHE (4027).
**Notes.** This specimen was deposited at the CNHE by Steve Curran as the holotype of the new species *Pseudaustrobdella cairae*, but their description was not published.

***Stibarobdella macrothela* (Schmarda, 1861) Llewellyn, 1966**

**Site of infection.** Skin.
**Locality.** TAMAULIPAS: Matamoros: *Ginglymostoma cirratum* (CNHE). VERACRUZ: Isla de Sacrificios: Elasmobranquio no determinado (CNHE).
**Specimens in collections.** CNHE (1640, 5572).

**Host-parasite list**

**Selachii**
ALOPIIDAE
***Alopias pelagicus* Nakamura**
*Litobothrium aenigmaticum* (C)
*Litobothrium amplifica* (C)
*Litobothrium daileyi* (C)
*Litobothrium nickoli* (C)
*Paronatrema vaginicola* (T)

***Alopias superciliosus*** Lowe
*Litobothrium amplifica* (C)
*Litobothrium daileyi* (C)
*Litobothrium janovyi* (C)
CARCHARHINIDAE
***Carcharhinus brachyurus*** Günther
*Liomos winteri* (M)
***Carcharhinus leucas*** (Müller & Henle)
*Cathetocephalus resendezi* (C)
*Cathetocephalus thatcheri* (C)
*Callitetrarhynchus gracilis* (C)
Eutetrarhynchidae gen. sp. (C)
*Phoreiobothrium* sp. (C)
*Platybothrium angelbahiense* (C)
*Platybothrium* sp. (C)
*Otobothrium* sp. (C)
*Paraorygmatobothrium* sp. (C)
***Carcharhinus limbatus*** Müller & Henle
*Staphylorchis pacifica* (T)
***Negaprion brevirostris*** (Poey)
*Floriceps caballeroi* (C)
***Prionace glauca*** (Linnaeus)
*Anthobothrium* sp. (C)
*Helicometrina nimia* (T)
*Nybelinia* sp. (C)
*Paronatrema vaginicola* (T)
*Phyllobothrium* sp. (C)
*Platybothrium auriculatum* (C)
*Prosobothrium armigerum* (C)
GINGLYMOSTOMATIDAE
***Ginglymostoma cirratum*** (Bonnaterre)
*Pedibothrium brevispine* (C)
*Pedibothrium manteri* (C)
*Stibarobdella macrothela* (H)
HETERODONTIDAE
***Heterodontus francisci*** (Girard)
*Acanthobothrium bajaensis* (C)
*Acanthobothrium puertecitense* (C)
*Acanthobothrium santarosaliense* (C)
*Acanthobothrium* sp. (C)
*Echinocephalus pseudouncinatus* (N)
*Nybelinia anthicosum* (C)

*Otodistomum veliporum* (T)
***Heterodontus mexicanus*** Taylor & Castro-Aguirre
*Otodistomum veliporum* (T)
HEXANCHIDAE
***Notorynchus cepedianus*** (Péron)
*Floriceps saccatus* (C)
SPHYRNIDAE
***Sphyrna lewini*** (Griffith & Smith)
*Loimosina parawilsoni* (M)
*Platybothrium tantulum* (C)
***Sphyrna mokarran*** (Rüppell)
*Phoreiobothrium* sp. (C)
***Sphyrna zygaena* (L.)**
*Otobothrium* sp. (C)
*Parachristianella dimegacantha* (C)
*Platybothrium tantulum* (C)
SQUATINIDAE
***Squatina californica*** Ayres
*Otodistomum veliporum* (T)
*Paraberrapex manifestus* (C)
TRIAKIDAE
***Galeorhinus galeus*** (Linnaeus)
*Staphylorchis pacifica* (T)
***Mustelus californicus*** Gill
*Calicotyle californiensis* (M)
*Ptychogonimus megastomum* (T)
***Mustelus henlei*** (Gill)
*Calliobothrium evani* (C)
*Calliobothrium riseri* (C)
*Orygmatobothrium* sp. (C)
*Otodistomum veliporum* (T)
***Mustelus lunulatus*** Jordan & Gilbert
*Calliobothrium evani* (C)
*Probolitrema richiardii* (T)
*Ptychogonimus megastomum* (T)
***Triakis semifasciata* Girard**
*Dollfusiella litocephalus* (C)
**Undetermined shark**
*Staphylorchis pacifica* (T)
Undetermined Elasmobranchii
*Staphylorchis pacifica* (T)
*Stibarobdella macrothela* (H)

**Batoidea**

ANACANTHOBATIDAE

***Anacanthobatis folirostris*** Bigelow & Schroeder

*Calicotyle kroyeri* (M)

DASYATIDAE

***Dasyatis americana*** Hildebrand & Schroeder

*Dendromonocotyle octodiscus* (M)

*Rhinebothrium maccallumi* (C)

***Dasyatis brevis*** (Garman)

*Acanthobothrium bullardi* (C)

*Acanthobothrium dasi* (C)

*Acanthobothrium rajivi* (C)

*Acanthobothrium soberoni* (C)

*Acanthobothroides* sp. (C)

*Anaporrhutum euzeti* (T)

*Anthocephalum currani* (C)

*Listrocephalos kearni* (M)

*Parachristianella trygonis* (C)

*Probolitrema richiardii* (T)

*Prosorhynchus truncatus* (T)

*Pseudochristianella elegantissima* (C)

*Rhinebothrium* sp. (C)

***Dasyatis dipterura* (Jordan & Gilbert)**

*Anthocephalum currani* (C)

***Dasyatis longa*** (Garman)

*Acanthobothrium cleofanus* (C)

*Acanthobothrium* sp. (C)

*Anaporrhutum euzeti* (T)

*Anthocephalum lukei* (C)

*Anthocephalum michaeli* (C)

*Dasyonchocotyle dasyatis* (M)

*Listrocephalos wittingtoni* (M)

*Parachristianella dimegacantha* (C)

*Probolitrema richiardii* (T)

*Pseudochristianella elegantissima* (C)

*Pseudochristianella nudiscula* (C)

*Pterobothrioides carvajali* (C)

*Rhinebothrium* sp. (C)

*Scalithrium* sp. (C)

***Himantura pacifica*** Beebe & Tee-Van

*Echinocephalus jenzeni* (N)

MYLIOBATIDAE

***Aetobatus narinari*** Euphrasen
- *Decacotyle floridana* (M)

***Mobula japanica*** (Müller & Henle)
- *Fellicocestus mobulae* (C)
- *Healyum harenamica* (C)
- *Healyum pulvis* (C)
- *Hemionchos maior* (C)
- *Hemionchos mobulae* (C)
- *Hemionchos striatus* (C)
- *Quadcuspibothrium francisi* (C)
- *Syncoelium vermilionense* (T)

***Mobula munkiana*** Notarbartolo-di-Sciara
- *Anaporrhutum euzeti* (T)
- *Hemionchos mobulae* (C)
- *Parachristianella trygonis* (C)

***Mobula* sp.**
- *Fellicocestus mobulae* (C)

***Mobula thurstoni*** (Lloyd)
- *Hemionchos striatus* (C)
- *Mobulocestus lepidoscolex* (C)
- *Mobulocestus mollis* (C)
- *Mobulocestus nephriditis* (C)
- *Nagmia cisloi* (T)
- *Syncoelium vermilionense* (T)

***Myliobatis californica*** Gill
- *Aberrapex senticosus* (C)
- *Anaporrhutum euzeti* (T)
- *Caulobothrium opisthorchis* (C)
- *Caulobothrium* sp. (C)
- *Echeneibothrium* sp. (C)
- *Echinobothrium fautleyae* (C)
- *Echinobothrium mexicanum* (C)
- *Echinocephalus pseudouncinatus* (N)
- *Hemionchos striatus* (C)
- *Probolitrema richiardii* (T)

***Myliobatis longirostris*** Applegate & Fitch
- *Anaporrhutum euzeti* (T)
- *Echinobothrium mexicanum* (C)
- *Probolitrema richiardii* (T)
- *Pseudochristianella nudiscula* (C)

***Rhinoptera bonasus*** (Mitchill)
*Benedeniella posterocolpa* (M)
*Euzetia lamothei* (M)
*Glyphobothrium zwerneri* (C)
***Rhinoptera steindachneri*** Evermann & Jenkins
Dasybatotreminae gen. sp. (M)
*Denarycotyle gardneri* (M)
*Duplicibothrium cairae* (C)
*Duplicibothrium paulum* (C)
*Echinobothrium fautleyae* (C)
*Heterocotyle* sp. (M)
Monocotylidae gen. sp. (M)
*Phyllobothrium* sp. (C)
*Rhinoptericola megacantha* (C)
*Rhinoptericola* sp. (C)
*Serendip danbrooksi* (C)
*Tylocephalum* sp. (C)
NARCINIDAE
***Diplobatis ommata*** (Jordan & Gilbert)
*Acanthobothrium dollyae* (C)
*Acanthobothrium maryanskii* (C)
*Acanthobothrium royi* (C)
*Anaporrhutum euzeti* (T)
***Narcine entemedor*** Jordan & Starks
*Anaporrhutum euzeti* (T)
*Nagmia rodmani* (T)
RAJIDAE
***Dipturus olseni*** Bigelow & Schroeder
*Calicotyle kroyeri* (M)
***Raja velezi*** Chirichigno
*Echeneibothrium* sp. (C)
RHINOBATIDAE
***Rhinobatos glaucostigma*** Jordan & Gilbert
*Anoplocotyloides papillatus* (M)
*Spinuris mexicana* (M)
***Rhinobatos lentiginosus*** Garman
*Paramonilicaecum* gen. sp. (T)
***Rhinobatos leucorhynchus*** Günther
*Echinobothrium rayallemangi* (C)
*Probolitrema richiardii* (T)
***Rhinobatos productus*** Ayres
*Anaporrhutum euzeti* (T)
*Pseudochristianella nudiscula* (C)

*Spinuris lophosoma* (M)
***Zapteryx exasperata*** (Jordan & Gilbert)
*Anaporrhutum euzeti* (T)
Piscicolidae gen. sp. (H)
*Pseudochristianella nudiscula* (C)
*Spinuris zapterygis* (M)
UROTRYGONIDAE
***Urobatis concentricus*** Osburn & Nichols
*Listrocephalos guberleti* (M)
*Echinobothrium hoffmanorum* (C)
***Urobatis jamaicensis*** Cuvier
*Dendromonocotyle octodiscus* (M)
***Urobatis*** sp.
*Listrocephalos guberleti* (M)
*Probolitrema richiardii* (T)
***Urolophus halleri*** Cooper
*Acanthobothrium olseni* (C)
*Acanthobothrium parviuncinatum* (C)
*Acanthobothrium* sp. (C)
*Anaporrhutum euzeti* (T)
*Anthocephalum duszynskii* (C)
*Calicotyle urobati* (M)
*Dollfusiella cotezensis* (C)
*Echinobothrium hoffmanorum* (C)
*Listrocephalos guberleti* (M)
*Mecistobothrium myliobati* (C)
*Onchobothrium* sp. (C)
*Oncomegas paulinae* (C)
*Phyllobothrium hallericola* (C)
*Phyllobothrium* sp. (C)
*Prochristianella minima* (C)
*Prochristianella multidum* (C)
*Rhinebothrium chollaensis* (C)
*Rhinebothrium gravidum* (C)
*Rhinebothrium urobatidium* (C)
***Urolophus maculatus*** (Garman)
*Acanthobothrium* sp. (C)
*Anaporrhutum euzeti* (T)
*Anthocephalum michaeli* (C)
*Calicotyle urobati* (M)
*Echinobothrium hoffmanorum* (C)
*Listrocephalos guberleti* (M)
*Parachristianella parva* (C)

*Pleorchis magniporus* (T)
*Probolitrema richiardii* (T)
*Rhinebothrium* sp. (C)
***Urotrygon simulatrix* Miyake & Eachran**
*Parachristianella dimegacantha* (C)
***Urotrygon* sp.**
*Escherbothrium molinae* (C)
"Mantarraya gris"
*Dendromonocotyle cortesi* (M)

## Discussion

To date, 132 helminth taxa (110 named species and 22 taxa not assigned to species) have been reported as parasites of elasmobranch species in Mexico. Seventy-three of these taxa are represented by holotypes from Mexican waters. All of these taxa have been collected in the adult stage (132). Thus, the richness of helminth species parasitizing elasmobranchs distributed in Mexican waters represents 7.2% of the worldwide species richness for this group (see Caira et al. 2012).

The 132 taxa parasitize 48 taxa of elasmobranchs (4 of them not assigned to species), within 15 families; Myliobatidae (8 species) and Urotrygonidae (6) being the families with the highest number of species sampled, due to the fact that100% and 60% respectively of the species of these two families recorded in Mexico, have been studied for helminths. In addition, helminths have been reported from 9 of the 12 orders of elasmobranchs in Mexican waters; no records are available for Squaliformes, Orectolobiformes (Selachii) or Rhinobatiformes (Batoidea). Fifteen of the 23 families of sharks have not been reported as hosts of helminths, as well as half of the families of rays. From the 204 known species of elasmobranchs recorded in Mexican waters, only 26% of them have been studied for helminths; thus, only 18.3% and 32.6% of shark and ray species, respectively, have been examined for, and found to host, helminths (Table 1). This value is similar to that found by Randhawa and Poulin (2010), who established that only 317 species (26%) of this globally distributed group of hosts have been examined for intestinal parasites (specifically tapeworms).

The species of elasmobranchs with the higher parasite species richness are *Urolophus halleri* (with 19 taxa), *Dasyatis longa* (14) and *Dasyatis brevis* (13). However, 8 shark and 9 ray species have been recorded only once as hosting helminths. In total, Batoidea is parasitized by 109 taxa of helminths and Selachii by 52, of which 56% and 61%, respectively, are cestode species. The mean value of species harbored by a host is 2.8 for sharks and 3.8 for rays; these traits are in accordance with the findings reported by Randhawa and Poulin (2010), who noted that, on average, batoids harbor significantly more species of tapeworms than sharks.

*Anaporrhutum euzeti* and *Probolitrema richardii* (Trematoda) are the species with the broadest host spectrum; the former species is associated with 11 species of rays

**Table 2.** Sampled localities for elasmobranchs as hosts of helminths in Mexico.

| **Baja California** | N | W |
|---|---|---|
| 1) Bahía de Los Ángeles† | 28°54'31" | 113°29'47" |
| 2) Bahía San Felipe | 28°42'00" | 112°35'00" |
| 3) Bahía San Francisquito | 29°45'05" | 114°18'36" |
| 4) Bahía de San Quintín | 30°27'09" | 115°56'54" |
| 5) Ensenada | 31°51'14" | 116°37'45" |
| 6) Isla Ángel de la Guarda (Puerto Refugio) | 29°26'26" | 113°34'25" |
| 7) Isla Rasa | 28°49'01" | 112°58'25" |
| 8) Isla San Esteban | 28°41'39" | 112°31'30" |
| 9) Playa María | 31°52'18" | 116°39'31" |
| 10) Puertecitos | 30°20'59" | 114°38'27" |
| **Baja California Sur** | | |
| 11) Bahía Almejas | 24°31'00" | 111°39'50" |
| 12) Bahía de La Paz | 24°14'30" | 110°28'08" |
| 13) Bahía de Santa Inés | 27°02'55" | 111°58'37" |
| 14) Bahía Magdalena | 25°20'00" | 112°05'00" |
| 15) Boca de Álamo | 23°53'51" | 109°48'12" |
| 16) El Comitán | 24°08'00" | 110°25'00" |
| 17) Isla Magdalena | 24°15'00" | 111°30'00" |
| 18) Laguna Guerrero Negro (Ojo de Liebre) | 27°51'21" | 114°14'28" |
| 19) Las Barrancas | 26°00'30" | 112°12'17" |
| 20) Loreto | 26°01'00" | 111°19'50" |
| 21) Punta Abreojos | 26°27'45" | 112°43'48" |
| 22) Punta Arena | 24°04'00" | 109°50'00" |
| 23) Punta Belcher | 25°20'00" | 112°05'00" |
| 24) San Isidro | 23°53'00" | 109°47'00" |
| 25) San José del Cabo | 23°04'49" | 109°40'49" |
| 26) Santa María | 27°24'53" | 112°18'17" |
| 27) Santa Rosalía | 27°20'04" | 112°15'35" |
| **Campeche** | | |
| 28) Bancos de Campeche | 19°53'03" | 90°31'43" |
| 29) Ciudad del Carmen | 19°51'33" | 90°31'35" |
| 30) Estuario Champotón | 19°20'56" | 90°41'18" |
| **Chiapas** | | |
| 31) Laguna Mar Muerto (El Paredón) | 15°59'00" | 94°00'00" |
| **Colima** | | |
| 32) Manzanillo | 19°04'54" | 104°19'31" |
| **Guerrero** | | |
| 33) Bahía de Acapulco | 16°49'21" | 99°52'55" |
| **Jalisco** | | |
| 34) Bahía de Chamela | 19°33'15" | 105°06'45" |
| 35) Puerto Vallarta | 20°35'48" | 105°15'00" |
| **Nayarit** | | |
| 36) Punta Mita | 20°46'38" | 105°30'46" |
| 37) San Blás | 21°32'00" | 105°17'22" |

| | | |
|---|---|---|
| **Oaxaca** | | |
| 38) Golfo de Tehuantepec | 15°45'26" | 96°07'21" |
| **Quintana Roo** | | |
| 39) Blanquizal | 18°16'03" | 87°54'12" |
| 40) Holbox | 21°34'05" | 86°14'32" |
| 41) Isla Contoy | 20° 48'25" | 86° 47'15" |
| 42) Isla Cozumel | 20°24'10" | 86°55'40" |
| 43) Xcalak | 18°19'32" | 87°44'49" |
| **Sinaloa** | | |
| 44) Bahía Santa María | 25°02'38" | 108°05'14" |
| 45) Mazatlán | 23°14'03" | 106°27'40" |
| **Sonora** | | |
| 46) Bahía de Guaymas | 27°54'45" | 110°52'41" |
| 47) Bahía de San Carlos | 27°56'36" | 111°03'44" |
| 48) Laguna de Agiabampo | 26°21'54" | 109°13'05" |
| 49) Puerto Peñasco | 31°18'33" | 113°31'30" |
| 50) Puerto Peñasco (Bahía Cholla) | 31°20'00" | 113°36'45" |
| **Tamaulipas** | | |
| 51) Matamoros | 25°52'00" | 97°30'00" |
| **Veracruz** | | |
| 52) Isla de Sacrificios | 19°10'32" | 96°05'50" |
| 53) Playa Chachalacas | 19°22'00" | 96°22'00" |
| **Yucatán** | | |
| 54) Ría Lagartos | 21°36'08" | 88°08'51" |

[†]These numbers correspond with the position of localities on Figure 1.

from three localities, and the latter has been found in 7 species of rays and one shark from three localities. Higher host specificity was shown by cestodes; 62 of the 76 nominal species of this group were specialists for a particular species of elasmobranch. These results are in accordance with Caira and Jensen (2014) who noted that the majority of tapeworm species are extremely host-specific, exhibiting species-specific (i.e., oioxenous) associations with their hosts. However, more conclusive results can be obtained only by increasing the sampling of this group of vertebrates on both coasts of Mexico, through comprehensive studies in which complete necropsies of elasmobranchs are conducted, avoiding partial analysis of a particular site of infection or organ system, which is a common trait of the research in this field according to Caira et al. (2012).

*Dendromonocotyle octodiscus* had the widest geographic distribution, being found in 7 localities; this monogenean is followed by *Echinobothrium fautleyae*, *Anthocephalum michaeli* and *Staphylorchis pacifica*, which are distributed in 5 localities each, as well as *Symcallio evani* and *Calicotyle urobati*, recorded in 4 locations each. *Acanthobothrium* is the most specious genus, as it is represented by 14 species parasitizing 6 species of elasmobranchs.

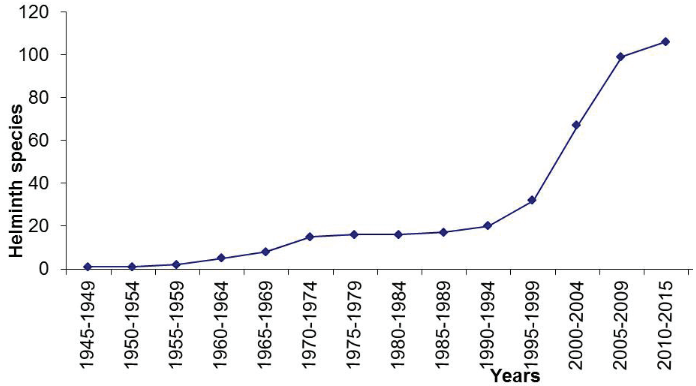


**Figure 2.** Cumulative curve of helminth species recorded in Mexico over 70 years of research.

Along with the increase in the number of species described worldwide, the number of helminth species parasitizing sharks and rays recorded in Mexico has increased in the past 2 decades, after slow growth from 1945, when Caballero y Caballero (1945) described the first species associated with this group of hosts (*Staphylorchis pacifica*). Between 1945 and 1994, only 20 species were reported in this group of hosts in the country. From 1995 to the present, this number increased more than 400%, rising to 107 species (Figure 2). According to Caira and Jensen (2014), approximately 250 species were erected over the past 2 decades; 36 of them were collected from elasmobranchs inhabiting Mexican waters.

The helminthological record of elasmobranchs distributed in Mexico is asymmetrically constituted in terms of the helminth groups represented, the hosts studied and the geographical distribution of the sampling sites. Cestodes are the most widely represented group, with 76 named species and 18 not assigned to species. The main reasons that explain this asymmetry can be summarized in two points: 1) the great diversity of cestodes associated with elasmobranchs, as nine of the 19 orders included in this Class infect this group of hosts, and eight are even exclusive parasites of them (Caira et al. 2014b); cestodes are by far the most diverse group of metazoan parasites of elasmobranchs, representing more than half of the described species for this host group (Caira et al. 2012); 2) the particular interest of a research team lead by Janine N. Caira from the University of Connecticut to inventory the fauna of tapeworm parasites of sharks and rays distributed in the Gulf of California, through the project "A systematic survey of the metazoan parasites of elasmobranchs from the Sea of Cortez" between 1993 and 1994. As a result of this project, more than 45 species of cestode were recorded in this area of Mexico, 36 of which were described as new species. The most intensively studied host group is Batoidea, with 32% of the species in the country harboring at least 1 species of helminth; on the other hand, only 18% of the species of sharks caught in Mexico have been reported as hosting helminths. To determine if this could represent a bias in sampling and not a reflection of the real richness of the helminths in the different groups of hosts, more sampling efforts are necessary. Likewise, the specific richness of helminths is concentrated in two states, i.e., Baja California Sur (69 helminth species reported to date) and Baja California (54), both located in the Gulf of California, up to now, the most intensively sampled region of Mexico.

In addition to the 132 helminth taxa recorded so far in elasmobranchs inhabiting Mexican waters, another 8 taxa of helminths were found in this group of hosts: 2 acanthocephalans, *Corynosoma* sp. (Méndez 2005) and *Gorgorhynchoides bullocki* (Monks et al. 2009), and 6 nematodes, *Anisakis simplex*, *Hedruris* sp. (Méndez 2005), *Anisakis* sp., *Hysterothylacium* sp., *Terranova* sp. (Pérez-Ponce de León et al. 1999), and *Mexiconema cichlasomae* (Moravec et al. 1998). However, their presence in elasmobranchs is considered accidental; elasmobranchs can be infected through 2 ways: 1) ingestion of prey acting as intermediate hosts for almost completely developed larvae and 2) ingestion of definitive hosts constituting an accidental, probably postcyclic transmission (Moravec et al. 1998; Anderson 2000; Weaver and Smales 2014).

In spite of the great amount of information generated in the last 20 years, new records of the helminth fauna of Elasmobranchii in Mexico remain scarce and fragmentary. To date, 81.7% of sharks and 67.4% of rays distributed in Mexican waters lack helminthological studies. Completing the helminthological inventory for this group of vertebrates is a major challenge, as recent estimates establish the number of species to be described associated with these hosts at approximately 3600, considering only the tapeworms (Randhawa and Poulin 2010). Only through efforts such as the one conducted by Caira and collaborators in the Gulf of California will a comprehensive understanding of these host-parasite associations be achieved.

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# Additions to the Limoniidae and Pediciidae fauna of Morocco, with an updated checklist (Diptera, Tipuloidea)

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### Abstract

Eighteen species of Limoniidae and two species of Pediciidae are recorded for the first time in Morocco, of which 15 species are new to North Africa. An updated checklist of Moroccan short-palped craneflies (Limoniidae and Pediciidae) is appended, containing 73 species in 25 genera.

### Keywords

Morocco, new records, North Africa, Short-palped crane flies, updated checklist

## Introduction

Limoniidae and Pediciidae belong to the Tipuloidea together with Cylindrotomidae and Tipulidae. The Limoniidae comprise more than 741 species already known in the Westpalaeartic region, from which only 102 species are known to occur in Morocco (49), Algeria (32), Tunisia (9), Libye (1) and Egypt (11). The Pediciidae are the second smallest family of the Tipuloidea, with 77 recognized taxa in the Westpalaeartic of which only 4 were recorded from North Africa, in Morocco (Driauach et al. 2013, Oosterbroek 2015).

Adults of Limoniidae and Pediciidae are associated with moist environments. They are usually collected from damp vegetation along the borders of various water bodies and from damp forests (Starý et al. 2005, De Jong et al. 2008). Their immature stages are usually found in a wide range of aquatic and semi-aquatic habitats (Reusch and Oosterbroek 1997).

Limoniidae and Pediciidae are poorly known in Morocco. They only received sporadic faunistic records made by European entomologists (Pierre 1922a, b, Pierre 1924, Séguy 1941a, b, Vaillant 1956, Starý 1971, Starý 1974, Savchenko et al. 1992, Eiroa 2000, Pârvu et al. 2006, Starý 2006, Starý and Oosterbroek 2008, Starý 2009, Starý 2011).

Only a single attempt was made to summarize the knowledge of the Moroccan fauna in the first checklist of the short-palped crane flies of Morocco (Driauach et al. 2013) and very recent research also revealed a new species, *Baeoura staryi* from Morocco including an identification key of the genus *Baeoura* in the West Palaearctic species (Driauach and Belqat 2015).

Prior to the present study, there were 53 species recorded from Morocco (Oosterbroek 2015). New findings increase the number of Moroccan Limoniidae and Pediciidae to 73. Altogether 44 species of Limoniidae and Pediciidae are collected and recorded in this work. Of these, 18 species of Limoniidae and two species of Pediciidae are recorded for the first time in Morocco of which 15 species are new to North Africa.

## Material and methods

The material presented in this work come from field campaigns carried out by the authors between 2011 and 2015 in 77 sites distributed over a mountainous area (Rif, High Atlas, Middle Atlas, Beni Snassen) and arid area (Sahara) of Morocco. A total of 564 specimens of short-palped crane flies (515 specimens of Limoniidae and 49 specimens of Pediciidae) were identified. Adults were mostly collected by sweeping vegetation with entomological hand net whereas other specimens were reared in the laboratory from the substratum taken from several zones of water bodies margins and kept under the laboratory conditions. Fresh material was stored in 70% alcohol. For identification, the male terminalia, if necessary, were prepared by boiling in a solution of 10% KOH and preserved in glycerine. All the specimens were collected by the authors and are deposited in the Diptera Collection of the Faculty of Sciences, University Abdelmalek Essaâdi, Tétouan, Morocco. The Distribution as it is given for individual species is based on Oosterbroek (2015).

The checklist of Moroccan short-palped craneflies is updated. New records are marked with an asterisk (*) and bold set text. A brief description of the material examined is given and the distribution of the identified species is provided, together with notes on the breeding sites (Table 1).

**Table 1.** Sampling sites (in alphabetic order) for the adults of Limoniidae and Pediciidae in Moroccan regions, 2011–2015, with localities, geographical coordinates and altitudes.

| Site | Locality | Province | Altitude (m) | Geographical coordinates | Type of habitat |
|---|---|---|---|---|---|
| **Rif Mts** | | | | | |
| Tributary of Oued El Fondak | Aïn Lahcen, Khandek Melouka | Tétouan | 291 | 35°33.544'N, 05°34.995'W | River of the middle course |
| Tributary of Oued El Kebir | Route de Beni Yedder | Chefchaouen | 145 | 35°25.864'N, 05°27.610'W | River of the middle course |
| Tributary of Oued Hachef | Dar Chaoui | Tanger-Assila | 47 | 35°31.458'N, 05°43.771'W | Small stream |
| Tributary of Oued Majjou | Nord Majjou | Chefchaouen | 786 | 35°06.730'N, 05°11.309'W | Small stream |
| Tributary of Oued Ouara | Khandek Romana, Jbel Khizana | Chefchaouen | 1003 | 35°02.579N 05°12.872'W | River of the middle course |
| Tributary of Oued Taïda | Sidi Brahim Ben Arrif | Larache | 860 | 35°20.398'N, 05°32.471W | River of the higher course |
| Tributary of Oued Zarka | Zarka | Chefchaouen | 128 | 35°31.220'N, 05°20.461'W | Stream |
| Aïn Afersiw | Mezine | Chefchaouen | 746 | 35°05.945'N, 05°20.439'W | Spring |
| Aïn Bab Tariouente | Ânasar | Chefchaouen | 1440 | 35°01.110'N, 05°00.586'W | Spring and stream |
| Aïn Boughaba | Jbel Bou Bessoui, Cedar Forest | Chefchaouen | 1526 | 34°58.779'N, 04°46.366'W | Spring |
| Aïn Chouk | Aïn Chouk | Larache | 5 | 35°09.803'N, 06°06.473'W | Marsh |
| Aïn El Ma Bared | Bouzthate | Chefchaouen | 1246 | 35°00.333'N, 05°12.105'W | Spring and irrigation canal |
| Aïn El Maounzil | Parc National de Talassemtane | Chefchaouen | 1106 | 35°04.577'N, 05°10.406'W | Spring and brook |
| Aïn El Malâab | Parc National de Talassemtane | Chefchaouen | 1278 | 35°05.509'N, 05°09.443'W | Spring and brook |
| Aïn Quanquben | Jbel Bou Bessoui | Chefchaouen | 1600 | 34°57.750'N, 04°40.783'W | Spring |
| Aïn Mâaze | Jbel Bouhachem | Larache | 1294 | 35°14.381'N, 05°26.316'W | Spring |
| Aïn Ras El Ma | Majjou | Chefchaouen | 856 | 35°06.873'N, 5°11.388'W | Spring |
| Aïn Sidi Brahim Ben Arrif (Site 1) | Route moulay Abdessalam | Larache | 882 | 35°20.415'N, 05°32.535'W | Small stream |
| Aïn Sidi Brahim Ben Arrif (Site 2) | Route moulay Abdessalam | Larache | 897 | 35°20.398'N, 05°32.712'W | Spring and brook |
| Aïn Takhninjoute | Bab Rouida, Parc National de Talassemtane | Chefchaouen | 1512 | 35°06.881'N, 05°08.270'W | Spring |
| Âounsar Aheramen | Majjou | Chefchaouen | 855 | 35°06.162'N, 05°10.739'W | Spring |
| Barrage Ajras | Aïn Lahcen | Tétouan | 49 | 35°33.833'N, 05°29.902'W | Barrage |
| Barrage Moulay Bouchta | Larbaa Beni Hassan | Tétouan | 364 | 35°15.864'N, 05°21.221'W | Barrage |
| Cascade Chrafate | Chrafate | Chefchaouen | 820 | 35°03.997'N, 05°06.434'W | Waterfall |
| Cascade Zarka | Zarka | Tétouan | 147 | 35°31.211'N, 05°20.477'W | Waterfall |
| Daya Aïn Jdioui | Aïn Jdioui | Tanger-Assilah | 5 | 35°34.074'N, 05°55.499'W | Pond |

| Site | Locality | Province | Altitude (m) | Geographical coordinates | Type of habitat |
|---|---|---|---|---|---|
| Daya Amsemlil | Jbel Bouhachem | Tétouan | 1059 | 35°15.596'N, 05°25.917'W | Bog |
| Daya El Ânassar | Bab Berred | Chefchaouen | 1183 | 35°00.788'N, 04°57.419'W | Bog |
| Daya Fifi | Fifi | Chefchaouen | 1202 | 35°1.367'N, 05°12.335'W | Bog |
| Daya Jbel Zemzem | Jbel Zemzem | Tétouan | 233 | 35°45.553'N, 05°22.340'W | Pond |
| Daya Mezine | Mezine | Chefchaouen | 778 | 35°06.104'N, 5°21.177'W | Pond |
| Daya Mghara | Dar Chaoui | Tétouan | 44 | 35°31.471'N, 05°42.963'W | Pond |
| Daya Mtahen | Jbel Bouhachem | Larache | 966 | 35°16.195'N, 05°26.158'W | Pond |
| Daya near Aïn Afersiw | Mezine | Chefchaouen | 716 | 35°06.069'N, 5°20.337'W | Pond |
| Daya Tazia | Route Moulay Abdessalam | Larache | 721 | 35°20.814'N, 05°33.139'W | Pond |
| Daya Rmali | El Malâab, Parc National de Talassemtane | Chefchaouen | 1276 | 35°05.563'N, 05°09.488'W | Pond |
| Daya Afrate | Tanaqoub | Chefchaouen | 600 | 35°05.634'N, 05°26.028'W | Bog |
| Lac Badriouen | Badriouen | Tanger | 34 | 35°42.083'N, 05°52.116'W | Lack |
| Maison forestière | Parc National de Talassemtane | Chefchaouen | 1674 | 35°08.076'N, 05°08.262'W | Spring and brook |
| Marj El Kheyl | Jbel Bouhachem | Tétouan | 989 | 35°15.985'N, 05°26.209'W | Marshland |
| Oued Aârate | Dardara | Chefchaouen | 269 | 35°07.381'N, 05°17.456'W | River of the middel course |
| Oued Aârkoub | Aârkoub | Chefchaouen | 140 | 35°15.943'N, 04°50.631'W | River of the middel course |
| Oued Abou Bnar | Douar Abou Bnar, Parc National de Talassemtane | Chefchaouen | 1254 | 35°10.977'N, 05°08.005'W | River of the higher course |
| Oued Amsa | Amsa | Tétouan | 13 | 35°31.593'N, 05°13.821'W | Downstream of the river |
| Oued El Kanar | Stehate | Chefchaouen | 50 | 35°17.233'N, 04°59.639'W | Downstream of the river |
| Oued Amsemlil (Site 1) | Jbel Bouhachem | Larache | 1059 | 35°15.614'N, 05°25.943'W | River of the higher course |
| Oued Amsemlil (Site 3) | Jbel Bouhachem | Larache | 1134 | 35°15.635'N, 05°26.134'W | River of the higher course |
| Oued at 15 Km from Fifi | Bouzthate | Chefchaouen | 1256 | 35°00.805'N, 05°12.365'W | River of the higher course |
| Oued Azila | Azila, Jbel Tidghine | Al Hoceïma | 1601 | 34°52.028'N, 04°32.609'W | River of the higher course |
| Oued El Kebir | Route Vers Beni Yedder | Tétouan | 89 | 35°27.351'N, 05°25.796'W | River of the middel course |
| Oued El Koub | Souk El Had | Chefchaouen | 124 | 35°01.298'N, 05°25.333'W | Waterfall |
| Oued Farda | Akchour | Chefchaouen | 394 | 35°14.147'N, 05°10.576'W | River of the higher course |
| Oued Jnane Niche | Jnane Niche | Chefchaouen | 36 | 35°17.019'N, 04°51.233'W | River of the middel course |
| Oued Loukous | Souk El Had | Chefchaouen | 148 | 35°01.317'N, 05°25.246'W | River of the middle course |
| Oued Kelâa | Akchour | Chefchaouen | 303 | 35°14.268'N, 05°10.452'W | River of the higher course |

| Site | Locality | Province | Altitude (m) | Geographical coordinates | Type of habitat |
|---|---|---|---|---|---|
| Oued Madissouka. | Madissouka, Parc National de Talassemtane | Chefchaouen | 1367 | 35°10.622'N, 05°08.400'W | River of the higher course |
| Oued Majjou (Hafa meqlouba) | Majjou | Chefchaouen | 825 | 35°06.175'N, 05°10.836'W | River of the higher course |
| Oued Majjou (Pont) | Majjou | Chefchaouen | 786 | 35°06.730'N, 05°11.309'W | River of the higher course |
| Oued Majjou (Zaouiet El habtiyne) | Zaouiet El Habtiyne | Chefchaouen | 800 | 35°06.784'N, 05°11.784'W | River of the higher course |
| Oued Mlila | Route de Ouazzane | Ouazzane | 172 | 35°02.086'N, 05°22.133'W | River of the middle course |
| Oued Ouara | Ikedjiouen | Chefchaouen | 680 | 35°3.987'N, 05°14.05'W | River of the middle course |
| Oued Ouringa | Jebha | Chefchaouen | 17 | 35°11.438'N, 04°41.150'W | Downstream of the river |
| Oued Sidi Yahya Aârab | Sidi Yahya Aârab | Chefchaouen | 62 | 35°17.545'N, 04°53.503'W | River of the middel course |
| Oued Taïda | Taïda | Larache | 505 | 35°22.250'N, 05°32.355'W | River of the middel course |
| Oued Tamerte | Belota | Chefchaouen | 114 | 34°57.607'N, 05°32.061'W | River of the middel course |
| Oued Tizekhte | Larbaa Beni Hassan | Tétouan | 728 | 35°18.600'N, 05°23.097'W | Stream of the middle course |
| Oued Tkaraâ | Jbel Bouhachem | Larache | 959 | 35°16.063'N, 05°25.829'W | River of the higher course |
| Oued Zarka | Kitane | Tétouan | 52 | 35°32.412'N, 05°20.393'W | River of the middel course |
| Oued Zendoula | 15 km North Ouazzane | Ouazzane | 108 | 34°55.707'N, 05°31.942'W | River of the middel course |
| Seguia Mtahen | Mtahen, Jbel Bouhachem | Larache | 983 | 35°16.226'N, 05°26.212'W | Small stream |
| **Beni Snassen** | | | | | |
| Grotte du Chameau | Zegzel, Beni Snassen | Berkan | 427 | 34°50.447'N, 02°21.532'W | Irrigation canal |
| Oued Tafoughalt | Tafoughalt, Beni Snassen, | Berkan | 751 | 34°48.941'N, 02°24.471'W | River of the Middle course |
| Oued Beni Ouachekradi | Beni Snassen | Berkan | 534 | 34°50.974'N, 02°16.217'W | River of the middle course |
| **Middel Atlas Mts** | | | | | |
| Barrage Allal El Fassi | Gîte Aït Ayoub | Sefrou | 537 | 33°55.446'N, 4°40.558'W | Dam |
| **High Atlas Mts** | | | | | |
| Oued Sidi Fares | Sidi Fares, Parc National de Toubkal | Marrakech | 1807 | 31°14.029'N, 07°52.813'W | River of the higher course |
| Assif Haouz | Imlil, Parc National De Toubkal | Marrakech | 1896 | 31°07.424'N, 7°55.083'W | Stream |
| **Sahara** | | | | | |
| Oued Zag | Zag | Assa-Zag | 400 | 28°01.344'N, 09°17.926'W | River of the middle course |

## Systematic part

### Limoniidae: Chioneinae

### *Cheilotrichia* (*Empeda*) *cinerascens* (Meigen, 1804)

**Material examined.** Oued Ouara, 1♂, 2♀♀, 23.XI.2012; Oued Amsa, 2♂♂, 13.XII.2013; Oued Majjou (Hafa meqlouba), 1♂, 10.V.2014; Grotte du Chameau, 1♂, 3♀♀, 24.XI.2014; Aïn Quanquben, 1♂, 28.IV.2015 (sweep net).

**Distribution.** Europe, Morocco, Georgia, Turkey, Cyprus, Israel, Iran. First record from Beni Snassen.

### *Cheilotrichia* (*Empeda*) *fuscohalterata* (Strobl, 1906)

**Material examined.** Daya Fifi, 1♂, 23.XI.2012; Tributary of Oued El Fondak, 2♂♂, 10.II.2013, 1♂, 10.IV.2013; Barrage Ajras, 2♂♂, 10.II.2013; Daya Mghara, 6♂♂, 14.II.2013; Oued Zarka, 1♂, 23.II.2015; Oued Tizekhte, 1♂, 2♀♀, 28.II.2015 (sweep net).

**Distribution.** Italy, Portugal, Spain, First records from Morocco and North Africa.

### *Cheilotrichia* (*Empeda*) *minima* (Strobl, 1898)

**Material examined.** Oued Zendoula, 2♂♂, 31.V.2013; Oued Jnane Niche, 1♂, 1♀, 25.IV.2015 (sweep net).

**Distribution.** Known from Europe (except for the north) and the south of the Palaearctic as far east as Kyrgyzstan. First records from Morocco and North Africa.

### *Ellipteroides* (*Ellipteroides*) *lateralis* (Macquart, 1835)

**Material examined.** Daya Afrate, 3♂♂, 18.IV.2015 (sweep net).

**Distribution.** Southern part of Europe, Morocco, Lebanon, Israel and Turkey. First record from the Rif.

### *Erioconopa diuturna* (Walker, 1848)

**Material examined.** Daya Jbel Zemzem, 1♂, 23.IV.2014; Oued Mezine, 4♂♂, 18.IV.2015; Aïn Bab Tariouente, 2♂♂, 2♀♀, 28.IV.2015 (sweep net).

**Distribution.** Europe, Morocco, Turkey. First record from the Rif.

### *Erioptera* (*Erioptera*) *fuscipennis* Meigen, 1818

**Material examined.** Aïn El Ma Bared, 2♂♂, 30.III.2012; Oued Amsemlil (Site 1), 1♂, 1♀, 27.IV.2012; Oued Majjou (Pont), 2♂♂, 15.V.2012; Oued Tkaraâ, 1♂, 30.V.2013; Daya near Aïn Afersiw, 3♂♂, 1♀, 11.VI.2013; Aïn Afersiw, 3♂♂, 1♀, 11.VI.2013; Oued Abou Bnar, 2♂♂, 18.V. 2014; Oued Majjou (Zaouiet El habtiyne), 9♂♂, 6♀♀, 12.VII.2014; Daya Amsemlil, 2♂♂, 2.XI.2014; Daya Aïn Jdioui, 12♂♂, 5♀♀, 28.III.2015; Daya Afrate, 2♂♂, 2♀♀, 18.IV.2015; Oued Mezine, 1♂, 18.IV.2015 (sweep net).

**Distribution.** Europe, Morocco, Algeria, Azerbaijan, Iran.

### *Erioptera* (*Erioptera*) *lutea lutea* Meigen, 1804

**Material examined.** Aïn Boughaba, 1♂, 24.V.2013 (sweep net).

**Distribution.** Europe, Transcaucasia, Turkey, Israel, ?Iran, Russia, Kazakhstan, Uzbekistan, Tajikistan, Kyrgyzstan. First records from Morocco and North Africa.

### *Gonomyia* (*Gonomyia*) *abscondita* Lackschewitz, 1935

**Material examined.** Maison forestière, 3♂♂, 21.IV.2015 (sweep net).

**Distribution.** Austria, Bulgaria, Czech Rep., Finland, Germany, Great Britain, Italy, Netherlands, Romania, Slovakia, Sweden, Ukraine. First records from Morocco and North Africa.

### *Gonomyia* (*Gonomyia*) *subtenella* (Meigen, 1818),

**Material examined.** Oued Beni Ouachekradi, 2♂♂, 30.XII.2014, 1♀, 8.I.2015 (reared); Oued Jnane Niche, 1♂, 25.IV.2015; Oued Sidi Yahya Aârab, 1♂, 3♀♀, 25.IV.2015 (sweep net).

**Distribution.** Czech Rep., Lithuania, Macedonia, Slovakia, Sweden, Morocco (High Atlas), Georgia, Azerbaijan, Iran. First records from Rif and Beni Snassen.

### *Gonomyia* (*Gonomyia*) *sicula* Lackschewitz, 1940

**Material examined.** Oued El Kebir, 1♂, 17.IV.2013; Daya Jbel Zemzem, 7♂♂, 23.IV.2014; Aïn Sidi Brahim Ben Arrif (Site 2), 1♂, 25.IV.2014; Daya Tazia, 1♂, 25.IV.2014; Aïn El Maounzil, 1♂, 21.IV.2015 (sweep net).

**Distribution.** Italy, Spain, Tunisia, Turkey. First records from Morocco.

### *Hoplolabis* (*Parilisia*) *sororcula* (Lackschewitz, 1940)

**Material examined.** Barrage Ajras, 3♂♂, 1♀, 10.II.2013; Oued Ouringa, 1♂, 19.IV.2013; Oued El Kanar, 1♂, 14.VI.2013; Oued Majjou (Hafa meqlouba), 1♂, 10.V.2014 (sweep net).

**Distribution.** France, Germany, Italy (incl. Sicily), Portugal, Romania, Slovakia, Spain, Switzerland, Ukraine, Morocco. First records from the Rif.

### *Idiocera* (*Idiocera*) *ampullifera* (Stary, 1979)

**Material examined.** Oued Zag, 1♂, 1♀, 22.V.2015 (sweep net).

**Distribution.** Egypt, Israel. First record from Morocco.

### *Idiocera* (*Idiocera*) *pulchripennis* (Loew, 1856)

**Material examined.** Oued Jnane Niche, 1♂, 2♀♀, 19.IV.2013, 3♂♂, 1♀, 14.VI.2013; Oued Aârkoub, 1♂, 19.IV.2013, 1♂, 16.III.2014; Aïn Jdioui, 4♂♂, 2♀♀, 28.III.2015 (sweep net).

**Distribution.** Southern part of Europe, Canary Islands, Morocco, Algeria, Egypt, Transcaucasia, Turkey, Cyprus, Israel, Iran, Central Asia. First record from the Rif.

### *Idiocera* (*Idiocera*) *sziladyi* (Lackschewitz, 1940)

**Material examined.** Oued Zarka, 1♂, 22.V.2014 (light trap).

**Distribution.** Europe (except for northern countries), Algeria, Egypt, Yemen. First record from Morocco.

### *Ilisia maculata* (Meigen, 1804)

**Material examined.** Oued Tafoughalt, 2♂♂, 25.XI.2014; Oued Majjou (Hafa meqlouba), 1♂, 27.IV.2015 (sweep net).

**Distribution.** Europe, Transcaucasia, Turkey , Iran. First records from Morocco and North Africa.

### *Molophilus* (*Molophilus*) *obscurus* (Meigen, 1818)

**Material examined.** Tributary of Oued Ouara, 1♂, 27.IV.2013, 5♂♂, 6.V.2014; Tributary of Oued Majjou, 2♂♂, 03.V.2013; Daya El Ânassar, 1♂, 24.V.2013; Daya Jbel Zemzem, 2♂♂, 23.IV.2014; Aïn El Ma Bared, 1♂, 1♀, 6.V.2014; Daya Rmali, 1♂, 17.V.2014; Oued Amsemlil (Site 1), 1♂, 2.XI.2014; Aïn Mâaze, 1♂, 1.XI.2014; Daya Afrate, 9♂♂, 18.IV.2015; Daya Mtahen, 1♂, 25.V.2015 (sweep net).

**Distribution.** Europe, Morocco (High Atlas), Transcaucasia, Turkey, Cyprus, Lebanon, Israel. First records from the Rif.

### ***Molophilus* (*Molophilus*) *propinquus propinquus* (Egger, 1863)**

**Material examined.** Oued Ouara, 1♂, 7.I.2013 (reared); Oued Farda, 3♂♂, 24.IV.2013; Tributary of Oued Taïda, 1♂, 25.IV.2014; Oued Majjou (Hafa meqlouba), 1♂, 27.IV.2015 (sweep net).

**Distribution.** Europe, Morocco (High Atlas), Georgia, Turkey. First records from the Rif.

### ***Molophilus* (*Molophilus*) *testaceus* Lackschewitz, 1940**

**Material examined.** Daya Amsemlil, 1♂, 23.V.2012, 1♂, 1♀, 30.V.2013; Daya Mtahen, 2♀♀, 23.V.2012; Marje El kheyl, 3♂♂, 5♀♀, 30.V.2013; Oued Tkaraâ, 1♂, 30.V.2013; Daya near Aïn Afersiw, 1♂, 11.VI.2013; Daya Mezine, 1♂, 11.VI.2013; Daya Tazia, 1♂, 25.IV.2014; Daya Rmali, 1♂, 17.V.2014 (sweep net). Daya Amsemlil, 1♂, 4♀♀, 9.VII.2013; Daya near Aïn Afersiw, 4♂♂, 1♀, 2.VII.2013 (reared).

**Distribution.** Only known from Portugal and Spain. First records from Morocco and North Africa.

### ***Symplecta* (*Symplecta*) *hybrida* (Meigen, 1804)**

**Material examined.** Barrage Ajras, 1♂, 10.II.2013; Barrage Allal El Fassi, 1♂, 15.IV.2014; Oued El Kanar, 1♀, 14.VI.2013, 1♂, 25.IV.2015; Oued Aârkoub, 1♂, 16.III.2014; Oued Jnane Niche, 1♀, 25.IV.2015 (sweep net).

**Distribution.** Nearctic (Canada, USA, Greenland); widespread in Palaearctic, including North Africa, Central Asia, Mongolia, as far east as North Korea and Japan; Oriental (India, Nepal, Pakistan). First records from the Rif and the Middle Atlas.

### ***Symplecta* (*Trimicra*) *pilipes* (Fabricius, 1787)**

**Material examined.** Tributary of Oued Hachef, 2♂♂, 2♀♀, 14.II.2013; Oued Aârkoub, 1♂, 19.IV.2013; Oued El Kanar, 2♂♂, 2♀♀, 14.VI.2013; Oued Mezine, 1♂, 18.IV.2015 (sweep net); Aïn Chouk, 1♂, 3.II.2015 (reared).

**Distribution.** The only cosmopolitan cranefly, widespread in all zoogeographical regions. First records from the Rif.

### *Tasiocera* (*Dasymolophilus*) *murina* (Meigen, 1818)

**Material examined.** Oued Farda, 1♂, 28.III.2013; Oued Amsemlil (Site 3), 4♂♂, 30.V.2013 (sweep net).

**Distribution.** Europe, Transcaucasia, Turkey. First records from Morocco and North Africa.

## Limoniidae: Limnophilinae

### *Austrolimnophila* (*Austrolimnophila*) *latistyla* Stary, 1977

**Material examined.** Oued Majjou (Hafa meqlouba), 1♂, 27.VI.2015 (sweep net).

**Distribution.** Croatia, France, Greece, Italy, Portugal, Spain. First record from Morocco and North Africa.

### *Dicranophragma* (*Brachylimnophila*) *nemorale* (Meigen, 1818)

**Material examined.** Aïn Sidi Brahim Ben Arrif (Site 1), 1♂, 25.IV.2014; Tributary Oued Ouara, 2♀♀, 27.IV.2013; Oued Majjou (Hafa meqlouba), 1♂, 10.V.2014 (sweep net).

**Distribution.** Widespread in the Palaearctic. First records from the Rif.

### *Euphylidorea crocotula* (Seguy, 1941)

**Material examined.** Aïn Bab Tariouente, 3♂♂, 28.IV.2015 (sweep net).

**Distribution.** Spain (Granada), Morocco (High Atlas). First record from the Rif.

### *Euphylidorea* (*Euphylidorea*) *dispar* (Meigen, 1818)

**Material examined.** Oued at 15 Km from Fifi, 1♀, 26.III.2014 (sweep net).

**Distribution.** Widespread in Europe, recorded from Russia. First records from Morocco and North Africa.

### *Hexatoma* (*Hexatoma*) *bicolor* (Meigen, 1818)

**Material examined.** Oued at 15 Km from Fifi, 1♂, 27.IV.2013, 1♀, 26.III.2014, 3♂♂, 6.V.2014; Tributary of Oued Ouara, 1♀, 27.IV.2013; Oued Madissouka, 1♂, 18.V.2014; Oued Majjou (Hafa meqlouba), 1♀, 10.V.2014; Aïn Quanquben, 1♂, 28.IV.2015; Oued Tkaraâ, 2♂♂, 7.V.2015; Oued Tamerte, 1♂, 1♀, 7.V.2015 (sweep net).

**Distribution.** Europe, Georgia, Turkey. First records from Morocco and North Africa.

### ***Pseudolimnophila*** **(*Pseudolimnophila*)** ***sepium*** **(Verrall, 1886)**

**Material examined.** Daya Tazia, 11♂♂, 8♀♀, 23.IV.2012; Aïn Sidi Brahim Ben Arrif (2), 2♂♂, 2♀♀, 17.IV.2013; Aïn Afersiw, 1♀, 11.VI.2013; Oued Majjou (Hafa meqlouba), 1♂, 10.V.2014 (sweep net). Daya Tazia, 1♂, 2♀♀, 14.V.2013; Oued Taïda, 1♂, 12.V.2014; Aïn Sidi Brahim Ben Arrif (Site 2), 2♂♂, 1♀, 26.V.2014 (reared).

**Distribution.** Europe, Morocco (Rif), Transcaucasia, Turkey, Turkmenistan, Kyrgyzstan, Afghanistan.

## Limoniidae: Limoniinae

### ***Dicranomyia*** **(*Dicranomyia*)** ***affinis*** **(Schummel, 1829)**

**Material examined.** Oued Amsemlil (Site 1), 1♂, 27.IV.2012; Daya Mtahen, 10♂♂, 1♀, 27.IV.2012; Oued Tkaraâ, 1♂, 27.IV.2012; Marj El Kheyl, 2♀♀, 30.V.2013; Aïn Sidi Brahim Ben Arrif (Site 2), 5♂♂, 1♀, 17.IV.2013, 3♂♂, 1♀, 25.IV.2014; Aïn Sidi Brahim Ben Arrif (Site 1), 10♂♂, 5♀♀, 25.IV.2014; Daya Tazia, 14♂♂, 4♀♀, 25.IV.2014; Aïn El Maounzil, 6♂♂, 4♀♀, 21.IV.2015; Aïn El Malâab, 1♂, 1♀, 21.IV.2015; Daya near Aïn Afersiw, 5♂♂, 3♀♀, 18.IV.2015; Daya Mtahen, 4♀♀, 7.V.2015; Aïn Bab Tariouente, 4♂♂, 28.IV.2015 (sweep net).

**Distribution.** Great Britain, Ireland, Poland, Morocco (High Atlas).

### ***Dicranomyia*** **(*Dicranomyia*)** ***chorea*** **(Meigen, 1818)**

**Material examined.** Oued Sidi Fares, 1♂, 7.V.2011; Oued Farda, 2♂♂, 2♀♀, 13.II.2013, 7♂♂, 4♀♀, 23.IV.2013; Oued Kelâa, 3♂♂, 13.II.2013; Aïn Ras El Ma, 1♂, 3.V.2013; Oued Jnane Niche, 2♂♂, 2♀♀, 25.IV.2015; Cascade Chrafate, 1♂, 2♀♀, 28.IV.2015; Maison forestière, 2♀♀, 21.IV.2015; Oued El Kanar, 1♂, 25.IV.2015 (sweep net). Tributary of Oued Zarka, 4♂♂, 1♀, 9.XII.2013 (reared).

**Distribution.** Nearctic (Canada), Europe, Morocco, Transcaucasia, Turkey, Cyprus, Israel, Iran. First records from the Rif.

### *Dicranomyia* (*Dicranomyia*) *goritiensis* (Mik, 1864)

**Material examined.** Oued Majjou (Hafa meqlouba), 1♂, 3.V.2013, 2♂♂, 10.V.2014; Cascade Chrafate, 1 ♀, 24.V.2013; Oued El Koub, 1♂, 31.V.2013; Cascade Zarka, 1♂, 14.XI.2013; Âounsar Aheramen, 1♂, 2♀♀, 10.V.2014, 2♂, 27.IV.2015; Maison forestière, 1♂, 21.IV.2015 (sweep net).

**Distribution.** Europe, Morocco, Algeria, Georgia, Turkey, Israel. First records from the Rif.

### *Dicranomyia* (*Dicranomyia*) *longicollis* (Macquart, 1846)

**Material examined.** Oued Aârate, 4♂♂, 3♀♀, 26.III.2014; Barrage Moulay Bouchta, 2♂♂, 5.IV.2014; Barrage Allal El Fassi, 1♂, 15.IV.2014; Oued El Kebir, 1♀, 23.IV.2014 (sweep net).

**Distribution.** Portugal, Spain, Morocco, Algeria. First records from the Rif and the Middle Atlas.

### *Dicranomyia* (*Dicranomyia*) *modesta* (Meigen, 1818)

**Material examined.** Oued Farda, 1♂, 24.IV.2013; Oued El Kanar, 4♂♂, 3♀♀, 14.VI.2013, 5♂♂, 10♀♀, 25.IV.2015; Oued Jnane Niche, 2♂♂, 16.III.2014; Oued Sidi Yahya Aârab, 4♂♂, 1♀, 25.IV.2015 (sweep net).

**Distribution.** Nearctic (Canada, USA, Greenland), widespread over Palaearctic, including Central Asia and Mongolia, as far east as Far East of Russia and Japan. First records from Morocco and North Africa.

### *Dicranomyia* (*Dicranomyia*) *novemmaculata* (Strobl, 1906)

**Material examined.** Tributary of Oued el Fondak, 1♂, 10.II.2013; Oued Aârate, 9♂♂, 4♀♀, 26.III.2014 (sweep net).

**Distribution.** Gibraltar, Portugal, Spain, Algeria. First records from Morocco.

### *Dicranomyia* (*Melanolimonia*) *hamata* Becker, 1908

**Material examined.** Tributary of Oued El Kebir, 2♂♂, 1♀, 18.III.2011; Oued Aârate, 2♂♂, 26.III.2014; Aïn Sidi Brahim Ben Arrif (Site 1), 2♂♂, 1♀ 25.IV.2014; Daya Tazia, 1♂, 25.IV.2014 (sweep net).

**Distribution.** France, Portugal, Spain, Turkey. First records from Morocco and North Africa.

***Dicranomyia* (*Dicranomyia*) *ventralis* (Schummel, 1829)**

**Material examined.** Lake Badriouen, 2♂♂, 26.IV.2011 (sweep net).

**Distribution.** Europe, Egypt, Azerbaijan, Turkey, Israel, Iran, Russia, Kazakhstan, Kyrgyzstan, Afghanistan, North Korea, India. First records from Morocco and North Africa.

***Dicranomyia* (*Melanolimonia*) *morio* (Fabricius, 1787)**

**Material examined.** Seguia Mtahen, 1♂, 30.V.2013; Daya near Aïn Afersiw, 2♀♀, 11.VI.2013; Daya Jbel Zemzem, 1♂, 23.IV.2014 (sweep net).

**Distribution.** Europe, Morocco, Transcaucasia, Turkey, Iran, Mongolia. First records from the Rif.

***Dicranoptycha fuscescens* (Schummel, 1829)**

**Material examined.** Oued Mlilah, 3♀♀, 31.V.2013; Oued Loukous, 4♂♂, 1♀, 6.V.2015; Oued Zendoula, 1♀, 6.V.2015 (sweep net).

**Distribution.** Europe, Morocco, Algeria, Transcaucasia, Turkey, Cyprus, Lebanon, Israel, ?Kazakhstan, Mongolia.

***Helius* (*Helius*) *hispanicus* Lackschewitz, 1928**

**Material examined.** Oued Amsemlil (Site 3), 1♀, 30.V.2013 (sweep net).

**Distribution.** Great Britain, Spain, Portugal, Morocco (High Atlas), Georgia, Azerbaijan, Turkey, Cyprus, Iran. First record from the Rif.

***Helius* (*Helius*) *pallirostris* Edwards, 1921**

**Material examined.** Aïn Chouk, 1♂, 1♀, 25.II.2015 (reared).

**Distribution.** Europe, Tunisia, Azerbaijan, Israel, Iran, Central Asia. First record from Morocco.

***Limonia nubeculosa* (Meigen, 1804)**

**Material examined.** Tributary of Oued El Fondak, 1♀, 10.II.2013; Aïn Ras El Ma, 1♀, 3.V.2013; Aïn Boughaba, 3♂♂, 4♀♀, 24.V.2013; Oued Azila, 1♀, 27.VI.2013; Âounsar Aheramen, 2♂♂, 1♀, 10.V.2014; Aïn Takhninjoute, 1♂, 17.V.2014; Maison forestière, 1♂, 4♀♀, 17.V.2014; Oued Madissouka, 1 ♂, 18.V.2014; Aïn Quanquben, 1♀, 28.IV.2015 (sweep net).

**Distribution.** Nearctic (Canada, USA), widespread over Palaearctic, including North Africa, Central Asia, and Mongolia, as far east as Far East of Russia, North Korea, and Japan. Recorded from Morocco in the High Atlas. First records from the Rif.

### *Limonia phragmitidis* (Schrank, 1781)

**Material examined.** Aïn Quanquben, 1 ♂, 27.VI.2013 (sweep net).

**Distribution.** Widespread in the West Palaearctic, also known from Russia, Kazakhstan and Kyrgyzstan. Recorded from Morocco (Middle Atlas). First record from the Rif.

## Pediciidae

### *Dicranota* (*Paradicranota*) *landrocki* Czizek, 1931

**Material examined.** Oued Ouara, 2♂♂, 22.XI.2013; Assif Haouz, 1♂, 17.IV.2014; Oued Taïda, 1♀, 25.IV.2014; Aïn Sidi Brahim Ben Arrif (Site 2), 2♂♂, 25.IV.2014; Âounsar Aheramen, 1♂, 10.V.2014, 1♂, 27.IV.2015; Oued Tizekhte, 1♂, 28.II.2015; Oued Mezine, 1♂, 18.IV.2015; Maison forestière, 32♂♂, 2♀♀, 21.IV.2015; Aïn Bab Tariouente, 1♂, 28.IV.2015 (sweep net).

**Distribution.** Europe, Russia (North Caucasus), Morocco (Rif), Transcaucasia Lebanon, Tajikistan. First record from the High Atlas.

### *Dicranota* (*Ludicia*) *claripennis* (Verrall, 1888)

**Material examined.** Oued Amsemlil (Site 1), 1♂, 16.XII.2013; Maison forestière, 1♂, 21.IV.2015 (sweep net).

**Distribution.** Austria, Belgium, France, Germany, Great Britain, Ireland, Italy, Netherlands, Spain. First records from Morocco and North Africa.

### *Tricyphona* (*Tricyphona*) *immaculata* (Meigen, 1804)

**Material examined.** Maison forestière, 1♂, 21.IV.2015; Daya Mtahen, 1♂, 7.V.2015 (sweep net).

**Distribution.** Europe, Transcaucasia, Turkey, Lebanon, West Siberia, Central Asia. First records from Morocco and North Africa.

## Discussion

Altogether a number of 570 specimens, most of which (521) belongs to the Limoniidae distributed in 20 genera, whereas only 49 specimens belong to the Pediciidae distributed in 2 genera.

This study provides an important contribution to the short-palped crane flies fauna of North Africa and Morocco, particularly to the Rif region where 35 species were recorded from the the first time. The most abundant species was *Dicranomyia* (*Dicranomyia*) *affinis*, with 86 specimens, followed successively by, *Erioptera* (*Erioptera*) *fuscipennis* (56), *Dicranota* (*Paradicranota*) *landrocki* (45), *Dicranomyia* (*Dicranomyia*) *chorea* (35), *Pseudolimnophila* (*Pseudolimnophila*) *sepium* (32), *Dicranomyia* (*Dicranomyia*) *modesta* (30) and *Molophilus* (*Molophilus*) *testaceus* (29). The most frequently collected species were *Erioptera* (*Erioptera*) *fuscipennis* (12 sites), *Dicranomyia* (*Dicranomyia*) *affinis* (11 sites), *Molophilus* (*Molophilus*) *obscurus* (10 sites), followed by *Dicranomyia* (*Dicranomyia*) *chorea*, *Limonia nubeculosa* and *Dicranota* (*Paradicranota*) *landrocki*, all three collected in 9 sites.

18 species of Limoniidae and 2 species of Pediciidae are newly recorded from Morocco, of which 15 species are provided to be new to North Africa. Those new records extend the distribution of the species to most southern localities of the western Palearctics in Africa. *Idiocera* (*Idiocera*) *ampullifera* represents the most southern record (Sahara) of the Limoniidae in Morocco.

Including our results, the total number of species of Limoniidae and Pediciidae known from Morocco now increases from 53 species to 73 (see chekclist).

These new data of crane flies fauna reflect the variety of suitable habitats prospected, such as are springs, mountain rivers and streams, and stagnant water mainly belonging to forest areas. This suggests that many more species and subspecies can yet be found in Morocco, that enjoys a wide variety of geographical and climatic properties.

### Check-list of the Moroccan short-palped crane flies

Limoniidae

**Chioneinae**

*Baeoura ebenina* Starý, 1981

*Baeoura staryi* Driauach & Belqat, 2015

*Cheilotrichia* (*Empeda*) *cinerascens* (Meigen, 1804)

***Cheilotrichia* (*Empeda*) *fuscohalterata* (Strobl, 1906)***

***Cheilotrichia* (*Empeda*) *minima* (Strobl, 1898)***

*Ellipteroides* (*Ellipteroides*) *lateralis* (Macquart, 1835)

*Ellipteroides* (*Protogonomyia*) *alboscutellatus* (Von Roser, 1840)

*Ellipteroides* (*Protogonomyia*) *hutsoni* (Stary, 1971)

*Erioconopa diuturna* (Walker, 1848)

*Erioconopa symplectoides* (Kuntze, 1914)

*Erioptera* (*Erioptera*) *fuscipennis* Meigen, 1818
***Erioptera* (*Erioptera*) *lutea lutea* Meigen, 1804***
*Erioptera* (*Erioptera*) *transmarina* Bergroth, 1889
***Gonomyia* (*Gonomyia*) *abscondita* Lackschewitz, 1935***
*Gonomyia* (*Gonomyia*) *subtenella* Savchenko, 1972
***Gonomyia* (*Gonomyia*) *sicula* Lackschewitz, 1940***
*Gonomyia* (*Gonomyia*) *tenella* (Meigen, 1818)
*Hoplolabis* (*Parilisia*) *obtusiapex* (Savchenko, 1982)
*Hoplolabis* (*Parilisia*) *punctigera* (Lackschewitz, 1940)
*Hoplolabis* (*Parilisia*) *sororcula* (Lackschewitz, 1940)
*Idiocera* (*Euptilostena*) *jucunda* (Loew, 1873)
***Idiocera* (*Idiocera*) *ampullifera* (Stary, 1979)***
*Idiocera* (*Idiocera*) *pulchripennis* (Loew, 1856)
***Idiocera* (*Idiocera*) *sziladyi* (Lackschewitz, 1940)***
***Ilisia maculata* (Meigen, 1804)***
*Molophilus* (*Molophilus*) *ibericus* Stary, 2011
*Molophilus* (*Molophilus*) *obscurus* (Meigen, 1818)
*Molophilus* (*Molophilus*) *propinquus propinquus* (Egger, 1863)
***Molophilus* (*Molophilus*) *testaceus* Lackschewitz, 1940***
*Symplecta* (*Symplecta*) *hybrida* (Meigen, 1804)
*Symplecta* (*Trimicra*) *pilipes* (Fabricius, 1787)
***Tasiocera* (*Dasymolophilus*) *murina* (Meigen, 1818)***

Dactylolabinae
*Dactylolabis* (*Dactylolabis*) *symplectoidea* Egger, 1863

Limnophilinae
***Austrolimnophila* (*Austrolimnophila*) *latistyla* Stary, 1977***
*Dicranophragma* (*Brachylimnophila*) *adjunctum* (Walker, 1848)
*Dicranophragma* (*Brachylimnophila*) *nemorale* (Meigen, 1818)
*Eloeophila maroccana* Stary, 2009
*Euphylidorea* (*Euphylidorea*) *crocotula* (Seguy, 1941)
***Euphylidorea* (*Euphylidorea*) *dispar* (Meigen, 1818)***
*Euphylidorea* (*Euphylidorea*) *lineola* (Meigen, 1804)
***Hexatoma* (*Hexatoma*) *bicolor* (Meigen, 1818)***
*Hexatoma* (*Hexatoma*) *gaedii* (Meigen, 1830)
*Pseudolimnophila* (*Pseudolimnophila*) *sepium* (Verrall, 1886)

Limoniinae
*Dicranomyia* (*Dicranomyia*) *affinis* (Schummel, 1829)
*Dicranomyia* (*Dicranomyia*) *chorea* (Meigen, 1818)
*Dicranomyia* (*Dicranomyia*) *didyma* (Meigen, 1804)
*Dicranomyia* (*Dicranomyia*) *goritiensis* (Mik, 1864)
*Dicranomyia* (*Dicranomyia*) *longicollis* (Macquart, 1846)

*Dicranomyia* (*Dicranomyia*) *lutea* Meigen
*Dicranomyia* (*Dicranomyia*) *mitis* (Meigen, 1830)
***Dicranomyia* (*Dicranomyia*) *modesta* (Meigen, 1818)***
***Dicranomyia* (*Dicranomyia*) *novemmaculata* (Strobl, 1906)***
***Dicranomyia* (*Dicranomyia*) *ventralis* (Schummel, 1829)***
*Dicranomyia* (*Glochina*) *sericata* (Meigen, 1830)
***Dicranomyia* (*Melanolimonia*) *hamata* Becker, 1908***
*Dicranomyia* (*Melanolimonia*) *morio* (Fabricius, 1787)
*Dicranomyia majuscula* Pierre, 1924
*Dicranoptycha fuscescens* (Schummel, 1829)
*Geranomyia caloptera* (Mik, 1867)
*Geranomyia obscura* Strobl, 1900
*Helius* (*Helius*) *hispanicus* Lackschewitz, 1928
***Helius* (*Helius*) *pallirostris* Edwards, 1921***
*Limonia flavipes* (Fabricius, 1787)
*Limonia hercegovinae* (Strobl, 1898)
*Limonia macrostigma* (Schummel, 1829)
*Limonia nubeculosa* Meigen, 1804
*Limonia phragmitidis* (Schrank, 1781)

Pediciidae
**Pediciinae**
*Dicranota* (*Dicranota*) *bimaculata* (Schummel, 1829)
*Dicranota* (*Dicranota*) *irregularis* Pierre, 1922
*Dicranota* (*Paradicranota*) *candelisequa* Stary, 1981
*Dicranota* (*Paradicranota*) *landrocki* Czizek, 1931
***Dicranota* (*Ludicia*) *claripennis* (Verrall, 1888)***
***Tricyphona* (*Tricyphona*) *immaculata* (Meigen, 1804)**

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# First Alaskan records and a significant northern range extension for two species of Diplura (Diplura, Campodeidae)

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### Abstract

Species in the class Diplura are recorded from Alaska for the first time. Two species, *Tricampa rileyi* Silvestri from Dall and Prince of Wales Islands in the Alexander Archipelago of Southeast Alaska and *Metriocampa allocerca* Conde & Geeraert from near Quartz Lake, southeast of Fairbanks, both in the family Campodeidae, are documented based on recently collected specimens deposited in the University of Alaska Museum Insect Collection. A brief review of the history of the documentation of the Alaskan soil microarthropod fauna is provided, as well as discussion of possible glacial refugia.

### Keywords

Diplura, Campodeidae, Alaska, new record, soil microarthropods, Protura, Symphyla, Pauropoda, refugium

## Introduction

Documentation of the Alaskan entomofauna has accelerated in the twenty-first century, with over 1.2 M specimens cataloged into the UAM Insect Collection since the year 2000 (http://arctos.database.museum/saved/UAM-Insects-since-2000). However, relatively little historic or current attention has been directed at the soil microarthropod fauna of Alaska, which likely contains many new records and species. This is expected, in part, because much of Alaska remained glacier-free during the Tertiary when most of what is now Canada was buried under glaciers (Ives 1974, Matthews 1975, Behan 1978, Pielou 1991). Alaska acted as a refugium for taxa, many of which are potentially endemic (360 arthropod species), some of which were presumably eliminated or prevented from dispersing by glaciers elsewhere. These endemics are often wingless, blind, soil dwelling species such as the beetles *Chionotyphlus alaskensis* (Smetana 1986), *Alaocybites egorovi* Grebennikov (2010) (known in Alaska from a fossil), and *Pinodytes borealis* (Peck & Cook, 2011), new taxa of which continue to be found (e.g. a flightless mecopteran *Caurinus tlagu* Sikes & Stockbridge, 2013). All fifteen species of Protura known from Alaska have yet to be documented occuring outside of Alaska, and are thus potential endemics (Nosek 1977, 1980, Allen 2007). The Pauropod (Myriapoda) fauna of Alaska comprises nine potentially endemic species (Scheller 1986). A single species, among the 11 known for Alaska, of soil centipede (Myriapoda: Geophilomorpha), *Escaryus paucipes* Chamberlin 1946, is a potential Alaskan endemic (Weber 1949). The UAM Insect collection has 10 specimens of Symphyla (Myriapoda) from the northern region of Alaska's Prince of Wales Island (http://arctos.database.museum/saved/AK-Symphyla), but these remain unidentified and there are no published records of Symphyla from Alaska that we are aware of. Although we have found no published records of Diplura from Alaska there are records of a Dipluran identified as *Tricampa* sp. from Prince of Wales Island recorded in unpublished documents (conference proceedings and US Forest Service reports) prepared by Carlson (1994, 1997, 2005). We have fewer records of potentially endemic Collembola – only two of over 200 species known for Alaska (*Spinonychiurus alaskensis* Pomorksi & Kaprus, 2015 and *Arneria filiformis* Pomorski, 2000). We have started, but not yet finished, summarizing the mite fauna of Alaska based on the works of Behan (1978) and Marshall et al. (1987), in which potential Alaskan endemics are certain to exist. These advances in our understanding of the soil fauna of Alaska demonstrate the progress made since Hilton's (1931a, b) focused efforts to collect Diplura, Protura, Symphyla, and Pauropoda from Alaska, during which he failed to recover any of these taxa except Pauropoda.

This unique Beringian fauna has received relatively little entomological attention historically (Riegert 1999). No study has yet focused exclusively on these potential endemics. These low-vagility organisms, like those adapted to alpine zones, are of particular concern in a changing climate because dispersal to maintain their ideal conditions is difficult. We know the northernmost latitudes are warming and drying more rapidly than any other region on Earth (Serreze et al. 2000) and alarming ecological and physical changes are being seen in Alaska (Chapin et al. 2006). The boreal forest, which dominates much of this northern landscape, making up about 17% of the earth's land surface area (Bonan 1992), is the coldest forested biome on Earth and is filled with organisms adapted to low temperatures. Alaska has warmed about 2 °C since the 1950s and 3.5 °C in the interior during the winter (US Global Change Research Program, National Assessment 2001). The growing season has lengthened by about two weeks, shrubs are invading the tundra and alpine zones, fires are more frequent and intense, permafrost and glaciers are melting, and Alaska's climate is shifting beyond the physiological optimum for one of its dominant boreal forest species, *Picea glauca*, white spruce (Veblen and Alaback 1996, Stone et al. 2002, Lawrence and Slater 2005, Sturm et al. 2005, McGuire et al. 2009, Beck et al. 2011, Juday et al. 2015). Additionally, the increase in extremes of warm temperatures in the boreal forest are associated with rapid maturation and increasingly large outbreaks of wood- and leaf-feeding insects, which increase stress to already moisture-stressed trees. Melting permafrost allows precipitation to drain, thereby drying the soil and further stressing the trees, making them more likely to burn. Post-fire successional trajectories have already begun to shift (Juday et al 2015) – this region will gradually lose its conifers which will be replaced perhaps by something like an aspen parkland – a combination of patches of aspen within a shrub grassland (Hogg and Hurdle 1995). We sit on the edge of this enormous ecological transition unlike anything modern humans have experienced before. It is therefore with great urgency that we document the current entomofauna of Alaska. We herein report on the first records of two species of Diplurans never before documented from Alaska.

## Methods

Specimens of *Tricampa rileyi* were collected primarily by forceps but also one specimen was recovered using Berlese funnels as part of two projects: Effects of forestry practices on ecological indicator species in the Tongass National Forest, Prince of Wales Island, Alaska and Baseline Community Surveys of Alpine and Subalpine Habitats in Southeast Alaska. As part of the Tongass sampling, BioQuip collapsible Berlese funnels were used with ~ 1m$^2$ of leaf/moss litter sifted prior to running under 40 watt bulbs for 48h. The Berlese funnel sample came from a low elevation (41-45 m) old growth forest site. The rest of the specimens (n = 40) came from alpine and subalpine habitats between 540 and 881 m elevation. Details of habitat composition and links to photos of each habitat are available in the results below. Specimens of *Metriocampa allocerca* (n = 6) were collected by forceps in a mid-elevation (308 m) spruce forest as part of a general survey of the Quartz Lake entomofauna.

Preparation of specimens for study involved removing specimens from the ethyl alcohol in which they had been stored and mounting them on standard microscope slides. A small drop of mounting medium (polyvinyl alcohol) was placed on the slide, the specimen was then placed in the medium and positioned, and a cover slip added. The slides were allowed to dry for five days on a warm slide dryer. Specimens were then studied at 200× and 400× using a Leica DMKB compound microscope with phase contrast lighting.

All specimens are deposited in the University of Alaska Museum Insect Collection and their data are available online at the links provided below. The data are also shared with iDigBio and GBIF.

## Results

The two Alaskan species may easily be separated by the number of macrochaeta on the pronotum. Species in the genus *Tricampa* have three macrochaeta (median anterior, *ma*, lateral anterior, *la*, lateral posterior, *lp*) (Fig. 1) while species in the genus *Metriocampa* have only two pronotal macrochaetae (*ma*, *lp*) (Fig. 2). Figure 3 shows the known distribution of the two species. The following list gives most of the specimen data for these species in Alaska – complete data are available at the links provided (Table 1).

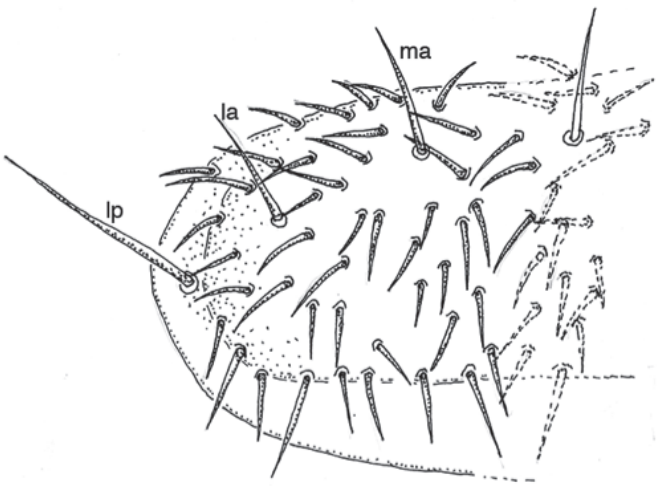


**Figure 1.** *Tricampa rileyi* Silvestri, pronotum.

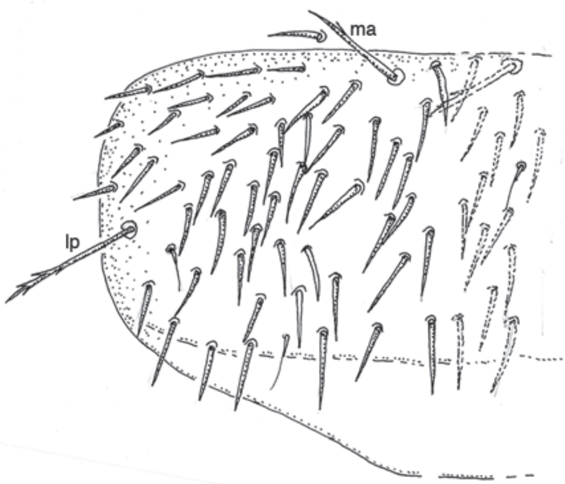


**Figure 2.** *Metriocampa allocerca* Conde & Geeraert, pronotum.

**Figure 3.** Distribution of *T. rileyi* and *M. allocerca*: *T. rileyi,* circles = previous distribution, triangle = Alaska; *M. allocerca*, square = previous distribution, diamond = Alaska distribution.

## Discussion

*Tricampa rileyi* Silvestri, 1993 is the most widely distributed among the four North American species in this genus (Allen 1994, 2002). It ranges from Louisiana north to Illinois and Iowa, west into Colorado, Utah, Wyoming, Montana, Washington, and has been recorded from Alberta (Banff), Canada (Allen 2002; Schwaninger 1996). Collections reported herein extend this range into southern Alaska. *Metriocampa allocerca* Conde & Geeraert, 1962 has been recorded from only the type locality in Montana. The new record given here is from just southeast of Fairbanks, Alaska.

Species in both *Metriocampa* and *Tricampa* have been described from the Eastern Hemisphere. Three species of *Metriocampa* are known from Japan and China. One species belonging to the genus *Tricampa* has been recorded from Australia. Diplura found primarily in western North America have not been thoroughly studied nor has

**Table 1.** Specimen data.

| | | |
|---|---|---|
| ***Tricampa rileyi* Silvestri** | | |
| Data including habitat photos, available online at: http://dx.doi.org/10.7299/X7JH3M91 | | |
| **ALASKA: Dall Island** | | |
| RTA-2014-1 | UAM100110978, UAM:Ento:231681 | |
| Dall IsI. | 54.99670°N | 133.00807°W |
| subalpine forest, *Abies lasiocarpa*, *Tsuga mertensiana* | | |
| 1 male, 4 females | | |
| RTA-2014-5 | UAM100110991, UAM:Ento:231694 | |
| Dall IsI. | 54.99605°N | 133.02089°W |
| heath, *Empetrum nigrum*, *Philodoce glanduliflora* | | |
| 1 specimen SEM | | |
| RTA-2014-6 | UAM100110790, UAM:Ento:233667 | |
| Dall IsI | 54.99670°N | 133.00807°W |
| floodplain meadow, under rocks | | |
| 3 females | | |
| RTA-2014-7 | UAM100111001, UAM:Ento:231631 | |
| Dall IsI. | 54.99617°N | 133.00932°W |
| flood meadow, *Athyrium*, *Rubus spectabilis* | | |
| 1 male 3 females | | |
| RTA-2014-16 | UAM100111083, UAM:Ento:232379 | |
| Dall IsI | 54.99555°N | 133.01039°W |
| flood meadow, *Athyrium*, *Caltha leptosepala* | | |
| 3 males, 5 females | | |
| **ALASKA: Prince of Wales Island** | | |
| RTA-2014-2 | UAM100110963, UAM:Ento:217660 | |
| Staney Creek | 55.79901°N | 133.11782°W |
| old growth, SEM 1 specimen | | |
| RTA-2014-3 | UAM100180086, UAM:Ento:233675 | |
| nr Black Lk | 55.58988°N | 132.89034°W |
| rocky heath, *Cassiope mertensiana*, *Luetkea pectinata*, *Harrimanella stelleriana* | | |
| 1 male | | |
| RTA-2014-4 | UAM100111153, UAM:Ento:232680 | |
| nr Black Lk | 55.58988°N | 132.89548°W |
| wet meadow, near bear dung, *Caltha Leptosepala*, *Athyrium filix-femina* | | |
| 1 female | | |
| RTA-2014-8 | UAM100180094, UAM:Ento:233702 | |
| nr Black Lk | 55.590299°N | 132.88896°W |
| meadow, *Nephrophyllidium crista-galli*, *Anemone narcissiflora* | | |
| 1 male 2 females | | |
| RTA-2014-17 | UAM100111137, UAM:Ento:232618 | |
| nr Black Lk | 55.58964°N | 132.88783°W |
| rocky meadow, *Nephrophyllidium crista-galli*, *Luetkea pectinata* | | |
| 2 males, 1 female | | |
| RTA-2014-19 | UAM100111147, UAM:Ento:232651 | |
| nr Black Lk | 55.58898°N | 132.88927°W |
| wet meadow, *Luetkea pectinata*, *Caltha leptosepala* | | |
| 4 males, 4 females | | |

| ***Metriocampa allocerca* Conde & Geeraert** | | |
|---|---|---|
| Data, including habitat photo, online at: http://dx.doi.org/10.7299/X7P84C1R | | |
| A video taken by the first author of this species at the Quartz Lake site is available at: https://youtu.be/my25LhHNFbg | | |
| **ALASKA: Quartz Lake** | | |
| RTA-2014-18 | UAM100046686, UAM:Ento:241928 | |
| Quartz Lake | 64.22086°N | 145.80301°W |
| *Picea*, moss carpet, firepit, under rotting logs, rocks | | |
| 3 males, 3 females | | |

the Asian dipluran fauna. It is highly likely that other North America/Asian biogeographic relationships will emerge as the faunae in the two regions become better known. Neither genus is known to occur in Europe but among the diverse European Diplura fauna none of the species have been recorded as far north (64°N) as the locality given for *Metriocampa* here (Paclt 1957). Lagerlöf and Andrén (1991) report on unidentified diplurans from central Sweden (60°N) and Reuter (1895) reported *Campodea staphylinus* Westwood from Kirkkonummi (Kyrkslätt) and Helsinki, Finland (60°N). These new northern records not only add additional distributional and biogeographic data to our knowledge of this group but also add to our knowledge about the environments and habitats Diplura are able to inhabit.

It could be argued that the rarity of diplurans in Alaska may result from a lack of effort spent using appropriate methods of capture. That is, if appropriate effort were expended, they would not be considered rare. We feel this is unlikely, due primarily to the state-wide collecting efforts of the first author, using the same methods which resulted in these two discoveries. Although as yet undocumented dipluran populations may occur in Alaska, given the effort to date, we expect there to be few.

Given that Prince of Wales Island was mostly buried under an ice sheet during the maximum of the late Wisconsin glaciation 26,000 to 13,000 $^{14}$C years BP (Carrara et al. 2007) and had been repeatedly buried by ice during the Pleistocene, the presence of these low vagility organisms seems unusual. However, there exists considerable biological and geological evidence that suggests ice-free refugia in the Alexander Archipelago may have existed during this time, allowing organisms to survive in relative isolation, and re-seed the region after deglaciation (Carrara et al. 2007). Twenty seven of the 108 mammal species or subspecies occurring in southeastern Alaska are endemic to the area (Cook et al. 2001). *Tricampa rileyi* was recovered from regions that were reconstructed as under ice by Carrara et al. (2007, fig. 3). Post deglaciation dispersal to these sites from ice-free refugia is the most likely explanation.

The *M. allocerca* specimens were found at a site, Quartz Lake, that has received recent archeological study. Wooller et al. (2012) document the earliest human occupation of these sites at 13,100–12,700 cal yr BP and cite dates for the origin of the gravel terrace which formed the lake to between 140,000 and 40,000 years ago. The disjunct nature of this species with an interior Alaska population and a population in Montana is similar to that of *Thanatophilus coloradensis* (Wickham, 1902) (Coleoptera: Silphidae) – a species known from interior Alaska and northern British Columbia in the north, and from elevations above tree line in Colorado, New Mexico, Utah, Montana and Wyoming in the south, with no intervening records (Anderson and Peck 1985). Genetic data would be needed to determine if this pattern is due to recent dispersal or ancient vicariance.

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