

New records of the rare calcareous sponge *Paragrantia waguensis* Hôzawa, 1940

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Abstract

Paragrantia waguensis Hôzawa is reported from coastal reefs of the island of Okinawa. This rare species was previously known only from Central Japan, Mie Prefecture. It has peculiar apopylar tetractine spicules, so far unique among Calcarea. We present in situ images of the species and a full description including SEM images of skeletal structure and spicule complement. The status of *Paragrantia* as a separate genus of the family Grantiidae distinct from *Grantia* Fleming is confirmed on the basis of a morphological and molecular comparison with the European type species of *Grantia*, *G. compressa* (Fabricius).

Keywords

Porifera, Calcarea, Leucosolenida, Grantiidae, Japan, apopylar spicules

Introduction

We report here the recent collection (2006, 2014) of a rare and curious calcareous sponge species from Okinawa, *Paragrantia waguensis* Hôzawa (1940), at considerable distance (1500 km) from its type locality in eastern mid-Japan (Mie Prefecture).

The species was first collected in Okinawa in 2006 and mentioned briefly in a paper reporting its chemistry (Tianero et al. 2009). Subsequently (2014), one of us (BWH) collected the same species slightly to the north of the 2006 locality.

Paragrantia Hôzawa (1940) was erected as a new genus in the family Grantiidae Dendy (1893) (Porifera, Calcarea, Calcaronea, Leucosolenida) on the basis of a curious structure in the exhalant openings of its type species, *P. waguensis*. The genus and its type species were not seriously evaluated since they were described in 1940: the genus was assigned to the synonymy of the genus *Scypha* Gray (1821) in Burton's literature review of the Calcarea (1963: 448) and the type species was considered a junior synonym of the boreal widespread species *Scypha compressa* (Fabricius, 1780) (= *Grantia compressa*), meriting only the comment that its general appearance resembled that of the well-known *Grantia compressa*. Burton (*l.c.*) simply summarized Hôzawa's description as a 'named form' under the synonyms of *Scypha compressa*, in the process omitting to provide information on its diactines (see below). Burton's (1963) 'decisions' about the classification of the Calcarea were severely criticized at the time, and his synonymy assignments were generally considered unacceptable.

The major revision of the Calcarea presented in the framework of the Systema Porifera project (Borojevic et al. 2002a, b, c) ignored the genus entirely, as was the case in the published preview of the subclass Calcaronea of Borojevic et al. (2000). Only in the 'Annotated List of unrecognizable sponge taxa and unavailable names' added as an appendix to the Systema Porifera (Hooper and Van Soest 2002: 1701–1706) the name *Paragrantia* is mentioned as a 'possible synonym of *Grantia*'. The World Porifera Database (Van Soest et al. 2015) assigned the species on that basis to the 'accepted' combination *Grantia waguensis*.

Despite Hôzawa's excellent description and generally good illustrations of *Paragrantia waguensis*, we feel induced by its subsequent lack of proper evaluation, to once again draw attention to it. Here, we fully describe our specimens, providing in situ photos, compare it with Hôzawa's description (unfortunately the type material itself, kept in the Tôhoku University Museum (TUMC), was not available to us), and pose the question of the validity of the genus *Paragrantia*. For that purpose, we compared our specimens with a representative sample of the type species of the genus *Grantia*, *G. compressa* (Fabricius, 1780) and performed a molecular sequence analysis of the two species and some of its assumed relatives.

Methods

Specimens were collected using SCUBA by J. Tanaka (University of the Ryukyus) in 2006, and by BWH, as a guest of JDR, in 2014, subsequently identified by NJDV and RWMVS, and registered in the RMNH collection. For our comparison with *Grantia compressa*, we used a specimen from Roscoff, W coast of France, incorporated in the ZMA collection, now housed in Naturalis Biodiversity Center.

Specimens were sectioned by hand: cross sections perpendicular to the surface and tangential sections of the outer surface (cortical region), the inner surface (atrial region), and the oscular fringe. The sections were air dried, mounted on stubs, and sputter coated for examination under SEM. Spicules were dissociated using household bleach, washed five times in distilled water, and subsequently plated on glass slides for light-microscopic measurements and on SEM stubs for examination and micrographing under SEM.

Measurements of the spicules are given as smallest-*average*-largest of 25 spicules of each distinct type.

To verify the conclusions from the morphological comparison of *Paragrantia waguensis* with *Grantia compressa*, a 28S rDNA sequence (430 bp) of *Paragrantia waguensis* (sample RMNH Por. 9317) was provided by the Naturalis Barcode Laboratory. DNA was extracted using the NucleoMag 96 Tissue kit by Macherey-Nagel on a Thermo Scientific Kingfisher Flex magnetic bead extraction robot with a final elution volume of 150 µl. The forward and reverse of the C2-D2 region of the nuclear ribosomal 28S was amplified (Forward primer 5'GAAAAGAAGCTTTGRARAGAGAGT 3' and Reverse primer 5'TCCGTGTTTCAAGACGGG 3'). Template was diluted ten times before amplification and added with 18.8 µl of ultrapure MQ water, 2.5 µl PCR buffer, 0.5 µl dNTP (containing 2.5 mM) and 0.25 µl Taq (5 units per µl) to a total reaction volume of 25 µl. PCR cycling consisted of an initial denaturation step at 94 °C for 3 mins, followed by 40 cycles each consisting of 15 secs at 95 °C, 30 secs at 50 °C, 40 secs at 72 °C, and a final extension of 5 mins at 72 °C. Bidirectional sequencing was performed at BaseClear (<http://www.baseclear.com/>). Sequences were edited manually with Sequencher 4.10.1 (Gene Codes Corporation).

With the obtained sequence we performed a BLAST analysis provided by the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), and downloaded a representative set of partial 28S sequences of calcareous species showing up in the BLAST result. The set of sequences included a sequence of *Grantia compressa* provided by Manuel et al. (2004), 14 other available Leucosolenida-sequences submitted by various research groups belonging to Grantiidae, Sycettidae, Jenkinidae, Amphoriscidae and Lelapiidae. We added as outgroup sequence the calcareous *Pericharax heteroraphis* (recently revised and renamed as *Pericharax orientalis* Van Soest & De Voogd, 2015). The combined dataset of 17 sequences (see Table 1) was then aligned using ClustalW, trimmed to approximately equal numbers of basepairs (407 bp), and subsequently analyzed phylogenetically, using the MEGA package vs 06.6 for Mac (<http://www.megasoftware.net/megamac.php>). For the phylogeny reconstruction we chose the Maximum Likelihood statistical method with a Bootstrap method set at 100 replicates. As Substitution Model we chose the Tamura-Nei model and – based on model testing algorithm in MEGA – we used GTR+G as Evolutionary Model. Further parameters were used in their default settings.

The systematic classification generally follows the Systema Porifera (Hooper and Van Soest 2002), chapter on Leucosolenida (Borojevic et al. 2002b).

Table 1. Leucosolenida species of which partial 28S sequences were downloaded from the NCBI website (<http://www.ncbi.nlm.nih.gov/>) and were used for an evaluation of the phylogenetic relationships of *Paragrantia waguensis* and *Grantia compressa*. From left to right columns list genus, species and family names, accession numbers of the sequences, and literature sources. The results of the phylogenetic analysis are represented in Fig. 8.

Genus	Species	Family	Accession number	Source
<i>Paragrantia</i>	<i>waguensis</i>	Grantiidae	KT277668.1	present study
<i>Grantia</i>	<i>compressa</i>	Grantiidae	AY563538.1	Manuel et al. 2004
<i>Ute</i>	<i>ampullacea</i>	Grantiidae	JQ272226.1	Voigt et al. 2012
<i>Leucandra</i>	<i>nicolae</i>	Grantiidae	JQ272268.1	Voigt et al. 2012
<i>Leucandra</i>	<i>aspera</i>	Grantiidae	AY563535.1	Manuel et al. 2004
<i>Leucandra</i>	<i>sp.</i>	Grantiidae	JQ272265.1	Voigt et al. 2012
<i>Aphroceras</i>	<i>sp.</i>	Grantiidae	AM181001.1	Dohrmann et al. 2006
<i>Teichonopsis</i>	<i>cylindrica</i>	Grantiidae	JQ272264.1	Voigt et al. 2012
<i>Synute</i>	<i>pulchella</i>	Grantiidae	JQ272274.1	Voigt et al. 2012
<i>Sycon</i>	<i>capricorn</i>	Sycettidae	AM181000.1	Dohrmann et al. 2006
<i>Scypha (=Sycon)</i>	<i>raphanus</i>	Sycettidae	AY563537.1	Manuel et al. 2004
<i>Anamixilla</i>	<i>torresi</i>	Jenkinidae	AY563536.1	Manuel et al. 2004
<i>Leucascandra</i>	<i>caveolata</i>	Jenkinidae	JQ272259.1	Voigt et al. 2012
<i>Paraleucilla</i>	<i>magna</i>	Amphoriscidae	JQ272267.1	Voigt et al. 2012
<i>Paraleucilla</i>	<i>sp.</i>	Amphoriscidae	AY563540.1	Manuel et al. 2004
<i>Grantiopsis</i>	<i>heroni</i>	Lelapiidae	AY563539.1	Manuel et al. 2004

Results

Phylum Porifera

Class Calcarea

Subclass Calcaronea

Order Leucosolenida

Family Grantiidae

Genus *Paragrantia*

Paragrantia waguensis Hôzawa, 1940

Figs 1–5

Paragrantia waguensis Hôzawa, 1940: 40, pl. V figs 8–11, text-fig. 4; Burton 1963: 448, text-fig. 274 as named form of *Scypha compressa* (not: *Spongia compressa* Fabricius, 1780).

Grantia waguensis; Van Soest et al. 2015 on-line.

Material. Naturalis Biodiversity Center, reg. nr. RMNH Por. 9317 (five individuals), Japan, South Kuroshio ecoregion, Okinawa, Manza, approximately 26.5°N, 127.8°E, vertical rocky wall, 25–30 m, coll. B.W. Hoeksema, 10 August 2014; Naturalis Biodiversity Center, reg.nr. RMNH Por. 3901 (three individuals), Japan, South Kuroshio

ecoregion, Okinawa, Onna village, approximately 26.5°N, 127.8°E, coral reef slope, 20–55 m, coll. J. Tanaka, 6 May 2006.

Syntype, 8 specimens (not seen) Tôhoku University Museum, reg.nr. TUMC 110908, Japan, Central Kuroshio ecoregion, Mie Prefecture, Wagu, approximately 34.25°N, 136.8°E, coll. S. Hôzawa, July 1933.

Description. Cup-shaped or tubular specimens (Fig. 1a–e), usually being a single rounded ‘person’ in life, but larger individuals may be somewhat elliptical, and occasionally consisting of two or three budded individuals. The cups or tubes have a narrow attachment to the substratum but there is no clear stalk. Outer surface pearly white and smooth, without any visible inhalant structures. The rim is pale purple in color and distinctly fringed. Algae or detritus may stick to the rim. Inside the cup, most specimens are likewise smooth and white, but one of the specimens is mottled greenish due to encrusting algae growing on its inner surface (Fig. 1d). A faint punctate inner surface pattern may be discernible in some individuals, representing the peculiar exhalant chambers characteristic for this species. Size of individuals may vary from 1 to 4 cm in height, 0.5–4 cm in diameter, thickness of the walls up to 1.5 mm. Consistency firm, somewhat flexible, but breakable under pressure. In preserved condition, the shape of the individuals alters notably: the specimens collapse and may become folded and compressed.

Aquiferous system. No histological slides were made, but the structure of the skeleton suggests it is syconoid (as was also the case in Hôzawa’s material). There is no evidence of branching choanocyte chambers. Subcortical lacunae are present, regularly distributed and apparently serving as inhalant reservoirs.

Skeleton of the walls. (Figs 2–4) In cross section from external side to inside: a fairly thick cortical skeleton of relatively large triactines (Fig. 2a), an articulate tubar skeleton (Fig. 3a) consisting of three or more rows of sagittal triactines with centrifugally directed unpaired actines, subsequently a layer of subatrial strongly sagittal triactines, and an atrial skeleton (Fig. 4a–b) of tetractines and triactines, containing the three-dimensionally rounded atrial exhalant chambers (Fig. 4b) supported and covered by inwardly directed small butterfly-shaped tetractines (Figs 4c–c1) with peculiarly swollen and ornamented apical actines (see below). These small apopylar tetractines in addition to the normal atrial triactines (Fig. 4d) and tetractines (Fig. 4e) are so far unique among the *Calcarea*.

Skeleton of the fringe. (Fig. 5a–b) The main support of the fringe consists of a palisade of long thick diactines tangentially covered outside and inside by sagittal triactines and tetractines differing morphologically from those of the walls.

Spicules. Cortical triactines, tubar triactines, subatrial triactines, atrial triactines, atrial tetractines, apopylar tetractines, diactines from the fringe, triactines from the fringe, tetractines from the fringe.

Cortical triactines (Fig. 2b), mostly slightly sagittal, with unpaired actines generally longer than paired actines, but not infrequently all actines are about the same length with the sagittal morphology only expressed by a wider angle between the paired actines; unpaired actines 48–196.6–315 × 6–15.3–24 µm, paired actines 40–162.4–242 × 9–14.7–23 µm.

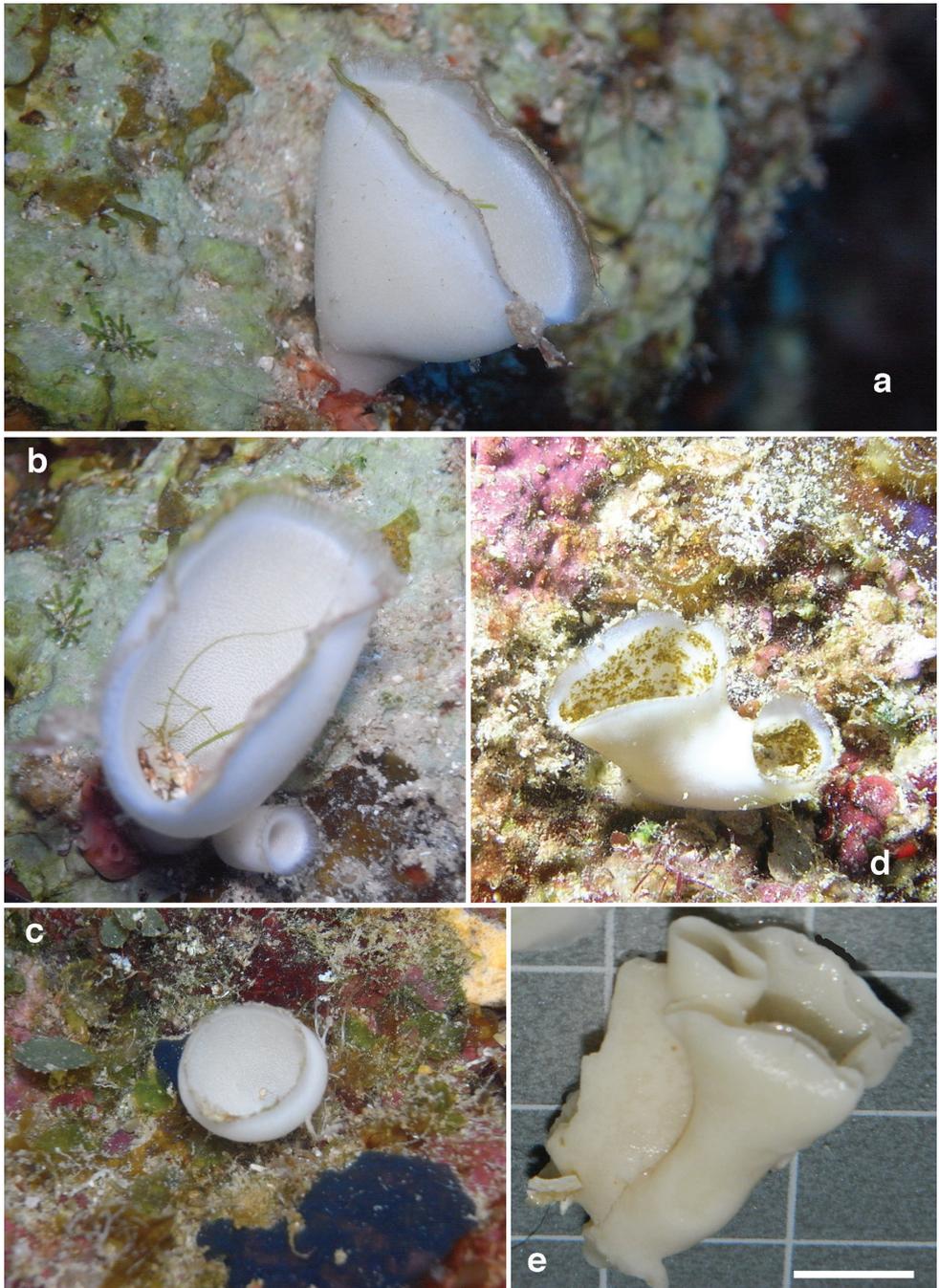


Figure 1. Habitus of *Paragrastia waguensis*. **a** *in situ* photo of oval cup-shaped individual at Manza, Okinawa (photo B.W. Hoeksema of RMNH Por. 9317) **b** ditto and small tubular rounded bud (photo B.W. Hoeksema) **c** ditto of small rounded cup-shaped individual (B.W. Hoeksema) **d** budded individual showing mottled algal growth, from Onna village, Okinawa (photo J. Tanaka) **e** preserved specimens from Manza, RMNH Por. 3901) (scale bar = 1 cm).

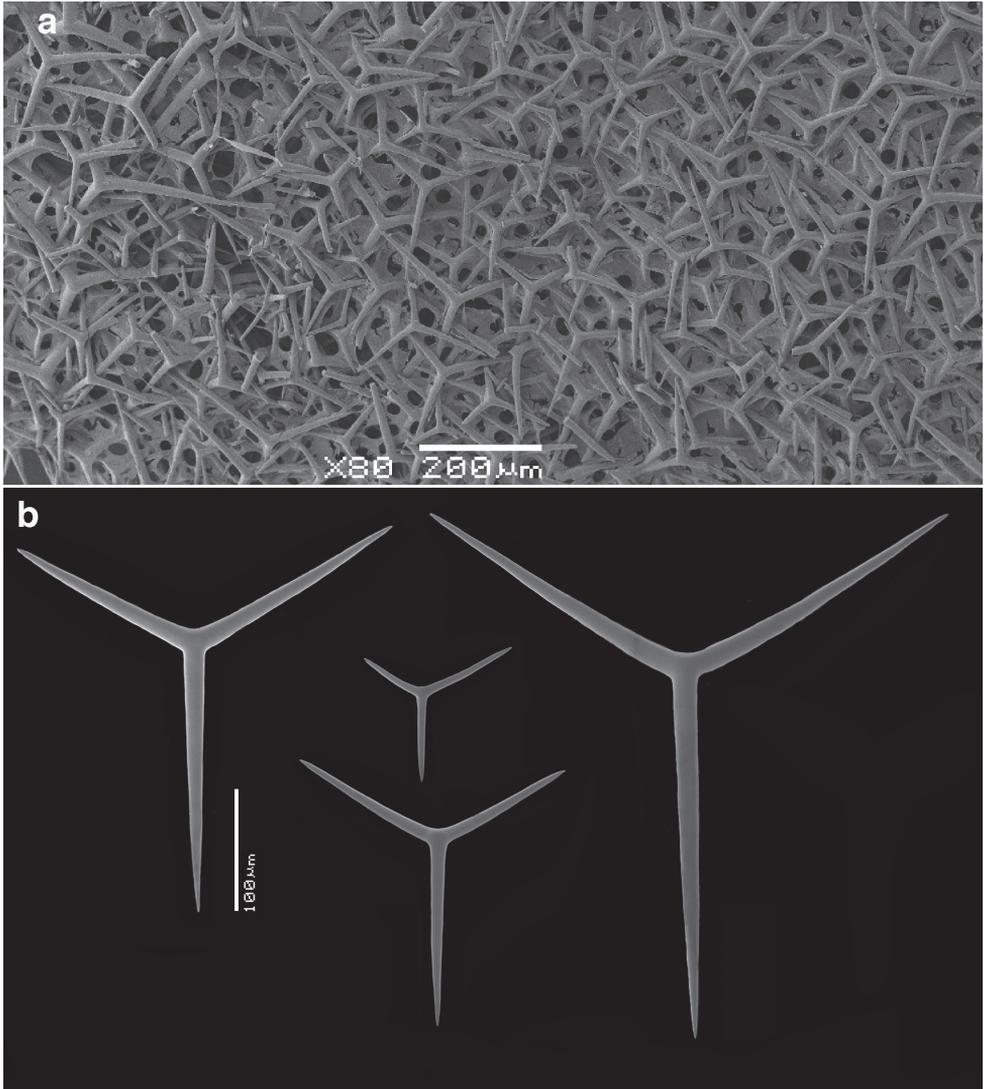


Figure 2. SEM images of cortical region of *Paragrantia waguensis* and its cortical spicules. **a** overview of cortical surface showing dense layer of cortical triactines **b** various sizes and shapes of cortical triactines.

Tubar triactines (Fig. 3b), sagittal, unpaired actines usually longer than paired actines, with paired actines often having a wide angle with the unpaired actines, frequently rather ‘oxhorn shaped’, the ends curved slightly inwards; unpaired actines $93\text{--}195.2\text{--}270 \times 8\text{--}13.6\text{--}21 \mu\text{m}$, paired actines $92\text{--}148.2\text{--}241 \times 9\text{--}13.4\text{--}21 \mu\text{m}$.

Subatrial triactines (Fig. 3c), strongly sagittal, with paired actines wide angled, occasionally at right angles with the unpaired actines, but often not in the same plane, unpaired actines much longer than paired actines; unpaired actines $207\text{--}242.3\text{--}291 \times 6\text{--}8.3\text{--}10 \mu\text{m}$, paired actines $61\text{--}85.0\text{--}111 \times 6\text{--}8.4\text{--}10 \mu\text{m}$.

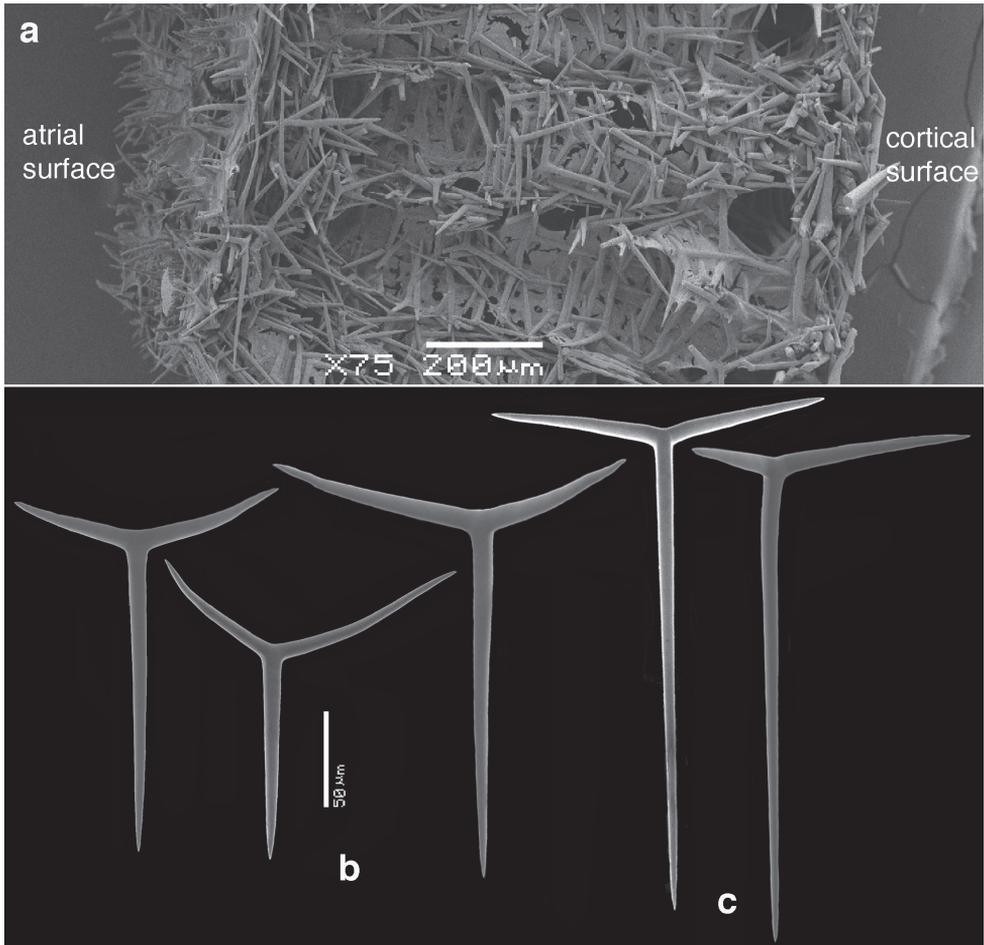


Figure 3. SEM images of cross section of *Paragrantia waguensis* and its choanosomal spicules. **a** overview of cross section showing skeleton of tubar chambers and subatrial skeleton **b** various sizes and shapes of tubar triactines **c** subatrial triactines.

Atrial triactines (Fig. 4d), strongly sagittal, with unpaired actines longer than the paired actines, but differentiated from the subatrial triactines by longer paired actines; unpaired actines $150\text{--}247.5\text{--}354 \times 8\text{--}9.6\text{--}11 \mu\text{m}$, paired actines $114\text{--}171.1\text{--}261 \times 7\text{--}10.3\text{--}12 \mu\text{m}$.

Atrial tetractines (Fig. 4e), sagittal, with unpaired and paired actines not strongly different in length, but occasionally the paired actines are slightly longer, and apical actines conical; unpaired actines $42\text{--}118.7\text{--}226 \times 7\text{--}9.7\text{--}10 \mu\text{m}$, paired actines $105\text{--}132.7\text{--}171 \times 7\text{--}9.0\text{--}10 \mu\text{m}$, apical actines $30\text{--}37.3\text{--}63 \times 6\text{--}8.6\text{--}12 \mu\text{m}$.

Atrial exhalant chamber tetractines (apopylar tetractines) (Figs 4c, 4c1), butterfly-shaped, sagittal, with short conical unpaired actines, distinctly longer paired actines, and with the apical actines swollen and ornamented with irregular spines, curved slightly inwards, likened to ‘torches’ by Hôzawa (1940: 42); unpaired actines 14--

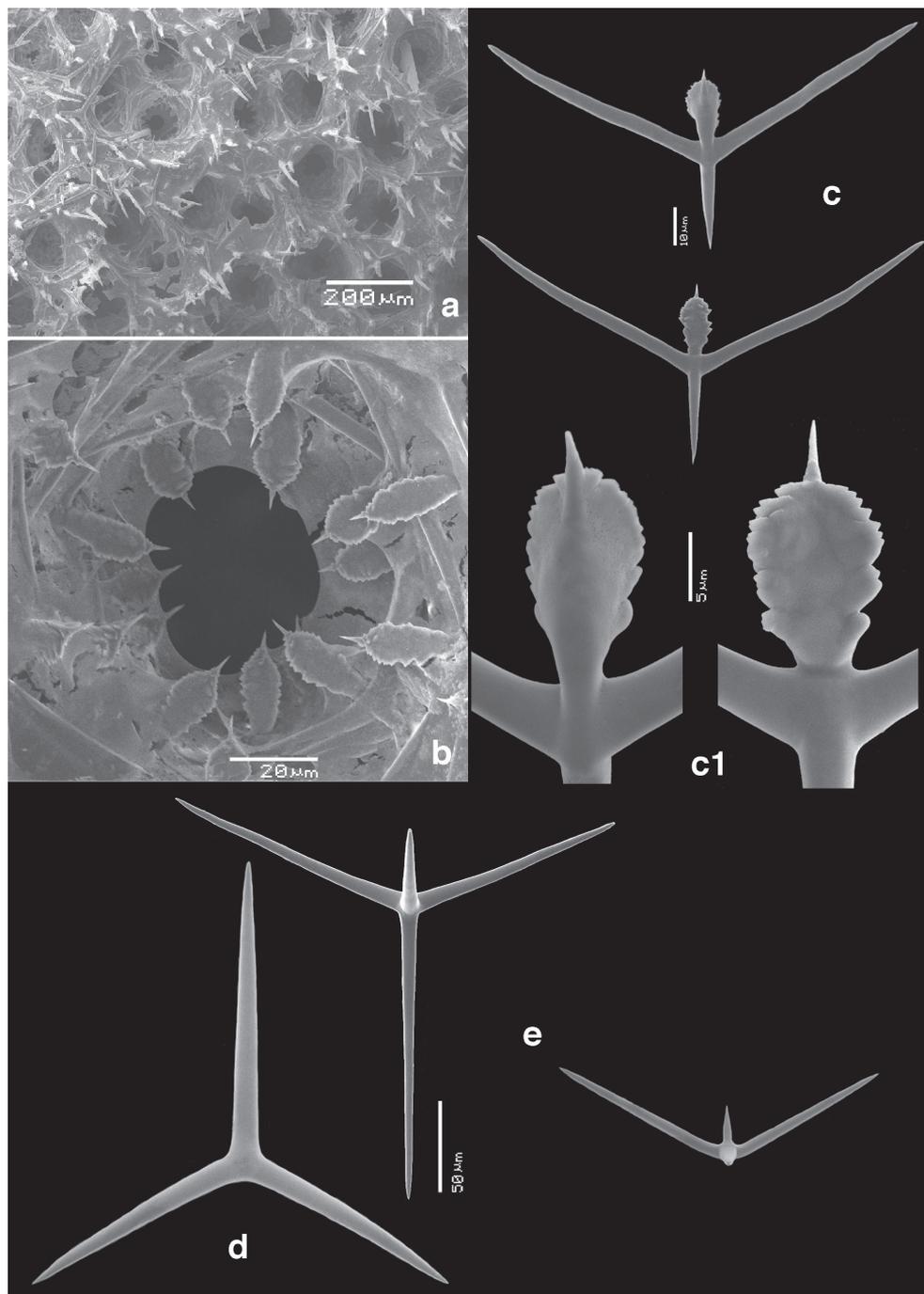


Figure 4. SEM images of atrial region of *Paragrantia waguensis* and its atrial spicules. **a** overview of atrial surface with atrial chambers **b** detail of atrial apopylar chambers showing the position of the apopylar spicules **c** apopylar spicules **c1** details of apical actines of **c** **d** atrial triactine **e** atrial tetractines.

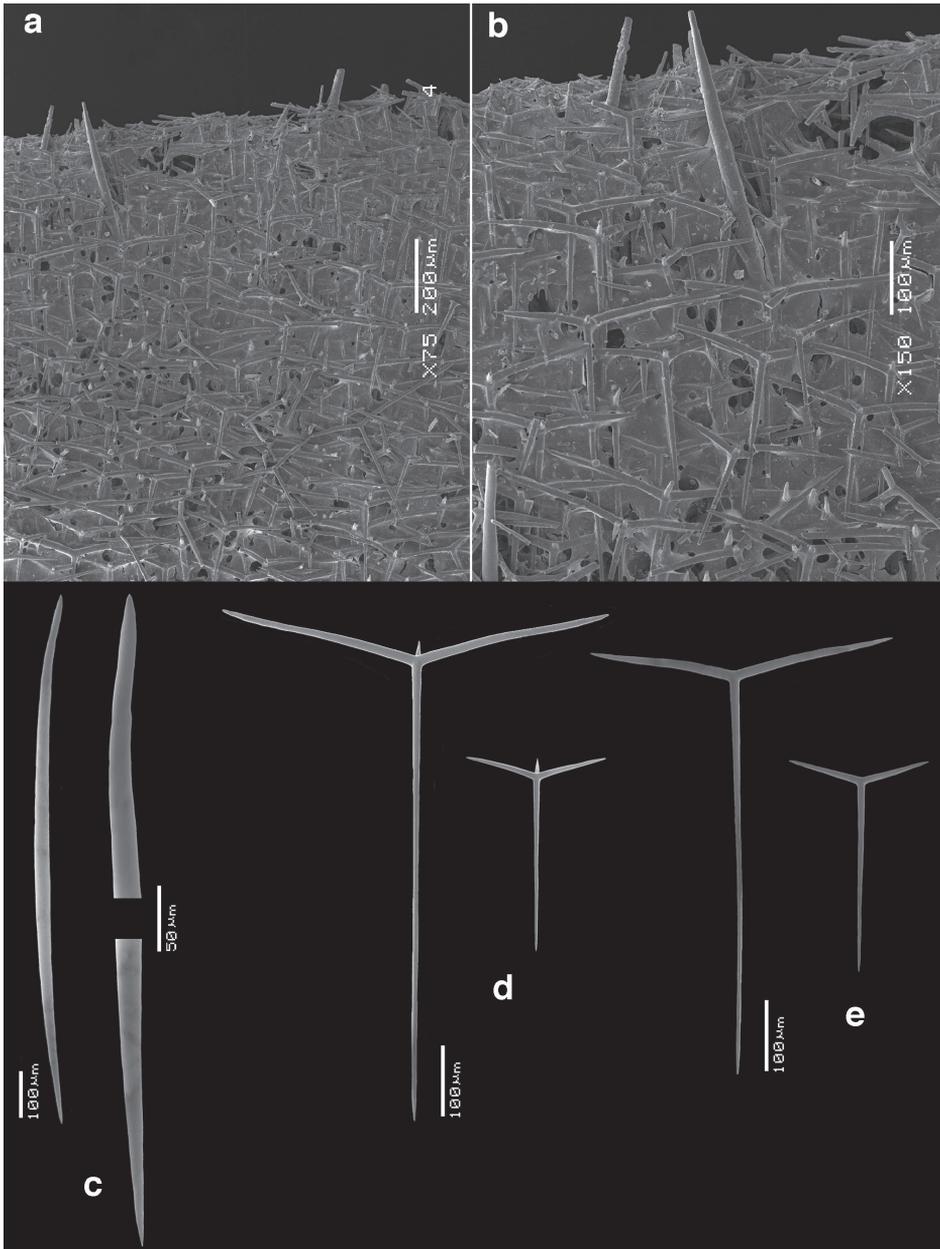


Figure 5. SEM images of the fringe of *Paragrantia waguensis* and its fringe spicules. **a** overview of fringe inner surface **b** detail of the same showing presence and position of the spicules **c** diactines **d** tetractines **e** triactines.

23.6–39 × 3–3.8–5 μm, paired actines 27–64.1–78 × 3–4.1–5 μm; apical actines 13–19.4–24 × 5–8.2–10 μm.

Diactines (Fig. 5c) from the fringe, fusiform, sharply pointed, 360–703.2–990 × 12–21.6–29 μm.

Tetractines (Fig. 5d) from the fringe, strongly sagittal, unpaired actines longer than the paired actines, which are widely flaring, and apical actines conical, sharply pointed; unpaired actines 165–284.8–528 × 9–10.6–15 µm, paired actines 81–167.6–273 × 8–10.3–12 µm, apical actines 16–39.3–76 × 5–7.2–10 µm.

Triactines (Fig. 5e) from the fringe, strongly sagittal, with much longer unpaired actines than paired actines (similar to subatrial triactines), 156–223.5–279 × 6–8.8–10 µm, paired actines 63–84.8–105 × 7–9.2–10 µm.

Ecology. No data were provided by Hôzawa, but the Okinawa specimens were from a steep reef slope at 20–55 m depth, growing among coralline and turf algae, and encrusting sponges.

Distribution. Warm-temperate (Mie Prefecture) and subtropical (Okinawa) regions of Japan.

Discussion

Comparison with Hôzawa's specimens

The habit of Hozawa's specimens is described from eight preserved individuals. A photo is given of two specimens (Hôzawa 1940: pl. V fig. 8) having a fusiform shape ending in a terminal oscule. This is unlike our own cup-shaped individuals. However, we assume that like in our own specimens the habit was changed rather dramatically into laterally compressed, partially 'branched' individuals after preservation. We here assume that the live habit was likely more cup-shaped/tubular. The Tōhoku University Museum was unable to grant our request for a loan of the type material (e-mail of Mr. Jun Nemoto, technical staff of the Tōhoku University Museum), but we are confident from Hôzawa's description and illustrations, and the changes we observed in our own material between live and preserved specimens (cf. Fig. 1a–d and Fig. 1e), that the features are sufficiently similar to consider both groups of specimens as belonging to the same species.

The general structure of the skeleton and the overall diversity and sizes of the spicules likewise match closely (as can be observed from Table 2), so microscopical features between the two sets of specimens also confirm that they belong to the same species.

Nevertheless, there are a few clear differences:

- The unpaired actines of the atrial tetractines in Hôzawa's specimens were given as large as up to 390 µm, whereas in our specimens they were only up to 226 µm.
- The apical actines of the apopylar tetractines in Hôzawa's specimens were given as having a width of 12–18 µm, whereas ours were only 5–10 µm.
- The tetractines of the fringe in Hôzawa's specimens were smaller and thinner: e.g. unpaired actines were 150–420 × 8 µm, whereas in our specimens these measured 165–528 × 9–15 µm.

We believe that these differences are too small to consider them as evidence for specific distinction.

Table 2. *Paragraptia waagenis*, spicule size data (micrometers). The data of the type specimens from Wagu (taken from Hózáwa 1941) are compared to those of specimens from Okinawa (our own measurements of RMNH Por. 9317 and 3901 combined), divided into three sets, a. spicules of the cortex and the chamber layer, b. spicules of the atrial region, and c. spicules of the fringe.

a. Spicules of the cortex and the chamber layer									
cortical triactines		tubar triactines		paired		subatrial triactines			
unpaired	paired	unpaired	paired	unpaired	paired	unpaired	paired	unpaired	paired
Type specimens: Wagu	80-420 x 8-20	50-220x4-16	140-225x10-16	90-160x10-16	180-350x8-10	65-110x8-10			
Okinawa	48-315x6-24	40-242x9-23	93-270x8-21	92-241x9-21	207-291x6-10	61-111x6-10			
b. Spicules of the atrial region									
atrial triactines		atrial tetractines		apopylar triactines					
unpaired	paired	unpaired	paired	unpaired	paired	unpaired	paired	unpaired	paired
Type specimens: Wagu	100-390x8-10	70-200x8-10	100-390x10	115-170x10	40-90x12	20-25x4	56-70x4	20x12-18	apical
Okinawa	150-354x8-11	114-261x7-12	42-226x7-10	105-171x7-10	30-63x6-12	14-39x3-5	27-78x3-5	13-24x5-10	
c. Spicules of the fringe									
Fringe diactines		Fringe triactines		Fringe tetractines					
		unpaired	paired	unpaired	paired	unpaired	paired	unpaired	paired
Type specimens: Wagu	300-880x14-28	340x10	150x12	150-420x8	150-200x10	20-40x10			
Okinawa	360-990x12-29	156-279x6-10	63-105x7-10	165-528x9-15	81-273x8-12	16-76x5-10			

Comparison with *Grantia compressa*

By its possession of a cortical skeleton, an articulated choanosomal skeleton, and lack of pseudosagittal spicules, the present species fits the family Grantiidae. In order to decide whether Hôzawa was right in establishing a new genus for his species *waguensis*, it is necessary to know the properties of the other genera of the family. The syconoid aquiferous system and the absence of long longitudinally arranged diactines (except in the fringe) limits the generic relationships of *waguensis* to *Grantia* Fleming (1828), *Sycandra* Haeckel (1872) and *Teichonopsis* Dendy & Row (1913). All three share the general structure of the skeleton with the present species. The latter two genera are monotypic and are distinguished on unique features, the presence of a special atrial network of tissue strands supported by small diactines (*Sycandra*), or an elaborate shape (*Teichonopsis*). *Sycandra utriculus* (Schmidt, 1869) has a similar structure and spiculation as our specimens (and many *Grantia* species), but the peculiar atrial network forms a unique distinction. *Teichonopsis labyrinthica* (Carter, 1878), vaguely resembles preserved specimens of the present species, but the spiculation differs clearly by the lack of any tetractines and the presence of brushes of small oxeas on the cortical and atrial surfaces. *Grantia* itself, in contrast, has approximately 40 accepted species (Van Soest et al. 2015), with considerable variability of habit and skeletal characters. In order to be able to judge whether the unique features of *waguensis* merit a separate genus status like *Sycandra utriculus* and *Teichonopsis labyrinthica*, or whether it can be assigned to *Grantia* s.l., we here compare our observations on *waguensis* with those of the type species of *Grantia* (and indeed the type of the family Grantiidae), *Grantia compressa*, the well known Purse Sponge of intertidal rocky coasts of Northern Europe. Remarkably, neither the Systema Porifera, chapter on Leucosolenida (Borojevic et al. 2002b), nor its preview publication (Borojevic et al. 2000), presented a proper description and illustration of this important species. We chose a specimen from Roscoff, W coast of France (intertidal, coll. D.A.G. Buizer, February 1977), in the collections of Naturalis Biodiversity Center, reg. nr. ZMA Por. 04159, as our object for comparison.

The sample we studied consisted of a cluster (Fig. 6a) of smaller and larger oval, laterally flattened, individuals, 0.5–4 cm in largest dimension, less than 0.5 cm thick, with terminal small oscules without visible rim. Color off-white, both in situ and in preserved condition. No change in shape when preserved. Skeleton (Fig. 6b) as usual for the family consisting of a cortex, tubar skeleton, and atrial skeleton. The cortical skeleton contains clusters or bouquets of club-shaped diactines overlying a thin (?single) layer of triactines. The tubar skeleton is very regular, made up of a row of sagittal triactines arranged with the unpaired angle pointing outwards. Subatrial triactines are overlying the atrial skeleton. There are rounded lacunae both subdermally and subatrially. The atrial skeleton is made up of atrial tetractines and triactines in variable proportions, the apical actines of the tetractines protrude into the atrial lumen. Apopyles do not have special spicules or skeletal specialization (Figs 6c–d). Spicules (Fig. 7) include cortical diactines (Figs 7a, a1) of 150–350 × 11–15 µm,

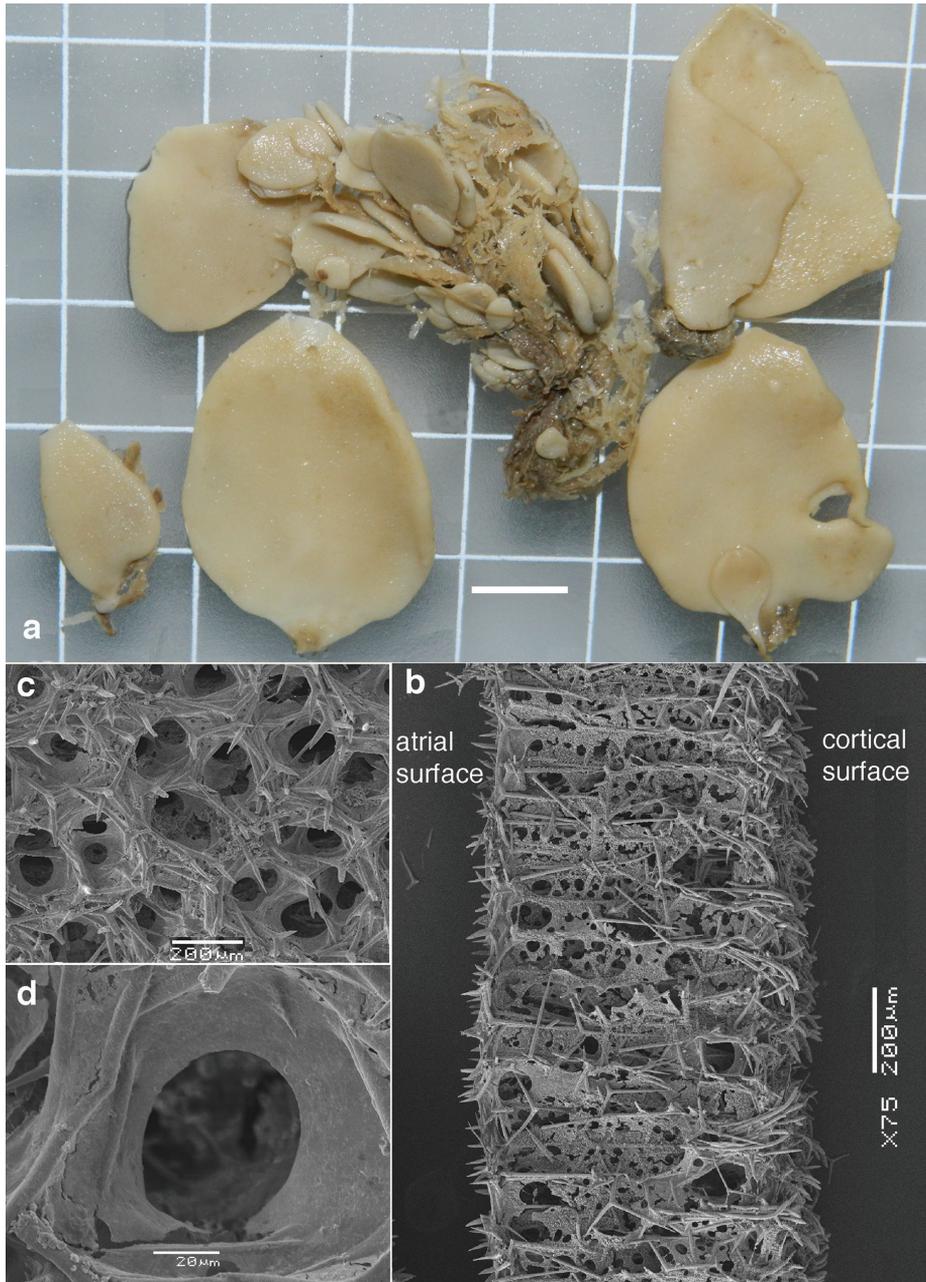


Figure 6. Habitus and skeleton of *Grantia compressa* from Roscoff, W coast of France (ZMA Por. 04159). **a** preserved specimens showing leaf-like habitus (scale bar = 1 cm) **b** SEM image of cross section of the leaf-like wall, showing the cortical brushes of club-shaped diactines and cortical triactines, rows of tubar triactines ending near the atrial region with subatrial triactines, closed off by mixed layer of atrial triactines and tetractines with the apical actines of the tetractines protruding beyond the atrial surface **c** overview of atrial surface and atrial chambers with protruding apical actines of the atrial tetractines **d** detail of atrial chamber lacking differentiated spicules.

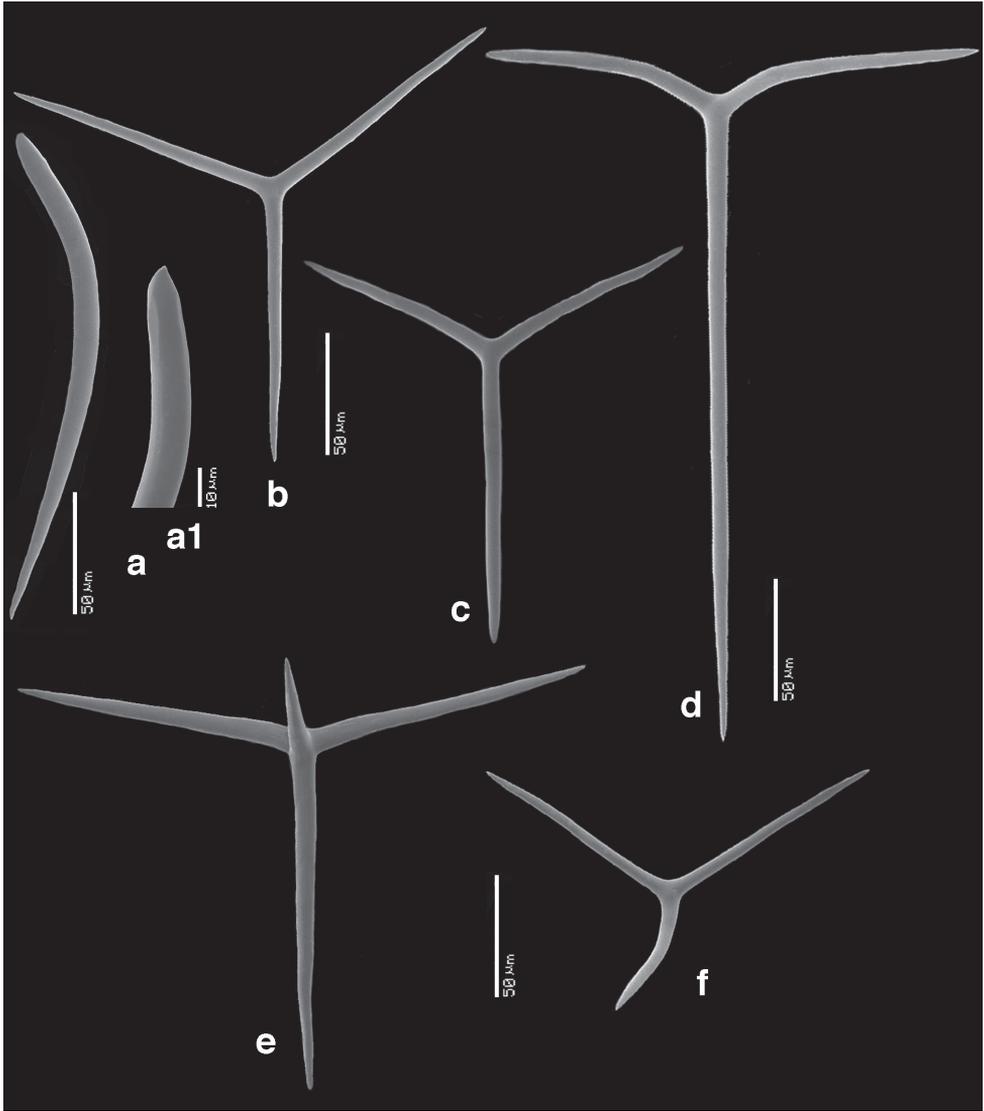


Figure 7. SEM images of the spicules of *Grantia compressa* (ZMA Por. 4159, from Roscoff, W coast of France). **a** club-shaped cortical diactine **a1** detail of head **b** cortical triactine **c** tubar triactine **d** subatrial triactine, e. atrial tetractines, f. atrial triactine.

cortical triactines (Fig. 7b) $88\text{--}140 \times 7\text{--}10 \mu\text{m}$, tubar triactines (Fig. 7c) $165\text{--}200 \times 7\text{--}9 \mu\text{m}$ (unpaired) and $88\text{--}102 \times 7.5\text{--}9 \mu\text{m}$ (paired), subatrial triactines (Fig. 7d) $255\text{--}325 \times 9\text{--}10 \mu\text{m}$ (unpaired) and $115\text{--}168 \times 9\text{--}10 \mu\text{m}$ (paired), atrial tetractines (Fig. 7e) $110\text{--}128 \times 7.5\text{--}10 \mu\text{m}$ (unpaired), $76\text{--}118 \times 7\text{--}9 \mu\text{m}$ (paired) and $25\text{--}105 \times 7\text{--}11 \mu\text{m}$ (apical), and atrial triactines (Fig. 7f) $48\text{--}108 \times 7.5\text{--}10 \mu\text{m}$ (unpaired) and $102\text{--}126 \times 8\text{--}9 \mu\text{m}$ (paired).

Grantia compressa and *Paragrantia waguensis* differ in the following features:

- In life, *G.c.* is a flattened, purse-shaped sponge, *P.w.* is a cup or wide-mouthed tube.
- *G.c.* lacks a distinct fringe, *P.w.* has a clear, differently colored, fringe containing special spicules with sizes not occurring in the rest of the body.
- Cortical skeleton of *G.c.* has club-shaped diactines in clusters, lacking in *P.w.*
- Cortical skeleton of *G.c.* is thin and contains small triactines, *P.w.* has a thick cortical skeleton including much larger triactines.
- Tubar triactines, subatrial triactines, atrial tri- and tetractines of *G.c.* are all smaller and thinner than those of *P.w.*
- A special apopylar skeleton and spicules are lacking from *G.c.* and is the dominant feature in *P.w.*

To date the World Porifera Database (Van Soest et al. 2015) lists 41 accepted species of the genus *Grantia* (including '*G.*' *waguensis*). The accepted status of many of those species is uncertain, as there has been no recent revision of the genus and the names were taken more or less uncritically from Burton's (1963) monograph. The most recent addition to the genus, *Grantia kempfi*, was made by Borojevic and Peixinho (1976). Among the species of *Grantia* s.l. there appears to be a wide variety of shapes and skeletal features, possibly divisible into distinct types, which may eventually lead to the distinction of subgenera or genera, leaving the genus name *Grantia* restricted to those species that share the properties of the above described *G. compressa*. Candidate species for such a restricted *Grantia* appear to be *G. cupula* (Haeckel, 1872), *G. extusarticulata* (Carter, 1886), *G. fistulata* Carter (1886), *G. foliacea* Breitfuss (1898), *G. stylata* Hôzawa (1929), *G. tenuis* Urban (1908), *G. aculeata* Urban (1908), *G. transgrediens* Brøndstedt (1931) and *G. uchidai* Hôzawa & Tanita (1941).

Other, not further specified groups of species may be distinguished e.g. on the possession of long protruding diactines ('hairy' *Grantia*'s), or those lacking tetractines, etc.

All these species do not have the apopylar specialization of *P. waguensis*. However, a few species assigned to the genus *Grantia* do appear to have at least a special category of atrial spicules, next to the usual atrial tri- and tetractines, viz. smaller tetractines in the apopylar region in *G. atlantica* Ridley (1881) as redescribed by Borojevic and Peixinho (1976), and in *G. nipponica* Hôzawa (1918). Furthermore, special apopylar spicules in the form of small diactines with serrated apices occur in *G. ramulosa* Dendy (1924). Possibly, these species could be united within *Paragrantia* by expanding its definition to include special apopylar spicules without specifying their shape. Such a decision is beyond the goals of the present study.

Sequence data on *Paragrantia waguensis* and *Grantia compressa*

A further differentiation between the two type species of the genera *Paragrantia* and *Grantia* was obtained from sequences. The molecular classification of the Calcarea and

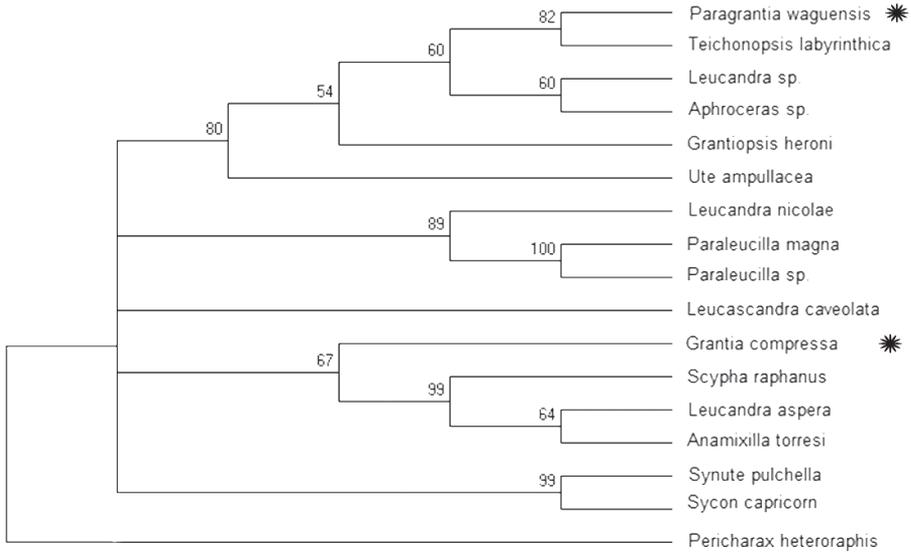


Figure 8. Phylogenetic tree (Maximum Likelihood, 50% majority consensus) of selected 28S partial sequences of calcareous sponge species belonging to the order Leucosolenida, including *Paragrantia waguensis* and *Grantia compressa* (asterisks). Sequences were downloaded from the NCBI website (<http://www.ncbi.nlm.nih.gov/>). The accession numbers and sources for the sequences are listed in Table 1. Bootstrap support values are based on 100 replicates. The species were selected on the basis of a BLAST search using a 430 bps 28S partial sequence of *Paragrantia waguensis* (specimen RMNH Por. 9317), provided by the Naturalis Biodiversity Center DNA laboratory. Sequences were aligned, trimmed and analyzed using MEGA 6.06 for Mac (<http://www.megasoftware.net/megamac.php>).

its subclasses, orders and families is still in its early stages (see e.g. Voigt et al. 2012), so it is not straightforward to submit sequences and draw conclusions about likely affinity of various calcareous sponges. However, 28S sequences of *Grantia compressa* were submitted to Genbank by Manuel et al. (2004), so that gave us the opportunity to compare it with our new molecular data on *Paragrantia waguensis* (cf. above in the Methods section). We obtained from an analysis of 17 aligned Calcaronea sequences (see Table 1), using the program MEGA, a provisional phylogenetic tree (Maximum Likelihood, 50% majority consensus). The tree (cf. Fig. 8) shows moderately significantly that the two species compared here are only distantly related. *Paragrantia waguensis* was shown to have *Teichonopsis cylindrica* as its nearest relative, and *Grantia compressa* was retrieved in an isolated position near *Anamixilla*, *Scypha* (= *Sycon*) *raphanus* and *Leucandra aspera*.

Conclusion

Until a revision of *Grantia* along the lines sketched above has been made – preferably guided by independent molecular markers of the studied taxa – we propose to

maintain *Paragrantia* as a separate genus, so far monotypic with *P. waguensis* as the only species. Its status is comparable to other such genera (*Sycandra* and *Teichonopsis*), recognizable by unique features, as in this case the unique butterfly-shaped apopylar tetractines, for which we introduce the term ‘aliactines’ (from ala (L.) = wing).

We propose here the following definition (modified from Hôzawa 1940: 43):

Genus *Paragrantia* Hôzawa, 1940

Type species. *Paragrantia waguensis* Hôzawa, 1940 (by monotypy)

Syconoid Grantiidae with cortical skeleton of triactines, articulate tubar skeleton composed of aligned triactines, and an oscular fringe with giant diactines and sagittal tri- and tetractines. Atrial skeleton composed of subatrial triactines, and atrial triactines and/or tetractines. Choanocyte chambers connect with the atrial lumen through apopylar chambers lined with modified specialized tetractine spicules (aliactines).

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Lepidasthenia lobo sp. n. from Puerto Madryn, Argentina (Polychaeta, Polynoidae)

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Abstract

Among polychaetes, polynoids have the highest number of symbiotic species found living with a wide variety of marine invertebrates, including other polychaetes. *Lepidasthenia* Malmgren, 1867 and *Lepidametria* Webster, 1879 were regarded as synonyms but belong to different subfamilies, although both have species associated with thelepodid or terebellid polychaetes. In this contribution *Lepidasthenia lobo* sp. n. is described from several specimens associated with the thelepodid *Thelepus antarcticus* Kinberg, 1867, collected on a rocky shore near Puerto Madryn, Argentina. *Lepidasthenia lobo* sp. n. can be confused with *L. esbelta* Amaral & Nonato, 1982 because both live with *Thelepus*, are of similar sizes with similar pigmentation patterns, and have giant neurochaetae. However, in *L. lobo* sp. n. all eyes are of the same size, cephalic and parapodial cirri are tapered and mucronate, the second pair of elytra is larger than the third, the ventral cirri arise at the base of parapodia such that they do not reach chaetal lobe tips, and neuracilae are tapered. On the contrary, in *L. esbelta* the posterior eyes are larger than anterior ones, cephalic and parapodial appendages are swollen subdistally, the second and third pairs of elytra are of the same size, the ventral cirri arise medially such that their tips reach the neurochaetal lobe tips, and the neuracilae have falcate tips. Some comments about other genera in the Lepidastheniinae, a simplified key to its genera, and a key to *Lepidasthenia* species with giant neurochaetae are also included.

Keywords

Thelepodidae, Terebellidae, *Lepidametria*, symbiosis, giant chaetae, Lepidastheniinae

Introduction

The polychaete family Polynoidae has the highest number of species involved in symbiotic relationships (Okuda 1936, Pettibone 1953, Martín and Britayev 1998). They are associated with other invertebrates such as echinoderms, echinurans (Anker et al. 2005), enteropneusts, sipunculans, hermit crabs (Achari 1974), burrowing shrimps (Sato et al. 2001), mollusks, octocorals, and tube-dwelling polychaetes belonging to the families Capitellidae, Chaetopteridae, Maldanidae and Terebellidae (Gibbs 1969).

There are few detailed studies on the relationships between polynoids, and their thelepodid or terebellid hosts. From a physiological perspective, Morgan (1974) studied the interaction between the thelepodid *Thelepus crispus* Johnson, 1901 and the polynoid *Halosydna brevisetosa* Kinberg, 1856 which can also be free-living. His main results were that 1) each thelepodid hosts a single polynoid; 2) their body size is proportional to each other indicating a long-term relationship; and 3) free-living polynoids of the same species do not react to the thelepodids hosting commensal members of the same species, such that contact is made by random encounter. McDermott (2005) found that specimens of *Lepidametria commensalis* Webster, 1879 were found in 65% of the terebellid *Amphitrite ornata* (Leidy, 1855) tubes he collected (42% also had a pinnotherid crab), but no further details on terebellids were included because pinnotherid crabs were the main area of interest.

Gravier (1905), Potts (1910), and Fauvel (1917) regarded *Lepidasthenia* and *Lepidametria* as synonyms, but they have also been regarded as distinct genera (Salazar-Silva 2006, 2009). Their delineation has been confused and remains unsettled. For example, Day (1973:6) indicated that he had earlier (Day 1962:634) "... gave my reasons for regarding *Lepidametria* as a synonym of *Lepidasthenia*. Dr. Pettibone, who is making an intensive study of the Polynoidae informs me that *Lepidametria* is a valid genus and in deference to her opinion I have not changed the name of *Lepidametria commensalis*." Barnich and Fiege (2004) regarded *Lepidametria* as a valid genus in a recent revision, including a list of genera, key to genera, and comparative tables for Lepidastheniinae Pettibone, 1989. However, *Lepidametria* was not included in the subfamily because their parapodia differ from those of typical Lepidastheniinae; instead they placed it in the subfamily Lepidonotinae. Some additional comments are necessary for clarifying the current status of both genera and, once better defined, changes will be needed for the corresponding species lists in WoRMS (Fauchald 2014a, b).

There are no world-wide keys to species of *Lepidasthenia* Malmgren, 1867 or *Lepidametria* Webster, 1879. There are some keys available (Chamberlin 1919, Fauvel 1923, Seidler 1923), which are in need of updating, and there are some other later ones for species from Japan (Imajima and Hartman 1964), the temperate Eastern Pacific (Ruff 1995), and tropical America (Salazar-Silva 2009).

Some authors have dealt with the delineation of what we now regard as Lepidastheniinae, either by making direct comments on some morphological attributes or by

indirect indications of their relevance by including them in keys. Their ideas, especially when they are convergent, are followed in the following sections, especially those made by Seidler (1923), Averintsev and Ushakov (1977), Uschakov (1982), Hanley and Burke (1991), Salazar-Silva (2006, 2009), and Wehe (2006).

In this contribution, *Lepidasthenia loboï* sp. n. is described from specimens collected on a rocky shore nearby Puerto Madryn, Argentina, associated with the thelepodid *Thelepus antarcticus* Kinberg, 1867. Remarks on other genera in the Lepidastheniinae, together with some others about morphology, a simplified key to lepidastheniini genera, and a key to *Lepidasthenia* species with giant neurochaetae are also included.

Material and methods

Field sampling. Cerro Avanzado is a large coastal mountain nearby Puerto Madryn, Argentina, and the same name applies to the nearby shore. There, a long sandy beach is bordered by rocky outgroups; rocks are mudstones which are easily bored through or broken apart. A hammer was used to crack apart larger rocks into fist-sized portions which were brought to the Centro Nacional Patagónico (CENPAT) facilities where specimens were removed from these fragments. Polychaetes were placed in tap water to relax them, then fixed in a 10% formalin solution. After 24 h they were rinsed with tap water and were preserved in 70% ethanol.

Specimens. Polynoids, thelepodids and terebellids were identified at El Colegio de la Frontera Sur (ECOSUR). Plates were arranged by compressing a series of digital photographs with HeliconFocus.

Type specimens deposition. Type and non-type specimens were deposited in the following institutions:

- ECOSUR** Colección de Referencia, El Colegio de la Frontera Sur, Chetumal, México.
- LACM** Allan Hancock Foundation Polychaete Collection, Natural History Museum of Los Angeles County, Los Angeles, U.S.A.
- MACN** Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina.
- MZUSP** Museo de Zoología, Universidad Federal de São Paulo, Brazil.
- ZUEC** Museo de Zoología, Universidad Estadual de Campinas, Campinas, Brazil

Restrictions for keys. There is one key to Lepidastheniinae genera available (Barnich and Fiege 2004). The present key, by contrast, is simpler to follow as it does not require complete specimens. However, a restriction was made to include only those species provided with giant neurochaetae, which are also present in *Polynoe elegans* Grube, 1840, the type species for *Lepidasthenia*.

Results

Morphological characters

Body. The number of chaetigers can be useful but because this number changes as the animals grow, and their bodies are delicate and fragment easily, this cannot be a diagnostic feature. Likewise, pigmentation and its intensity might be size-dependent as well and vary according to how long specimens have been stored in alcohol. However, there are some interesting differences regarding pigmentation patterns involving a series of pigmented segments, their number, and the relative shape of segmental bands, although growth abnormalities or regeneration might slightly alter these patterns.

Prostomium. The shape and relative length of antennae are useful diagnostic features; antennae can be tapered or subdistally swollen. The relative size of eyes, being either of about the same size, or one pair markedly larger than the other, together with their position on the prostomial surface (variable in relation to lateral margins) are also useful. The relative length of antennae has not been used but could help to separate similar species, with caution as they can sometimes be lost or undergoing regeneration, such that observations on more than a single specimen are desirable.

First chaetiger. Some species have an anterior projection over the prostomium, and its surface and margins can be papillated or smooth. Further, in *Lepidametria* species there are notochaetae in the tentaculophore.

Parapodia. Parapodial cirri can be basally swollen, tapered or subdistally swollen, and the relative length and width of cirrophores *versus* cirrostyles are relevant as well. It is useful to note the relative size of dorsal and ventral cirri to each other, and to the tip of neurochaetal lobes. Parapodial surfaces are usually smooth in *Lepidasthenia*, often papillated in *Lepidametria*, and rugose, or markedly folded in *Perolepis*. The presence of parapodial papillae is used to group similar polynoid genera (Pettibone 1977), and we think their spatial arrangement could be useful to separate species as well.

Elytra. The body can have elytra along its length, or only through to medial chaetigers (*Branchipolynoe*). For those having elytra along the body, their relative size as an indication of how much of dorsum is covered, or if they abut successive or other elytra along the mid-dorsal line, are diagnostic features. In general, most elytra of *Lepidasthenia* are small, often markedly reduced from chaetigers 2–3, whereas in *Lepidametria* they can be of about the same size along the body, either touching along the middorsal line, or leaving a wide dorsal area uncovered. In the posterior region the elytra and cirri can be alternating, or elytra can be present on every three segments. Elytral pigmentation patterns are also useful since it can be solid or homogeneous, black, grayish or pale, have a pigmented spot near the junction or insertion region, or form a band from the insertion region.

Chaetae. Notochaetae are present in many *Lepidametria* species, at least along anterior chaetigers, but never present in *Lepidasthenia* species. Neurochaetae of different shapes and relative width are present in *Lepidasthenia*, although they are usually of decreasing length from the superior to the inferior most. They can be finely dentate with a single series of marginal teeth, or more frequently provided with a series of paired rounded stiff blades, each blade with finely denticulate margins, as originally indicated

by Potts (1910:344), by Moore (1903:420) who called them transverse combs, and by Chamberlin (1919:50) who referred to them as lanceolate scales. They have been regarded as spines as well, but when seen from the front, they have a wide distal area, not a narrower one as is usually the case for typical spines, and the margins are not smooth but crenulate or denticulate. Neurochaetal tips can be entire or bidentate, and sometimes there can be one pair of lateral subdistal spines or teeth; the relative size of distal teeth and the orientation of their tips can also be diagnostic. Most chaetae are yellowish and of similar width, but sometimes superior chaetae are markedly thicker and darker, honey or light brown in color; some authors have called them giant chaetae.

Systematics

Family Polynoidae Kinberg, 1856

Subfamily Lepidonotinae Willey, 1902

Lepidametria Webster, 1879

(incl. *Nectochaeta* von Marenzeller, 1892, *Harmopsides* Chamberlin, 1919, and *Bouchiria* Wesenberg-Lund, 1949).

Type species. *Lepidametria commensalis* Webster, 1879, by monotypy.

Diagnosis (modif. Webster 1879). Body long with up to 80 segments. Elytrae large, covering body or leaving a narrow dorsal surface uncovered; posterior region with elytra and cirri alternating every other segment. Tentaculophores with chaetae. Notopodia reduced, with fine notochaetae at least along anterior and median segments, rarely absent. Neuropodia projecting with several types of neurochaetae. Ventral surface often papillated.

Remarks. *Lepidasthenia* and *Lepidametria* were regarded as synonyms by Gravier (1905), Potts (1910), Fauvel (1917), and Day (1962). Gravier indicated that the main difference was the presence of notochaetae in the latter but at the same time, he apparently rejected this difference by indicating that “*Lepidasthenia elegans* Grube a précisément une rame dorsale rudimentaire”. [Transl.: *L. elegans* precisely has a rudimentary dorsal branch]. However, the diagnostic feature of missing notochaetae does not denote the presence of a notopodial lobe, but rather a complete absence of notochaetae. Consequently, we think that this is an important difference that can be used to easily separate these two genera. This same approach has been useful for separating other genera, and has been implied by the keys by Fauvel (1923:87), or directly by Fauchald (1977:59).

The pattern of the presence of elytra on posterior segments in *Lepidametria* and *Lepidasthenia* made Uschakov (1982), and Hanley and Burke (1991) regard them as distinct genera; i.e., *Lepidametria* has alternating elytra and cirri, whereas *Lepidasthenia* has elytra on every third segment in medial and posterior regions. Barnich and Fiege (2004) indicated they were following these conclusions and regarded *Lepidametria* as a

member of Lepidonotinae, not Lepidastheniinae. Their reason was that in *Lepidametria* (Barnich and Fiege 2004:864): “parapodia differ significantly in their shape from those of the members of the Lepidastheniinae. In the Lepidastheniinae neuropodia are well developed, rather elongate, and distinctly notched dorsally and ventrally, while in *Lepidametria* neuropodia are well developed, but shorter, and not distinctly notched, which is typical for the Lepidonotinae Willey, 1902”.

It is unfortunate that there is no redescription for the type species of *Lepidametria*. The only illustrations available do not show this lepidastheniin notch; however, one figure shows that their neuropodia are not short (Pettibone 1963:21, fig. 4g, k). In the original description Webster (1879:12) indicated (*italics added*): “Ventral ramus of foot stout, elongate, conical, *widely excavated for the transmission of the setae*, and obliquely truncated from above downward.” This phrase in *italics* could be taken as equivalent to a notched neuropodium, however. The presence of chaetae in the tentaculophore, another non-lepidastheniin feature, was overlooked by Webster, and by Pettibone (1963:20), who regarded *Lepidametria* as a valid genus. Gardiner (1976, fig. 1n) illustrated the presence of chaetae in the tentaculophores, but his figure was based upon non-topotype specimens. After the syntype material was examined by one of us (PSS), we concluded that in *L. commensalis* there are chaetae in the tentaculophore and that parapodia differ from those present in Lepidastheniinae. Consequently, *Lepidametria* does not belong in this subfamily but in Lepidonotinae, as previously indicated by Barnich and Fiege (2004).

Subfamily Lepidastheniinae Pettibone, 1989

Type genus. *Lepidasthenia* Malmgren, 1867, by original designation.

Diagnosis (Modif. Barnich and Fiege 2004). Prostomium with median and lateral antennae; lateral antennae terminal, ceratophores distinct. Tentaculophores without chaetae. Palps visible dorsally. Pharynx with jaws and border papillae. Dorsal tubercles indistinct. Dorsal cirrophores without filamentous organs, sometimes with lateral projections. Notopodia reduced, notochaetae usually missing. Neuropodia distally truncate, elongate, notched dorsally and ventrally, forming subequal anterior and posterior lobes; no supra-acicular processes.

Remarks. Pettibone (1989:301) listed six genera as belonging to Lepidastheniinae: *Alentiana* Hartman, 1942, *Benhamipolynoe* Pettibone, 1970, *Hyperhalosydna* Augener, 1922, *Lepidasthenia*, *Perolepis* Ehlers, 1908, and *Telolepidasthenia* Augener & Pettibone in Pettibone, 1970. The review by Barnich and Fiege (2004) is followed regarding the composition and affinities in Lepidastheniinae.

Key to genera of Lepidastheniinae Pettibone, 1989

- 1 Body with elytrae continued through posterior segments; sometimes reduced in size in medial and posterior segments **2**
- Body with elytra limited to anterior and medial regions..... **10**

2	Notochaetae present	3
–	Notochaetae absent; parapodial surface usually smooth	4
3	Lateral antennae with ceratophores as long as wide; dorsal cirri about three times longer than ventral ones (numerous segments, up to 90 pairs of elytra) ...	
 <i>Lepidastheniella</i> Monro, 1924	
–	Lateral antennae with ceratophores twice as long as wide; dorsal cirri 6–7 times longer than ventral ones (reduced number of segments, 15 pairs of elytra)	
 <i>Parahalosydna</i> Horst, 1915	
4	Elytra alternate with cirri in medial and posterior regions	5
–	Elytra present every third segment in medial and posterior regions	7
5	First chaetiger with a middorsal anterior projection over prostomium; neurochaetae unidentate with two subdistal teeth; elytra smooth	
 <i>Showapolynoe</i> Imajima, 1997	
–	First chaetiger without anterior projection; elytra with microtubercles.....	6
6	Neurochaetae mostly bidentate with series of 10–20 lamellae; elytral microtubercles along exposed area.....	
 <i>Hyperhalosydna</i> Augener, 1922	
–	Neurochaetae only unidentates with series of about 10 tiny lamellae; elytral microtubercles scattered	
 <i>Benhamipolynoe</i> Pettibone, 1970 (partim)	
7	Elytrophores elongated, pedunculate; ventral cirri sometimes irregularly swollen	
 <i>Perolepis</i> Ehlers, 1908	
–	Elytrophores short, not transformed into peduncles; ventral cirri tapered or subdistally swollen	8
8	Medial segments with large elytra, overlapping successive ones or approaching middorsally	9
–	Medial segments with tiny, non-overlapping elytra	
 <i>Lepidasthenia</i> Malmgren, 1867	
9	Eyes small, on prostomial upper surface; ventral parapodial surface papillate; all neurochaetae of a single type, with about 20 series of lamellae	
 <i>Telolepidasthenia</i> Pettibone, 1970	
–	Eyes large, on prostomial margins; ventral parapodial surface smooth; neurochaetae of three types: lamellate, denticulate and smooth.....	
 <i>Alentiana</i> Hartman, 1942	
10	Notochaetae present; parapodial surface smooth; neurochaetae with rows of large lamellae	
 <i>Pseudopolynoe</i> Day, 1962	
–	Notochaetae absent; parapodial surface rugose; neurochaetae with rows of tiny lamellae	
 <i>Benhamipolynoe</i> Pettibone, 1970 (partim)	

***Lepidasthenia* Malmgren, 1867**

Type species. *Polynoe elegans* Grube, 1840, by monotypy.

Diagnosis (modif. Seidler 1923). Body long with up to 150 segments. Elytra small, not covering each other, leaving dorsal region mostly uncovered; posterior region with

one pair of elytra every three segments. Each elytron rounded, margins entire, without tubercles, pale or pigmented. Tentaculophores without chaetae. Notopodia reduced, without notochaetae. Neuropodia projecting with several types of neurochaetae. Ventral surface usually smooth.

Remarks. There was some confusion regarding the presence of notochaetae, but in the original diagnosis for *Lepidasthenia*, Malmgren (1867:15–16) indicated: “Ramus superior pedis perminutus acicula sola praeditus, setis omnino carens.” [Transl. Notopodium with minute acicula, chaetae entirely lacking]. An extended diagnosis was provided by Barnich et al. (2012: 406–407).

It must be emphasized that what can be regarded as the *Lepidasthenia* elytra-cirri pattern in the posterior region is shared by *Perolepis* and *Telolepidasthenia*. However, in *Perolepis* species the integument is usually rugose, and at least the first elytriphores are hypertrophied into distinct peduncles or stems, whereas in *Lepidasthenia* the integument is smooth and all elytriphores are reduced. Furthermore, in *Telolepidasthenia* elytrae are large, covering most of the dorsum, and all neurochaetae are unidentate, whereas in *Lepidasthenia* only the first elytra are large enough to touch each other and the remaining ones are reduced exposing the dorsum, and the dentition of the neurochaetal tips is variable.

***Lepidasthenia lobo* sp. n.**

<http://zoobank.org/75B1A4B8-4684-49AD-87EA-922AFC4384F2>

Figures 1–2

Lepidasthenia esbelta: Barnich et al. 2012: 406–407 (*non* Amaral & Nonato, 1982).

Type material. Southwestern Atlantic, Argentina. Cerro Avanzado, 16 km southward from Puerto Madryn (42°49'S, 65°04'W), Golfo Nuevo. Holotype (ECOSUR 176), and 12 paratypes, rocky shore, intertidal, in mudstone, within tubes of *Thelepus antarcticus* Kinberg, 1867, coll. 27 Feb. 2013, J.M. Orensanz, N.E. González & S.I. Salazar-Vallejo [Paratypes: Two (ECOSUR 177), 45–64 mm long, 5–6 mm wide, 83–97 chaetigers; two paratypes (LACM 7040), 30–32 mm long, 4 mm wide, 63–77 chaetigers; two paratypes (MACN), 40–58 mm long, 4.0–4.5 mm wide, 78–90 chaetigers; three paratypes (MZUSP 2857), 12–42 mm long, 2–4 mm wide, 37–83 chaetigers; two paratypes (ZUEC 17781, 17782), 36–39 mm long, 4 mm wide, 79–80 chaetigers.

Additional material. Southwestern Atlantic, Argentina. One specimen (ECOSUR), San Antonio Oeste (40°44'S, 64°57'W), Golfo San Matías, 3 m, coll. 10 Oct. 2005, J.M. Orensanz (30 mm long, 4 mm wide, 70 chaetigers). Six anterior fragments (ECOSUR), Cerro Avanzado, 16 km southward from Puerto Madryn (42°49'S, 65°04'W), rocky shore, intertidal, in mudstone, with *Thelepus antarcticus* Kinberg, 1867, coll. 27 Feb. 2013, J.M. Orensanz, N.E. González & S.I. Salazar-Vallejo.

Description. Holotype (ECOSUR 176) twisted, almost complete (without anal cirri). Body 64 mm long, 5 mm wide (at chaetiger 1, without chaetae), 99 chaetigers.

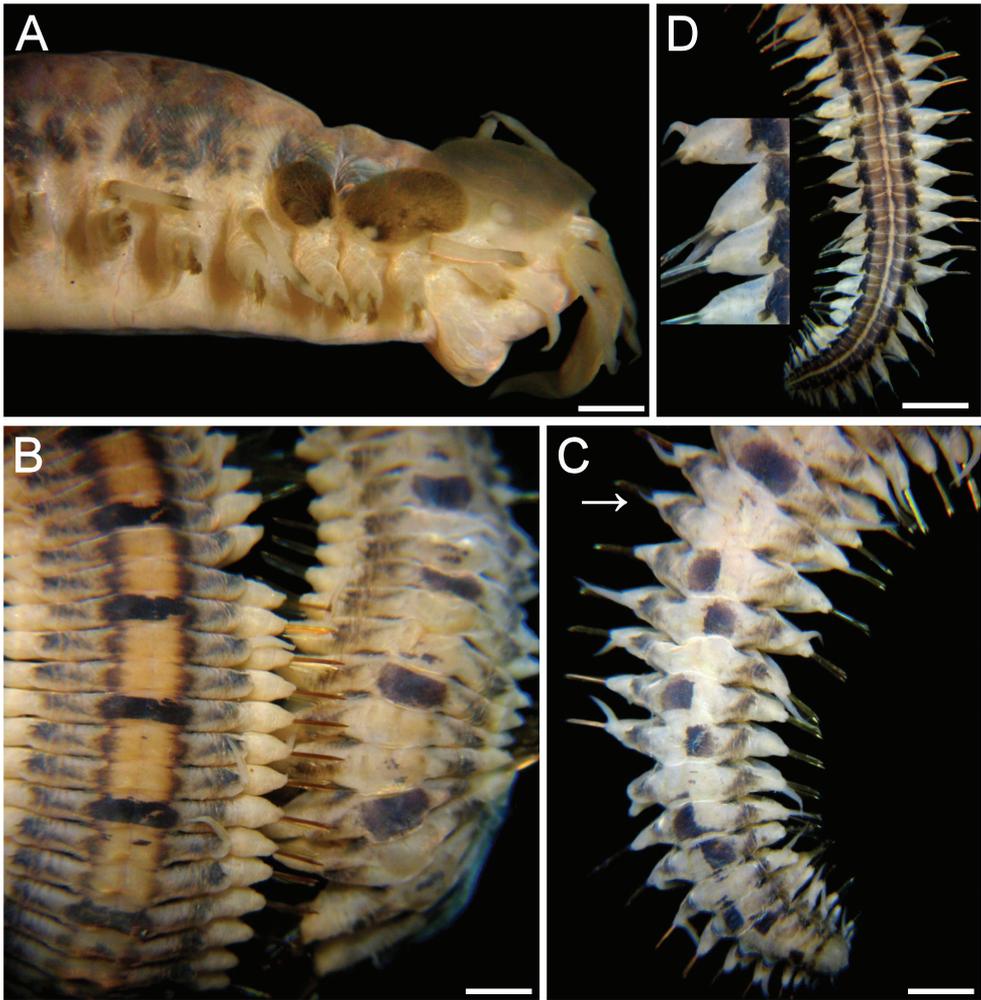


Figure 1. *Lepidasthenia loboï* sp. n., holotype (ECOSUR 176) **A** Anterior end, right lateral view **B** Dorsal surfaces, medial (left) and posterior (right) regions **C** Dorsal surface, posterior end (arrow points to an asymmetrical parapodium) **D** Ventral surface, posterior end (inset: close-up of right parapodia). Bars: 1.1 mm (**A**), 0.5 mm (**B**, **D**), 1.3 mm (**C**).

Antennae, palps and tentacular cirri pale. Dorsal cirri with blackish cirrophore, cirrostyles with subdistal blackish ring, tips pale (Fig. 1A). Dorsum with almost continuous thick, lateral, longitudinal dark-brown bands; bands continuous in chaetigers 1–4, medial areas paler; chaetiger 5 pale, alternating with blackish transverse band (Fig. 1B, C). First transverse band (chaetiger 6) slightly longer than corresponding segment, followed by irregular transverse bands occupying slightly more than half of segment length along 6 chaetigers, middorsal areas with irregular brownish spots, bands then alternating to chaetiger 21, thereafter darker bands every three segments but intermediate segments paler, maculated. First elytra greyish, largest (Fig. 1A); following ones

blackish, markedly smaller. Venter smooth; anterior third pale, posterior two-thirds with discontinuous darker, blackish bands along nephridial lobes areas; midventral region slightly less pigmented, ventral chord area paler (Fig. 1D). Far posterior segments with darker pigmentation ventrally. Nephridial papillae projecting, dark.

Prostomium with eyes black, medium-sized (as wide as antennal width), central on prostomium; anterior eyes more separated than posterior ones (Fig. 2A). Medial antenna slightly longer than laterals; ceratophores of similar width, slightly longer than wide; ceratostyles tapered, with long tips. Palps 3–4 times thicker than antennae, right one 2.5 times longer than medial antenna, left one regenerating. Segment 1 with tentacular cirri 1.3 times as long as, and slightly thicker than, antennae, tapered, with long tips mucro.

Elytra on segments 2, 4, 5, alternating with dorsal cirri to chaetiger 26, thereafter on every three segments but last 7 segments more irregular. First pair of elytra largest, covering prostomium and middorsal region, grayish, slightly darker around junction area, laterally with a paler, thin area. Second pair of elytra blackish, oval, less than half as large as first elytra, slightly overlapping anterior elytra, not covering middorsal region, laterally with a paler, thin area. Third pair of elytra blackish, subcircular, less than half as large as second elytra, non-overlapping with previous elytra, not covering middorsal region. Following elytra with same pigmentation, progressively reducing in size, up to chaetiger 20, about twice as large as junction area.

Parapodia sub-biramous throughout body. Notopodia reduced to a projecting, digitate lobe, reducing in size posteriorly. Neuropodia projecting lobes throughout body, neurochaetal lobes truncate or rounded. Dorsal cirri with cirrophores blackish, about as long as wide, cirrostyles tapered, with long tips, longer anteriorly, slightly reducing in length and pigmentation posteriorly, about twice as long as neuropodium. Ventral cirri small, tapered, basal-half blackish, tips mucronate, arising at base of parapodia, about as long as half neuropodial length.

Notopodia without notochaetae. Neurochaetae of different size and shape. Anterior chaetigers with about 15 neurochaetae per bundle, of similar width, smaller ventrally, each with bidentate tips, accessory tooth smaller, directed distally, and 10 or more series of subdistal lamellae (Fig. 2B, C). Medial chaetigers with one giant (thicker, more than twice as wide as other ones), brownish, superior neurochaeta with series of 5–6 tiny spines, tips unidentate, and about 10 thinner bidentate chaetae with series of 5–6 lamellae, becoming thinner and smaller ventrally (Fig. 2D, E). Posterior neuropodia with 1–2 slightly wider, superior chaetae and 4–5 thinner neurochaetae (Fig. 2F). Neuracillae hyaline, tapered.

Posterior region tapered; pygidium truncate, anus dorsal. Nephridial papillae from chaetiger 9; anterior region with papillae pale, smaller along anterior body half, progressively larger and darker in posterior body half.

Pharynx (observed in some paratypes) with marginal prismatic papillae, upper ones larger, 9 upper and 9 lower. Two pairs of dark brown jaws.

Etymology. This species name honors the late José María Orensanz, in recognition of his many contributions to the study of Southwestern Atlantic and Antarctic polychaetes, of his continued support of our research dreams, and for his participation

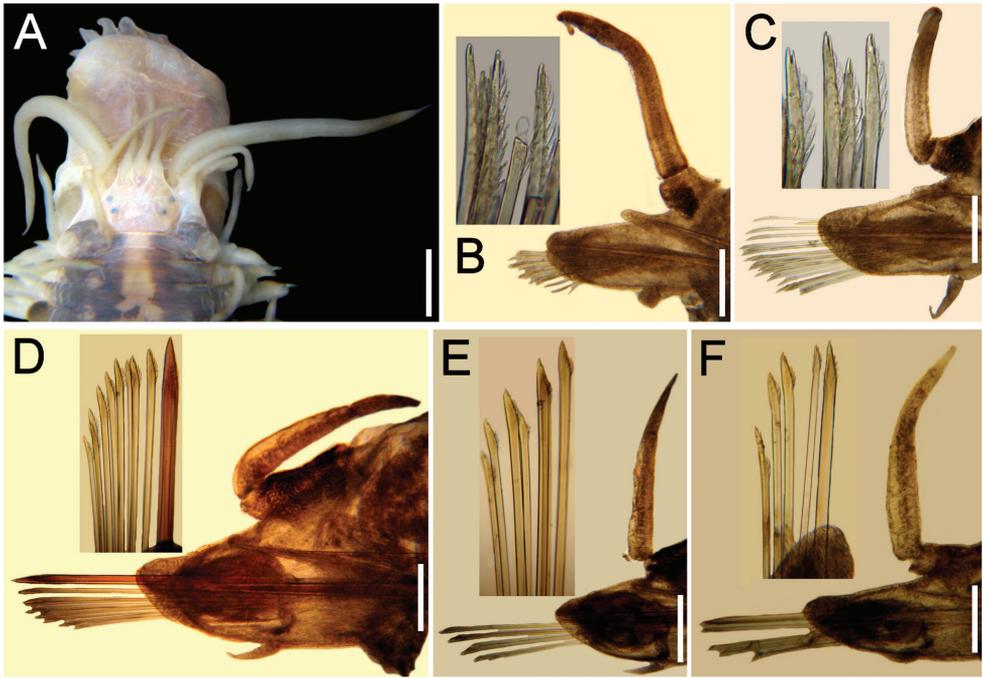


Figure 2. *Lepidasthenia lobo* sp. n. **A** Paratype (ECOSUR 177b), anterior end, dorsal view, first two pairs of elytra removed **B** Paratype (ECOSUR 177a), chaetiger 2, right parapodium, anterior view (inset: neurochaetal tips) **C** Same, chaetiger 9, right parapodium, anterior view (inset: neurochaetal tips) **D** Same, chaetiger 29, right parapodium, anterior view (inset: neurochaetae) **E** Same, chaetiger 59, right parapodium, anterior view, larger chaetae broken (inset: neurochaetae) **F** Same, chaetiger 80, right parapodium, anterior view (inset: neurochaetae). Bars: 0.1 mm (**A**), 40 μ m (**B–D**), 45 μ m (**E**), 25 μ m (**F**).

in the field trip that collected the species. The specific epithet is derived from his nickname, Lobo, and is a noun in apposition.

Type locality. Cerro Avanzado rocky shore, intertidal, Puerto Madryn (42°49'S, 65°04'W), Golfo Nuevo, Argentina.

Variation. Paratypes 12–64 mm long, 2–6 mm wide, 37–99 chaetigers. Smallest specimen with transverse bands restricted to anterior region; larger specimens more heavily pigmented and showing variation in the amount of spots or darkening of paler areas between successive transverse bands. Intensity of pigmentation increased in larger specimens, and in some (including holotype), posterior region had an irregular pattern probably due to imperfect regeneration, which is rather uncommon in other errant polychaetes (Yáñez-Rivera and Méndez 2014).

Remarks. *Lepidasthenia lobo* sp. n. has been confused with *L. esbelta* Amaral & Nonato, 1982, described from southern Brazil because both live with *Thelepus*, have similar size and pigmentation patterns, and giant neurochaetae. However, they differ in several diagnostic features such as the size of eyes, the type of cephalic and parapodial appendages, size of anterior elytra, topology of parapodial cirri, and tips of neuro-

ciculae. In *L. lobo* sp. n. eyes are of the same size, cephalic and parapodial cirri have long tapered tips, second pair of elytra is larger than third, ventral cirri arise basally to neuropodia such that they do not reach the tips of the chaetal lobe, and neuracaculae are tapered. On the contrary, in *L. esbelta* posterior eyes are larger than the anterior ones, cephalic and parapodial appendages are subdistally swollen, the second and third pairs of elytra are of the same size, ventral cirri are medially placed such that their tips reach the tips of the neurochaetal lobe, and the neuroacaculae have falcate tips.

Another species has been recorded from Brazil by Nonato and Luna (1970), and by Amaral and Nonato (1982): *Lepidasthenia virens* (Blanchard in Gay, 1849). These records indicate a lepidastheniian without notochaetae that resembles *L. lobo* because of the type of antennae and tentacular cirri, although palps are shorter than antennae, and by the relative size and position of parapodial cirri, although they illustrated a mature female with hypertrophied dorsal cirrophores. They gave no further detail and the affinities between these two species cannot be clarified. However, two issues deserve comments.

First, *L. virens* was described briefly with material from Calbuco (41°46'S, 73°08'W), Chiloé, Chile. The description and illustration indicates that elytra are large enough to touch each other along the body but while leaving the middorsal surface uncovered (Blanchard 1849:16, Pl. 1, Fig. 2: "... dejando descubierta la porción del medio del dorso, y en cuanto á la longitud del cuerpo apenas si se cubren"). Ehlers (1901:54, Pl. 3, Figs 10–16) described *L. irregularis* with material from the same locality; this species has elytra touching each other, leaving the middorsal surface bare, and notochaetae are present in anterior parapodia. If *L. virens* and *L. irregularis* are synonyms then they both belong in *Lepidametria* by having notochaetae and large elytra overlapping or touching successive ones.

Second, Hartman (1939: 46) noticed this synonymy but because she studied material from a more tropical region, her illustrations do not match Ehlers' ones. Her specimens have no notochaetae, and neurochaetae are very abundant (ca. 20 per bundle *vs* about 10 per bundle). Consequently, the Eastern tropical Pacific material belongs to another, probably undescribed species, and they differ from true *L. virens* (? = *L. irregularis*).

Ecological notes. *Thelepus antarcticus* Kinberg, 1867 builds its tubes with a mucoid protein forming a semi-transparent matrix with attached fragments of shells or other calcareous fragments. Tubes run inside rock crevices or fractures and are difficult to track individually because they break when the rock is fragmented. There were 34 *T. antarcticus* specimens plus six belonging to two other terebellid species, making it the most frequent thelepodid (or terebellid) in the rocky intertidal environment. About half of *L. lobo* specimens remained inside *Thelepus* tubes, whereas the others left the tubes as the rock was broken. In total, there were 19 *L. lobo* specimens taken at Cerro Avanzado, and there were polynoids in only one-third of the *Thelepus* tubes, half the rate of association between *T. crispus* and *H. brevisetosa* found by Morgan (1974). It would be interesting to conduct a more detailed study to find out what are the functional relationships between *T. antarcticus* and *L. lobo* sp. n. Some specimens exhibited regeneration of palps, antennae, or both, indicating there must be some in-

teractions with other invertebrates, possibly other scale-worms. Some of the anterior fragments were dissected for gut contents but none were found.

Distribution. The specimens were found in two localities in two southern Argentina Gulfs: Cerro Avanzado, Puerto Madryn, Golfo Nuevo, and San Antonio Oeste, Golfo San Matías, but might co-occur with *Thelepus antarcticus* throughout its distribution. Kinberg (1867: 345) described *T. antarcticus* from the intertidal in York Bay, Bucket Island, Magellan Strait. Hartman (1966:109) and Rozbaczylo et al. (2006: 83) regarded it as a junior synonym of *T. plagiostoma* (Schmarda, 1861: 41), described from New Zealand. However, this synonymy was not based upon a study of type material so these two species must be regarded as distinct until a future comparison involving type specimens indicates otherwise. The distribution of *T. antarcticus* would correspond to Patagonian shores, along southern Chile and Argentina, in intertidal to shallow water bottoms.

Key to species of *Lepidasthenia* Malmgren, 1867 with giant neurochaetae

- 1 Anterior eyes larger than posterior ones **2**
- Anterior eyes smaller or subequal to posterior ones **3**
- 2 Dorsal cirri subdistally swollen; ventral cirri digitate
 *L. elegans* (Grube, 1840), Mediterranean Sea
- Dorsal cirri tapered; ventral cirri basally swollen, tapered
 *L. ornata* Treadwell, 1937, Western Mexico*
- 3 Dorsal cirri subdistally swollen; ventral cirri tapered, surpassing neurochaetal lobe tip; neuroaciculae falcate *L. esbelta* Amaral & Nonato, 1982, Brazil
- Dorsal and ventral cirri tapered, ventral cirri short, not reaching neurochaetal lobe tip; neuraciculae tapered, straight *L. lobo* sp. n., Patagonia

* A junior synonym of *L. virens* (Blanchard in Gay, 1849) fide Hartman (1956: 271); they are probably different. *Lepidasthenia virens* was described from Chiloé, southern Chile, whereas *L. ornata* is from western Mexico. If *L. virens* is the same as *L. irregularis* Ehlers, 1901, both described from the same locality in Chile, and having large notopodia, the latter provided with notochaetae, then both belong in *Lepidametria*.

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A new genus and species of pennatulacean octocoral from equatorial West Africa (Cnidaria, Anthozoa, Virgulariidae)

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Abstract

A new genus and species of sea pen or virgulariid pennatulacean from the Gulf of Guinea in the tropical eastern Atlantic is described, and a key to the genera of the Virgulariidae is included. The new genus and species described here adds to the previously described five other genera of the family. It is distinguished by unique sclerite and polyp leaf characters from the superficially-similar genus *Virgularia*, which lacks conspicuous sclerites in the polyp leaves and coenenchyme (other than minute oval bodies that are generally <0.01 mm in length).

Keywords

Pennatulacea, Virgulariidae, sea pens, new genus and species, Gulf of Guinea, West Africa, Nigeria, key to the virgulariid genera

Introduction

The biogeographic region of the Mediterranean Sea and Atlantic coast of Africa is home to several apparently endemic octocoral genera, including the pennatulaceans *Amphibelemon* López-González, Gili & Williams, 2000 and *Crassophyllum* Tixier-Durivault, 1961 (Williams 2011: 4, fig. 2), as well as the alcyonacean *Nidaliopsis* Kükenthal, 1906 (Verseveldt and Bayer 1988: 63–66). Four colonies of a third such

pennatulacean has recently been revealed after over thirty years of storage in a museum marine invertebrate collection. These colonies from the Niger River Delta of Nigeria represent the only known specimens from the Niger River delta of a previously undescribed genus and species of sea pen.

Five genera were previously included in the family Virgulariidae (Williams, 1995: 122–125) – *Acanthoptilum* Kölliker, 1870; *Scytaliopsis* Gravier, 1906; *Scytalium* Herklotz, 1858; *Stylatula* Verrill, 1864; and *Virgularia* Lamarck, 1816. The new genus described here adds an additional genus. Consequently, a total of six genera are here recognized as comprising the family Virgulariidae.

Virgulariid sea pens are known to range from intertidal habitats to approximately 1200 m in depth (Williams 2011: 6, Fig.4). The ecological importance of sea pens has recently been increasingly recognized. Baillon et al. (2012) have shown that at least several species of deep-water pennatulaceans in the northwest Atlantic – including *Anthoptilum grandiflorum* (Verrill, 1879) and *Pennatula aculeata* Danielssen, 1860 – can act as nurseries for larval fish such as *Sebastes* spp., or can provide habitat for other fish species. In regards to virgulariid sea pens, often abundant constituents of endangered, impacted or protected soft bottom habitats such as *Stylatula elongata* (Gabb, 1863) in San Francisco Bay and the estuary regions of central California (Mooi et al. 2007: 27). Behavioral observations of species in the genus *Virgularia* regarding their unique ability of rapidly withdrawing the entire colony into the soft substratum, have been recorded for over 400 years. Darwin (1845: 98–100) describes his observation of the withdrawal and reappearance of a Patagonian species, and refers to a similar observation by Captain James Lancaster in Indonesia in 1601. Likewise, Ambroso, et al. (2013) describe their in situ observations of withdrawal behavior in *Virgularia mirabilis*.

It is the aim of this paper to describe a new genus and species of pennatulacean octocoral previously unknown to science, to name the new genus in recognition of the significant career contributions of a prominent octocoral systematist, and to differentiate the new genus from all other genera in the family based on morphological comparisons.

Materials and methods

Material for this study was revealed during a survey of the pennatulacean octocoral collection at the Museum Support Center (MSC), Smithsonian Institution, Washington DC (National Museum of Natural History, Department of Invertebrate Zoology) in April of 2013. An examination of the material showed that it represented a previously-undescribed genus and species of sea pen. The material was processed as a loan, and laboratory work was conducted at the California Academy of Sciences, San Francisco.

Scanning electron micrographs were made in the Academy's SEM laboratory with a LEO 1450 VP scanning electron microscope after coating the sclerites on a standard SEM pin stub mount (12.7 mm in diameter and 8 mm pin height) with gold/palladium.

Abbreviations used in text

USNM United States National Museum

NMNH National Museum of Natural History, Smithsonian Institution, Washington, D.C.

CASIZ California Academy of Sciences Invertebrate Zoology, San Francisco, California.

Systematic account

Family Virgulariidae Verrill, 1868

Genus *Grasshoffia* gen. n.

<http://zoobank.org/913459D9-D166-4A6E-B998-F8991C19BED4>

Figures 1–5

Generic diagnosis. Virgulariid pennatulaceans with polyp leaves rolled or convoluted; 20–26 polyps per polyp leaf; polyp leaves and coenenchyme contain rod-like, somewhat three-flanged sclerites, with parallel sides, broadly triangular at each end, 0.02 to 0.06 mm in length.

Type species. *Grasshoffia virgularioides* by original designation.

Etymology. The genus is named for Dr. Manfred Grasshoff in recognition of his important contributions to the systematics of octocorals, particularly gorgonians and pennatulaceans. He is currently Honorary Scientist at the Senckenberg Research Institute and Natural History Museum, Frankfurt, for his significant contributions to the taxonomy and evolution of octocoral cnidarians. From 1969–2001, Dr. Grasshoff headed the Marine Invertebrates Section at the Institute, where his main research objectives were the taxonomy of octocorals and the evolutionary biology of coelenterates, as well as more general aspects of evolution and phylogeny. From 1972 to 1989 he published several papers on deep-sea pennatulaceans from European and North Atlantic waters (Grasshoff 1972, 1973, 1982).

Grasshoffia virgularioides sp. n.

<http://zoobank.org/6D86C220-3DA9-49EE-98E2-E67EEA3290C1>

Figures 1–5

Species diagnosis. Virgulariid sea pens superficially resembling some species of *Virgularia*. Axis circular in transverse section, extending throughout virtually entire colony length. Polyp leaves variously rolled, funnel-shaped, or semicircular in shape with conspicuous, somewhat narrowed basal stalks. Sclerites of polyps leaves and coenenchyme rod-like with parallel sides and mostly deltoid apices, inconspicuously three-flanged. Preserved colony color cream-white in ethanol.

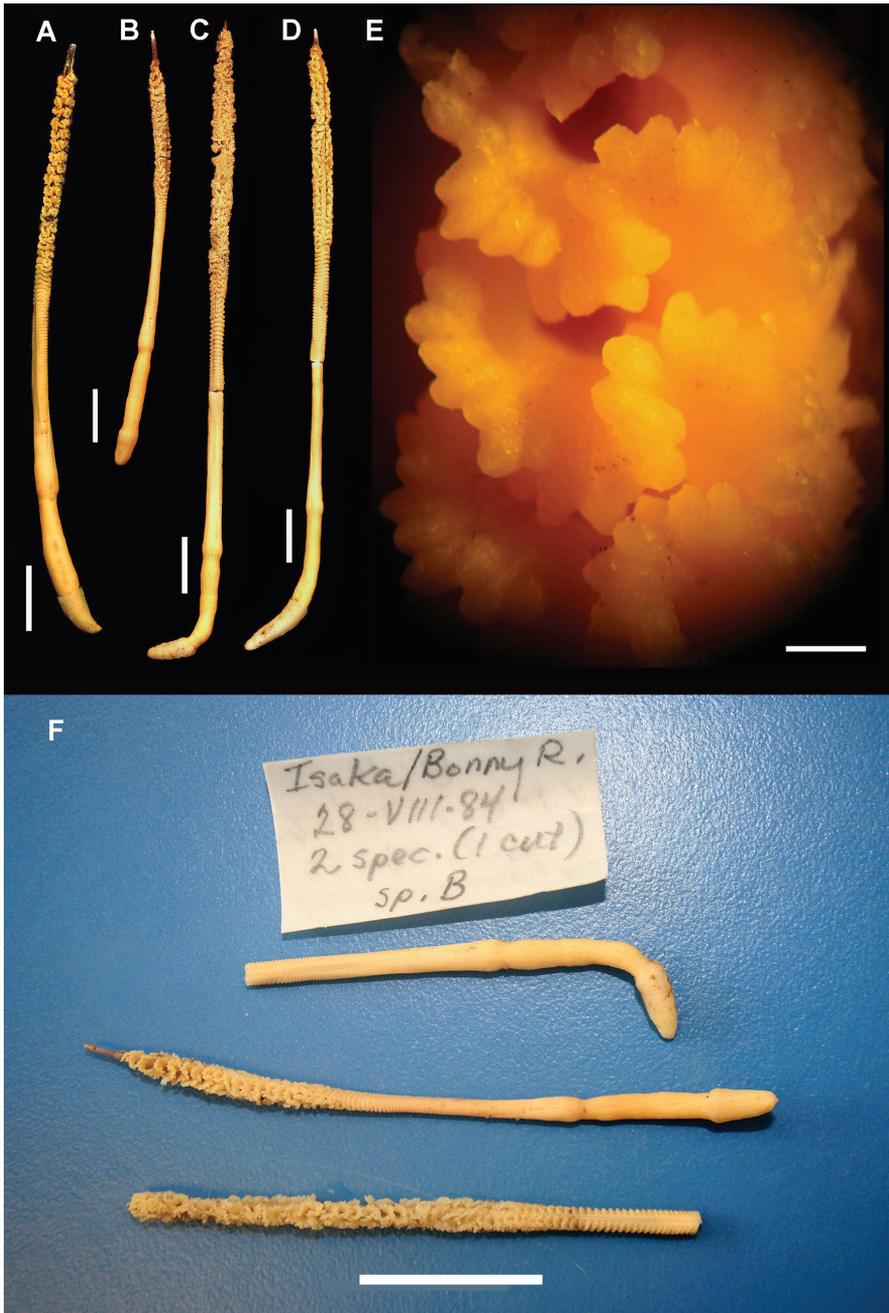


Figure 1. *Grasshoffia virgularioides* gen. & sp. n. External morphology. **A** Holotype (USNM 1205583) **B** Paratype 2 (USNM 1231550) **C** Paratype 1 (USNM 1231549) **D** Paratype 3 (USNM 1205580) **E** Detail of rachis of holotype showing polyps on convoluted polyp leaves **F** Material from the Smithsonian's Museum Support Center invertebrate zoology collections. Top, USNM 1231549, Paratype 1 (peduncle); Middle, USNM 1231550, Paratype 2 (entire specimen); Bottom: USNM 1231549, Paratype 1 (rachis). Scale bar: 20 mm (**F**); 0.5 mm (**E**); 20 mm (**F**); 10 mm (**A–D**).

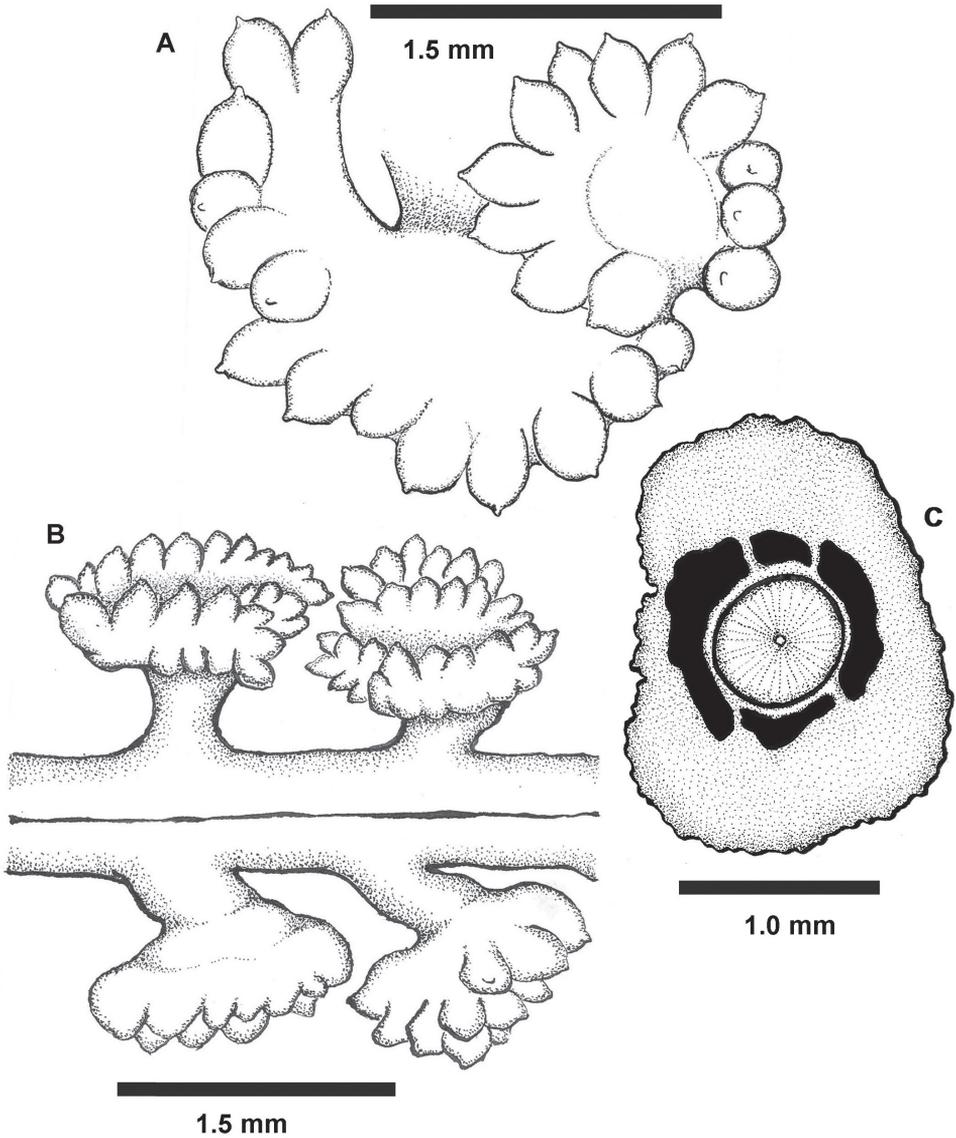


Figure 2. *Grasshoffia virgularioides* gen. & sp. n. Diagrams of polyp leaves from the holotype. **A** A single polyp leaf showing convoluted overall shape and numerous, bulbous, retracted polyps with acute apical tips **B** Dorsal side of the rachis showing congested placement of polyps on sub-circular polyp leaves attached to the rachis by narrow, neck-like stalks **C** Transverse section of paratype 1 (USNM 1231549) at the proximal-most level of the rachis, showing the circular axis and four surrounding longitudinal canals. Scale bar: 1.0 mm (**C**); 1.5 mm (**B**).



Figure 3. *Grasshoffia virgularioides* gen. & sp. n. Scanning electron micrographs of sclerites from a polyp leaf and polyp walls of the holotype. Scale bar: 0.01 mm.

Type material. Holotype: USNM 1205583, North Atlantic Ocean, Gulf of Guinea, Nigeria, Isaka, Bight of Bonny, Niger Delta, Bonny River; depth not recorded; 28 August 1984; wet-preserved 70% ethanol; one whole colony. Paratype 1: USNM 1231549, same data as holotype; one colony in two pieces 122 mm in total

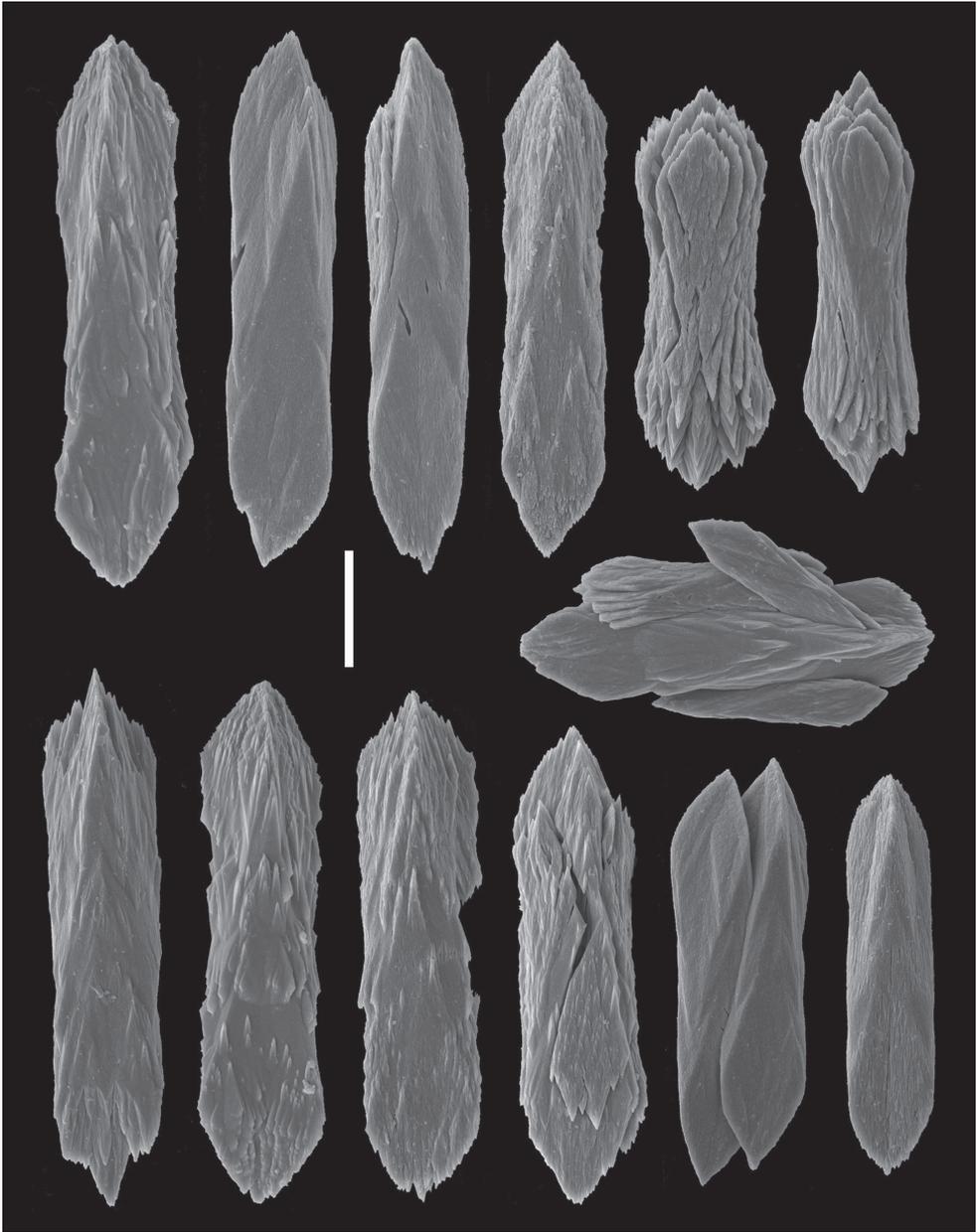


Figure 4. *Grasshoffia virgularioides* gen. & sp. n. Scanning electron micrographs of peduncle sclerites from the holotype. Scale bar: 0.01 mm.

length. Paratype 2: USNM 1231550, same data as holotype; one colony 82 mm in length. Paratype 3: USNM 1205580, same data holotype; one colony in two pieces 119 mm in total length.

Description. *Morphology* (Figures 1–2). The holotype is 98 mm in length. The axis extends throughout the length of the colony and is exposed for 5 mm at the distal-most region of the rachis. The axis is circular to broadly elliptical in cross section (Figure 2C), mottled brown and white in color (presumably due to a mixed content of calcitic and proteinaceous material), composed of narrow wedges of axial material radiating outward from the center, and is approximately 0.80–0.90 mm in diameter. The rachis is also approximately 0.80–0.90 mm in width and is densely congested with polyp leaves; there is approximately 1.5 mm of bare rachis between adjacent proximal bases of the polyp leaves. The polyp leaves are funnel-shaped or mushroom-shaped in lateral view. They broaden distally where the polyps reside, and have neck-like bases that narrow proximally and serve to attach the polyp leaves to the rachis (Figure 2B). The appearance of the polyp leaves are conspicuously rolled or convoluted (some are horseshoe-shaped), with approximately fourteen to twenty-six polyps per leaf (Figures 1A, 2A). The polyps are contractile and non-retractile, urn-shaped or teardrop-shaped and approximately 0.50–0.60 mm in diameter. Siphonozooids not apparent on the rachis or polyp leaves.

Sclerites (Figures 3–4). The distal region of the polyp leaves and the tissues of the peduncle contain numerous, small, rod-like sclerites that are prismatically-shaped with straight parallel sides, more-or-less three-flanged, mostly broadly-triangular in shape at each end, and vary in length from 0.02 to 0.06 mm.

Color (Figure 1). The color of the wet-preserved colonies is cream-white throughout.

Etymology. The specific epithet is derived from the genus *Virgularia* and the suffix *-oidea* (likeness of form); in reference to the superficial resemblance of the colonies to some species of the genus *Virgularia*.

Habitat and distribution. Habitat not known. Known only from the type locality – Niger River Delta, Nigeria, Gulf of Guinea, West Africa. Depth not recorded (Figure 5).

Differential diagnosis. *Virgularia* and *Scytaliopsis* do not have sclerites in the rachis or polyp leaves. *Scytalium* has ovoid plate-like sclerites in the polyp walls and polyp leaves that are not three-flanged. *Grasshoffia* has prismatically-shaped rod-like sclerites in the polyp leaves and coenenchyme that are indistinctly three-flanged and have broadly-triangular ends. Species of *Stylatula* have a fan-like armature of large spindles at the base of each polyp leaf, and most species of *Acanthoptilum* have a cluster of non-aligned spindles at the base of each polyp leaf. *Virgularia*, *Scytalium*, *Stylatula*, and *Acanthoptilum* generally have flattened polyp leaves that are variously-shaped, while *Grasshoffia* has strongly curved, rolled, often horseshow-shaped to funnel-shaped polyp leaves. *Stylatula macphersoni* López-González, Gili & Williams, 2001, has sclerites in the body walls of the autozooids that are similar in shape to coenenchymal sclerites in *Grasshoffia virgularioides* (López-González et al. 2001; 67, Fig. 4A).

Remarks. The coenenchyme covering the rachis is extremely thin, and therefore the rachis and axis diameters are virtually equal. The polyp leaves are distinctly rolled or conspicuously curved, perhaps due to contraction in the wet preserved type material, as the appearance of the living colonies is not known. Siphonozooids were not observed in the preserved type material, possibly due to the congested and contracted state of the polyp leaves along the rachis.



Figure 5. Map of the Gulf of Guinea, West Africa, showing type locality of *Grasshoffia virgularioides* gen. and sp. n. (arrow).

Discussion

Historical perspective

The family name “Virgularidae” was first proposed by Verrill (1868: 382), who included the genera *Virgularia* Lamarck, 1816 and *Stylatula* Verrill, 1864. Subsequently, Kölliker (1869: 123) used the family name “Virgulariaceae” to include the genera *Virgularia*, *Sylatula*, *Pavonaria* Kölliker, 1869, *Scytalium*, *Acanthoptilum*, *Funiculina* Lamarck, 1816, and *Halipterus* Kölliker, 1866. Gray (1860 and 1870) removed *Funiculina* from the Virgulariaceae and named the family “Funiculineae” in 1860, changing it to “Funiculinidae” in 1870. Kölliker (1880: 37) defined the family “Virgularidae” to include *Virgularia*, *Dübenia* Koren & Danielssen, 1877, *Stylatula*, *Acanthoptilum*, *Pavonaria*, and *Scytalium*. Jungersen (1904) placed *Pavonaria* in a separate family Pavonaridae. Bals (1910) agreed, but named the family Balticinidae instead of Pavonaridae. Kükenthal and Broch (1911) and Kükenthal (1915) divided the family Virgulariidae into two subfamilies – Pavonariinae to include *Pavonaria*, and Virgulariinae to include *Acanthoptilum*, *Scytaliopsis*, *Scytalium*, *Stylatula*, *Virgularia*. The family Virgulariidae was included by Kükenthal (1915) in the nominal and no longer recognized ranks – Suborder Subselliflorae and Section Pennatulina junciformia, and at the same time the family Halipteridae (Pavonaridae) was treated as a subfamily of the Virgulariidae. The revision of Williams (1995) treated the Halipteridae and Virgulariidae as separate families. Hickson (1916) recognized these two families – Virgulariidae

for *Virgularia* and *Stylatula* and Pavonariidae for *Osteocella* Gray, 1870 and *Pavonaria*. Williams (1990, 1995, 2015) recognized *Halipteris* (a genus of seven species) as the valid name for *Balticina* Gray, 1870, *Göndul* Koren & Danielssen, 1883, *Lygomorpha* Koren & Danielssen, 1877, *Microptilum* Kölliker, 1880, *Norticina* Gray, 1870, *Osteocella*, *Pavonaria*, *Stichoptilum* Grieg, 1887, and *Verrillia* Stearns, 1873. Williams (1995: 120) established the family Halipteridae to include the sole genus *Halipteris*, and recognized the Virgulariidae to include the five genera *Acanthoptilum*, *Scytaliopsis*, *Scytalium*, *Stylatula*, and *Virgularia*.

Taxonomic perspective

The Virgulariidae is here defined as follows (modified from Williams 1990: 86–87, 95 and Williams 1995: 97–98). Pennatulacean octocorals; usually elongate and slender to vermiform, feather-like in appearance in life, usually < 500 mm in length; axis well-developed and present throughout the length of the colony, round to quadrangular in transverse section; proximal portions of adjacent autozooids fused forming conspicuous polyp leaves (flattened expansions, often wing-like) that emanate laterally along the rachis in two opposite longitudinal series; polyp leaves thin and often translucent; polyps without calyces; rachis rod-shaped; peduncle slender and vermiform; siphonozooids present on polyp leaves or on rachis between polyp leaves; sclerites are spindles or rods often three-flanged, plates, minute ovals, or absent. The family is circumglobal in distribution with a depth range of 0–1100 m, and contains six genera – *Acanthoptilum*, *Scytaliopsis*, *Scytalium*, *Stylatula*, *Virgularia*, and *Grasshoffia* gen. n.

Key to the genera of the family Virgulariidae

- 1 Sclerites are present in the rachis and polyp leaves 2
- Sclerites are absent in the rachis and polyp leaves 5
- 2 Sclerites of the polyp leaves are conspicuous spindles or needle-like spindles (0.20–1.50 mm in length) 3
- Sclerites of the polyp leaves are small oval-shaped plates or slightly three-flanged rods (0.02–0.04 mm in length) 4
- 3 Needle-like sclerites form a strong fan-shaped armature at the base of each polyp leaf *Stylatula*
- Spindle-like sclerites form a weak cluster at the bases of the polyp leaves (but not as a fan), or they are scattered in the polyp leaves and autozooids (but not as basal clusters) *Acanthoptilum*
- 4 Numerous ovoid plate-like sclerites are present in the rachis and polyps leaves; sclerites red in color *Scytalium*

- Numerous prismatically-shaped rod-like sclerites, which are indistinctly three-flanged, are present in the polyp leaves and peduncle; sclerites colorless..... *Grasshoffia* **gen. n.** (Figs 1–4)
- 5 The polyps of a single polyp leaf are of equal size; number of polyps per polyp leaf are highly variable (3–100 or more) *Virgularia*
- The polyps on the inner portion of a single polyp leaf are smaller in size than those of the outer portion of the leaf; polyps per polyp leaf are few (4–7 in number)..... *Scytaliopsis*

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First record of the plant bug genus *Paramiridius* Miyamoto & Yasunaga (Heteroptera, Miridae, Mirinae) from Indochina, with descriptions of two new species from Laos

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Abstract

The mirine plant bug genus *Paramiridius*, previously known only from a single Taiwanese species, is reported from Indochinese Laos for the first time and redefined. Two additional species, *P. indochinensis* and *P. laomontanus*, are described as new to science. The female genitalic structures of the genus are documented for the first time. Habitus illustrations, figures of male genitalia, and key are provided for all three known *Paramiridius* species.

Keywords

Paramiridius, new species, taxonomy, key, Indochina, Laos, Heteroptera, Miridae, Mirinae, Mirini

Introduction

The mirine plant bug genus *Paramiridius* was proposed by Miyamoto and Yasunaga (1992) to accommodate a single species known only from Taiwan, *P. tigrinus* Miyamoto & Yasunaga. The genus is readily recognized by the moderate to rather large body with the conventional mirine shape and typical color pattern (yellow or yellowish green dorsum with dark stripes and maculae). However, no subsequent information has been available since the original description.

During recent field investigations undertaken by Seoul National University, 21 specimens, we perceived as belonging to *Paramiridius*, were collected. Upon closer examination, we can confirm that these Lao specimens represent two undescribed species of *Paramiridius*, herein we describe them as new to science. The present discovery also represents a range extension of the genus in Indochina. *Paramiridius* is redefined and diagnosed, and a key to all known species is provided. The female genitalic structures are examined and figured for the first time.

Materials and methods

All type specimens are deposited in the collection of Insect Biosystematics Laboratory, Research Institute for Agriculture and Life Science, Seoul National University, Korea (SNU). Digital images used in this paper were captured using a Diagnostic Instruments Insight Camera 14.2 Color Mosaic, with a SPOT Insight System. Specimens were dissected and observed under a Leica S8APO stereoscopic microscope.

All measurements (mean and range) are in millimeters. Terminology of the male and female genitalia primarily follows Yasunaga and Schwartz (2007), but some additional terms, such as ‘lateral lobal sclerite’ and ‘median lobal sclerite’ (Fig. 3), are used to indicate the taxonomic characters properly.

Results

Genus *Paramiridius* Miyamoto & Yasunaga

Paramiridius Miyamoto & Yasunaga, 1992: 93 (gen. n.), type species: *Paramiridius tigrinus* Miyamoto & Yasunaga, 1992: 94, original designation; Schuh 1995: 861 (cat.); Kerzhner and Josifov 1999: 136 (cat.).

Diagnosis. *Paramiridius* can be distinguished from other known mirine genera by the following combination of characters: moderate to rather large size; sparsely distributed vestiture; weakly shining, matte dorsum with noticeable dark pattern (yellow with dark stripes and maculae as in Fig. 1); generally slender antenna; six or eight dark stripes on pronotum; always wholly darkened mesoscutum; endosoma with a spicule, two (lateral

and median) lobal sclerites; apically situated secondary gonopore; posterior wall of bursae with distinct interramal lobe and rather narrowed interramal sclerite; and thick-rimmed sclerotized ring with a developed dorsal labiate plate. For further diagnostic characters, see Miyamoto and Yasunaga (1992).

Distribution. Indochina (Laos), Taiwan.

Biology. Unknown; almost all available specimens were collected using UV light traps. Two females of *P. laomontanus* were found on *Castanea* sp. (Fagaceae).

Discussion. The original authors (Miyamoto and Yasunaga 1992) mentioned *Paramiridius* is similar in some external characters to two western Palearctic genera, *Miris* Fabricius and *Miridius* Fieber. Nonetheless, the relationships with these genera are now considered only superficial, on the basis of completely different structures exhibited in the male genitalia.

The present work suggests *Paramiridius* is more probably related to *Lygocoris* Reuter, based on sharing the following characters: apically tuberculate phallosome; presence of a single spicule and apically situated secondary gonopore on endosoma; and similar shape of female sclerotized rings and posterior wall. However, *Paramiridius* is readily distinguished from *Lygocoris* by the unique dark pattern on the dorsum which is nearly matte and glabrous, the different shape of the parameres, and the posterior wall of bursae lacking a lateral lobe (for principal diagnostic characters of *Lygocoris*, see Yasunaga 1991).

There are quite a few mirines superficially similar to *Paramiridius*. To demonstrate more reliable systematic position of the genus, further comprehensive revision is required, including the acquisition of DNA sequence data for representatives of all related genera, a long-run task far beyond the scope of this study.

Key to *Paramiridius* species

- 1 Basal two-third part of antennal segment II and almost entire scutellum yellowish brown; known only from Taiwan ... ***tigrinus* Miyamoto & Yasunaga**
- Antennal segment II wholly infuscate, without pale portions; scutellum mesally with a dark stripe; known from Laos **2**
- 2 Body elongate oval, subparallel-sided; dark, mesal stripe on scutellum broad and continuous from base to apex (Fig. 1); all coxae pale, immaculate ***indochinensis* sp. n.**
- Body nearly ovoid, short; dark, mesal stripe on scutellum narrow, obliterated at apical 1/2–1/3 (Fig. 1); each coxa with a few, dark, small spots ***laomontanus* sp. n.**

***Paramiridius indochinensis* sp. n.**

<http://zoobank.org/9EA50EC3-729E-4EB7-B76F-71A77956B484>

Figs 1–4

Diagnosis. Recognized by the characters given in the key, and the tapered hypophysis of the right paramere (Fig. 2), the developed, curved endosomal spicule (Fig. 3), and

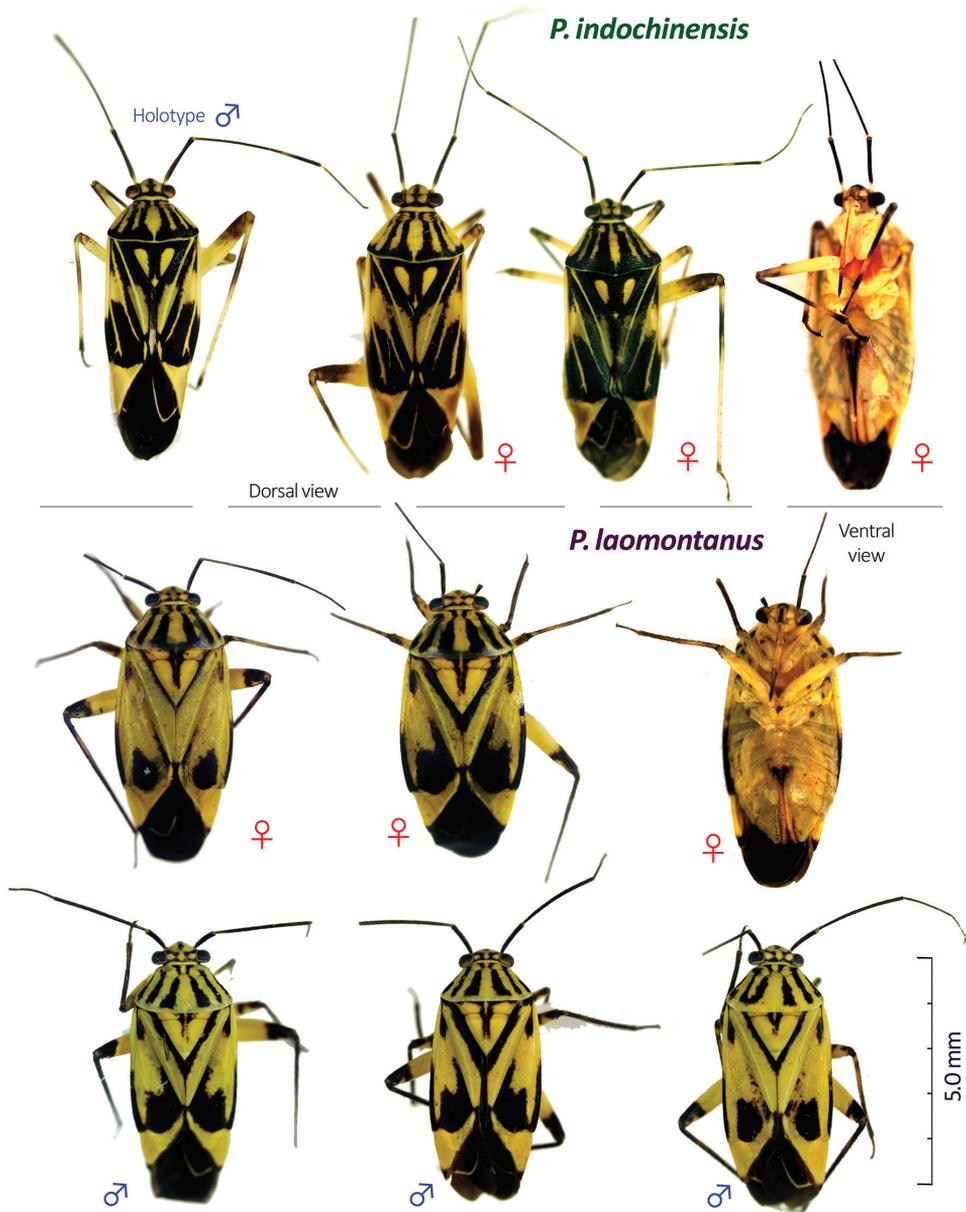


Figure 1. Dorsal and ventral habitus images of two new *Paramiridius* species from Laos.

the wide, squared interrampal lobe (Fig. 2). Most closely related to *P. tigrinus*, from which this new species can be distinguished by the preceding diagnostic characters.

Description. *Coloration:* Body generally yellow; dorsum with black-striped patterns (Fig. 1). Head yellow, medially black; eye margin black. Antenna almost entirely dark brown without pale portions. Labium shiny yellowish brown; segment IV

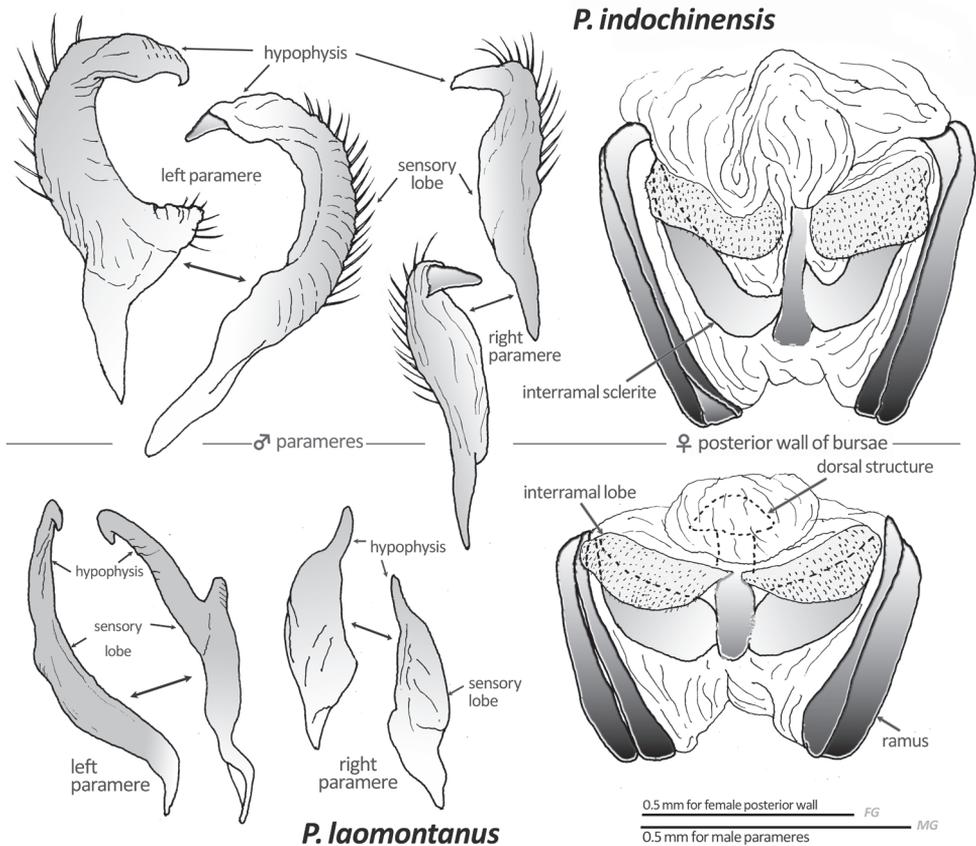


Figure 2. Male parameres and female posterior wall of two new *Paramiridius* species from Laos.

darkened. Pronotum yellow, with three pairs of black stripes (each pair fused together posteriorly); and with narrowly yellow posterior margin. Mesoscutum wholly black. Scutellum medially black; lateral part and apex yellow. Hemelytron widely blackish brown, with three pairs of yellow stripes each along claval vein, claval suture and R+M vein (Fig. 1); posterior half of clavus, anterior quarter to half part of corium, anterior two-third of embolium and entire cuneus yellow. Coxa pale yellow; leg yellow; each femur with more or less darkened apical part; each tibia yellow, with a dark, subbasal annulation and darkened apex; all tarsi brown.

Structure and vestiture. As in generic description provided by Miyamoto and Yasunaga (1992). Body elongate, parallel-sided; dorsal vestiture generally short, simple, and only sparsely distributed. Head vertical; vertex apparently wider than an eye in dorsal view. Labium reaching middle part of metacoxa.

Male genitalia (Figs 2, 3): Similar to type species of the genus, *P. tigrinus*. Left paramere with a sharp, triangular subbasal protuberance; hypophysis of right paramere developed, claw-like (Fig. 2). Endosomal spicule broad, curved at basal one third, somewhat flattened apically (Fig. 3).

Female genitalia (Figs 2, 4): Bursa copulatrix as in Fig. 4; dorsal labiate plate ventro-medially produced. Interramal lobe wide and squared; interramal sclerite narrowed (Fig. 2).

Measurements ♂/♀: Total body length 5.88/ 6.42–7.02; head width across eyes 1.09/ 1.13–1.17; vertex width 0.49/ 0.50–0.52; lengths of antennal segment I–IV 1.25, 3.43, 1.32, ?/ 1.24–1.26, 2.67–2.81, 1.71–1.76, 0.42–0.44; labial length 2.10/ 2.18–2.34; mesal pronotal length including collar 1.23/ 1.29–1.40; basal pronotal width 1.96/ 2.09–2.25; width across hemelytron 2.06/ 2.21–2.43; cuneal length 1.13/ 1.18–1.22; cuneal width 0.72/ 0.81–0.92; lengths of metafemur, tibia and tarsus 3.06, 4.21, 0.75/ 3.08–3.19, 4.38–4.56, 0.73–0.77.

Etymology. Named for its occurrence in the Indochina.

Distribution. Laos (Xiang Khoang Province).

Type material. Holotype ♂: **LAOS:** Xiang Khoang Prov., Kham Dist., Phosabou National Protected Area, Namchack Village, [N19°50'57" E103°47'51", 670m alt.], light trap, 2 May, 2015, Oh, 1♂ (SNU). **Paratypes: LAOS:** Same data as for holotype, 3♀♀ (SNU).

***Paramiridius laomontanus* sp. n.**

<http://zoobank.org/2B495EF2-9E41-4900-8008-EA6CFB6A1CF4>

Figs 1–4

Diagnosis. Recognized by the characters in the key, and the modified shape of the parameres (Fig. 2), and the well-developed lateral lobe and short spicule on the endosoma (Fig. 3). By these characters, *P. laomontanus* can be readily distinguished from other congeners.

Description. *Coloration:* Body yellow, often tinged with green, with black maculae and stripes. Head yellow, with paired, symmetrical, dark maculae on vertex; frons with a black stripe medially; clypeus darkened basally. Antenna dark brown to black; extreme bases of segments II and III white. Labium dark brown, except for yellowish segment II. Pronotum greenish yellow, with three pairs of black stripes not reaching pale posterior margin of pronotum. Mesoscutum and scutellum yellow, with symmetrical, dark patterns. Hemelytron pale green or greenish yellow, with variable black patterns as in Fig. 1; inner margin of clavus, and apical half of embolium blackish brown. Coxa pale yellowish brown, with a dark spot basally; leg yellowish brown; pro- and mesofemur with dark brown spots; apex of each femur more or less darkened; all tibiae and tarsi dark brown.

Structure and vestiture: Body rather ovoid; dorsal surface with sparsely distributed, simple, pale, short setae. Eye small, contiguous to pronotal collar; vertex wide. Antenna generally slender. Labium comparatively broad, extending to apex of mesocoxa. Pronotum rather tumid, not carinate laterally. Hemelytron shallowly and roughly punctate, almost glabrous.

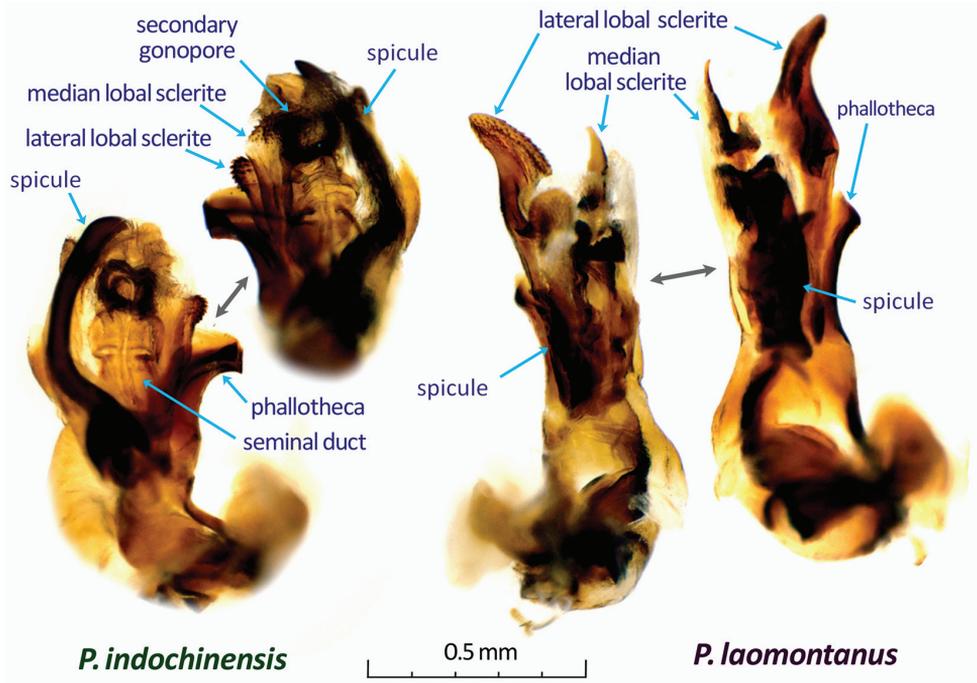


Figure 3. Male endosoma of two new *Paramiridius* species from Laos.

Male genitalia (Figs 2, 3): Parameres glabrous; left paramere slender and nearly straight, with a protuberance at middle and an apically hooked hypophysis; right paramere simple (Fig. 2). Endosoma with a broadened, thin spicule, a tapered median lobal sclerite, and an apically developed, brush-shaped lateral lobal sclerite (Fig. 3).

Female genitalia (Figs 2, 4): Bursa copulatrix similar to that of the preceding species, but dorsal labiate plate more developed (Fig. 4). Posterior wall of bursae with rounded interramal sclerite and rather broadened interramal sclerite (Fig. 2).

Measurements ♂/♀: Total body length 5.29–5.88/ 5.98–6.53; head width across eyes 1.16–1.26/ 1.22–1.30; vertex width 0.55–0.62/ 0.58–0.66; lengths of antennal segment I–IV 0.78–0.82, 2.10–2.15, 0.97–1.15, 0.66–0.72/ 0.81–0.82, 2.12–2.26, 1.18–1.28, 0.67; labial length 1.49–1.69/ 1.63–1.78; mesal pronotal length including collar 1.17–1.27/ 1.24–1.39; basal pronotal width 1.93–2.14/ 2.13–2.29; width across hemelytron 2.11–2.34/ 2.46–2.74; cuneal length 1.10–1.21/ 1.08–1.20; cuneal width 0.73–0.84/ 0.79–0.93; lengths of metafemur, tibia and tarsus 2.02–2.28, 2.83–3.15, 0.71–0.77/ 2.20–2.57, 3.08–3.37, 0.73–0.77.

Etymology. Named for its occurrence in mountain of Laos.

Distribution. Laos (Xiang Khoang Province).

Type material. Holotype ♂: LAOS: Xiang Khoang Prov., Kham Dist., Phosabous National Protected Area, Namchack village, [N19°50'57", E103°47'51", 670m

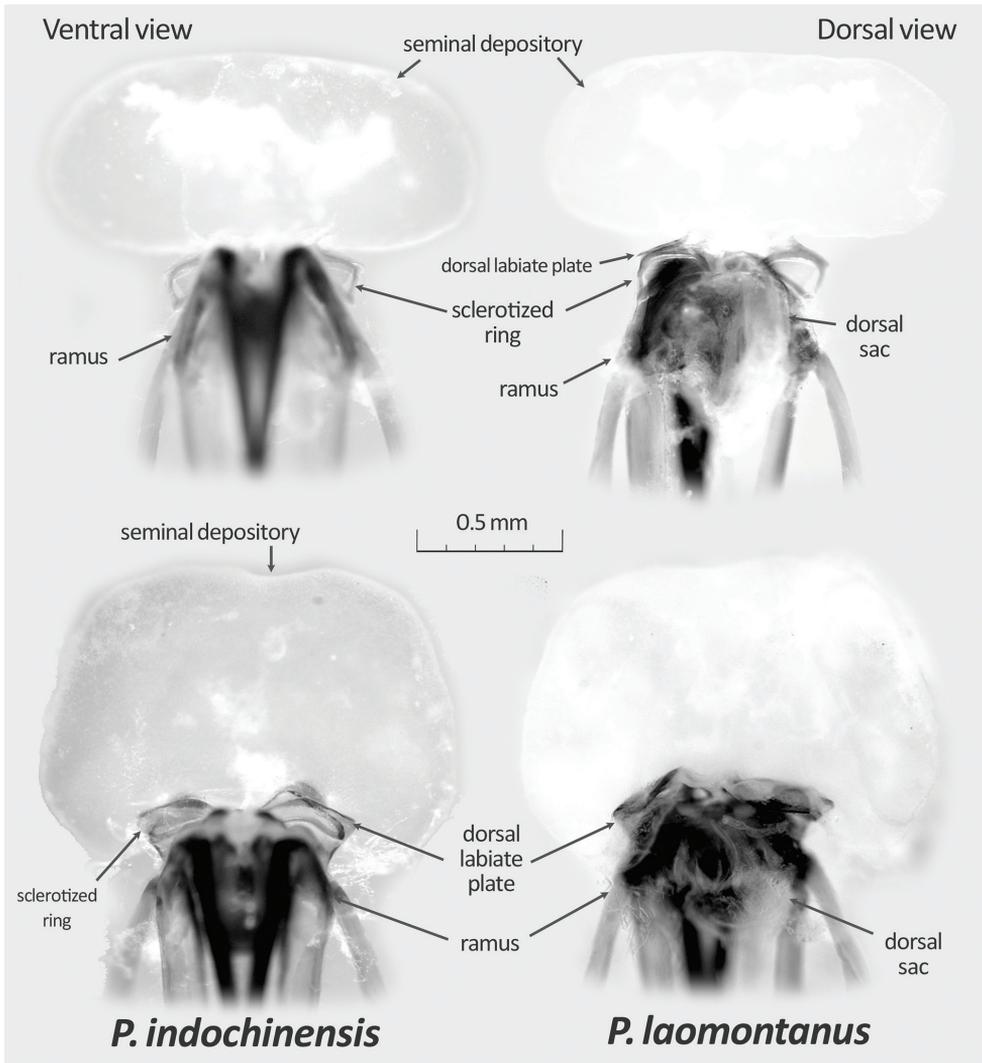


Figure 4. Female bursa copulatrix of two new *Paramiridius* species from Laos.

alt.], light trap, 2 May, 2015, Oh, 1♂ (SNU). **Paratypes: LAOS:** Xiang Khoang Prov., Kham Dist., Phosabous National Protected Area, Tha Village Middle School, light trap, 1 May, 2015, Oh, 3♂♂ 3♀♀ (SNU). Same data as for holotype, 8♂♂ (SNU). Namchack village, [N19°50'57", E103°47'51", 670m alt.], sweeping *Castanea* sp., 2 May, 2015, Oh, 2♀♀ (SNU).

***Paramiridius tigrinus* Miyamoto & Yasunaga**

Paramiridius tigrinus Miyamoto & Yasunaga, 1992: 94 (sp. n.); Schuh 1995: 861 (cat.); Kerzhner and Josifov 1999: 136 (cat.).

Diagnosis. Recognized by the characters mentioned in the key, and the broader yellow posterior margin of the pronotum, the slender, blunt-tipped hypophysis of the right paramere, and the straight endosomal spicule (See Miyamoto and Yasunaga 1992).

Distribution. Taiwan.

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A list of oribatid mites (Acari, Oribatida) of Vietnam

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Abstract

A species list of identified oribatid mite taxa (Acari, Oribatida) in the fauna of Vietnam is provided. During 1967–2015, a total of 535 species/subspecies from 222 genera and 81 families was registered. Of these, 194 species/subspecies were described as new for science from Vietnam.

Keywords

Oribatid mites, species list, fauna, Vietnam

Introduction

The first data on oribatid mite fauna (Acari, Oribatida) of Vietnam were presented by Balogh and Mahunka (1967). Subsequent studies of oribatids during the next 40 years were fragmentary in character, and the main papers on descriptions of new taxa and new records are the following: Rajski and Szudrowicz (1974), Golosova (1983, 1984), Vu et al. (1985, 1987), Jeleva and Vu (1987), Mahunka (1987, 1988, 1989), Niedbala (1989, 2000, 2004), Vu (1990, 1993, 1994), Starý (1993), Vu and Thi (1995), Krivolutsky (1998), Vu and Nguyen (2000, 2005), and Pavlichenko (2001).

Until 2007, only 195 species were recorded in Vietnam. Vu (2007) summarized this data, but his paper omitted more than 50 species, and did not consider synonyms and modern systematics.

A significant increase on oribatid taxonomic knowledge for Vietnam was contributed by the author and colleagues, which is based on material collected during joint Russian-Vietnamese expeditions in 2006–2014: Ermilov and Anichkin (2010, 2011a-k, 2012a-d, 2013a-g, 2014a-g), Ermilov (2011, 2013), Ermilov et al. (2011a-c, 2012a-f, 2013a-c, 2014a-f), Niedbała and Ermilov (2013, 2014), Anichkin et al. (2014). At present, Vietnamese oribatid mites have been actively studied by several researchers (Ermilov 2015a, b; Ermilov and Anichkin 2015a, b; Ermilov and Bayartogtokh 2015; Ermilov and Corpuz-Raros 2015; Fernandez et al. 2015a, b; Vu et al. 2015).

The objective of this paper is to list all identified oribatid mite taxa known from Vietnam to date (1 September 2015), to present primary references (in square brackets) on descriptions of new species or new findings for each species, and to provide the subsequent faunistic and taxonomic studies.

General taxonomic system of Oribatida used in this paper mostly follows that of Subías (2004, online version 2015), Weigmann (2006), Norton and Behan-Pelletier (2009), and Schatz et al. (2011).

Species list

Acaronychidae

- Loftacarus siefi* Lee, 1981 [Vu et al. 2014]
- Stomacarus ciliosus* Luxton, 1982 [Vu et al. 2014]
- S. leei* Mahunka, 1989 [Vu et al. 2014]

Ctenacaridae

- Ctenacarus araneola* (Grandjean, 1932) [Ermilov and Anichkin 2013e]

Cosmochthoniidae

- Cosmochthonius* (*Cosmochthonius*) *lanatus* (Michael, 1885) [Vu et al. 1985]

Sphaerochthoniidae

- Sphaerochthonius splendidus* (Berlese, 1904) [Ermilov et al. 2012c]

Hypochthoniidae

- Eohypochthonius* (*Eohypochthonius*) *crassisetiger* Aoki, 1959 [Ermilov et al. 2012c]
- E.* (*Eohypochthonius*) *gracilis* Jacot, 1936 [Nguyen and Vu 2012]
- Malacoangelia remigera* Berlese, 1913 [Ermilov et al. 2012b]

Lohmanniidae

- Annectacarus unilateralis* Hammer, 1973 [Ermilov and Anichkin 2015a]
- Bedoslomannia anneae* Fernandez, Theron, Rolland & Castillo, 2015 [Fernandez et al. 2015a]
- Haplacarus pairathi* Aoki, 1965 [Nguyen and Vu 2012]
- Javacarus* (*Javacarus*) *kuehnelti* Balogh, 1961 [Vu et al. 1985]
- Lohmannia* (*Lohmannia*) *javana* Balogh, 1961 [Vu et al. 1985]

- L. (Carolohmannia) monosetosa* Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
Meristacarus africanus annobonensis Pérez-Íñigo, 1969 [Ermilov and Anichkin 2015a]
M. madagascarensis obscurus Aoki, 1965 [Golosoova 1983]
M. sundensis Hammer, 1979 [Ermilov and Anichkin 2011i]
Mixacarus (Mixacarus) exilis Aoki, 1970 [Ermilov and Anichkin 2011i]
M. (Mixacarus) foliifer Golosoova, 1984 [Golosoova 1984]
Paillacarus aciculatus (Berlese, 1908) [Vu et al. 1987]
P. benenensis Vu, Ermilov & Dao, 2010 [Vu et al. 2010]
P. cornutus Sarkar & Subías, 1984 [Ermilov and Anichkin 2011i]
P. gueyae (Pérez-Íñigo, 1989) [Nguyen and Vu 2012]
P. hirsutus (Aoki, 1961) (= *Papillacarus arboriseta* Jeleva & Vu, 1987 – Ermilov et al. 2011a) [Golosoova 1983]
P. indistinctus Ermilov, Anichkin & Wu, 2012 [Ermilov et al. 2012b]
P. luteus Ermilov, 2015 [Ermilov 2015b]
P. polygonatus Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011i]
P. polysetosus Ermilov, Anichkin & Wu, 2012 [Ermilov et al. 2012b]
P. ramosus Balogh, 1961 [Ermilov and Anichkin 2011i]
P. undirostratus Aoki, 1965 [Vu and Nguyen 2000]
P. whitteni Fernandez, Theron, Rolland & Leiva, 2015 [Fernandez et al. 2015b]
Paulianacarus (Paulianacarus) vietnamese Fernandez, Theron, Rolland & Castillo, 2015 [Fernandez et al. 2015a]

Nehypochthoniidae

- Nehypochthonius porosus* Norton & Metz, 1980 [Ermilov and Anichkin 2013e]

Epilohmanniidae

- Epilohmannia crassisetosa* Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012b]
E. cylindrica (Berlese, 1904) [Vu et al. 1985]
E. dimorpha Wallwork, 1962 [Nguyen and Vu 2012]
E. minuta pacifica Aoki, 1965 [Ermilov et al. 2012c]
E. ornata Mahunka, 1993 [Vu et al. 2014]
E. spathulata Aoki, 1970 [Golosoova 1983]
Epilohmannoides xena (Mahunka, 1983) [Vu et al. 2012]

Protoplophoridae

- Arthrophlophora vulpes* Berlese, 1916 [Ermilov et al. 2012c]

Mesoplophoridae

- Apoplophora minuscula* Niedbała, 2013 [Niedbała and Ermilov 2013]
A. pantotrema (Berlese, 1913) [Golosoova 1983]

Oribotritiidae

- Austrotritia lebronneci* (Jacot, 1934) [Niedbała and Ermilov 2014]
A. saraburensis Aoki, 1965 [Niedbała 2004]

Indotritia javensis Sellnick, 1923 [as *Indotritia completa* Mahunka, 1987 – Mahunka 1987]

Oribotritia bulbifer (Mahunka, 1987) [Ermilov et al. 2012c]

O. paraaokii Niedbała, 2000 [Niedbała 2000]

O. submolesta Niedbała, 2000 [Niedbała 2000]

Euphthiracaridae

Acrotritia aokii (Niedbała, 2000) [Niedbała 2000]

A. ardua (Koch, 1841) [Vu et al. 1985]

A. duplicata (Grandjean, 1953) [Vu et al. 1985]

A. hyeroglyphica (Berlese, 1916) [as *Rhysotritia hauseri* Mahunka, 1991 – Vu et al. 2012]

A. otabeitensis (Hammer, 1972) [Starý 1993]

A. paraganulata Niedbała, 2014 [Niedbała and Ermilov 2014]

A. proxima Niedbała, 2013 [Niedbała and Ermilov 2013]

A. sinensis Jacot, 1923 [as *Rhysotritia rasile* Mahunka, 1982 – Vu et al. 1987]

A. vestita (Berlese, 1913) [as *Rhysotritia comteae* Mahunka, 1983 – Niedbała 2000]

Euphthiracarus (*Euphthiracarus*) *foveolatus* Aoki, 1980 [Niedbała 2004]

E. (*Euphthiracarus*) *labyrinthicus* Starý, 1993 [Starý 1993]

E. (*Euphthiracarus*) *quasitakahashii* Niedbała, 2014 [Niedbała and Ermilov 2014]

E. (*Euphthiracarus*) *parareticulatus* Niedbała, 2000 [Niedbała 2000]

E. (*Euphthiracarus*) *vietnamicus* Starý, 1993 [Starý 1993]

E. (*Parapocsia*) *medius* Niedbała, 2014 [Niedbała and Ermilov 2014]

Mesotritia maerkeli Sheals, 1965 [Niedbała and Ermilov 2014]

M. spinosa Aoki, 1980 [Starý 1993]

Microtritia minima (Berlese, 1904) [Niedbała 2000]

M. tropica Märkel, 1964 [Starý 1993]

Synichotritiidae

Sabahtritia dongnaiensis Niedbała, 2013 [Niedbała and Ermilov 2013]

Steganacaridae

Arphthiracarus parasentus Niedbała, 2000 [Niedbała 2000]

A. tubulus (Hammer, 1972) [Ermilov et al. 2012c]

Atropacarus (*Atropacarus*) *phyllophorus* (Berlese, 1904) [Vu et al. 1985]

A. (*Atropacarus*) *striculus* (Koch, 1935) [Niedbała 2000]

A. (*Hoplophorella*) *cucullatus* (Ewing, 1909) [Ermilov et al. 2012c]

A. (*Hoplophorella*) *hamatus* (Ewing, 1909) [as *Hoplophorella cuneiseta* Mahunka, 1988 – Mahunka 1988; also as *Atropacarus floridae* Jacot, 1933 – Niedbała 2000]

A. (*Hoplophorella*) *stilifer* (Hammer, 1961) [Niedbała 2000]

A. (*Hoplophorella*) *vitrinus* (Berlese, 1913) [Golosoova 1983]

Austrophthiracarus evexus (Niedbała, 2000) [Niedbała 2000]

Austrophthiracarus pullus (Niedbała, 1989) [Ermilov et al. 2012c]

A. sentus Niedbała, 1989 [Niedbała 1989]

Plonaphacarus kugohi (Aoki, 1959) [Niedbała 2000]

- P. insignitus* Niedbala, 1989 [Niedbala 1989]
P. scrupeus Niedbala, 1989 [Niedbala 1989]
Protophthiracarus finitima Niedbala, 2002 [Vu 2013]
Steganacarus (Rhacaplacarus) spinus Niedbala, 2014 [Niedbala and Ermilov 2014]

Phthiracaridae

- Hoplophthiracarus clavatus* Niedbala, 2014 [Niedbala and Ermilov 2014]
H. pakistanensis Hammer, 1977 [Nguyen and Vu 2012]
H. stigmosus Niedbala, 2000 [Niedbala 2000]
Notophthiracarus nitidus (Pérez-Íñigo & Baggio, 1988) [Nguyen and Vu 2012]
N. perparvus Niedbala, 1989 [Niedbala 1989]
Phthiracarus abstemius Niedbala, 1989 [Niedbala 1989]
P. crispus Hammer, 1972 [Niedbala 2000]
P. invenustus Niedbala, 2000 [Niedbala 2000]
P. paucus Niedbala, 1991 [Niedbala 2000]
P. pygmaeus Balogh, 1958 [Niedbala 2000]

Trhypochthoniidae

- Afronothrus incisivus* Wallwork, 1961 [Ermilov et al. 2012a]
Allonothrus russeolus Wallwork, 1960 [Nguyen and Vu 2012]
Archegozetes longisetosus Aoki, 1965 [Vu 1990]

Malaconothridae

- Malaconothrus dorsofoveolatus* Hammer, 1979 [Ermilov et al. 2012c]
Tyrphonothrus dilatatus (Ermilov, Anichkin & Tolstikov, 2014) [Ermilov et al. 2014a]
T. angustirostrum (Hammer, 1966) [Nguyen and Vu 2012]
T. geminus (Hammer, 1972) [Ermilov et al. 2012c]
T. variosetosus (Hammer, 1971) [Ermilov et al. 2012b]

Nothridae

- Nothrus baviensis* Krivolutsky, 1998 [Krivolutsky 1998]
N. gracilis Hammer, 1961 [Vu et al. 2012]
N. montanus Krivolutsky, 1998 [Krivolutsky 1998]
N. oceanicus Sellnick, 1959 [Vu et al. 2014]
N. shapensis Krivolutsky, 1998 [Krivolutsky 1998]

Crotoniidae

- Heminothrus apophysiger* Hammer, 1969 [Ermilov and Anichkin 2014d]

Nanhermanniidae

- Cosmohermannia robusta* (Aoki, 1994) [Ermilov and Anichkin 2011i]
Cyrthermannia vicinicornuta Aoki, 1965 [Ermilov and Anichkin 2011i]
Masthermannia mammillaris (Berlese, 1904) [Ermilov and Anichkin 2011i]
Nanhermannia thainensis Aoki, 1965 [Vu 1993]

Hermanniiidae

- Phyllhermannia bimaculata* Hammer, 1979 [Ermilov and Anichkin 2014f]
P. forsteri Balogh, 1985 [Vu et al. 2014]
P. gladiata Aoki, 1965 [Vu and Nguyen 2005]

P. javensis Hammer, 1979 [Nguyen and Vu 2012]

P. similis Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]

Hermanniellidae

Hermanniella bugiamapensis Ermilov & Bayartogtokh, 2015 [Ermilov and Bayartogtokh 2015]

H. aristosa Aoki, 1965 [Ermilov and Anichkin 2014d]

H. orbiculata Hammer, 1979 [Vu et al. 2014]

H. thani Mahunka, 1987 [Mahunka 1987]

Plasmobatidae

Plasmobates asiaticus Aoki, 1973 [Ermilov and Anichkin 2014d]

Neolioididae

Neoliodes theleproctus (Hermann, 1804) [Vu et al. 1985]

Platyliodes japonicus Aoki, 1979 [Nguyen and Vu 2012]

Pherolioididae

Pheroliodes longiceps Balogh & Mahunka, 1966 [Vu et al. 2014]

Licnodamaeidae

Hexachaetoniella dispersa (Balogh, 1985) [Nguyen and Vu 2012]

Pedrocortesella dongnaiensis Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014f]

P. pulchra Hammer, 1961 [Nguyen and Vu 2012]

P. temperata Balogh, 1985 [Nguyen and Vu 2012]

P. vietnamica Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014f]

Gymnodamaeidae

Arthrodamaeus vietnamicus Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011b]

Gymnodamaeus adpressus (Aoki & Fujikawa, 1971) [Vu et al. 2008]

Damaeidae

Belba corynopus (Hermann, 1804) [Vu 1993]

Metabelba orientalis Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]

Tectodamaeus heterotrichus Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]

Cepheidae

Sphodrocephus tuberculatus Mahunka, 1988 [Mahunka 1988]

Astegistidae

Cultroribula bicuspidata Mahunka, 1978 [Ermilov et al. 2012c]

C. lata Aoki, 1961 [Golosoova 1983]

Furcoppia (*Furcoppia*) *cattienica* Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012b]

F. (*Furcoppia*) *parva* Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]

Peloppiidae

Austroceratoppia japonica Aoki, 1984 [Ermilov and Anichkin 2014d]

Ceratoppia bipilis (Hermann, 1804) [Ermilov and Anichkin 2014d]

C. crassiseta Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]

Gustaviidae

Gustavia longicornis (Berlese, 1904) [Ermilov and Anichkin 2014d]

Zetorchestidae

Zetorchestes equestris Berlese, 1908 [Golosoova 1993]

Z. saltator Oudemans, 1915 [Vu 1990]

Z. transvaalensis Coetzee, 1989 [Nguyen and Vu 2012]

Z. phylliferus Mahunka, 1983 [Vu et al. 2014]

Ctenobelbidae

Ctenobelba (*Berndamerus*) *bugiamapensis* Ermilov, Shtanchaeva, Subías & Anichkin, 2014 [Ermilov et al. 2014f]

Amerobelbidae

Roynortonia vietnamica Ermilov, 2011 [Ermilov 2011]

Eremulidae

Austroeremulus glabrus Mahunka, 1985 [Nguyen and Vu 2012]

Eremulus avenifer Berlese, 1913 [Balogh and Mahunka 1967]

E. flagellifer Berlese, 1908 [Nguyen and Vu 2012]

E. spinosus Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011d]

E. truncatus Hammer, 1971 [Golosoova 1983]

Mahunkana bifurcata (Mahunka, 1987) [Mahunka 1987]

M. japonica (Aoki & Karasawa, 2007) [Ermilov et al. 2012c]

Reteremuloides bifurcatus Mahunka, 1989 [Ermilov and Bayartogtokh 2015]

Damaeolidae

Fosseremus laciniatus (Berlese, 1905) [Ermilov et al. 2012c]

Gressittolus marginatus Balogh, 1970 [Ermilov et al. 2012c]

Hungarobelbidae

Costeremus ornatus Aoki, 1970 [Vu et al. 2014]

Eremobelbidae

Eremobelba bella Hammer, 1982 [Nguyen and Vu 2012]

E. bellicosa Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]

E. breviseta Balogh, 1968 [Ermilov et al. 2012c]

E. capitata Berlese, 1913 [Vu et al. 1985]

E. hamata Hammer, 1961 [Nguyen and Vu 2012]

E. japonica Aoki, 1959 [Vu et al. 2011]

Heterobelbidae

Heterobelba stellifera stellifera Okayama, 1980 [Ermilov et al. 2012a]

H. stellifera formosana Aoki, 1990 [Vu et al. 2014]

Basilobelbidae

Basilobelba maidililae Fernandez, Theron, Rolland & Leiva, 2015 [Fernandez et al. 2015b]

B. parmata Okayama, 1980 [Ermilov et al. 2012b]

B. retiaria (Warburton, 1912) [Ermilov and Anichkin 2013g]

Xyphobelba hamanni Csiszár, 1961 [Ermilov and Anichkin 2014d]

Platyameridae

Gymnodampia crassisetiger (Aoki, 1984) [Ermilov and Anichkin 2014d]

Caleremaeidae

Epiereumus bidupensis Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]

Eremellidae

Eremella vestita Berlese, 1913 [Vu and Nguyen 2000]

Arceremaeidae

Tecteremaeus incompletus Mahunka, 1988 [Ermilov and Bayartogtokh 2015]

T. hauseri Mahunka, 1982 [Ermilov et al. 2012c]

Oppiidae

Acroppia processigera (Balogh & Mahunka, 1967) [Nguyen and Vu 2012]

Arcoppia arcualis (Berlese, 1913) [Balogh and Mahunka 1967]

A. corniculifera (Mahunka, 1978) [Nguyen and Vu 2012]

A. hammerae Rodríguez & Subías, 1984 [Vu and Nguyen 2005]

A. incerta Balogh & Balogh, 1983 [Vu et al. 2014]

A. longisetosa Balogh, 1962 [Vu 1990]

A. robustia (Berlese, 1913) [Balogh and Mahunka 1967]

A. serrutala (Balogh & Mahunka, 1980) [Ermilov and Bayartogtokh 2015]

A. viperea (Aoki, 1959) [as *Arcoppia baloghi* Rodríguez & Subías, 1984 – Vu and Nguyen 2005]

A. waterhousei Balogh & Balogh, 1983 [Nguyen and Vu 2012]

Belloppia shealsi Hammer, 1968 [Vu et al. 2012]

Berniniella (*Berniniella*) *bicarinata* (Paoli, 1908) [Vu et al. 1985]

Brachioppiella (*Brachioppiella*) *biseriata* (Balogh & Mahunka, 1975) [Vu et al. 2012]

Congoppia deboissezoni (Balogh & Mahunka, 1966) [Vu et al. 2012]

Cryptoppia elongata Csiszár, 1961 [Vu et al. 1985]

Cycloppia restata (Aoki, 1963) [Ermilov and Anichkin 2013f]

Discoppia (*Cylindroppia*) *cylindrica* (Pérez-Íñigo, 1965) [Ermilov et al. 2012b]

Elaphoppia quadripilosa (Balogh, 1961) [Ermilov and Anichkin 2014d]

Helioppia sol (Balogh, 1959) [Vu et al. 2012]

Karenella (*Karenella*) *acuta* (Csiszár, 1961) [Vu et al. 1985]

Kokoppia dendricola (Jeleva & Vu, 1987) [Jeleva and Vu 1987]

Lanceoppia (*Lancelalmoppia*) *becki* Hammer, 1968 [Nguyen and Vu 2012]

L. (Lanceoppia) translucens (Mahunka, 1985) [Vu et al. 2014]

Lasiobelba (*Lasiobelba*) *kuehnelti* (Csiszár, 1961) [Vu et al. 1985]

L. (Lasiobelba) remota Aoki, 1959 [Balogh and Mahunka 1967]

L. (Lasiobelba) vietnamica (Balogh, 1983) [as *Oppia remota* Aoki, 1959 – Balogh and Mahunka 1967]

Lineoppia microseta Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011g]

Lyroppia dongnaiensis Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013f]

Microppia minus (Paoli, 1908) [Ermilov et al. 2012b]

Multioppia (*Multioppia*) *pseudoglabra* Ermilov, 2015 [Ermilov 2015b]

- M. (Multiopppia) tamdao* Mahunka, 1988 [Mahunka 1988]
Multipulchroppia pectinata (Balogh & Mahunka, 1967) [Balogh and Mahunka 1967]
M. similis (Hammer, 1979) [Nguyen and Vu 2012]
Neoamerioppia (Neoamerioppia) vietnamica (Mahunka, 1988) [Mahunka 1988]
Oppiella (Oppiella) nova (Oudemans, 1902) [Golosova 1983]
Oxybrachioppia barbata (Choi, 1986) [Ermilov et al. 2012c]
Oxyoppia (Aciculoppa) clavata (Aoki, 1983) [Vu et al. 2008]
Pulchroppia elegans Hammer, 1979 [Ermilov et al. 2012c]
P. granulata Mahunka, 1988 [Mahunka 1988]
P. roynortoni Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011g]
Ramusella (Ramusella) chulumaniensis (Hammer, 1958) [Ermilov et al. 2012b]
R. (Ramusella) clavipectinata (Michael, 1885) [Vu et al. 1985]
R. (Ramusella) pocsi Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
R. (Insculptoppia) elliptica (Berlese, 1908) [Ermilov et al. 2012c]
R. (Insculptoppia) insculpta (Paoli, 1908) [Vu 1993]
Ramuselloppia vietnamica Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013b]
Striatoppia lanceolata Hammer, 1972 [Ermilov et al. 2013a]
S. madagascarensis Balogh, 1961 [Vu et al. 2012]
S. opuntiseta Balogh & Mahunka, 1968 [Mahunka 1988]
S. papillata Balogh & Mahunka, 1966 [Vu et al. 1985]
Taiwanoppia (Taiwanoppia) hungarorum (Mahunka, 1988) [Mahunka 1988]

Granuloppiidae

- Gigantoppia zryanini* Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011e]
Granuloppia kamerunensis Mahunka, 1974 [Nguyen and Vu 2012]
G. vietnamensis Ermilov & Bayartogtokh, 2015 [Ermilov and Bayartogtokh 2015]
Hammerella (Parawoasella) bayartogtokhi Ermilov, Shtanchaeva, Subías & Anichkin, 2012 [Ermilov et al. 2012f]

Machuellidae

- Machuella lineata* Hammer, 1973 [Ermilov et al. 2012b]

Suctobelbidae

- Allosuctobelba vietnamensis* Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
Novosuctobelba (Novosuctobelba) vietnamica Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
Suctobelbata bituberculata Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013c]
Suctobelbella (Suctobelbella) elegantissima (Hammer, 1979) [Golosova 1983]
S. (Suctobelbella) finlayi (Balogh & Mahunka, 1980) [Nguyen and Vu 2012]
S. (Suctobelbella) latirostris (Strenzke, 1950) [Vu 1990]
S. (Suctobelbella) longicuspis Jacot, 1937 [Nguyen and Vu 2012]
S. (Flagrosuctobelba) elegantula (Hammer, 1958) [Ermilov et al. 2012c]

- S. (Flagrosuctobelba) paralleloidentata* Hammer, 1979 [Ermilov et al. 2012c]
S. (Flagrosuctobelba) semiplumosa (Balogh & Mahunka, 1967) [Balogh and Mahunka 1967]
S. (Flagrosuctobelba) subtrigona (Oudemans, 1900) [Vu et al. 2012]
S. (Ussuribata) bivittata (Hammer, 1979) [Ermilov and Anichkin 2014d]
S. (Ussuribata) multituberculata Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
S. (Ussuribata) phylliformis Ermilov, Shtanchaeva & Subías, 2014 [Ermilov et al. 2014d]
S. (Ussuribata) sexsetosa (Hammer, 1979) [Ermilov and Anichkin 2013d]
S. (Ussuribata) variosetosa (Hammer, 1961) [Golosova 1983]
Suctobelbilla minima Hammer, 1979 [Golosova 1983]
S. multituberculata Hammer, 1979 [Ermilov et al. 2012c]
S. scutata Hammer, 1972 [Ermilov and Anichkin 2014d]
S. transrugosa (Mahunka, 1986) [Nguyen and Vu 2012]

Oxyameridae

- Oxyamerus aokii* Balogh, 1968 [Ermilov and Anichkin 2014d]
O. hyalinus Hammer, 1979 [Ermilov and Anichkin 2014d]
O. truncatus Hammer, 1979 [Ermilov and Anichkin 2014d]

Dampfeliidae

- Dampfella angusta* Hammer, 1979 [Ermilov and Anichkin 2014d]

Otocepheidae

- Basiceramerus igorotus* Corpuz-Raros and Gruèzo, 2011 [Ermilov and Anichkin 2013g]
Eurostocepheus aquilinus Aoki, 1965 [Ermilov et al. 2012c]
Dolicheremaeus aokii Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
D. baloghi Aoki, 1967 [Ermilov and Bayartogtokh 2005]
D. bartkei Rajski & Szudrowicz, 1974 [Rajski and Szudrowicz 1974]
D. bruneiensis Aoki, 1967 [Ermilov and Bayartogtokh 2005]
D. bugiamapensis Ermilov, Anichkin & Wu, 2012 [Ermilov et al. 2012a]
D. capillatus (Balogh, 1959) [Nguyen and Vu 2012]
D. contactus Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013d]
D. damaeoides (Berlese, 1913) [Ermilov and Anichkin 2014d]
D. donacunarensis Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014g]
D. dwalteri Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014g]
D. insolitus Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
D. junichiaokii Subías, 2010 (= *Dolicheremaeus magnus* Aoki, 2006) [Ermilov and Anichkin 2013g]
D. inaequalis Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
D. lineolatus Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
D. orientalis (Aoki, 1965) [Vu 1994]
D. ornatus Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
D. philippinensis Aoki, 1967 [Vu et al. 2014]

- D. sabahnus* Mahunka, 1988 [Nguyen and Vu 2012]
Fissicepheus (Fissicepheus) elegans Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
F. (Fissicepheus) striganovae Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
Leptotocepheus murphyi (Mahunka, 1989) [Ermilov and Bayartogtokh 2015]
Megalotocepheus (Archegotocepheus) crinitus Berlese, 1905 [Ermilov and Anichkin 2014d]
M. (Archegotocepheus) singularis (Mahunka, 1988) [Nguyen and Vu 2012]
Otocepheus (Otocepheus) spatulatus Mahunka, 2000 [Ermilov and Anichkin 2013c]
O. (Acrotocepheus) duplicornutus duplicornutus Aoki, 1965 [Vu and Nguyen 2005]
O. (Acrotocepheus) duplicornutus discrepans Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
O. (Acrotocepheus) excelsus Aoki, 1965 [Ermilov and Anichkin 2013d]
O. (Acrotocepheus) triplicicornutus Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
O. (Acrotocepheus) vietnamicus Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011e]
Papillocepheus primus Ermilov, Anichkin & Tolstikov, 2014 [Ermilov et al. 2014b]
Pseudotocepheus setiger (Hammer, 1972) [Ermilov et al. 2013a]
Umashtanchaeviella plethotricha Ermilov, Anichkin & Tolstikov, 2014 [Ermilov et al. 2014c]

Carabodidae

- Aokiella florens* Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
A. rotunda Hammer, 1979 [Ermilov et al. 2012b]
A. xuansoni Vu, Ermilov & Dao, 2010 [Vu et al. 2010]
Austrocarabodes (Austrocarabodes) alveolatus Hammer, 1973 [Vu et al. 2014]
A. (Austrocarabodes) falcatus Hammer, 1973 [Nguyen and Vu 2012]
A. (Austrocarabodes) szentivanyi (Balogh & Mahunka, 1967) [Balogh and Mahunka 1967]
A. (Austrocarabodes) vaucheri Mahunka, 1984 [Nguyen and Vu 2012]
A. (Uluguroides) polytrichus Balogh & Mahunka, 1978 [Vu et al. 2015]
Carabodes (Klapperiches) mikhaetandreorum Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013g]
C. (Klapperiches) samoensis Balogh & Balogh, 1986 [Ermilov and Anichkin 2013d]
C. (Klapperiches) strinovichi Balogh & Mahunka, 1978 [Vu et al. 2014]
C. (Phyllocarabodes) inopinatus (Mahunka, 1985) [Nguyen and Vu 2012]
C. (Phyllocarabodes) schatzii Subías, 2010 [as *Phyllocarabodes ornatus* Balogh, 1986 – Nguyen and Vu 2012]
Chistyakovella insolita Ermilov, Aoki & Anichkin, 2013 [Ermilov et al. 2013c]
Gibbicepheus (Gibbicepheus) baccanensis Jeleva & Vu, 1987 [Jeleva and Vu 1987]
G. (Gibbicepheus) fenestralis Hammer, 1979 [Golosova 1983]
G. (Gibbicepheus) latohumeralis Hammer, 1982 [Ermilov and Anichkin 2015a]

Yoshiobodes (Yoshiobodes) irmayi (Balogh & Mahunka, 1969) [as *Yoshiobodes aokii* Mahunka, 1987 – Ermilov and Anichkin 2013c]

Y. (Yoshiobodes) neotrichorostralis Ermilov, Shtanchaeva, Subías & Anichkin, 2014 [Ermilov et al. 2014e]

Y. (Dongnaiobodes) biconcavus Ermilov, Shtanchaeva, Subías & Anichkin, 2014 [Ermilov et al. 2014e]

Y. (Dongnaiobodes) hexasetosus Ermilov, Shtanchaeva, Subías & Anichkin, 2014 [Ermilov et al. 2014e]

Nippobodidae

Nippobodes monstruosus (Jeleva & Vu, 1987) [Jeleva and Vu 1987]

Tectocephidae

Tectocephus elegans Ohkubo, 1981 [Vu et al. 2014]

T. minor Berlese, 1903 [as *Tectocephus cuspidentatus* Knülle, 1954 – Vu 1993]

T. velatus (Michael, 1880) [Golosova 1983]

Tegezozetes tunicatus tunicatus Berlese, 1913 [Ermilov and Anichkin 2013f]

T. tunicatus breviclava Aoki, 1970 [Vu 2013]

Tegeocranellidae

Tegeocranellus martinezi Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]

Microtegeidae

Microtegeus borhidii Balogh & Mahunka, 1974 [Ermilov et al. 2012a]

M. cardosensis Pérez-Íñigo, 1985 [Vu et al. 2014]

M. cornutus Balogh, 1970 [Nguyen and Vu 2012]

M. quadristriatus Mahunka, 1984 [Nguyen and Vu 2012]

M. reticulatus Aoki, 1965 [Vu 1990]

Cymbaeremaeidae

Scapheremaeus ascissuratus Ermilov & Anichkin, 2015 [Ermilov and Anichkin 2015b]

S. cellulatifer Mahunka, 1987 [Mahunka 1987]

S. crassus Mahunka, 1988 [Mahunka 1988]

S. fisheri Aoki, 1966 [Ermilov et al. 2012c]

S. foveolatus Mahunka, 1987 [Mahunka 1987]

Licneremaeidae

Licneremaeus licnophorus (Michael, 1882) [Vu et al. 2014]

L. polygonalis Hammer, 1971 [Ermilov et al. 2013a]

Phenopelopidae

Eupelops forsslundi (Balogh, 1959) [Vu et al. 1987]

Nesopelops intermedius Hammer, 1979 [Ermilov and Anichkin 2013g]

Eremaozetidae

Mahunkaia bituberculata (Mahunka, 1983) [Nguyen and Vu 2012]

Idiozetidae

Idiozetes javensis Hammer, 1979 [Ermilov et al. 2012a]

Limnozetestidae

Limnozetestes pustulatus (Mahunka, 1987) [Mahunka 1987]

Microzetidae

Berlesezetes ornatissimus (Berlese, 1913) [as *Berlesezetes auxiliaris* Grandjean, 1936 – Mahunka 1988]

Caucasozetes frankeae Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011e]

Kaszabodes velatus Mahunka, 1988 [Mahunka 1988]

Schalleriella vietnamica Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011a]

Achipteriidae

Achipteria (*Achipteria*) *curta* Aoki, 1970 [Vu 1993]

Anachipteria (*Anachipteria*) *svetlanae* Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]

Austrachipteria phongnhae Ermilov & Vu, 2012 [Ermilov and Vu 2012]

Campachipteria distincta (Aoki, 1959) [Vu and Nguyen 2000]

C. uenoi Aoki, 1995 [Ermilov and Anichkin 2014d]

Plakoribates asiaticus Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013a]

Tegoribatidae

Ceratobates cangioensis Ermilov & Anichkin, 2015 [Ermilov and Anichkin 2015a]

Tegoribates americanus Hammer, 1958 [Vu et al. 2014]

Oribatellidae

Novoribatella minutisetarum Engelbrecht, 1986 [Nguyen and Vu 2012]

Ophidiotrichus ussuricus Krivolutsky, 1971 [Vu et al. 2014]

Oribatella (*Oribatella*) *gerdweigmanni* Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012d]

O. (Oribatella) illuminata Hammer, 1961 [Vu et al. 2014]

O. (Oribatella) prolongata Hammer, 1961 [Vu et al. 2014]

O. (Oribatella) sculpturata Mahunka, 1987 [Mahunka 1987]

O. (Oribatella) umaetluisorum Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012a]

Heterozetidae

Farchacarus philippinensis (Corpuz-Raros, 1979) [Golosoza 1983]

Ceratozetidae

Ceratozetes (*Ceratozetes*) *bicornis* Hammer, 1967 [Golosoza 1983]

C. (Ceratozetes) gracilis (Michael, 1884) [Golosoza 1983]

C. (Ceratozetes) mediocris Berlese, 1908 [Golosoza 1983]

Fuscozetes fuscipes (Koch, 1844) [Vu 1990]

Lepidozetes trifolius (Fujikawa, 1972) [Vu et al. 2014]

Sphaerozetes bugiamapensis Ermilov, Anichkin & Wu, 2013 [Ermilov et al. 2013b]

Punctoribatidae

Allozetes africanus Balogh, 1958 [Golosoza 1983]

A. pusillus (Berlese, 1913) [Mahunka 1988]

Lamellobates molecula (Berlese, 1916) [Golosoza 1983; also as *Lamellobates palustris* Hammer, 1958 – Vu et al. 1985; as *Lamellobates hauseri* Mahunka, 1977 – Mahunka 1988]

L. ocularis Jeleva & Vu, 1987 [Jeleva and Vu 1987]

Paralamellobates misella (Berlese, 1910) [as *Paralamellobates schoutedani* (Balogh, 1959) – Vu et al. 1985; also as *Paralamellobates ceylanicus* (Oudemans, 1916) – Mahunka 1988]

Punctoribates hexagonus Berlese, 1908 [Vu 1990]

P. punctum (Koch, 1839) [Pavlichenko 1991]

Chamobatidae

Chamobates (Chamobates) javensis (Hammer, 1979) [Golosoova 1983]

Mochlozetidae

Mochlozetes ryukyuensis Aoki, 2006 [Ermilov and Anichkin 2013d]

Unguizetes asiaticus Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012b]

U. cattienensis Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011e]

U. clavatus Aoki, 1967 [Golosoova 1983]

U. latus Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013d]

U. sphaerula (Berlese, 1905) [Ermilov et al. 2012c]

Uracrobates (Uracrobates) magniporosus Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]

Oribatulidae

Paraphauloppia gracilis (Hammer, 1958) [Nguyen and Vu 2012]

Zygoribatula pennata Grobler, 1993 [Nguyen and Vu 2012]

Z. prima Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011k]

Z. undulata Berlese, 1916 [as *Zygoribatula longiporosa* Hammer, 1953 – Vu et al. 2012]

Sellnickiidae

Sellnickia caudata (Michael, 1908) [Ermilov et al. 2012c]

Caloppiidae

Zetorchella latior (Berlese, 1913) [Ermilov et al. 2012c]

Z. reticulata (Willmann, 1933) [Ermilov and Anichkin 2013g]

Schelorbitidae

Areozetes incertus Balogh, 1970 [Vu et al. 2014]

Cordiozetes olahi (Mahunka, 1987) [Mahunka 1987]

Euschelorbitates (Euschelorbitates) samsinaki Kunst, 1958 [Vu 1990]

E. (Trischelorbitates) clavatus (Mahunka, 1988) [Mahunka 1988]

Exorbitatula (Multorbitates) longior (Hammer, 1958) [Ermilov and Anichkin 2014d]

Fijibates aelleni (Mahunka, 1988) [Ermilov et al. 2012c]

F. rostratus Hammer, 1971 [Ermilov et al. 2013a]

Liebstadia (Liebstadia) humerata Sellnick, 1928 [Vu 1993]

Perschelorbitates (Perschelorbitates) lanceolatus (Aoki, 1984) [Vu et al. 2008]

P. (Perschelorbitates) luminosus (Hammer, 1961) [Ermilov and Anichkin 2014d]

P. (Perschelorbitates) luteus (Hammer, 1962) [Nguyen and Vu 2012]

P. (Perschelorbitates) minutus (Pletzen, 1965) [Golosoova 1983]

Rhabdoribates siamensis Aoki, 1967 [Vu 1990]

Schelorbitates (Schelorbitates) crucisetus Jeleva & Vu, 1987 [Jeleva and Vu 1987]

S. (Schelorbitates) fimbriatus Thor, 1930 [Vu et al. 1985]

- S. (Scheloribates) flagellisetosus* Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014c]
S. (Scheloribates) kraepelini (Berlese, 1908) [Ermilov and Anichkin 2013e]
S. (Scheloribates) laevigatus (Koch, 1835) [Vu et al. 1985]
S. (Scheloribates) latipes (Koch, 1844) [Golosoova 1983]
S. (Scheloribates) pallidulus (Koch, 1844) [Vu et al. 1985]
S. (Scheloribates) parvus Pletzen, 1963 [Vu et al. 2015]
S. (Scheloribates) praeincisus praeincisus (Berlese, 1910) [Golosoova 1983]
S. (Scheloribates) praeincisus interruptus (Berlese, 1916) [Ermilov and Anichkin 2013g]
S. (Scheloribates) vulgaris Hammer, 1961 [Nguyen and Vu 2012]
S. (Bischeloribates) mahunkai Subías, 2010 [as *Philoribates heterodactylus* (Mahunka, 1988) – Nguyen and Vu 2012]
Tuberemaeus lineatus Balogh, 1970 [Nguyen and Vu 2012]
T. perforatoides Hammer, 1979 [Ermilov and Anichkin 2013d]
T. sculpturatus Mahunka, 1987 [Mahunka 1987]
T. singularis Sellnick, 1930 [Ermilov and Anichkin 2013g]
Vesiculobates silvaticus Hammer, 1979 [Ermilov and Anichkin 2014f]

Oripodidae

- Brachyoripoda foveolata* Balogh, 1970 [Ermilov et al. 2012c]
Cosmopirnodus tridactylus Mahunka, 1988 [Mahunka 1988]
Oripoda excavata Mahunka, 1988 [Mahunka 1988]
O. pinicola Aoki & Ohkubo, 1974 [Vu et al. 2008]
Subpirnodus mirabilis Mahunka, 1988 [Mahunka 1988]
Truncopes moderatus variabilis Aoki & Yamamoto, 2007 [Ermilov and Anichkin 2013f]
T. orientalis Mahunka, 1987 [Mahunka 1987]

Haplozetidae

- Acutozetes rostratus* Balogh, 1970 [Vu et al. 2014]
Indoribates (Indoribates) bicarinatus Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
I. (Indoribates) microsetosus Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011j]
I. (Indoribates) nobilis (Golosoova, 1984) [Golosoova 1984]
I. (Indoribates) punctulatus (Sellnick, 1925) [as *Indoribates panabokkei* (Balogh, 1970) – Ermilov and Anichkin 2013e]
Haplozetes vindobonensis (Willmann, 1935) [Ermilov et al. 2012c]
Lauritzenia (Incabates) major (Aoki, 1967) [Vu et al. 2014]
L. (Magnobates) glagellifer (Hammer, 1967) [Vu 1990]
Peloribates (Peloribates) gressitti Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
P. (Peloribates) guttatoides Hammer, 1979 [Ermilov and Anichkin 2014f]
P. (Peloribates) guttatus Hammer, 1979 [Vu et al. 2014]

- P. (Peloribates) kaszabi* Mahunka, 1988 [Mahunka 1988]
P. (Peloribates) paraguayensis Balogh & Mahunka, 1981 [Nguyen and Vu 2012]
P. (Peloribates) pseudoporosus Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
P. (Peloribates) rangiroaensis Hammer, 1972 [Ermilov and Anichkin 2011k]
P. (Peloribates) ratubakensis Hammer, 1979 [Vu et al. 2014]
P. (Peloribates) spiniformis Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011k]
P. (Peloribates) stellatus Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
P. (Peloribates) tatyanae Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
Perxylobates brevisetosus Mahunka, 1988 [Mahunka 1988]
P. crassisetosus Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011j]
P. guehoi Mahunka, 1978 [Nguyen and Vu 2012]
P. thanhoaensis Ermilov, Vu, Trinh & Dao, 2011 [Ermilov et al. 2011c]
P. vermisetus (Balogh & Mahunka, 1968) [Vu et al. 1985]
P. vietnamensis (Jeleva & Vu, 1987) [Jeleva and Vu 1987]
Protoribates (Protoribates) capucinus Berlese, 1908 [Golosoova 1983]
P. (Protoribates) cattienensis Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011j]
P. (Protoribates) dentatus (Berlese, 1883) [as *Protoribates monodactylus* (Haller, 1884) – Vu et al. 1985]
P. (Protoribates) gracilis (Aoki, 1982) [Vu and Nguyen 2000]
P. (Protoribates) lophotrichus (Berlese, 1904) [Vu et al. 1985]
P. (Protoribates) paracapucinus (Mahunka, 1988) [Ermilov et al. 2012c]
P. (Triaunguis) acutus (Hammer, 1979) [Golosoova 1983]
P. (Triaunguis) biscalpturatus (Mahunka, 1988) [Vu et al. 2014]
P. (Triaunguis) duoseta (Hammer, 1979) [Nguyen and Vu 2012]
P. (Triaunguis) heterodactylus Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011c]
P. (Triaunguis) maximus (Mahunka, 1988) [Mahunka 1988]
Setoxylobates (Setoxylobates) foveolatus Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
S. (Polyxylobates) diversiporosus (Hammer, 1973) [Ermilov and Anichkin 2015a]
Trachyoribates irregularis (Balogh & Mahunka, 1969) [Vu et al. 1985]
T. ovulum Berlese, 1908 [as *Rostrozetes foveolatus* Sellnick, 1925 – Golosoova 1983; also as *Rostrozetes areolatus* (Balogh, 1958) – Vu et al. 1985; as *Rostrozetes punctulifer* Balogh & Mahunka, 1979 – Vu et al. 1987; as *Rostrozetes trimorphus* Balogh & Mahunka, 1979 – Vu 1990]
Transoribates agricola (Nakamura & Aoki, 1989) [Ermilov and Anichkin 2014f]
Vilhenabates sinatus (Aoki, 1965) [Ermilov et al. 2012a]

Parakalummidae

- Neoribates (Neoribates) aurantiacus* (Oudemans, 1914) [Vu 1990]
N. (Neoribates) jacoti (Balogh & Mahunka, 1967) [Balogh and Mahunka 1967]

- N. (Neoribates) monodactylus* Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
N. (Neoribates) paratuberculatus Ermilov, Shtanchaeva & Subías, 2014 [Ermilov et al. 2014d]
N. (Neoribates) spindleformis Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012c]

Galumnidae

- Allogalumna (Allogalumna) bipartita* (Aoki & Hu, 1993) [Ermilov and Anichkin 2013c]
A. (Allogalumna) costata Mahunka, 1996 [Vu et al. 2014]
A. (Allogalumna) monodactyla Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014e]
A. (Allogalumna) multesima Grandjean, 1957 [Nguyen and Vu 2012]
A. (Allogalumna) paramachadoi Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
A. (Allogalumna) rotundiceps Aoki, 1996 [Ermilov and Anichkin 2014d]
A. (Allogalumna) upoluensis Hammer, 1973 [Vu et al. 2014]
A. (Globogalumna) biporosa Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012c]
Dimidiogalumna azumai Aoki, 1996 [Nguyen et al. 2013]
D. grandjeani Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014a]
Galumna (Galumna) aba Mahunka, 1989 [Mahunka 1989]
G. (Galumna) acutirostrum Ermilov & Anichkin, 2010 [Ermilov and Anichkin 2010]
G. (Galumna) coronata Mahunka, 1992 [Vu et al. 2015]
G. (Galumna) discifera Balogh, 1960 [Nguyen and Vu 2012]
G. (Galumna) flabellifera Hammer, 1958 [as *Galumna flabellifera orientalis* Aoki, 1965 – Vu 1990]
G. (Galumna) kebangica Ermilov & Vu, 2012 [Ermilov and Vu 2012]
G. (Galumna) khoii Mahunka, 1989 [Mahunka 1989]
G. (Galumna) lanceata (Oudemans, 1900) [Golosoova 1983]
G. (Galumna) levisensilla Ermilov & Anichkin, 2010 [Ermilov and Anichkin 2010]
G. (Galumna) microfissum Hammer, 1968 [Vu et al. 2014]
G. (Galumna) obvia (Berlese, 1914) [Vu 1993]
G. (Galumna) paracalcicola Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014e]
G. (Galumna) parakazakhstani Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014b]
G. (Galumna) paramastigophora Ermilov, 2015 [Ermilov 2015a]
G. (Galumna) pseudokhoii Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011h]
G. (Galumna) pseudotriquetra Ermilov, 2015 [Ermilov 2015a]
G. (Galumna) triquetra Aoki, 1965 [Vu 1990]

- G. (Galumna) triops* Balogh, 1960 [Vu et al. 2015]
- G. (Cosmogalumna) dongnaiensis* Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013f]
- G. (Cosmogalumna) praeoccupata* Subías, 2004 [Ermilov and Anichkin 2014d]
- G. (Cosmogalumna) tenensis* Ermilov, Vu & Nguyen, 2011 [Ermilov et al. 2011b]
- G. (Neogalumna) longilineata* Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
- G. (Neogalumna) seniczaki* Ermilov & Anichkin, 2010 [Ermilov and Anichkin 2010]
- G. (Neogalumna) tolstikovi* Ermilov & Anichkin, 2014 [Ermilov and Anichkin 2014d]
- Leptogalumna (Leptogalumna) ciliata* Balogh, 1960 [Ermilov and Anichkin 2011h]
- Notogalumna foveolata* Balakrishnan, 1989 [Ermilov and Anichkin 2013g]
- N. lagunaensis* Ermilov & Corpuz-Raros, 2015 [Ermilov and Corpuz-Raros 2015]
- Pergalumna (Pergalumna) altera* (Oudemans, 1915) [Vu et al. 1985]
- P. (Pergalumna) cattienica* Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011f]
- P. (Pergalumna) granulata* Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
- P. (Pergalumna) hauseri* Mahunka, 1995 [Ermilov and Anichkin 2013d]
- P. (Pergalumna) indistincta* Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011h]
- P. (Pergalumna) kotschyi* Mahunka, 1989 [Mahunka 1989]
- P. (Pergalumna) longisetosa* Balogh, 1960 [Vu et al. 2014]
- P. (Pergalumna) magnipora capillaris* Aoki, 1961 [Vu 1993]
- P. (Pergalumna) margaritata* Mahunka, 1989 [Mahunka 1989]
- P. (Pergalumna) mauritii* Mahunka, 1978 [Vu et al. 2014]
- P. (Pergalumna) montana* Hammer, 1961 [Vu et al. 2015]
- P. (Pergalumna) nuda* Balogh, 1960 [Vu et al. 2014]
- P. (Pergalumna) paraelongata* Ermilov & Anichkin, 2012 [Ermilov et al. 2012c]
- P. (Pergalumna) pseudosejugalis* Ermilov & Anichkin, 2012 [Ermilov and Anichkin 2012d]
- P. (Pergalumna) punctulata* Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
- P. (Pergalumna) taprobanica* Balogh, 1988 [Vu et al. 2014]
- P. (Pergalumna) yurtaevi* Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011f]
- Trichogalumna nipponica* (Aoki, 1966) [Ermilov et al. 2012c]
- T. subnuda* Balogh & Mahunka, 1967 [Balogh and Mahunka 1967]
- T. vietnamica* Mahunka, 1987 [Mahunka 1987]

Galumnellidae

- Galumnella (Galumnella) cellularis* (Balogh & Mahunka, 1967) [Balogh and Mahunka 1967]
- G. (Galumnella) geographica* Mahunka, 1995 [Vu et al. 2014]

- G. (Galumnella) microporosa* Ermilov & Anichkin, 2011 [Ermilov and Anichkin 2011f]
G. (Galumnella) tiunovi Ermilov & Anichkin, 2013 [Ermilov and Anichkin 2013d]
G. (Galumnella) paulinai Balogh, 1961 [Vu et al. 2014]
G. (Bagalumnella) scavatorum (Mahunka, 1994) [Vu et al. 2014]
Porogalumnella pulchella Aoki & Hu, 1993 [Ermilov and Anichkin 2014d]

Conclusion

The list of oribatid mites of Vietnam includes now 535 species/subspecies, 222 genera and 81 families. Of these, 194 species/subspecies were described as new for science from Vietnam; 94 species have been described by Ermilov and co-authors, 30 species by Balogh and Mahunka, 28 species by Mahunka, 21 species by Niedbala, 6 species by Jeleva and Vu, 4 species by Fernandez and co-authors, 3 species by Krivolutsky, 2 species by Golosova, 2 species by Starý, 2 species by Vu and co-authors, 1 species by Balogh, 1 species by Rajski and Szudrowicz.

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A review of the cleptoparasitic bee genus *Townsendiella* (Apidae, Nomadinae, Townsendiellini), with the description of a new species from Pinnacles National Park

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Abstract

The cleptoparasitic bee genus *Townsendiella* Crawford (Nomadinae, Townsendiellini) is a rare group restricted to the southwestern United States and adjacent Mexico, whose taxonomy and biology remain poorly known. This paper describes *Townsendiella ensifera* **sp. n.**, the first known record of this genus from Pinnacles National Park. A key to the species of the genus is provided. Several potential areas of future research are also discussed.

Keywords

Apoidea, taxonomy, new species, cleptoparasitism

Introduction

Townsendiella Crawford, the only member of the tribe Townsendiellini, are one of several (e.g., *Hexepeolus* Linsley and Michener, *Rhopalolemma* Roig-Alsina) rarely collected cleptoparasitic apid bees endemic to the southwestern United States and adjacent northern Mexico (Michener 2007). Originally monobasic (Crawford 1916), Linsley (1943) reviewed the genus and divided it into three monotypic subgenera: *Townsendiella* Crawford, 1916 (type species: *Townsendiella pulchra* Crawford, 1916),

Xeropasites Linsley, 1943 (type species: *Townsendiella rufiventris* Linsley, 1942), and *Eremopasites* Linsley, 1943 (type species: *Townsendiella californica* Michener, 1936). Michener (2000) later synonymized *Xeropasites* and *Eremopasites* with *Townsendiella* s. str. The genus has recently been included in phylogenetic studies, where it is apparently closely related to two other clades of Nomadinae: ((*Townsendiellini*+*Neolarini*)+*Biastini*) (Cardinal and Danforth 2013, Payne 2014). As this agrees with historical placement (Linsley 1943), the tribal position of *Townsendiellini* seems settled, pending any future data. An intensive study of the bees of Pinnacles National Park (Messinger and Griswold 2003) yielded specimens of *Townsendiella* that ran to *T. pulchra* in the available revisionary study (Linsley 1943), but appeared to differ from other known *T. pulchra*. Similar unexpected variation was subsequently observed in specimens of *T. rufiventris*.

Here we describe a new species, *Townsendiella ensifera* sp. n., and discuss the taxonomic status of *T. rufiventris*. A key to known species of *Townsendiella*, using a combination of novel and historic characters, is also provided. Though few in species, this group may prove useful as indicator species (Sheffield et al. 2013). Comments are given for future research directions regarding the biogeography and evolution of bee host choice in this group.

Materials and methods

A total of 443 specimens of *Townsendiella* from nine institutions were examined in this study, comprising both sexes of all four species (65 *T. ensifera*, 197 *T. pulchra*, 169 *T. rufiventris*, and 12 *T. californica*). The male genitalia of 12 *T. ensifera* and 12 *T. pulchra* were compared. Thorough examinations of maxillary palp morphology were also conducted (33 *T. ensifera*, 30 *T. pulchra*, 20 *T. rufiventris*, and 1 *T. californica*). A listing of specimens examined is provided as a supplementary file in reduced Darwin Core format. This file includes location descriptions for each specimen in the “locality” field, which when discussed throughout this text are given in double quotes.

Morphological terminology (e.g., T = tergum, S = sternum) follows Michener (2007), with two exceptions: the pseudopygidial area on T5 of *T. rufiventris* is called the lunule and the minute first maxillary palpomere of *T. rufiventris* is counted as a true palpal article rather than a tubercle (Linsley 1943). Images were taken with a Keyence VHF-500x Digital Microscope, and then processed using Photoshop CS5 Extended Version 12.0 (Adobe 2010, San Jose, CA). The map was generated with ArcMap10.2.2 (ESRI 2014, Redland, CA). All ecoregion calculations were based on the World Wildlife Fund (WWF) terrestrial ecoregions of the world (Olson et al. 2001).

Institutions that provided material, along with abbreviations used in the text and supplementary metadata, are as follows:

- AMNH** American Museum of Natural History, New York, NY, USA (J. Rozen, E. Wyman)
- CAS** California Academy of Sciences, San Francisco, CA, USA (N. Penny, B. Zuparko, V. Lee)
- EMEC** Essig Museum of Entomology, University of California, Berkeley, CA, USA (P. Oboyski)
- NPIC** U.S. National Pollinating Insects Collection, USDA-ARS Pollinating Insects Research Unit, Utah State University, Logan, UT, USA
- SDNHM** San Diego Natural History Museum, San Diego, CA, USA (J. Hung)
- UCDC** Bohart Museum of Entomology, University of California, Davis, CA, USA (L. Kimsey, S. Heydon)
- UCR** Entomology Research Museum, University of California, Riverside, CA, USA (D. Yanega)
- UCSD** University of California at San Diego, La Jolla, CA, USA (J. Hung)
- USNM** U.S. National Entomological Collection, National Museum of Natural History, Washington, D.C. (S. Brady, B. Harris)

Results

Morphological analysis strongly supported the separation of the Pinnacles specimens from *T. pulchra*. Numerous characters which separate this species, *T. ensifera*, from other species of *Townsendiella* were identified. The number of palpomeres and their form in the maxillary palpus were found to be instrumental in distinguishing *T. ensifera* from the morphologically similar *T. pulchra* (Fig. 1). Both male and female specimens of *T. ensifera* were consistent in the characters of the maxillary palpus. In light of the utility of this character, spreading of the mouthparts is strongly encouraged in specimens of *Townsendiella*. Genitalic and other differences are further discussed in the subsequent key and species description. The geographic distribution of *T. ensifera* is also somewhat useful. Based on current records, *T. ensifera* is only known from California, in the South Coast Range and Transverse Range, apparently absent from much of the range of the similar *T. pulchra* (Fig. 2). Only *T. ensifera* and the dissimilar, more southerly *T. rufiventris* are currently known from the area north and west of the Transverse Range and Sierra Nevada. However, the presence of a single *T. ensifera* near Riverside, California demonstrates that this species is not limited to that region.

A DiscoverLife identification guide has also been created for this group. This guide may be accessed at: <http://www.discoverlife.org/mp/20q?guide=Townsendiella>

Instructions for the use of these guides are included within the Very Handy Bee Manual (Droege 2012).

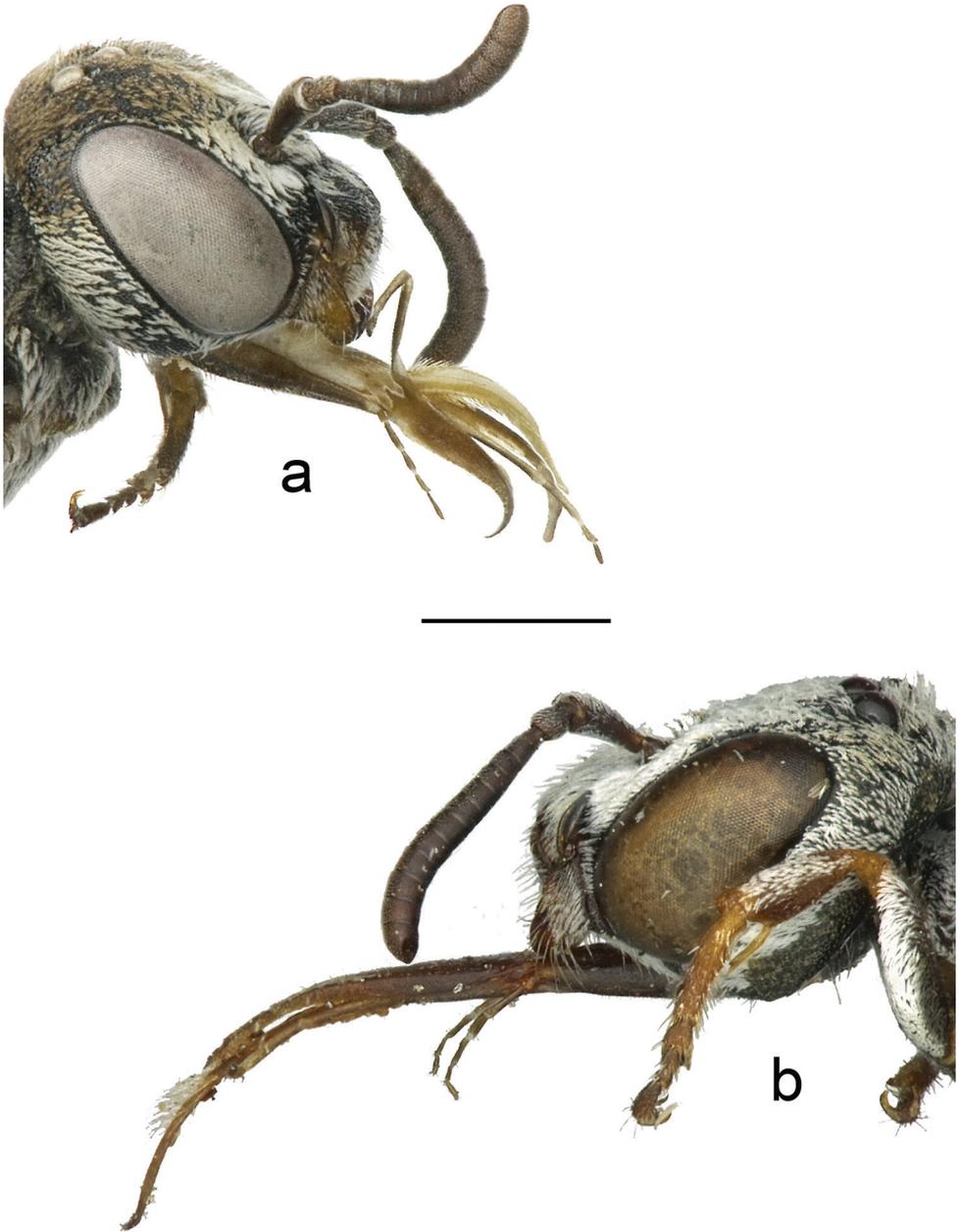


Figure 1. The male maxillary palp of **a** *T. ensifera* sp. n. (BBSL331567) and **b** *T. pulchra* (BBSL209492). The length of the terminal, fifth segment in *T. ensifera* sp. n. well exceeds that of the terminal, sixth segment in *T. pulchra*, likely due to the fusion of the fifth and sixth segments in the former. This character is reflected in both sexes. The scale bar represents 0.75 mm.

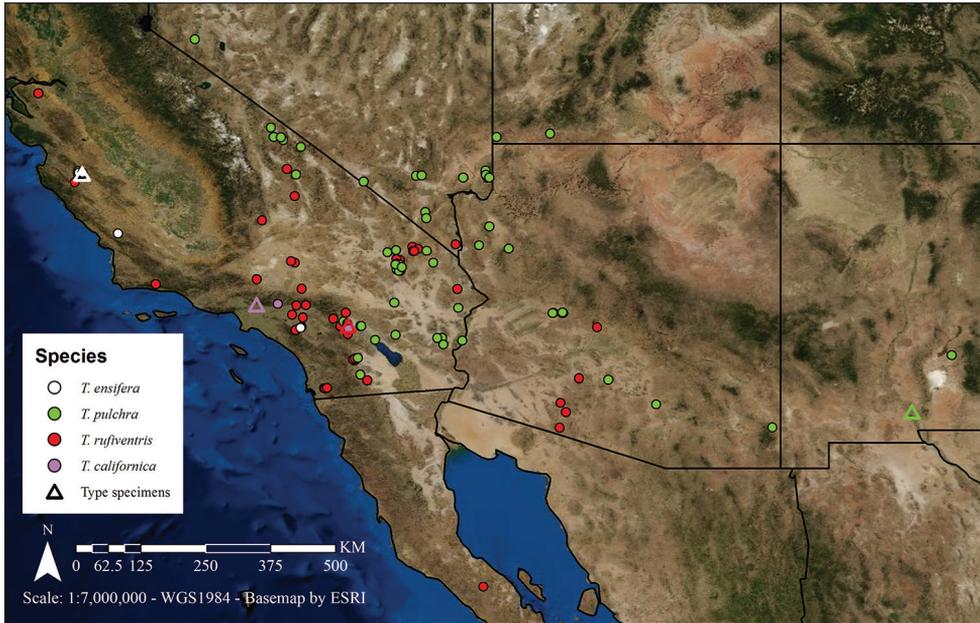


Figure 2. The distribution of *Townsendiella*. Species are color-coded as follows: *T. ensifera* sp. n. in white, *T. pulchra* in green, *T. rufiventris* in red, and *T. californica* in violet. The type locality for each species is given as a hollow triangle of its corresponding color.

Key to the species of *Townsendiella*

- 1 Metanotum not, or only slightly, produced medially, not produced into abrupt medial knob. **Female:** T5 without lunule (apical rim gradually and evenly concave; Fig. 3a). **Male:** gonoforceps only moderately flattened, opaque such that ventral spike is not visible in dorsal view; gonoforceps split apically, with long setae arising from tips **2**
- Metanotum with distinct, abrupt medial knob, clearly produced even if covered in thick pubescence. **Female:** T5 with apical lunule (depressed, finely-pitted, tessellate apicomедial region; Fig. 3b). **Male:** gonoforceps broadened and flattened, fully transparent such that ventral spike is visible in dorsal view; gonoforceps not split apically, with setae arising from tip short.....
 ***Townsendiella rufiventris* Linsley, 1942**
- 2 Marginal cell elongate, about equal in length to distance from tip of marginal cell to wing tip or longer; posterior margin of first submarginal cell longer than posterior margin of second submarginal cell, but clearly less than twice length of posterior margin of second submarginal cell; second submarginal cell with distal vein strongly curved, much longer than proximal vein; apical fasciae of T2–T4 incompletely divided medially or, when completely divided, only narrowly so. **Female:** mesoscutellum black..... **3**

- Marginal cell shorter, length less than that from tip of marginal cell to wing tip; posterior margin of first submarginal cell about twice as long as posterior margin of second submarginal cell, sometimes slightly shorter; second submarginal cell with distal vein only slightly curved, approximately same length as proximal vein; apical fasciae of T2–T4 strongly interrupted medially. **Female:** mesoscutellum red *Townsendiella californica* Michener, 1936
- 3 Maxillary palp with five palpomeres, terminal palpomere about equal in length to long second palpomere (Fig. 1a). **Female:** S5 with setae evenly covering segment throughout (may be worn off in older specimens); pygidial plate relatively flat and smooth, punctures distinct. **Male:** genital capsule in dorsal view with upper gonostylus small and short, distance from tip of upper gonostylus to tip of lower gonocoxite equal to twice maximum width of upper gonostylus or greater (Fig. 4a)..... *Townsendiella ensifera* sp. n.
- Maxillary palp with six palpomeres, terminal palpomere clearly shorter than long second palpomere, usually about equal to fifth palpomere (Fig. 1b). **Female:** S5 with apicomedial, asetose V- shaped patch, defined by slight integumental indent; pygidial plate craggy, punctures indistinct. **Male:** genital capsule in dorsal view with upper gonostylus large and long, distance from tip of gonostylus to tip of gonocoxite equal to about maximum width of upper gonostylus or less (rarely greater) (Fig. 4b) *Townsendiella pulchra* Crawford, 1916

***Townsendiella ensifera* sp. n.**

<http://zoobank.org/D80DEF1E-DFC2-4D3F-A539-1FC439A78A67>

Figs 1a, 3a, 4a, 5a, and 6–8

Type-locality. USA, California: San Benito County, Pinnacles National Park, East of Mount Defiance, 36.46060 -121.15210, Blue oak woodland, white pantrap, 29 May 1999, O. Messinger leg., host unknown.

Holotype. Female, pinned. Original label: “USA CA San Benito Co., / Pinnacles Natl. Mon., / East of Mount Defiance / 36°28.24’N 121°09.13’W [white typed label]” “White pantrap, burn / Blue oak woodland [white typed label]” “29 May, 1999 / O. Messinger [white typed label]” “NativeBeeSurvey / USDA, Logan, Utah / BBSL331869 [barcode label].”

Paratypes. Nine topo-typical specimens (1F8M); all deposited in the NPIC. Unique specimen identifiers are as follows: BBSL330902, BBSL331852, BBSL331878, BBSL331886, BBSL331887, BBSL331891, BBSL332126, BBSL332137, and BBSL332139. Selected specimen data for each paratype is available in the Suppl. material 1.

Other material. Additional records are detailed in the Suppl. material 1.

Diagnosis. Both males and females are most similar to *T. pulchra* and are separated easily from other *Townsendiella* by the absence of a medially projecting knob on



Figure 3. The female T5 of **a** *T. ensifera* sp. n. (BBSL331869) and **b** *T. rufiventris* (BBSL209499). The latter has a distinctly impressed, finely-pitted, and tessellate lunule medially in the pseudopygidial area, a feature unique within *Townsendiella*. The scale bar represents 0.5 mm.

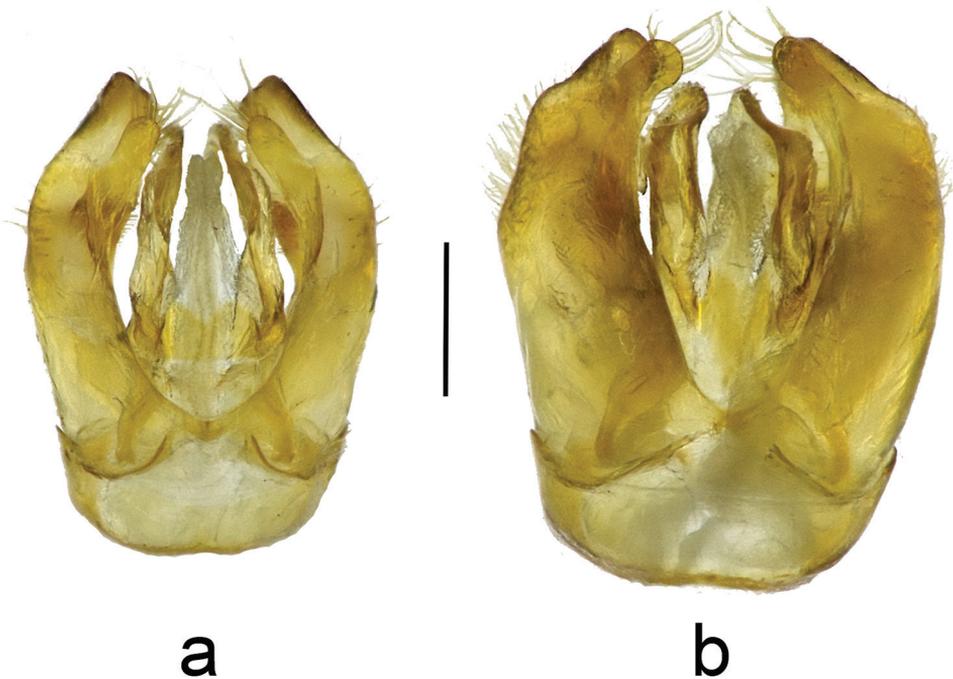


Figure 4. The male genitalia of **a** *T. ensifera* sp. n. (BBSL259479) and **b** *T. pulchra* (BBL340294). Dorsal view of genital capsule and gonostylus. The scale bar represents 0.25 mm.

the metanotum, the length of the marginal cell being about equal or greater than the distance from its posterior tip to the posterior tip of the wing, and the asymmetrical second submarginal cell with the longer, more curved distal vein. From *T. pulchra*,

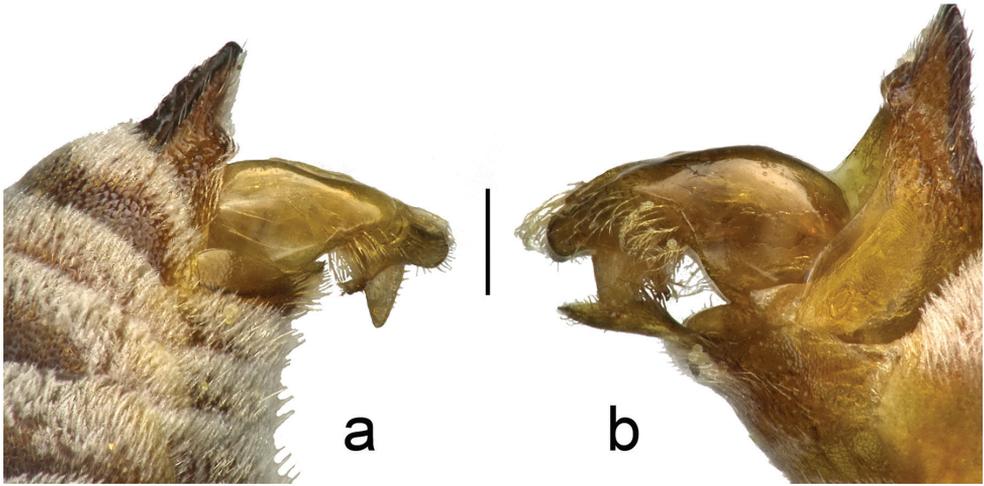


Figure 5. The male genitalia of **a** *T. ensifera* sp. n. (BBSL259415) and **b** *T. pulchra* (BBSL349670). Lateral view of gonocoxite, with focus on setae. The scale bar represents 0.25 mm.

it may be differentiated by the following characteristics: five maxillary palpomeres, the final palpomere almost as long as the second palpomere; male pygidial plate narrower, sharper at the tip; female S5 lacking a pubescent apicomedial area, setal density roughly even along rim; when viewing the genital capsule laid flat against a surface, with your view perpendicular to the surface: the tip of the male gonostylus is about its maximum width distant from the gonocoxite tip, while in *T. pulchra* it is almost equal; male gonocoxite with shorter, fewer, and less plumose setae present ventrolaterally (Fig. 5a) compared to *T. pulchra* (Fig. 5b); and male genital capsule smaller overall, with gonocoxites less expanded apically (Figs 4, 5).

Description. Female: Head: Pubescence dense, white, covering one-half to three-fourths of height of compound eye, becoming sparser, less plumose, and often off-white in coloration dorsally. Facial integument black, reddening, if present, limited to labrum and rim of clypeus. Punctation relatively dense throughout, integument shining between punctures. Mandible simple, basal three-fourths to four-fifths of integument dull orange to bright yellow, tip reddish-brown. Clypeus protuberant from anterior margin of compound eye by one-third to one-half of maximum compound eye width. Integument of antenna dark brown to black, sometimes light brown. Compound eyes slightly converging ventrally. Lateral ocellus distinctly closer to rear margin of head than compound eye, separated from rear margin by roughly 2–2.5× lateral ocellar diameter.

Mesosoma: Pubescence all white except for light brown areas of mesoscutum and mesoscutellum. Pubescence dense over mesosoma, except slightly less dense where brown, sparser on pronotum anteriorly, mesepisternum anteriorly, and propodeal triangle below metanotum. Mesoscutum with pubescence primarily light brown except along border, with two thin, longitudinal stripes of white setae intruding posteriorly from anterior margin for one-third to one-half of mesoscutal length. Mesoscutellum

with pubescence largely light brown, bordered by white, with anterior-directed stripe of white setae from posterior midline. Integument dark brown to black, often with pronotal lobe reddened, less commonly reddened ventrally or elsewhere on pronotum. Punctuation dense throughout mesosoma, except propodeal enclosure where absent or obscured by tessellation, integument otherwise relatively smooth and shiny. Tegula brown, slightly transparent, but obscured by dense setae.

Wings: Wings equal to 3.0–3.1× medial length of mesoscutum along longitudinal axis. Length of marginal cell slightly greater than distance from distal tip of marginal cell to apical tip of wing. Length of posterior margin of first submarginal cell greater than that of second, but clearly less than twice length of second. 2m-cu usually interstitial with juncture between first and second submarginal cells, or only slightly past this point, creating four-way intersection.

Legs: Integumental color variable, ranging from dark brown to light reddish-brown; tarsi typically black. White pubescence present ventrally on femora, variable in extent. Outer surfaces of tibiae densely clothed in white pubescence, densest on metatibia. Thicker, spine-like setae readily apparent on meso- and metatibiae, usually obscured by pubescence on protibia.

Metasoma: Pubescence white except in basal areas of terga, where it is slightly browned; brown setae obscured on T5 by white setae throughout. Pubescence denser and more branched apically on terga, creating distinct setal bands on T1–T4, each of which is usually thinner medially and thicker laterally, with V-shaped medial notch. Sternal pubescence primarily white but thinner and sparser in basal and lateral areas, appearing apically banded at some angles. S5 pubescence relatively even throughout. Integumental color of terga highly variable, ranging from nearly all black to nearly all red. T5 even across its rim, with simple setae extending posteriorly. Pygidial plate roughly square, only slighter wider at base than apex due to rounded posterior corners; entire rim slightly raised into carina, marked by darker, blackish integument there compared to pitted, redder interior.

Male similar to female, except as follows: **Head:** Pubescence all white, dense over much of lower face up to slightly below top of compound eye and on gena to about top of compound eyes, becoming less branched and, in some specimens, slightly off-white near vertex. Clypeus protuberant from anterior margin of compound eye by about half of compound eye width or slightly less. Paraocular area raised into flange adjacent to clypeus, this area impunctate and shiny. Integument of antenna dark brown to black, sometimes slightly lighter brown on apical segments.

Mesosoma: Integument dark brown to black, rarely with dark reddish-brown coloration on pronotum or venter.

Wings: Wings equal to 3.1–3.2× medial length of mesoscutum along longitudinal axis.

Legs: Integumental color variable, ranging from dark brown to light reddish-brown. Metabasitarsus and metatarsus more obscured by white, plumose setae than on other legs.

Metasoma: Pubescence white except in basal areas of terga, where slightly browned; brown setae intermixed with lighter setae in basal area of T6. Pubescence denser

and more branched apically on terga, creating distinct setal bands on T1–T4, each of which is usually thinner medially and thicker laterally, typically with those of T5 and T6 thicker, more uniform overall. S6 pubescence notably thinner than preceding sterna, not appearing apically banded. Integumental color of terga variable from dull reddish-brown to near black, more often black. Pygidial plate coming to acute point, sides roughly straight to weakly convex, heavily-sculpted medial region often raised, integumental color darker brownish around rim and reddened interiorly.

Etymology. The specific epithet, *ensifera*, is Latin for sword-bearing. This name is primarily a reference to the elongate, sword-shaped terminal maxillary palpomere, and secondarily a reference to its cleptoparasitic life history.

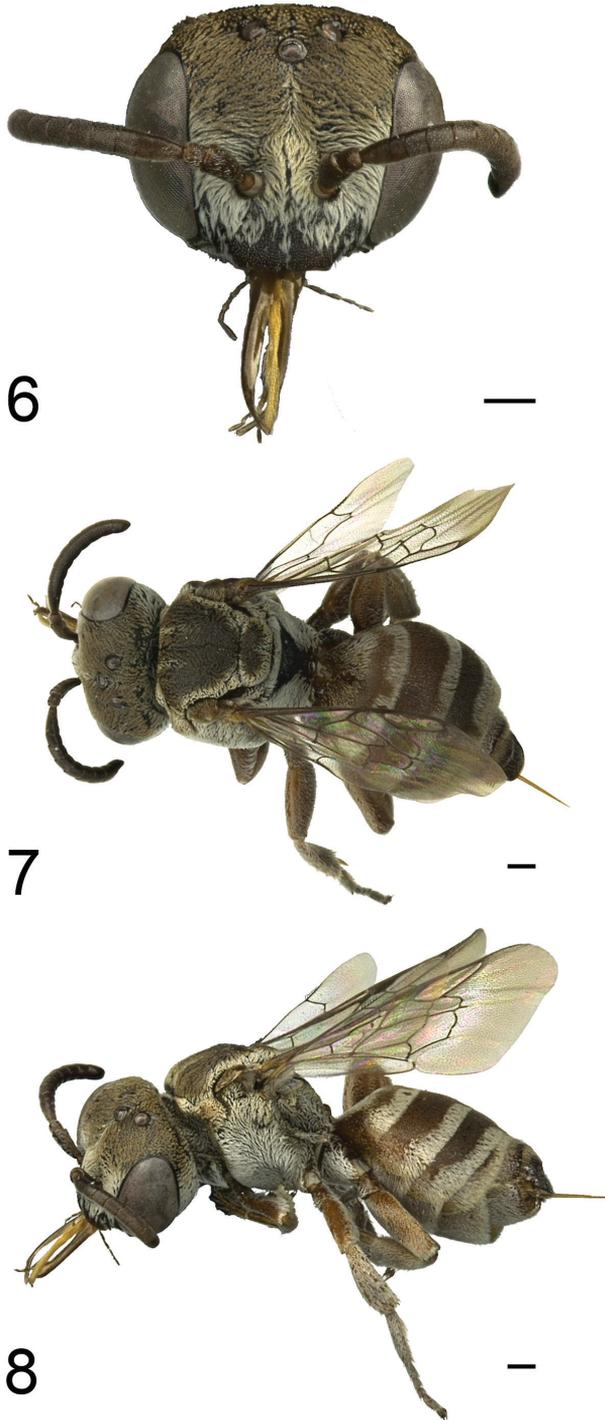
Distribution. *Townsendiella ensifera* is known primarily from the South Coast Range of California, where it has been found at two localities: Pinnacles National Park in San Benito County and San Luis Obispo County, 6 mi NE Santa Margarita. The only other record is a single specimen from south of the Transverse Range (“The Gavilan”) near Riverside, California. More collections are necessary to determine the true extent of this species’ range, although it presently appears more restricted than most other *Townsendiella*.

Phenology. Known to be active from early May to late August. Within Pinnacles National Park, where multiple collections took place, it was collected from early May to early July.

Bee hosts. There is no direct knowledge concerning the host of *T. ensifera*. Given the apparent preference of the similar *T. pulchra* for species of *Hesperapis* Cockerell and the extensive sampling of Pinnacles National Park, *Hesperapis* as a potential host may be inferred as a working hypothesis. Only two species of *Hesperapis*, *H. (Ambyapis) ilicifoliae* (Cockerell, 1910) and *H. (Panurgomia) regularis* (Cresson, 1878), have been recorded from Pinnacles. *Hesperapis regularis* is many times the body size (>12 mm in length) of *T. ensifera*, while *H. ilicifoliae* is about 5–6 mm in length, suggesting *H. ilicifoliae* is the better candidate as host. The likelihood of *H. ilicifoliae* as host increases when considering that it belongs to the same subgenus as *H. (A.) larreae* Cockerell, 1907, the host for *T. pulchra* (Rozen and McGinley 1991). Further support is derived from the synchrony of their flight periods; both *H. ilicifoliae* and *T. ensifera* fly from May to July, with the majority of records from June and July. It should also be noted that the specimen of *T. ensifera* from Gavilan, California was collected on *Adenostoma fasciculatum* Hook. & Arn., the plant which *H. ilicifoliae* specializes on, and in an area where *H. ilicifoliae* has been collected previously (Moldenke and Neff 1974, DiscoverLife 2014). In fact, *T. ensifera* has only been collected in areas where *H. ilicifoliae* is known. Based on all evidence, *H. ilicifoliae* is currently the most likely host for *T. ensifera*.

Floral hosts. Polygonaceae: *Eriogonum fasciculatum* Benth. and Rosaceae: *Adenostoma fasciculatum*.

Discussion. This species is much closer in body form to *T. pulchra* than the other species are to each other or to the pair of *T. ensifera* and *T. pulchra*. It is possible that these very distinct species led to a general expectation of great differentiation between species in the group and that the relatively minor differences between *T. ensifera* and *T. pulchra* were overlooked as a result.



Figures 6–8. General appearance of the female *T. ensifera* sp. n. holotype (BBSL331869). **6** Anterior view of face **7** Dorsal view of body **8** Lateral view of body. All scale bars represent 0.25 mm.

***Townsendiella californica* Michener, 1936**

Holotype. female, pinned; Altadena, California; 6-26-35 [26 June 1935]; deposited in CAS (Type#4544).

Diagnosis. This species is immediately separable from all other species by its wing venation. The marginal cell is the shortest of any *Townsendiella*, its maximum length significantly shorter than the distance from its apical tip to the apex of the wing. The posterior margin of the first submarginal cell is roughly twice the length of the second submarginal's, while in other species the first submarginal cell is closer to 1.5× the length of the second. The second submarginal cell forms a nearly symmetrical triangle with the distal vein nearly straight and nearly the same length as the proximal vein.

Distribution. *Townsendiella californica* has a relatively restricted range in comparison to the other species of the genus. It is currently known from localities along the southern edge of the Transverse Range and north of the nearby Mt. San Jacinto. Interestingly, these collections are all along the edge of the California montane chaparral and woodlands ecoregion. It may be that this species, its host, or both inhabit a very narrow ecological niche.

Phenology. The phenology of this species is difficult to ascertain due to few collection records, though it appears to be active from late April through June.

Bee hosts. Uncertain. This species is hypothesized to be cleptoparasitic on *H. (Zacesta) rufipes* (Ashmead, 1899) based on observation of *T. californica* flying over a nesting aggregation of the former (Michener 1936). Multiple attempts were made to confirm the host at the same site; the population has apparently been extirpated by urban sprawl from the Altadena, California area (Stage 1966).

Floral hosts. No floral records are known for this species.

Discussion. This exceptionally rare species is known from the fewest specimens of any *Townsendiella*. As such, much remains to be discovered regarding its distribution, host specificity, and environmental constraints.

***Townsendiella pulchra* Crawford, 1916**

Figs 1b, 4b, and 5b

Holotype. female, pinned; Las Cruces, New Mexico; 5.12 [12 May]; deposited in USNM (Type#20831).

Diagnosis. This species may be distinguished from *T. californica* and *T. rufiventris* by the combination of the following characteristics: long marginal cell, the maximum length of which is about equal to or longer than the distance from the marginal cell tip to the apex of the wing; metanotum lacking medial projection, only very gradually curved throughout its width; and the female lacking a lunule on T5. *Townsendiella pulchra* is more similar to *T. ensifera* than to the other species, and may be separated from it as presented in the latter's species account.

Distribution. Present from west-central Nevada to central New Mexico, and ranging southward to the Mexican border, *T. pulchra* has arguably the largest range in the genus. With both the most northerly and easterly collection sites, *T. pulchra* inhabits a number of different ecoregions, given here in order from most to least collection localities: Mojave Desert, Sonoran Desert, Chihuahuan Desert, Great Basin shrub steppe, and Colorado Plateau shrublands.

Phenology. *Townsendiella pulchra* has been collected primarily during April and May, although a small number of records exist from late March and June. Surprisingly, there are two records of this species from August near Portal, Arizona. It may be that this species is active during the fall in the Chihuahuan Desert, in time with monsoonal rains. Additional collections are necessary to investigate this possibility, though the collection of the Las Cruces type specimen in May demonstrates it does not fly exclusively in fall in the Chihuahuan Desert.

Bee hosts. This species is known to invade and oviposit within nests of *H. (A.) larreae*, mistakenly placed in *Hesperapis (Panurgomia)* in previous host associations (Michener 1936, Michener 2000, Rozen and McGinley 1991). The description of this behavior includes extensive notes on the interactions between these two species (Rozen and McGinley 1991).

Floral hosts. Asteraceae: *Baileya pleniradiata* Harv. & A. Gray, *Baileya* sp. Harv. & A. Gray ex Torr., *Chaenactis* sp. DC.; Boraginaceae: *Tiquilia hispidissima* (Torr. & A. Gray) A.T. Richardson; Fabaceae: *Psorothamnus arborescens* (Torr. ex A. Gray) Barneby, *Psorothamnus fremontii* (Torr. ex A. Gray) Barneby, *Psorothamnus schottii* (Torr.) Barneby, *Psorothamnus* sp. Rydb.; Zygophyllaceae: *Larrea tridentata* (DC.) Coville.

Discussion. This species is the best known of the *Townsendiella*, given the extensive life history work conducted on it and its host (Rozen and McGinley 1991). It will be interesting to see if all *Townsendiella* share similar life histories, once such information becomes available for the remaining species.

Townsendiella rufiventris Linsley, 1942

Fig. 3b

Holotype. female, pinned; Palm Springs, California; Mar 26, 1932; deposited in CAS (Type#14881).

Diagnosis. The female of this species may immediately be separated from the other *Townsendiella* by the presence of the lunule on T5 (apicomedial impressed rim with dense, fine punctures). Both females and males also have a strong medial production on the metanotum, which is not seen in other species. The male gonoforceps are quite distinctly flattened and relatively transparent, lacking a distinct gonostylus.

Distribution. The distribution of *T. rufiventris* is exceptionally broad among the *Townsendiella*, spanning from Baja California, Mexico, and the eastern Sonoran Desert,

extending northward through the coastal ranges nearly to the San Francisco Bay. As such, this species has both the most southerly and westerly collection events of any *Townsendiella*. It inhabits several ecoregions, given here in order of most to least collection localities: Mojave Desert, Sonoran Desert, California coastal sage and chaparral, California interior chaparral and woodlands, California montane chaparral and woodlands, and Baja California Desert.

Phenology. This species is known to fly from mid-March through July, although its phenology appears to differ throughout its range. Within the Mojave, it appears to be most active from late March through May. In the South Coast Range, however, it appears to be active in June and July. Further collections from its northern distribution are necessary to test this possibility.

Bee hosts. Interestingly, *T. rufiventris* appears to use the halictid genus *Conanthalictus* Cockerell as hosts, although prior publications have not listed hosts at the species-level (Linsley 1958, Rozen and McGinley 1991). A determination label by the late Paul D. Hurd with a date of 1963 from the Essig Museum gives the determination of *T. rufiventris* and states it was “flying about nest site of *Conanthalictus nigricans* Timb.” The label was placed before a series of *T. rufiventris* from “San Marcos Ranch HQ, Santa Inez Mts,” found near Santa Barbara Co., California. More recently, *T. rufiventris* has been collected northwest of San Bernardino, California (“N Sierra Ave”) invading the nests of *Conanthalictus bakeri* Crawford, 1907 (D. Yanega, unpublished observations, 30 April 2015). Circumstantial evidence has also been found in the association of high numbers of *T. rufiventris* at sites with *C. bakeri* (“Jamul CA” and “Spring Valley CA”) and *Conanthalictus wilmattae* Cockerell, 1936 (“Anza-Borrego, In-Ko-Pah Park” and “Anza-Borrego: Peña Spring”), all sites which yielded few to no *Hesperapis*, though no positive host associations were possible (J. Hung, unpublished observations, 14 May 2015).

Floral hosts. Asteraceae: *Lasthenia californica* DC. ex Lindl.; Boraginaceae: *Cryptantha intermedia* (A. Gray) Greene, *Cryptantha* sp. Lehm. ex G. Don, *Phacelia distans* Benth., *Phacelia* sp. Juss.; Onagraceae: *Chylismia munzii* (P.H. Raven) W.L. Wagner & Hoch.

Discussion. The possibility that *T. rufiventris* is two species was explored based on observations by Doug Yanega (pers. comm., 10 December 2013). The primary character investigated was the form of the lunule on the female T5, a finely-pitted, tessellate apicomedial depression filling the otherwise concave rim, the presence of which is a unique character for *T. rufiventris*. The species may be roughly split into two series, those with a flat apical rim on the lunule (series 1) and those with an apical lunule which projects farthest medially (series 2; allied with type of *T. rufiventris*). However, the reliability of the lunule as a character is questionable in light of its apparent flexibility, demonstrated by the variability in its angle relative to the rest of T5 across specimens of the same series. The area basal to the lunule is also variable, going from sparsely pitted and shiny in series 1 to densely, craggily pitted and dull in series 2, although numerous exceptions have been discovered. The proportion of black integument basal to the lunule also varies, with more in series 1 and less in series 2, but

exceptions to this have also been found. The male genitalia were also examined, using four males collected with females of series 1 and three specimens associated with series 2 females, but no diagnostic characters were detected. No characters from either sex which clearly and consistently delineate the two entities were discovered. A geographic split is also impossible; although series 2 is primarily found in southern California, the range of series 1 appears to completely envelop that of series 2. It must also be noted that females of both series 1 and series 2 were found from the same collection event thrice, casting further doubt on the existence of two species.

Discussion

It is now clear that the *Townsendiella* from Pinnacles National Park (*T. ensifera*) represent a species separate from *T. pulchra*. Consistent differences of the mouthparts, male genitalia, and other characters confirm this. The apparently allopatric distribution of these species further supports this distinction. Despite intense collection effort near Riverside, California, by P.H. Timberlake and others, only a single male of *T. ensifera* was collected (Fig. 2); no *T. pulchra* were detected. The closest record of *T. pulchra* is only 75 kilometers away, but this seemingly insignificant distance represents the transition from the California coastal sage and chaparral ecoregion to the northeastern limit of the Sonoran Desert ecoregion, a significant ecological leap.

The question of potential cryptic species within *T. rufiventris* warrants further investigation. It is possible that there may be two or more species, which would explain the high level of variation and the exceptions to the character patterns discovered. Given this high level of variation, there is inadequate material available at this time to determine whether or not *T. rufiventris* is a species complex. Future study using molecular techniques would be beneficial, but access to molecular-grade specimens is limited due to the rarity of this group. Although Michener (2000) previously rejected the subgenera of *Townsendiella*, hesitantly doing so as they were “unnecessary” in light of so few species, they may prove useful if *T. rufiventris* is found to be a species complex.

The biogeography and host evolution of *Townsendiella* are areas ripe for research. Although all species are found in the southwestern US and adjacent Mexico, the seemingly disjoint distributions of *T. ensifera* and *T. rufiventris* raise the questions of how and when they arrived in the South Coast Range. It may be that ancestral populations had much larger distributions, including the deserts and Mediterranean California, but were then separated by Neogene uplift or Pleistocene climate change, as they have apparently played a role in the diversification of other Hymenoptera (Wilson and Pitts 2010). An alternative explanation is dispersal, where both species have surmounted the incomplete barrier imposed by the Transverse Range and lower Sierra Nevada to become established in Mediterranean California. As Mediterranean vegetation occurs along the sides of the Transverse Range and patchily throughout it, it may be that avenues of dispersal are even currently available to this group. The directionality of dispersal under the latter hypothesis is another open question to be investigated.

Host evolution may prove to be an even more interesting area of research. Most host records are in the melittid genus *Hesperapis*, but *T. rufiventris* apparently attacks *Conanthalictus*. The most obvious question is how this host switch occurred. As *Townsendiella* is known to search for nesting sites, and oligoleges often nest near their host plants, it may be that the switch was facilitated by both *Conanthalictus* and *Hesperapis* nesting near a shared host plant (Rozen and McGinley 1991). Two possibilities are the plant genera *Nama* L. and *Phacelia* Juss., on both of which some *Conanthalictus* and *Hesperapis* specialize (Moldenke and Neff 1974, Rozen 1987, Stage 1966). Given that *C. bakeri*, *C. nigricans* Timberlake, 1961, and *C. wilmattae* are all specialists on *Phacelia*, the latter possibility seems more likely at present.

A second, related question is why only *T. rufiventris* possesses a lunule. As this species appears to be the only *Townsendiella* which attacks *Conanthalictus* nests, it may be that the lunule serves a special function for invading or ovipositing within the nests of that group. As *T. pulchra* apparently enters open nest cells, as evidenced by their oviposition into the cell wall (Rozen and McGinley 1991), there are many ways in which the female could be using the lunule, such as smoothing soil or applying secretions. More work is necessary to understand the evolution of these elusive bees.

Acknowledgements

We first thank Olivia Messinger and her assistants for their collecting efforts in Pinnacles National Park, for without their efforts this species would still be unknown, and Joan Meiners for her subsequent work in Pinnacles National Park. Our thanks are also extended to Doug Yanega for his input on *T. rufiventris* and James Hung for his comments on hosts of *T. rufiventris*. We further thank all specimen providers and institutions listed in the methods. The three anonymous reviewers are thanked for their comments. Anthony Auletta is thanked for his mastery of Latin and advice in its usage. Databasing of the specimens was supported by National Science Foundation grants DBI-0956388 and DBI-0956340. Finally, we thank Pinnacles National Park and Zion National Park for their interest in bees, funding, and logistical support of past inventory projects.

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Supplementary material I

Townsendiella specimen data

Authors: Michael C. Orr, Terry L. Griswold

Data type: species data

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Butterflies of the Bodoquena Plateau in Brazil (Lepidoptera, Papilionoidea)

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Abstract

Butterflies and moths are found in all terrestrial environments and require efforts for a better understanding of its mega-diversity. These taxa have been the subject of several studies involving phylogeny, ecology and environmental impacts. Nevertheless, several areas in the tropics remain unexplored, resulting in gaps in the taxonomic composition and distribution of butterflies in endemic environments. Therefore, a survey of the butterfly fauna of the Bodoquena Plateau in Brazil was conducted. This area consists of tropical Atlantic Forests, with marginal influences of Savannah, Chaco and Pantanal. Sampling was carried out in 20 locations using Van Someren Rydon traps and insect nets between November 2009 and April 2015. Active collection of individuals was conducted from 9:00 to 17:00h, totaling 240 hours of sampling effort. In total, we registered 768 individuals belonging to 146 species of 98 genera, six families and 18 subfamilies. Nymphalidae was the richest family (84 species), followed by Hesperidae (22 species), Riodinidae (14 species), Pieridae (12) Papilionidae (11 species) and Lycaenidae (five species). We sampled 239 nymphalids in traps, with 48 species, 30 genera, 15 tribes and five subfamilies. The most common species were *Eunica macris* (Godart, 1824), *Dynamine artemisia* (Fabricius, 1793) and *Memphis moruus* (Fabricius, 1775). Therefore, this study contributes to the knowledge of the Neotropical butterfly diversity and distribution, providing 37 new records and supporting the use of wildlife inventories as important tools for the knowledge of tropical forests biodiversity and conservation.

Keywords

Biodiversity inventory, conservation unit, Atlantic Forest

Introduction

Insects occupy a prominent position in biological studies on communities and habitats conservation due to its biodiversity and role in ecological processes (Elton 1973; Janzen 1987; Hölldobler and Wilson 1990; Gaston 1991; Wolda 1992, Groombridge 1992; Kato et al. 1995). Nevertheless, while insects are the most diverse group on the planet, accounting for more than half of the described living organisms, knowledge is still relatively scarce when compared to other groups (Teston and Corseuil 2002).

Butterflies and moths are found in all terrestrial environments and require efforts to better understand its mega-diversity (De Vries 1987). These taxa have been the subject of several studies involving phylogeny, ecology and environmental impacts (Brown Jr. 1996). Furthermore, the group predictably responds to environmental changes because of its microhabitat fidelity, thus facilitating rapid reactions to habitat degradation (Brown Jr. 1996). However, the natural history of most groups is still unknown, what limits conservation acts, since species respond individually to the effects of fragmentation and habitat loss (Summerville et al. 2001).

In Brazil, foreigners made the first studies on butterflies, and the first Brazilian to conduct studies was Adolpho Mabilde, who also was the first to put together a collection of Lepidoptera (Freitas and Marini-Filho 2011). Studies on butterflies were then concentrated in areas of Atlantic and Amazonian forests (Brown Jr. 1996; Uehara-Prado et al. 2004, 2009; Brown Jr. and Freitas 1999), with a few studies concentrating in the areas of Cerrado and semi-deciduous forests (Carneiro et al. 2008). For instance, there are few studies on the biodiversity of the Mato Grosso do Sul State (MS), which exhibits a set of unique endemic environments, such as the Pantanal, the Chaco and the Montaine forests of the Bodoquena Plateau.

The first studies to provide information about the butterfly fauna of MS were by Talbot (1928) and Travassos and Freitas (1941). Brown Jr. (1986) listed more than 1,000 species in a study conducted in the Pantanal region. After this study, others were carried out by Aoki and Sigrist (2006), Boff et al. (2008), Rech et al. (2009), Uehara-Prado (2009), Dolibaina et al. (2010), Aoki et al. (2012) and Bogiani et al. (2012), summing a total number of 291 species for the state. Furthermore, although this region is a priority area for studies of lepidopteran biodiversity (Freitas and Marini-Filho 2011), a large area is still unexplored.

Therefore, this study aimed to assess the diversity of butterflies of the Bodoquena Plateau, which is a conservation priority hotspot with great geological and biogeographical importance, but with insufficient data. The Bodoquena Mountains are part of the ecological corridor of Cerrado-Pantanal biodiversity, belonging to the core area of the endangered Atlantic Forest Reserve and the Pantanal Biospheres. This region has been highly threatened by tourist development and the increasing growth of agricultural practices in adjacent farms (Brazil 2007). The knowledge on the fauna of this region is scarce, except for frogs (Uetanabaro et al. 2007), macroinvertebrates (Escarpinati et al. 2011, 2013; Schulz et al. 2012), ants and wasps (Auko & Silvestre 2013; Silvestre et al. 2012; Silvestre et al. 2014).

Material and methods

Study area

The Serra da Bodoquena National Park (Parque Nacional da Serra da Bodoquena - PNSB) is the only conservation unit in the Mato Grosso do Sul State, located in central Brazil (21°8'2" to 2°38'26"S and 56°48'31" 56°44'28"W) (MMA 2002). It consists of two major geomorphological blocks with different characteristics: one to the north, with an area of 27.793 ha, and another to the south, with 48.688 ha (Figure 1) (Fundação Neotrópica 2002). This conservation unit has 300 km in length and width ranging from 20 to 50 km, and exhibits limestone rocks of the Corumbá Formation (Neoproterozoic III), with altitudes ranging from 450 to 800 meters (PCBAP 1997; Boggiani et al. 2000).

The vegetation is a mix of alluvial semi-deciduous forest (gallery forest), submontane deciduous forest (dry forest), wetlands, pasture and regenerating areas (Françoso et al. 2011). The predominant vegetation type is submontane deciduous forest. With an area of 764,81 km², the NBSP covers approximately 0.2% of the surface of MS, which corresponds to 16% of all Atlantic Forest remnants in the state. When considering submontane deciduous seasonal forests alone, more than 25% of its area is located in this protected ecological reserve (Brazil 2007).

Sampling procedures

Sampling was carried out in 20 locations (Table 1) using Van Someren Rydon traps and insect nets between November 2009 and April 2015. Individuals were collected with an insect net, following pre-existing trails along each locality from 9:00 to 17:00 h, totaling 240 hours of sampling effort. Each trail was sampled for 4 hours (~15-20 km), following a zigzag path along the trail. This method allowed us to sample individuals inside the forest, since we sampled along 5 m of each side of the trail. We also used bait traps with fermented banana and sugar cane juice randomly arranged at a height of two meters, with ten traps per location, totaling 200 traps. Traps were set at 10:00 h and removed eight days later at the same period of the day. Voucher specimens are deposited in the Lepidoptera Collection of the Biodiversity Museum (MuBio) of the Federal University of Grande Dourados (UFGD).

The species identification was performed with the aid of specialized bibliography (Brown Jr. 1992, Canals 2000, 2003, Casagrande 1995, D'Abreu 1981, 1987a, b, c, 1988, 1994, 1995, Glassberg 2007) and confirmed by specialists (see acknowledgments). The taxonomical classification follows the proposal of Warren et al. (2009) for HesperIIDae, and Lamas (2004) for other families. To confirm new records for the state, we consulted Talbot (1928), Travassos and Freitas (1941), Brown Jr. (1986), Aoki and Sigrist (2006), Rech et al. (2009), Uehara-Prado (2009), Dolibaina et al. (2010), Aoki et al. (2012) and Boggiani et al. (2012).

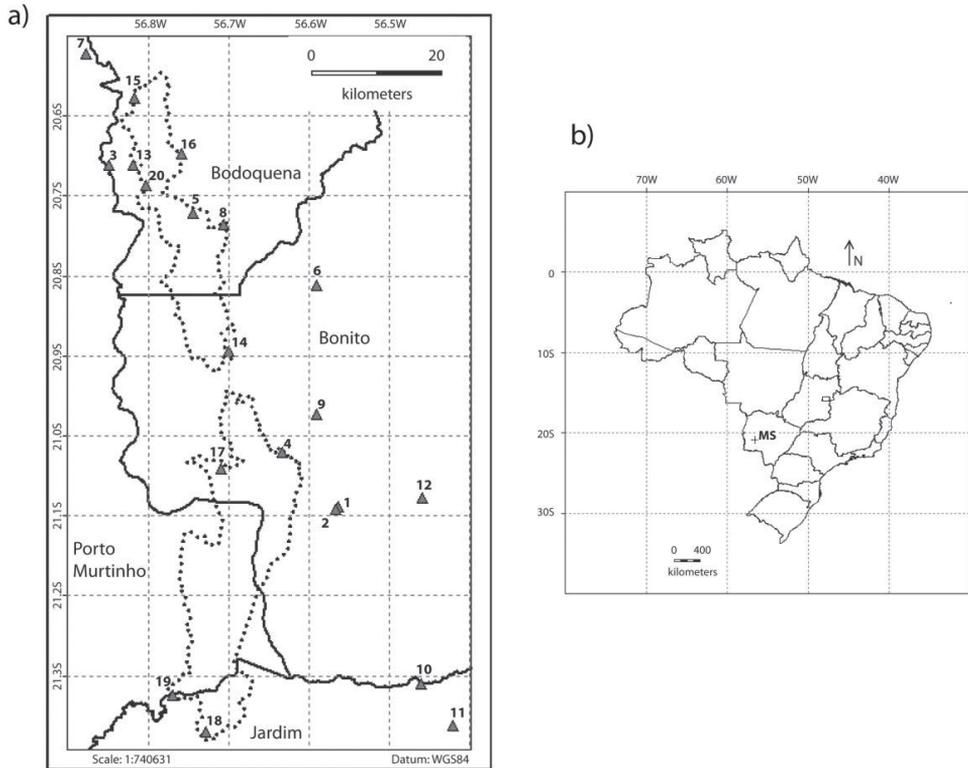


Figure 1. Sampling sites. Maps of the location of sampling sites in the Bodoquena Mountains in the Serra da Bodoquena National Park (a), and the location of the Bodoquena Plateau in Brazil (b).

The effectiveness of the survey was analyzed with individual and sample-based rarefaction curves (Gotelli and Colwell 2001). Sampling effort (by active collection and traps) and the number of individuals and species collected was utilized to obtain the rarefaction curves. All analyses were made with the EstimateS 9.1.0 software (Colwell et al. 2012). Richness was estimated for 80 and 200 random samples for active and trap sampling respectively, using the second order Jackknife estimator. Results are shown as mean \pm SD for observed and estimated richness.

Results and discussion

In total, 768 butterfly individuals were registered, belonging to 146 species in 98 genera, six families in 18 subfamilies (Appendix 1). Nymphalidae was the richest family (82 species), followed by Hesperiiidae (22 species), Riodinidae (14 species), Pieridae (12) Papilionidae (11 species) and Lycaenidae (6 species). 239 individuals were sampled in traps, with 48 species of 30 genera, 15 tribes and five subfamilies of Nymphalidae (Table 1). Before this study, 291 species were recorded for MS, from the literature

Table 1. Sampling sites in the Bodoquena Plateau, Mato Grosso do Sul State, Brazil.

Code	Sites	Geographic Coordinates	Height (m)	Sampling Date
1	Palmeirinhas II	21°11'5.57"S, 56°33'25.25"W	341	November 2009
2	Palmeirinhas I	21°11'16.01"S, 56°33'39.71"W	350	November 2009
3	Faz. California	20°42'5.17"S, 56°52'50.27"W	733	November 2009
4	Taquaral	21°06'27"S, 56°38'14"W	569	November 2009
5	As. Canaã	20°46'5.96"S, 56°45'43.09"W	214	November 2009
6	Faz. Pitangueiras	21°52'14"S, 56°35'19"W	469	November 2009
7	Kadwéu	20°32'41"S, 56°54'44"W	519	November 2009
8	Afluente Salobra	20°47'3.90"S, 56°43'7.37"W	447	November 2009
9	Faz. Morro Alto II	21°01'85.6"S, 56°37'47.6"W	528	November 2009
10	Rio da Prata	21°25'58.80"S, 56°26'31.34"W	255	March 2011
11	Buraco das Araras	21°29'37.2"S, 56°23'52.2"W	318	March 2011
12	Hotel Cabanas	21°10'15.44"S, 56°26'24.2"W	276	March 2011
13	Nascente do Gruta	20°42'6.72"S, 56°50'43.79"W	476	March 2011
14	Marambaia	20°57'53.60"S, 56°42'43.90"W	665	December 2013
15	Faz. Sol de Maio	20°36'18.00"S, 56°50'36.40"W	399	February 2013
16	Faz. Rancho Branco	20°41'6.20"S, 56°46'43.70"W	178	December 2013
17	Boqueirão	21°7'51.30"S, 56°43'19.30"W	542	December 2013
18	Santa Fé	21°30'5.32"S, 56°44'37.49"W	485	June 2013 February 2014
19	Ponte Rio Perdido	21°26'59.18"S, 56°47'28.01"W	422	February 2014
20	Ouro Verde	20°43'49.84"S, 56°49'43.98"W	487	March 2011

and museum collections (Talbot 1928, Travassos and Freitas 1941, Brown 1986, Aoki and Sigrist 2006, Rech et al. 2009, Uehara-Prado 2009, Dolibaina et al. 2010, Aoki et al. 2012, Bogiani et al. 2012). Here, we provide 37 new records for MS (Appendix 1), summing 328 species for the State.

The richest subfamilies were Satyrinae (26 espécies), Biblidinae (24 species), Pyrginae (12 species) and Nymphalinae (10 species). Most new records are represented by rare species with few individuals and low frequency. The most common species were *Eunica macris* (Godart, 1824), *Dynamine artemisia* (Fabricius, 1793) and *Memphis moruus* (Fabricius, 1775). The estimated richness for the Bodoquena Mountains was 83 species for the traps and 142 species for the active collection, while the observed richness was 60 species for the traps and 85 species for the active. Therefore, the results suggest that approximately 72.3% and 59.8% of the species richness of the region were collected with traps and active collection, respectively (Fig. 2). These results indicate that, although traps were more efficient, more species were collected with insect nets. Nevertheless, the rarefaction curves show that the lepidopteran richness in the Bodoquena Plateau may be greater than what we observed in this study.

Half of the listed species, (52.05% - 76 species) of the butterfly fauna consists of species with records in areas of Cerrado (Brown Jr. and Mielke 1967a, b) and 56.16% (82 species) from the Atlantic Forest (Brown Jr. and Freitas 2000). The vegetation mo-

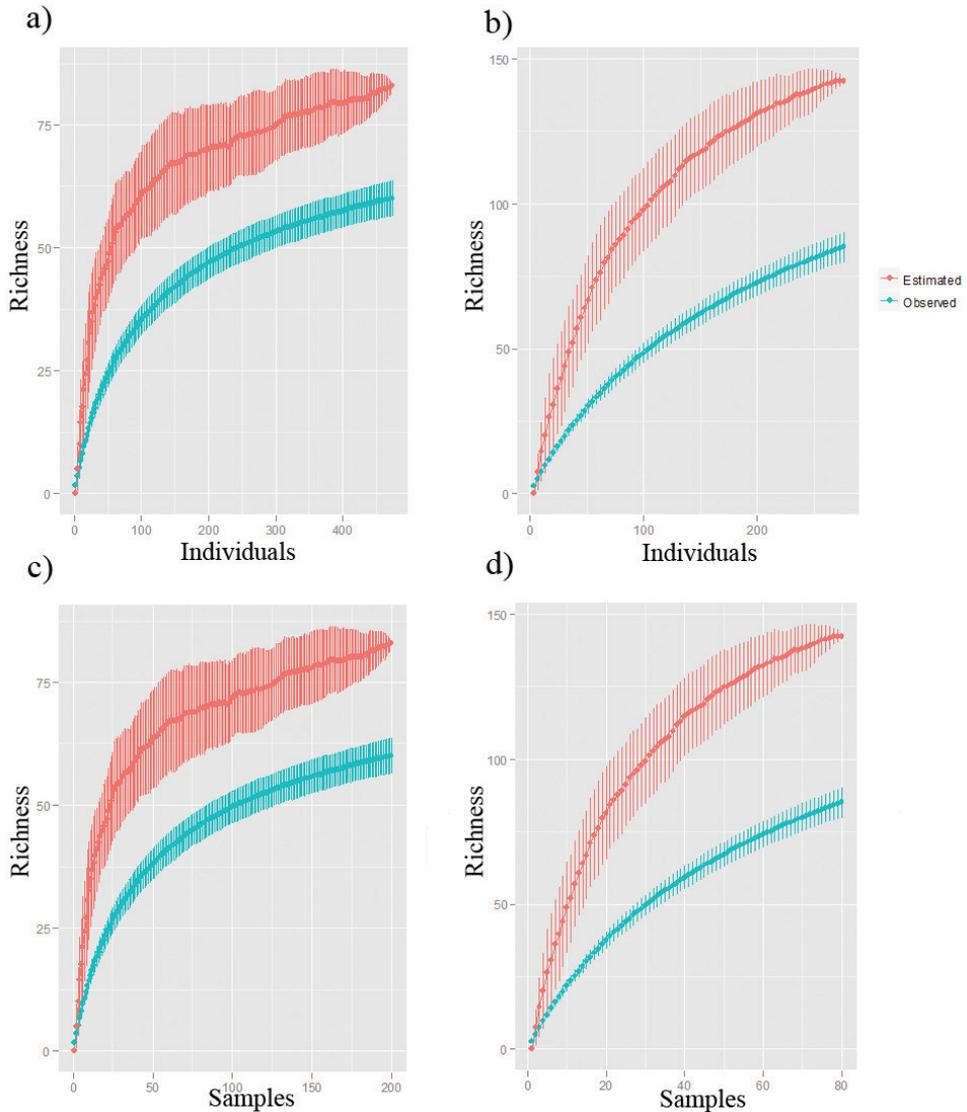


Figure 2. Butterfly richness in the Bodoquena Mountains. Observed and estimated richness of butterflies of the Bodoquena Plateau for both traps (**a, c**) and active collecting (**b, d**), in function of the number of individuals (**a, b**) and sampling effort (**c, d**).

saic found in the SBNP can explain this significant number of shared species among biomes. Most species recorded in the Bodoquena Plateau have a wide geographic distribution in Brazil, a fact evidenced in other studies conducted in the Cerrado (Brown Jr. and Mielke 1967a, b). Nevertheless, some rare species were found, such as *Leucochimona icare* (Hübner, [1819]) (Figure 3a, b), *Strymon mulucha* (Hewitson, 1867) (Figure 3d) and *Catocyclotis aemulius* (Fabricius, 1793) (Figure 3e, f). Moreover, two

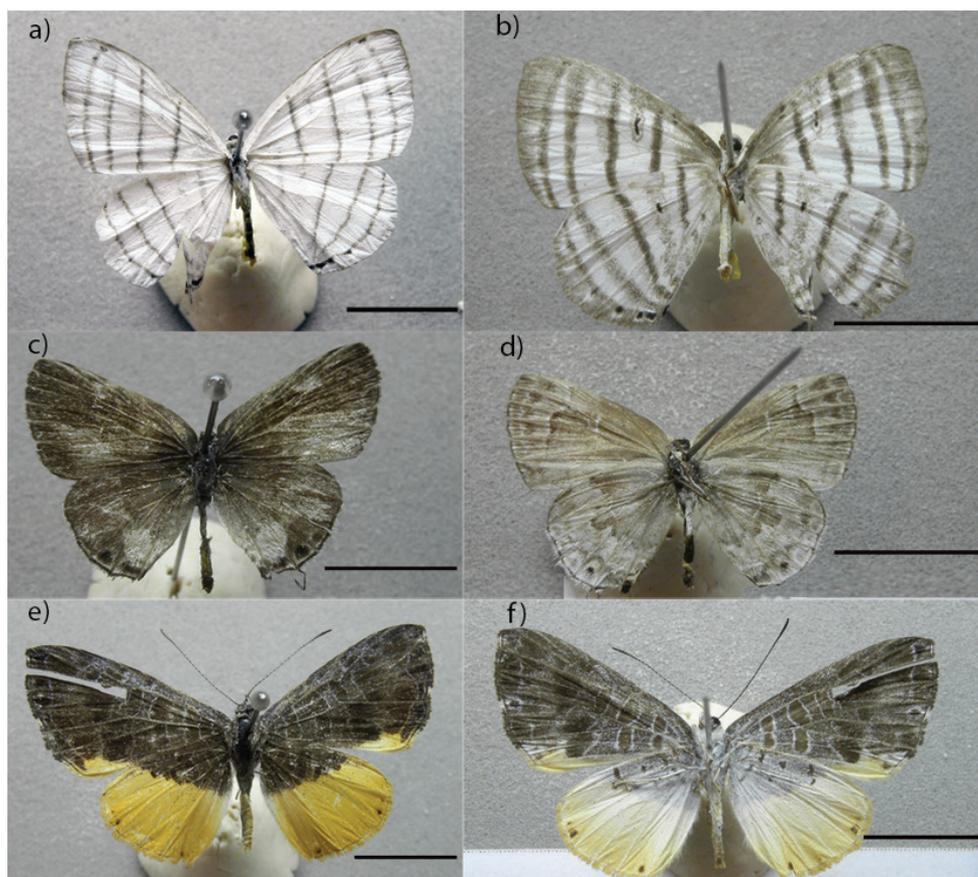


Figure 3. Rare species collected in the Bodoquena Mountains. *Leucochimona icare* (Hübner, [1819]) (a, b), *Strymon mulucha* (Hewitson, 1867) (c, d) and *Catocyclotis aemulius* (Fabricius, 1793) (e, f). Scale bars

new species of *Moneuptychia* (Nymphalidae) were found and are being described (André V. L. Freitas pers. comm.).

The percentage of 8.27% for HesperIIDae collected in the Bodoquena Plateau, are not in agreement with results of other inventories carried out in the Atlantic Forest (Mielke 1994; Carneiro et al. 2008; Dolibaina et al. 2011), in which hesperiids are usually more common. The HesperIIDae sub-sampling is common in butterflies inventories (Bonfanti et al. 2009; Vasconcelos et al. 2009; Ritter et al. 2011; Zacca and Bravo 2012), especially by their small size, cryptic color patterns and inefficient attraction by fermented fruit lures, thus hindering their collection (Brown Jr. and Freitas 1999; Caldas and Robbins 2003; Zacca and Bravo 2012). In this context, sub-sampling may be derived from different methodologies and approaches of each one of these studies, besides familiarity of the collector with each taxon.

Brown Jr. (1972) discusses and tests the methods used by Ebert (1970) in which the author shows that supplementation of methodologies, proper maintenance of re-

cords, performance of several collectors at the same time and knowledge about the behavior of groups guarantee a more complete and representative record of these species. Pinheiro-Machado and Silveira (2006) show that the mentioned method may vary according to the location and logistics, but the best results in number of species are achieved when various methods are employed.

Nymphalidae was the family with highest diversity independent of methodology. This result was expected since this is butterfly family with most species (De Vries 1987), as recorded in the studies by Iserhard and Romanowski (2004), Marchiori and Romanowski (2006), Sackis and Morais (2008), Iserhard et al. (2010) and Rosa et al. (2011) conducted in the state of Rio Grande do Sul. However, Brown Jr. and Freitas (2000) compiled records that resulted in more than 2,100 butterfly species in the Atlantic Forest. According to these authors, in Brazil, the Family HesperIIDae, Nymphalidae and Lycaenidae are the richest in species, followed by Riodinidae, Pieridae, and Papilionidae.

In this study, 32.19% of the species showed were singletons. In the study conducted by Dessuy and Morais (2007) in a forest fragment of Santa Maria, 26% of species collected were singletons, whereas in Sackis (2008) study, it represents 36% of the species. According Dessuy and Morais (2007) singletons are species that live in the habitat in which they are sampled and can be very difficult to find as it keeps in small populations. In addition, these species may be considered rare in one spot, but not in others, due to differences in the availability of food resources, host plants or microclimatic factors (Brown Jr. and Freitas 2003).

The results obtained in this study represent the only information on the butterfly species composition of the Bodoquena Plateau, an area considered of utmost priority for biodiversity conservation. Interestingly, 44.5% of the whole butterfly fauna of MS can be found in the Bodoquena Mountains, showing its great importance for population maintenance and butterfly conservation. Furthermore, the records of rare and new species add evidence to the role of large ecological reserves and conservation areas, as well as the importance of taxonomical surveys. Therefore, this study contributed to the knowledge on Neotropical butterfly species diversity and distribution, providing new records and supporting the use of wildlife inventories as important tools for the knowledge of tropical forests biodiversity and conservation.

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Appendix I. Butterfly species list for the Serra da Bodoquena National Park, including 20 occurrence sites (represented by codes, see Table 1). Taxa are presented according to family and subfamily. ♦: New records for the Mato Grosso do Sul State; * New species.

Family	Subfamily	Tribe	Species	Abundance		Occurrence site (codes)			
						Bonito	Bodoquena	Jardim	Porto Murtinho
Nymphalidae (N = 82)	Libytheinae		<i>Libytheana carinenta</i> (Cramer, 1777)	1				18	
	Apaturinae		<i>Doxocopa agathina</i> (Cramer, 1777)	14		4,14,17	13	18	
			<i>Doxocopa linda nitoris</i> Fruhstorfer, 1907 ♦	11		4		18	
			<i>Biblis hyperia</i> (Cramer, 1779)	16		4,14	13	18	
			<i>Callicore pygas</i> (Godart, [1824])	6		4		11,18	
			<i>Callicore sonna</i> (Godart, [1824])	1				11	
			<i>Haematera pyrane</i> (Hübner, [1819]) ♦	7		6,9	16	18	
			<i>Dynamine</i> sp.	4		2,4,9	8		
			<i>Dynamine aerata</i> (Butler, 1877) ♦	11		4,9			
			<i>Dynamine agacles</i> (Dalman, 1823)	1		9			
			<i>Dynamine artemisia</i> (Fabricius, 1793)	50		2,4,9,14,17	3,5,15		
			<i>Dynamine coenus</i> (Fabricius, 1793)	1				18	
			<i>Dynamine postverta</i> (Cramer, 1779)	9		4,9,14		18	
			<i>Dynamine postverta postverta</i> (Cramer, 1779)	9		2,4		18	
			<i>Eunica macris</i> (Godart, [1824]) ♦	25		4,6,14	3,5,13,16	18,19	
			<i>Eunica margarita</i> (Godart, [1824]) ♦	1		12			
			<i>Eunica tailla</i> (Herrich-Schäffer, [1855])	20		4,14		18,19	
			<i>Eunica bechina</i> (Hewitson, 1852)	2		14		18	
			<i>Hamadryas amphinome</i> (Linnaeus, 1767)	2				18,19	
			<i>Hamadryas arete</i> (Doubleclay, 1847)	1		4			
		<i>Hamadryas chloe</i> (Stoll, 1787)	1		14	12	18		
		<i>Hamadryas epinome</i> (Felder & Felder, 1867)	15		4,9,14	13	11,18		
		<i>Hamadryas februa</i> (Hübner, [1823])	21		4,6,12	3,20	11,18	7	
		<i>Hamadryas feronia</i> (Linnaeus, 1758)	1				11		
		<i>Hamadryas ipthime</i> (Bates, 1864) ♦	4		4		10		
		<i>Nica flavilla</i> (Godart, [1824])	2		6,12				
		Epiphelini							

Family	Subfamily	Tribe	Species	Abundance	Occurrence site (codes)			
					Bonito	Bodoquena	Jardim	Porto Murтинho
			<i>Temeris laothoe</i> (Cramer, 1777)	7	1,9	20	10,18,19	
			<i>Marpesia chiron</i> (Fabricius, 1775)	11	4,14,17	3, 16	18	
			<i>Marpesia petreus</i> (Cramer, 1776)	1	14			
			<i>Fountainea ryphea</i> (Cramer, 1775)	5	12		18	
		Cyrestini	<i>Memphis acidalia</i> (Hübner, [1819]) ♦	5	1,2,14	8		
	Cyrestinae		<i>Memphis moruus</i> (Fabricius, 1775)	33	1,4,12,14,17	8	11,18	
			<i>Zaretis isidona</i> (Cramer, 1779)	17	1,4,14	3,130	18,19	
		Preponini	<i>Archaeoprepona demophoon</i> (Linnaeus, 1758)	6	1,2,4,12		18	
			<i>Prepona pylene</i> Hewitson, [1854] ♦	1	4			
			<i>Heliconius erato phyllis</i> (Fabricius, 1775)	3	4	16		
	Heliconiinae	Heliconiini	<i>Dryas iulia</i> (Fabricius, 1775)	1		16		
			<i>Dryadula phaetusa</i> (Linnaeus, 1758)	1		13		
			<i>Agraulis vanillae maculosa</i> (Stichel, [1908])	1			11	
		Ithomiini	<i>Thyridia psidii</i> Linnaeus, 1758 ♦	1	17			
	Danaeinae	Danaini	<i>Lycorea halia pales</i> Felder & Felder, 1862	2		15		
			<i>Tithorea harmonia</i> (Cramer, 1777)	1	6			
		Limnitiini	<i>Adelpha iphicleola leucates</i> Fruhstorfer, 1915	6	14,17		18	
	Limnitiinae		<i>Adelpha mabea</i> (Felder & Felder, 1861) ♦	1			18	
			<i>Colobura dirce</i> (Linnaeus, 1758)	7	1,2,4,12,14	8		
		Nymphalini	<i>Historis odius</i> (Fabricius, 1775)	1	4			
			<i>Smyrna blomfieldia</i> (Fabricius, 1781)	54	4,14		18	
		Victorini	<i>Siproeta stelenes</i> (Linnaeus, 1758)	14	4,14		18	
			<i>Anartia jatrophae</i> (Linnaeus, 1763)	5	6,12		11,18	
	Nymphalinae		<i>Chlosyne lacinia saundersi</i> (Doubleday, [1847])	5	6	13	11,18	
		Melitaeini	<i>Orrilia ibira</i> (Kirby, 1900)	5	9,17	5		
			<i>Orrilia orithia</i> (Hewitson, 1864) ♦	2	1			
			<i>Tegosa claudina</i> (Eschscholtz, 1821)	8	4, 14	3,5,16		
		Junoniini	<i>Junonia evarete</i> (Cramer, 1779)	2			18	

Family	Subfamily	Tribe	Species	Abundance	Occurrence site (codes)			
					Bonito	Bodoquena	Jardim	Porto Murтинho
			<i>Cissia terrestris</i> (Butler, 1867)	8	1,2,4		18	
			<i>Hermeupyichia</i> sp.	3	14		19	
			<i>Magneupyichia ocnus</i> (Butler, 1867) ♦	2	2,4			
			<i>Manataria bercynia</i> (Hübner, [1821]) ♦	1	14			
			<i>Moneupyichia</i> sp.* ♦	6	4,14		18	
			<i>Moneupyichia</i> sp.2* ♦	3	14		18	
			<i>Pareupyichia ocirrhoe</i> (Fabricius, 1776)	4	2,9		18	
			<i>Pareupyichia ocirrhoe interjecta</i> (D'Almeida, 1952)	3	2,12			
			<i>Pareupyichia summandosa</i> (Gosse, 1880) ♦	3	1, 14			
			<i>Paryphthimoides grimon</i> (Godart, [1824]) ♦	1	14			
			<i>Paryphthimoides phronius</i> (Godart, [1824])	9	1,6,9,14	20	18,19	
			<i>Paryphthimoides polys</i> (Prittwitz, 1865)	14	1,4,9,14	20	18,19	
			<i>Postagygetis penolea</i> (Cramer, [1777])	4	1,2,6		18	
			<i>Tagyetina kereza</i> (Butler, 1869)	6	2	8,15		
			<i>Tagyetis</i> sp.	5	1,14	8		
			<i>Tagyetis laevis</i> Fabricius, 1793	7	4	8	18,19	
			<i>Tagyetis larua</i> Felder & Felder, 1867 ♦	2	1			
			<i>Tagyetis mermeria</i> (Cramer, 1776) ♦	1		15		
			<i>Tagyetis rufomarginata</i> Staudinger, 1888	1		8		
			<i>Tagyetis sylvia</i> Bates, 1866 ♦	1	14	8		
			<i>Tagyetis tripunctata</i> Weymer, 1907 ♦	1		8		
			<i>Tagyetis virgilia</i> (Cramer, [1776])	5	4,12		18	
			<i>Yphthimoides celmis</i> (Godart, [1824]) ♦	1	9			
			<i>Caligo illioneus</i> (Cramer, 1775) ♦	2	4		10	
			<i>Catoblepia berecynthia</i> (Cramer, 1777) ♦	5	1,2,4	7		
			<i>Eryphanis reevesii</i> (Doubleday, [1849]) ♦	3	12		10,18	
			<i>Opsiphanes invivata</i> (Hübner, [1808])	8	4,14		18	
			<i>Morpho helenor</i> (Cramer, 1776)	21	4,12	15,20	10,19	

Satyrini

Satyrinae

Brassolini

Morphini

Family	Subfamily	Tribe	Species	Abundance	Occurrence site (codes)		
					Bonito	Bodoquena	Jardim
Papilionidae (N = 11)	Leptocircini		<i>Protesilaus</i> sp.	1		16	
			<i>Heracles hectorides</i> (Esper, 1784) ♦	3		15	18
			<i>Heracles anchistades</i> (Esper, 1788)	4	6,9	16	
	Papilionini		<i>Heracles isidorus</i> (Doubleday, 1846) ♦	1	14		
			<i>Heracles androgeus</i> (Cramer, 1775) ♦	2		16	
			<i>Heracles asyralus asyralus</i> (Godart, 1819)	2		16	
			<i>Heracles thoas brasiliensis</i> (Rothschild & Jordan, 1906)	2	1	3	
			<i>Battus polydamas polydamus</i> (Linnaeus, 1758)	1	17		
			<i>Battus crassus</i> (Cramer, 1777)	1		16	
			<i>Parides lysander mattoossensis</i> (Talbot, 1928)	2	1,4		
			<i>Parides neophilus</i> (Geyer, 1837) ♦	2	1		19
Hesperiidae (N = 22)		Hesperiidae sp.	7	1	16		
		Hesperiidae sp. 1	2	1,6			
		Hesperiidae sp. 2	3	1,6	16		
		Hesperiidae sp. 3	4	1,6	16	18	
		Hesperiidae sp. 4	1			18	
		Hesperiidae sp. 5	1			18	
		<i>Urbanus</i> sp.	2	4		18	
		<i>Urbanus dorantes</i> (Stoll, 1790)	1	6			
		<i>Urbanus telus</i> (Hübner, 1821)	2	6			
		<i>Xeniades orchamus orchamus</i> (Cramer, 1777)	1	14			
Eudaminae		<i>Mylon maimon</i> (Fabricius, 1775) ♦	1			18	
		<i>Gorythion begga begga</i> (Prittwitz, 1868)	2	1			
		<i>Xenophanes tryxus</i> (Stoll, 1780)	2	1,14			
		<i>Pyrgus orcus</i> (Stoll, 1780)	7	1		18,19	
		<i>Pyrgus oilens</i> (Linnaeus, 1767)	10	1,6			
		<i>Heliopterus arsalte</i> (Linnaeus, 1758)	1			18	
Hesperiinae		<i>Heliopterus libra</i> Evans, 1944	2		16		
Pyrginae							

Family	Subfamily	Tribe	Species	Abundance	Occurrence site (codes)			
					Bonito	Bodoquena	Jardim	Porto Murтинho
Riodinidae (N = 14)			<i>Heliopetes omrina</i> (Butler, 1870)	8	1	16	18	
			<i>Antigonus nearctus</i> (Latreille, [1817]) ♦	1		3		
			<i>Antigonus erosus</i> (Hübner, [1812])	3		16	18	
			<i>Elbella</i> sp. ♦	1			19	
			<i>Myscelus amyntis epigona</i> Herrich-Schäffer, 1869	1			18	
			<i>Anarynythis meneria</i> (Cramer, 1776)	1	1			
			<i>Barbicornis basilis</i> Godart, [1824]	1	1			
			<i>Catoclyotis acmilius</i> (Fabricius, 1793) ♦	1	1			
			<i>Chalodeta theodora</i> (Felder & Felder, 1862)	1				18
			<i>Chamaelinnus briola meridionalis</i> (Lathy, 1932) ♦	1	17			
			<i>Emesis</i> sp.	6	14	3,16	18	
			<i>Hyphilaria thasus</i> (Stoll, 1780)	2			18	
			<i>Lasia agesilas agesilas</i> (Latreille, [1809])	1			18	
			<i>Leucobimona icare</i> (Hübner, [1819])	1	1			
Lycenidae (N = 6)		Pyrrhopygini	<i>Notheme erota</i> (Cramer, 1780)	2		5		
			<i>Nymphidium leucosia</i> (Hübner, [1806])	3	1			
			<i>Rethus perianther</i> (Cramer, 1777)	2		16		
			<i>Synargis bifasciata</i> (Mengel, 1902) ♦	1		16		
			<i>Synargis calyce</i> (Felder & Felder, 1862) ♦	1		16		
			<i>Leptotes cassius</i> (Cramer, 1775)	5	1,14,17	16		
			<i>Hemiargus hamo</i> (Stoll, 1790)	8	6	16	18	
			<i>Araucacus aetobus</i> (Sulzer, 1776)	3	17	16		
			<i>Strymon mulucha</i> (Hewitson, 1867)	3	1,6		18	
			<i>Strymon rufifascia</i> (Hewitson, 1877)	1			18	
			<i>Strymon ziba</i> (Hewitson, 1868) ♦	1	14			
			<i>Eurema</i> sp.	26	4,6	15,16		
			<i>Eurema elathea</i> (Cramer, 1777)	4	14		18	
			<i>Anteos clorinde</i> (Godart, [1824])	2		16		
Pieridae (N = 12)	Coliadinae							
	Coliadinae							
	Coliadinae							

Family	Subfamily	Tribe	Species	Abundance	Occurrence site (codes)			
					Bonito	Bodoquena	Jardim	Porto Murтинho
	Pierinae		<i>Ganyra phaloe endeis</i> (Godart, 1819) ♦	2		16		
	Coliadinae	Pierini	<i>Aphrissa statira statira</i> (Cramer, 1777)	2		16		
	Coliadinae		<i>Itaballia demophile</i> (Linnaeus, 1763)	6		16		
	Pierinae		<i>Glutophrissa drusilla</i> (Cramer, 1777)	4	9	3,16		
	Coliadinae		<i>Phoebis argante</i> (Fabricius, 1775)	8	6	16		
	Coliadinae		<i>Phoebis sennae</i> (Linnaeus, 1758)	2		16		
	Coliadinae	Pierini	<i>Pyrisitia leuce</i> (Boisduval, 1836)	1		16		
	Coliadinae		<i>Pyrisitia nise</i> (Cramer, 1775)	3	9	3,5		
	Coliadinae		<i>Rhabdodryas irrie</i> (Linnaeus, 1758)	1		16		

Embryogenesis and tadpole description of *Hyperolius castaneus* Ahl, 1931 and *H. jackie* Dehling, 2012 (Anura, Hyperoliidae) from montane bog pools

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Abstract

Tadpoles of *Hyperolius castaneus* and *H. jackie* were found in the Nyungwe National Park in Rwanda and adjacent areas. Tadpoles of both species were identified by DNA-barcoding. At the shore of a bog pool three clutches of *H. castaneus* of apparently different age, all laid on moss pads (*Polytrichum commune*, *Isotachis aubertii*) or grass tussocks (*Andropogon shirensis*) 2–5 cm above the water level, were found. One clutch of *H. castaneus* was infested by larval dipterid flies. The most recently laid clutch contained about 20 eggs within a broad egg-jelly envelope. The eggs were attached to single blades of a tussock and distributed over a vertical distance of 8 cm. A pair of *H. castaneus* found in axillary amplexus was transported in a plastic container to the lab for observation. The pair deposited a total of 57 eggs (15 eggs attached to the upper wall of the transport container, 42 eggs floated in the water). Embryogenesis of the clutch was monitored in the plastic container at 20 ± 2 °C (air temperature) and documented by photos until Gosner Stage 25. The description of the tadpole of *H. castaneus* is based on a Gosner Stage 29 individual from a series of 57 tadpoles (Gosner stages 25–41). The description of the tadpole of *H. jackie* is based on a Gosner Stage 32 individual from a series of 43 tadpoles (Gosner stages 25–41). Egg laying behavior and

embryogenesis are unknown for *H. jackie*. The labial tooth row formula for both species is 1/3(1) with a narrow median gap of the tooth row. Variation in external morphology was observed in size and labial tooth row formula within the species. With the tadpole descriptions of *H. castaneus* and *H. jackie*, 36 tadpoles of the 135 known *Hyperolius* species have been described, including five of the eleven *Hyperolius* species known from Rwanda.

Keywords

Cryptic species diversity, egg predation, egg laying behavior, frogfly, Nyungwe National Park, Rwanda

Introduction

The reed frog genus *Hyperolius* currently comprises 135 species (Frost 2015). Taxonomy of this genus is known to be complicated (e.g., Ahl 1931, Schiøtz 1975, 1999, Lötters et al. 2004, Rödel et al. 2010) because of high intraspecific variability, high interspecific morphological similarity, and sympatric distributions (e.g., Channing et al. 2013, Liedtke et al. 2014). Not surprisingly, the tadpoles of only 34 (24.8%) *Hyperolius* species have been described to date (Viertel et al. 2007, Channing et al. 2012, Conradie et al. 2013), a serious drawback for a reliable assessment of the presence of species in remote regions where adults are not easily caught (e.g. Greenbaum et al. 2013).

During our recent field work in Rwanda, we focussed on the estimation of *Hyperolius* diversity, specifically in the Nyungwe National Park (about 970 km² cloud forest, Plumptre et al. 2003; for a map see Dehling 2012: page 60, figure 4). Despite a century of taxonomic studies (Ahl 1931, Hinkel and Fischer 1990, 1995, Fischer and Hinkel 1992, Hinkel 1996, Sinsch et al. 2011, Dehling 2012) diversity of the cloud forest *Hyperolius* from that area is not yet clear. The checklist of Hinkel (1996) mentions *H. adolfriederici* Ahl, 1931, *H. alticola* Ahl, 1931, *H. castaneus* Ahl, 1931, *H. discodactylus* Ahl, 1931, *H. raveni* Ahl, 1931 and *H. viridiflavus francoisi* Laurent, 1951, several of which are now considered junior synonyms (Frost 2015). Our current view integrating morphological, bioacoustics and molecular data gives credit to the presence of only four species in the Nyungwe National Park: *H. castaneus*, *H. discodactylus*, *H. frontalis* Laurent, 1950 and the recently described *H. jackie* Dehling, 2012 (Sinsch et al. 2011, Dehling 2012, Greenbaum et al. 2013, Liedtke et al. 2014, Dehling unpubl. data). Analysing habitat preferences and distribution of these four species within the cloud forest and the adjacent areas now deforested and in agricultural use would be easier, if encountered tadpoles could be assigned to either taxon. Yet, none of the tadpoles are currently described (Channing et al. 2012). Consequently, we surveyed lentic water bodies for *Hyperolius* tadpoles of these four species at all localities where we previously detected the presence of either species by collection of specimens or based on advertisement calls (Sinsch et al. 2011, Dehling 2012, Greenbaum et al. 2013, Liedtke et al. 2014). This survey yielded a large number of tadpoles which we identified as those of *H. castaneus* and *H. jackie* by DNA-barcoding. Herein we describe the morphological features of the tadpoles and provide new information on the egg-laying behavior of *H. castaneus* and embryogenesis in their terrestrial clutches.

Methods

Study areas and field surveys

Presence of larval and adult individuals of *Hyperolius castaneus* and *H. jackie* was monitored in the Nyungwe National Park, Rwanda (Sinsch et al. 2011, Dehling 2012) and adjacent areas used for agriculture (Table 1). Daytime surveys (9.00–17.00) for tadpoles and nightly records (18.00–21.00) of calling males were conducted in March 2009, March and April 2011 and in March 2012. *Hyperolius castaneus* egg laying behavior was studied in the Uwasenkoko swamp. Tadpoles of *H. castaneus* were collected at the same site and additionally in the Karamba swamp together with those of *H. jackie* (Table 1). Additional tadpole specimens were collected from multiple localities in the Albertine Rift in Democratic Republic of Congo and Uganda. Museum acronyms are: UTEP = University of Texas at El Paso, ZFMK = Zoologisches Forschungsmuseum Alexander Koenig, Bonn (Appendix I).

Larval characters

The format of the tadpole description follows that of Viertel et al. (2007) but excludes description of oral cavities. Tadpoles were preserved in 5–10% formalin. Body measurements follow the primary landmarks defined by McDiarmid and Altig (1999: see figure 3.1 on page 26 for tadpole drawing with defined primary landmarks). In our descriptions, we use the terminology of Altig (1970) and McDiarmid and Altig (1999) with the labial tooth row formula (LTFR) written as a fraction in line with the rows with median gaps in parentheses. P1 = first posterior tooth row. Ecomorphological types for larvae follow McDiarmid and Altig (1999) and Orton (1953). Tadpoles were staged according to Gosner (1960). Preserved tadpoles were observed on tiny glass beads (1 mm) filled shallowly with water to allow proper positioning. Most measurements were taken to the nearest 0.1 mm using a stereomicroscope equipped with an

Table 1. Localities where *H. castaneus* and *H. jackie* adults (= A) and tadpoles (= T) were collected in Rwanda.

Locality	Latitude [°S], Longitude [°E]	Altitude [m a.s.l.]	<i>H. castaneus</i>		<i>H. jackie</i>	
			A	T	A	T
Gisakura	2.457, 29.092	1927	+	-	-	-
Kitabi	2.546, 29.426	2190	+	-	-	-
Nyungwe, stream	2.464, 29.101	1881	+	-	+	-
Nyungwe, Kamiranzovu	2.486, 29.153	1961	+	-	-	-
Nyungwe, Karamba	2.479, 29.112	1936	+	+	+	+
Nyungwe, Pindura	2.481, 29.228	2283	+	-	-	-
Nyungwe, Uwasenkoko	2.529, 29.354	2379	+	+	-	-

ocular micrometer, except for tail length, body length, body width, and greatest tail height, which were measured with a digital caliper held under the microscope.

Recorded measurements include: body length (distance from the tip of the snout to the body terminus, which is the junction of the posterior body wall with the tail axis); tail length (distance from the body terminus to the absolute tip of tail); total length (sum of body length and tail length); body width (measured at the widest point right behind the eyes); body height (at level of eye); eye diameter; interorbital distance (measured between the centers of the pupils); internarial distance (measured between the centers of the nostril indicated by reduced pigmentation when closed); distance between tip of snout and naris (from center of the naris to the middle of the snout); and distance between nostril and eye (from the center of nostril to the anterior edge of the eye); spiracle length (medially to opening); and spiracle tube width (at level of opening), and oral disc width (at middle between outer marginal papillae). Drawings of tadpoles were done with a camera lucida attached to a microscope. Descriptions of coloration in life are based on photos taken by JMD shortly after collection in the field.

DNA sampling and barcoding

We isolated DNA from the tail tip of the tadpole morphotypes, collected at the Karamba and Uwasenkoko localities (Table 1). DNA was used to sequence a fragment of the 16S mitochondrial rRNA gene, a suggested universal marker to barcode amphibians for species allocation (Vences et al. 2005). Protocols of DNA extraction, PCR, purification, and sequencing follow Dehling and Sinsch (2013) and Greenbaum et al. (2013). The obtained sequences were compared with our own sequences from adult frog specimens collected in southwestern Rwanda and are deposited in GenBank (Table 2). Editing and alignment were completed in MEGA5 (Tamura et al. 2011). Sequences were trimmed to the same length. The final alignment consisted of 548 base pairs. Calculations of pairwise distances and phylogenetic analysis (Maximum Likelihood) were carried out in MEGA5. A Maximum Likelihood analysis was run with 1000 bootstrap replicates using the GTR + G + I model and the Nearest-Neighbor-Interchange, as proposed by jModelTest 2 (Darriba et al. 2012) using the Akaike information criterion.

Results

Distribution and habitat preferences of *Hyperolius* spp. in the Nyungwe region

Based on call surveys and collection of adult specimens, *H. castaneus* populations were detected at seven localities, five inside the Nyungwe National Park, and two outside (Table 1). They occurred in sympatry with *H. discodactylus*, *H. jackie*, *Leptopelis karisimbensis* Ahl, 1929, *L. cf. kivuensis* 2 (sensu Portillo et al. 2015), *Phrynobatrachus acutirostris* Nieden 1912, “1913”, *P. cf. versicolor* Ahl, 1924, *Xenopus wittei* Tinsley, Ko-

Table 2. Samples of species used for molecular genetic analyses, their geographic origin, voucher specimens (T = tadpole, otherwise adult), GenBank accession numbers, and original source.

Species	Origin	Voucher	GenBank #	Source
<i>Afrivalus quadrivittatus</i>	Butare	JMD544	KT439195	This study
<i>Hyperolius castaneus</i>	Nyungwe National Park	ZMB 77537	JQ423936	Dehling 2012
<i>Hyperolius castaneus</i>	Uwasenkoko, Nyungwe National Park	ZFMK 97191, T	KT439194	This study
<i>Hyperolius castaneus</i>	Karamba	ZFMK 97192, T	KT439193	This study
<i>Hyperolius cinnamomeoventris</i>	Butare	ZMB 77533	JQ966568	Dehling 2012
<i>Hyperolius discodactylus</i>	Nyungwe National Park Rwanda	ZMB 77536	JQ966565	Dehling 2012
<i>Hyperolius jackie</i>	Karamba, Nyungwe National Park	ZMB 77481	JQ966571	Dehling 2012
<i>Hyperolius jackie</i>	Karamba, Nyungwe National Park	ZFMK 97194, T	KT439192	This study
<i>Hyperolius kivuensis</i>	Butare	ZMB 77532	JQ966567	Dehling 2012
<i>Hyperolius lateralis</i>	Butare	ZMB 77534	JQ966569	Dehling 2012
<i>Hyperolius rwandae</i>	Akagera wetland	ZMB 77225	JQ863713	Channing et al. 2013
<i>Hyperolius rwandae</i>	Butare	JMD 592	KT439191	This study
<i>Hyperolius viridiflavus</i>	Gitarama	ZMB 77535	JQ966570	Dehling 2012
<i>Leptopelis karissimbensis</i>	Uwasenkoko swamp, Nyungwe National Park	ZFMK 97188, T	KT439190	This study
<i>Leptopelis karissimbensis</i>	Uwasenkoko swamp, Nyungwe National Park	JMD 631	KT439189	This study
<i>Leptopelis cf. kivuensis 2</i>	Karamba, Nyungwe National Park	ZFMK 97189, T	KT439188	This study
<i>Leptopelis cf. kivuensis 2</i>	Karamba, Nyungwe National Pak	JMD 746	KM047142	Portillo et al. 2015

bel & Fischberg, 1979 and an undetermined species of *Amietia* Dubois, 1987 “1986”. *Hyperolius castaneus* tadpoles shared the same lentic water bodies with those of *H. jackie*, *Leptopelis karissimbensis* and *L. cf. kivuensis 2* (Fig. 1). *Hyperolius jackie* populations are currently known only from the type locality (a natural pond at Karamba, Nyungwe National Park), and a stream at the west end of the Nyungwe National Park (Table 1). Adults were found in sympatry with *H. castaneus*, *H. discodactylus*, *Leptopelis karissimbensis* and *Xenopus wittei*; and tadpoles syntopically with those of *H. castaneus* and *L. cf. kivuensis 2*. *Hyperolius discodactylus* tadpoles were found syntopically with tadpoles of *Phrynobatrachus acutirostris* in a slow flowing stream passing through the Uwasenkoko swamp.

Males of *Hyperolius castaneus* and *H. jackie* were observed vocalizing from shrubs and sedges bordering forest swamps. *Hyperolius castaneus* also called from the ground in moist swamp areas. While *H. jackie* never started vocalizing before dusk, *H. castaneus* gave advertisement calls throughout the day, but more frequently at night. Bog pools close to calling sites and containing tadpoles had a pH of 5.5–6.0 and a water depth varying from a few centimetres to a maximum of 35 cm (Fig. 1).



Figure 1. Tadpole habitats in the Nyungwe National Park. **A** Karamba swamp **B** Uwasenkoko swamp. For geographical details see Table 1. Photos by U. Sinsch.

Egg-laying behavior and embryogenesis of *H. castaneus*

The natural history observations reported here were made on 22 March 2012 between 13:00 and 16:00 hrs, at a small breeding pond forming part of the Uwasenkoko swamp (2379 m a.s.l.; Fig. 1B). During an initial survey of a 25 m² area, we located two males advertising at the ground and an unpaired female, all individuals staying 3–8 m apart from each other. At the shore of the pond we detected three clutches of different ages, laid on moss pads and grass tussocks 2–5 cm above the water level (Fig. 2). The first clutch mass was placed on a moss pad (*Polytrichum commune*, *Isotachis aubertii*) and consisted only of the gelatinous remains of the egg envelopes (Fig. 2A). According to the duration of embryogenesis (see below) we estimate the age of this clutch is at least seven days. The second clutch was found upon depressed blades of mainly *Andropogon shirensis* (Fig. 2B) and had a similar consistency to the first one. However, with the exception of three undeveloped eggs, it contained a large number of undetermined insect larvae, probably of parasitic dipterid flies. The third clutch was recently laid with about 20 eggs within the broad egg-jelly envelope. The eggs were attached to single blades of an *Andropogon shirensis* tussock and distributed over a vertical distance of 8 cm (Fig. 2C). The eggs had a black pole cap, whereas about two-thirds of the egg was yellowish. Within the shallow water adjacent to the clutches we observed > 50 *H. castaneus* tadpoles (Gosner stages 25–31, one metamorphic individual with lateral yellowish stripes of Stage 41) and > 15 *L. karissimbensis* tadpoles (Gosner stages 35–39). The developmental stage of most tadpoles indicated that they had hatched recently. We

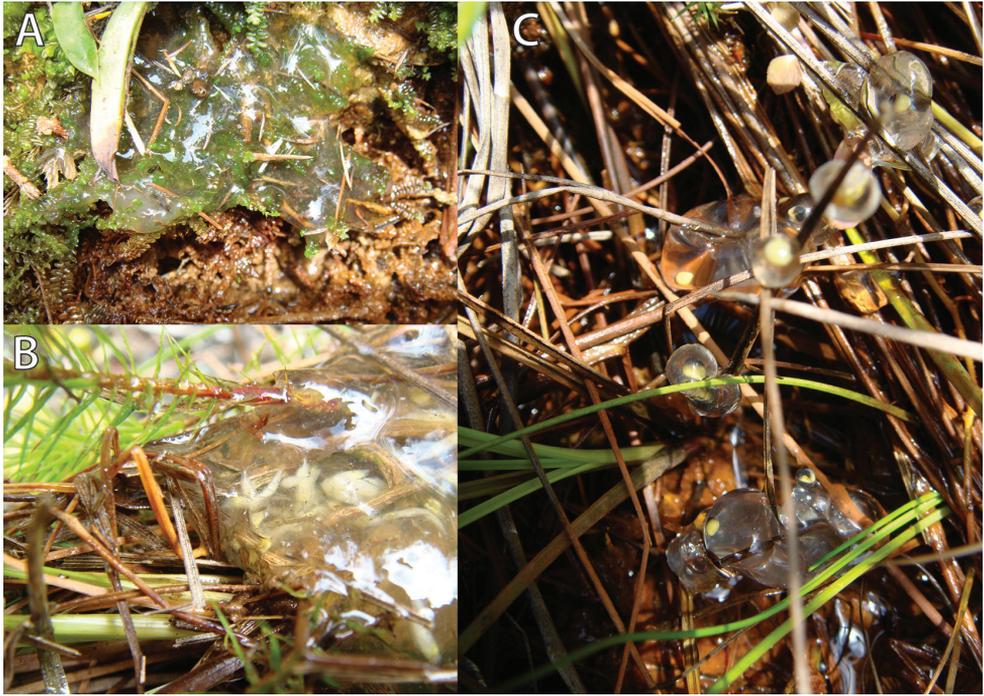


Figure 2. *Hyperolius castaneus* clutches of different age at the Uwasenkoko swamp. **A** Gelatinous clutch mass following hatching of tadpoles **B** Parasitized clutch mass with a few undeveloped eggs **C** Recently laid eggs attached to rush stalks. For further details see text. Photos by U. Sinsch.

conclude that a reproductive burst of several pairs had occurred 1–2 weeks prior to the survey, but that reproduction period is prolonged with little synchronisation among the several hundred local *H. castaneus* adults.

During the same survey we observed a pair in axillary amplexus on shore close to the open water surface (Fig. 3A). The male did not call and during the next two hours the pair moved occasionally along the shoreline. As the pair did not oviposit during this period, they were transferred into a small plastic container (5 cm diameter, 12 cm height, containing water to a height of 4 cm) and transported to the laboratory in Butare at 1643 m a.s.l. Reaching the laboratory two hours later we found that the pair had laid 15 eggs attached to the upper wall of the transport container and another 42 eggs were floating in the water (Fig. 3B). Eggs were deposited one by one using the egg-jelly envelope as glue for attachment to the wall and among single eggs. The pair, which already had finished amplexus, was removed from the box. Embryogenesis of the clutch was monitored in the same transport container at 20 ± 2 °C, but at a significantly higher air temperature compared to the native Uwasenkoko locality where daily fluctuations between 5 and 19 °C occur.

Six hours after oviposition the first eggs of the upper egg mass showed signs of cleavage (Gosner Stage 2; Fig. 4A). The egg envelope was not swollen by moisture uptake, but each single egg remained distinguishable. After 48 h most eggs were in a



Figure 3. **A** *Hyperolius castaneus* pair in amplexus at the Uwasenkoko swamp **B** Clutch laid in the transport box; 15 eggs attached to the upper container wall and 42 eggs within the water. For further details see text. Photos by U. Sinsch and M. Dehling.

stage of gastrulation (Gosner stages 10–13). After 5 d the most advanced embryos had reached Gosner Stage 19 (Fig. 4B), and after 6 d embryos reached Gosner Stage 22 and egg envelopes had fused to a single swollen gelatinous mass (Fig. 4C). Between 6 and 7 d following oviposition the egg-jelly became more fluid and the late embryos and early tadpoles of Gosner stage 24–25 started moving within the egg mass. At the end of day 7 the most advanced tadpoles had moved downwards within the egg-jelly, reaching the water level and beginning their free-swimming tadpole stage (Figs 4D, 5). In general, embryonic development of the 15 eggs was slightly asynchronous and two eggs did not seem to be fertilized (Fig. 5). In contrast, eggs deposited in water failed to develop further than Gosner Stage 10.

DNA-barcoding of tadpoles

DNA-sequences of representative specimens of the three morphologically distinct tadpole types collected in the Karamba pond and of the two tadpole types collected in the

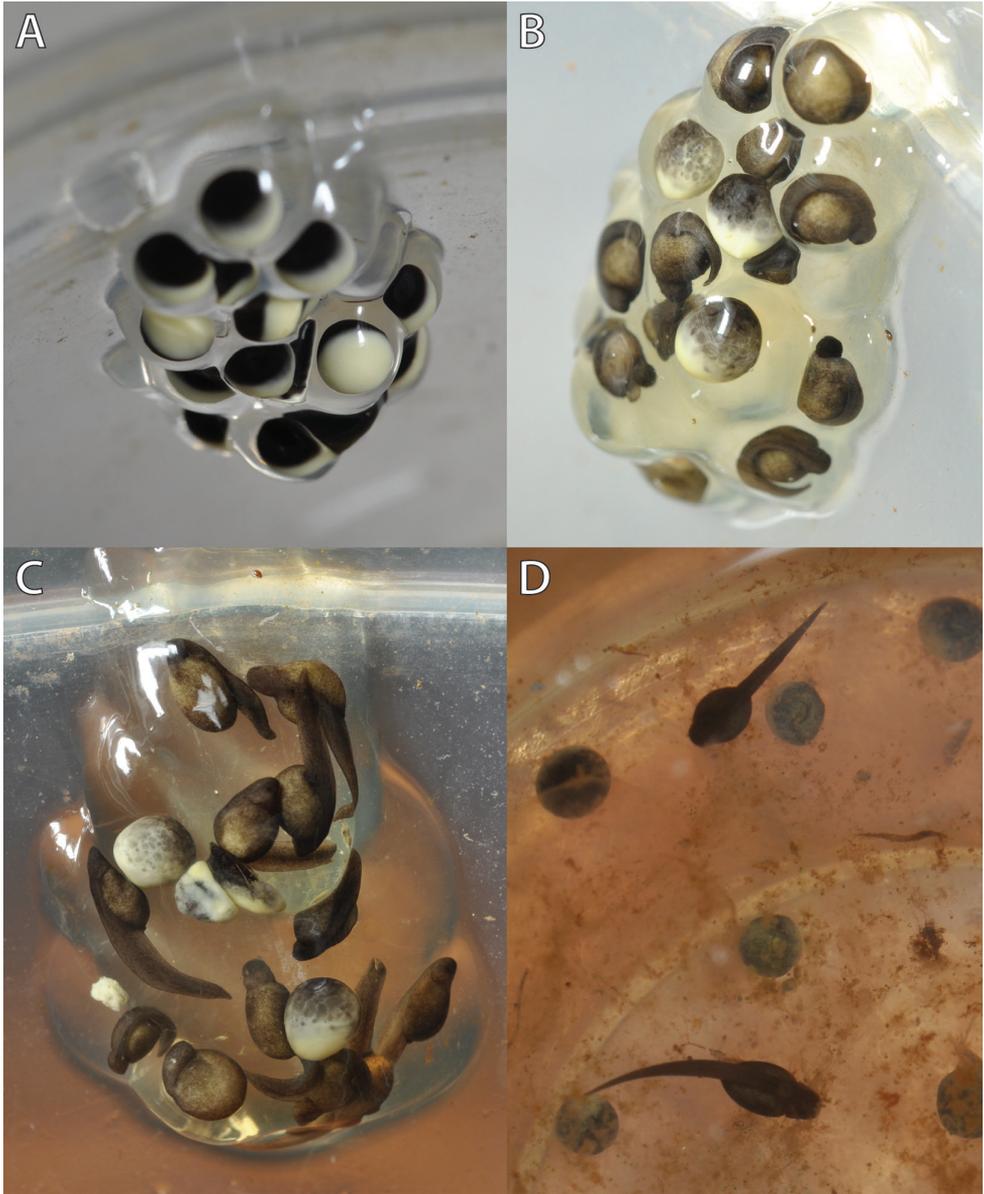


Figure 4. Embryogenesis of a *H. castaneus* clutch at 20 ± 2 °C. **A** Egg mass 4 cm above water level 6h following oviposition **B** 5d following oviposition **C** 6d following oviposition **D** 7d following oviposition; two hatchlings of the upper egg mass and undeveloped eggs within water. For further details see text. Photos by M. Dehling.

Uwasenkoko swamp were unequivocally associated (uncorrected p distance 0.0% between tadpole and corresponding adult sequence) with adult sequences of *H. castaneus*, *H. jackie*, *Leptopelis karissimbensis*, and *L. cf. kivuensis* 2 (Fig. 6).



Figure 5. Hatching of *H. castaneus* tadpoles from an egg mass attached 4 cm above water level. For further details see text. Photos by M. Dehling.

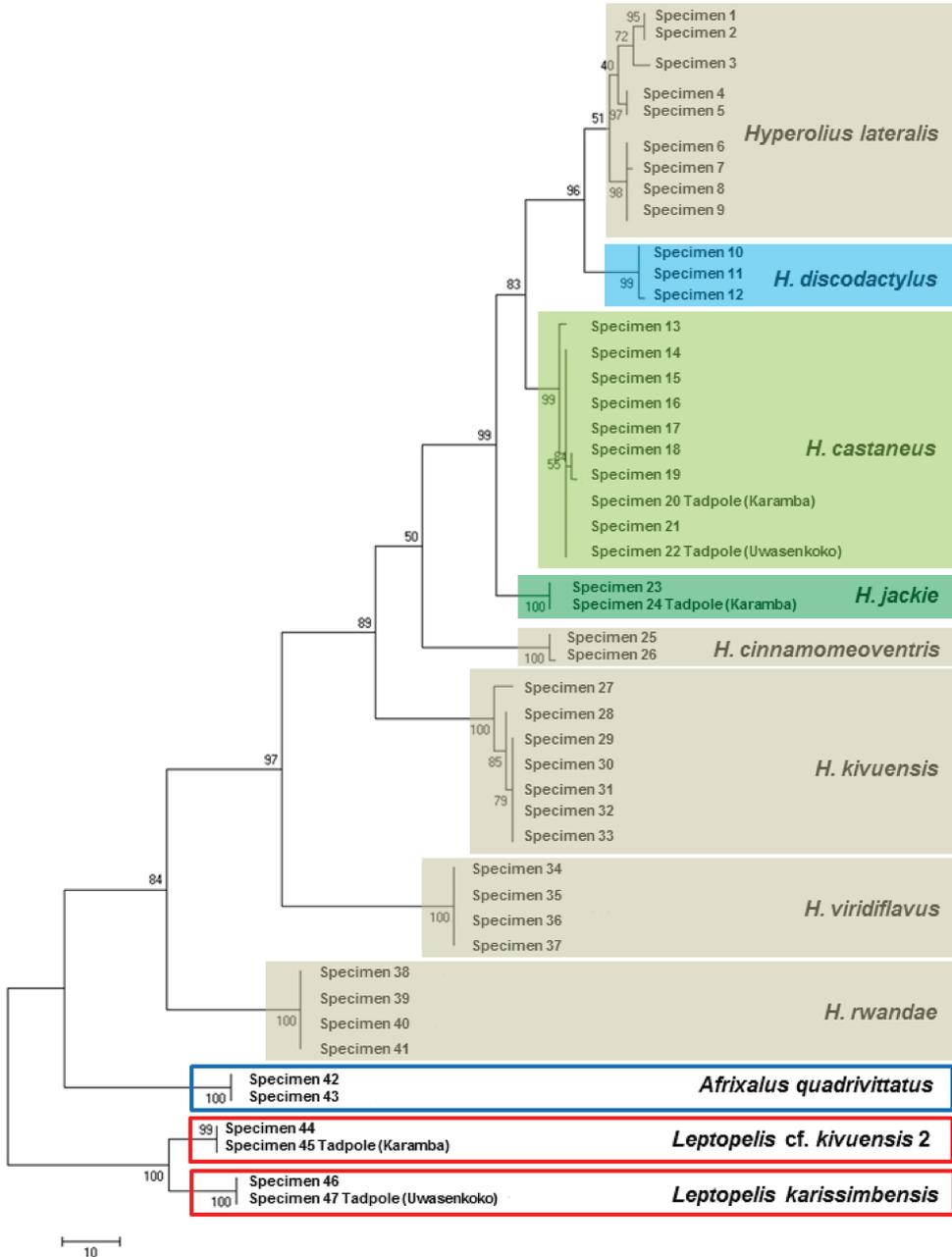


Figure 6. Maximum likelihood phylogram of Rwandan species in the genus *Hyperolius* with *Afrixalus quadrivittatus*, *Leptopelis karissimbensis* and *L. cf. kivuensis 2* as outgroups, based on comparison of 548 base pairs of the mitochondrial 16S rRNA gene. Included are 42 adult specimens collected in southwestern Rwanda, samples taken from GenBank and five tadpoles representing the morphotypes collected in the Karamba and Uwasenkoko swamps (specimen identification in Appendix I). Numbers above nodes are percentage support values from maximum likelihood. Only values above 50% are shown.

Tadpole of *Hyperolius castaneus* Ahl, 1931

The following description is based on a Stage 29 individual from the Uwasenkoko swamp, Rwanda (Figs 7A, B, ZFMK 97190, selected from a series of 52 tadpoles, Gosner stages 25–38, ZFMK 97191, and a series of 5 tadpoles, Gosner stages 34–41, ZFMK 97192 from Karamba, Figs 8–10). Exotrophous lentic benthic Type IV tadpole with following measurements (mm): total length 24.0, body length 9.0, tail length 15.0, body width 4.7, body height 3.6, eye diameter 1.0, interorbital distance 4.0, internarial distance 2.7, snout–naris–distance 1.9, distance–naris–eye 1.6, spiracle length 1.7, spiracle width 1.0, distance–snout–spiracle 6.4, tail muscle height at its beginning 2.4, tail muscle height at tail mid-length 1.8, greatest tail height 4.0, oral disc width 2.3. In dorsal view the body is elongated and ovoid and is widest at the level of the spiracle opening. The snout is rounded both in lateral and dorsal views. The interorbital distance is about twice the snout–naris distance, and internarial distance is 68% of interorbital distance. The eyes are positioned laterally, directed dorsolater-

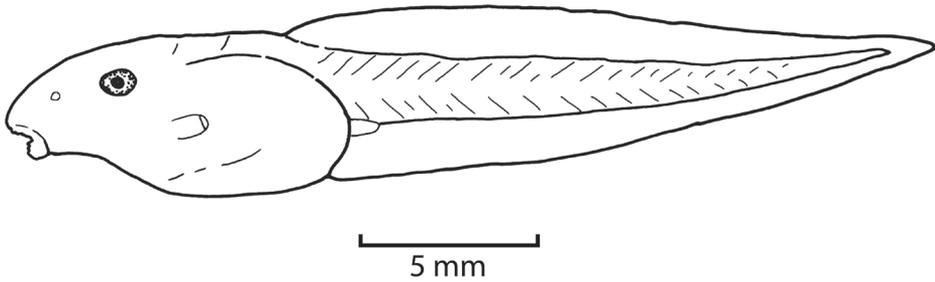
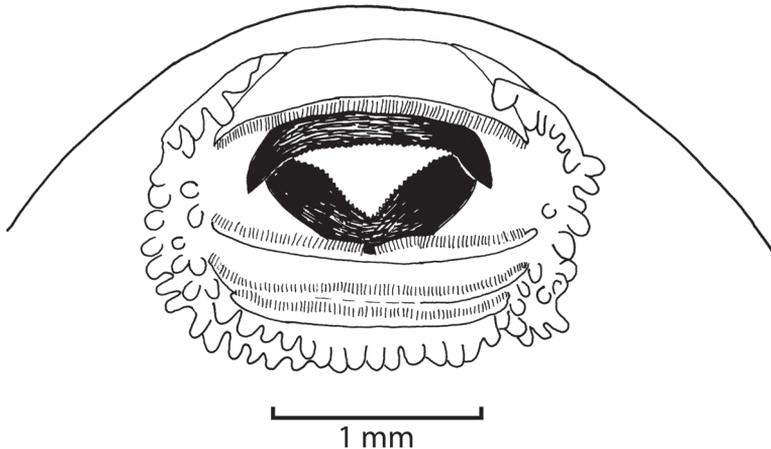
A**B**

Figure 7. Tadpole of *H. castaneus* (Stage 29, ZFMK 97190) in lateral view (**A**) and oral disc (**B**). Drawings by E. Lehr.

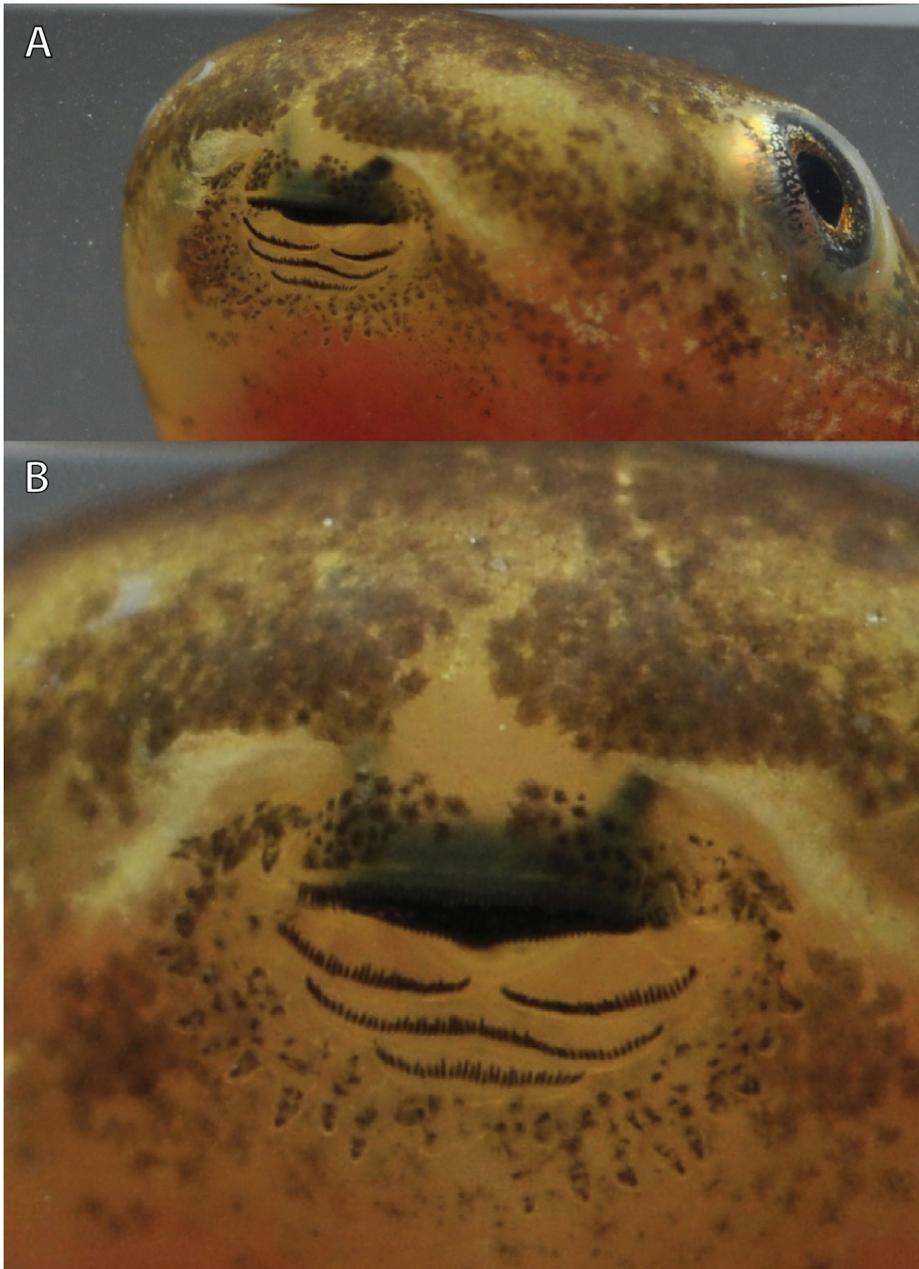


Figure 8. Oral disc in life of *H. castaneus* (Gosner Stage 40, from Karamba, ZFMK 97192) in overview (A) and close up view (B). Photos by M. Dehling.

ally, and are not visible in ventral view. The external nares are nearly round (slightly elongated horizontally), very small, and positioned laterally. They are more closely positioned to the eyes than to the snout (naris–eye–distance to snout–naris–distance

84%). In lateral view the body is highest at the mid-body length (approximately at the level of the spiracle opening). The body height is 40% of the body length, the body width is about half (52%) the length of the body, and the body height is 77% of the body width. The spiracle is single, sinistral, and attached to the body wall. Its shape is cylindrical and its length is about twice (170%) the eye diameter. The spiracle opening is rounded, directed posteriorly, and located at mid-body with its upper margin below the lower margin of the eye in lateral view. The length of the tail represents 63% of the total length. The tail is highest at about mid-tail and represents about a quarter (27%) of the tail length. The greatest tail height is located at the anterior quarter of the tail. The greatest tail height is slightly more than twice (225%) the body length, and slightly larger (111%) than the body height. The dorsal fin does not extend onto the body. Dorsal and ventral fins are about equal in height throughout their length. The tip of the tail is narrowly pointed and rounded. The height of the tail musculature at mid-body is about half (45%) of the maximum tail height. The vent tube is dextral, short, posteriorly directed, and linked to the tail musculature. The oral disc (Figs 7B, 8) is anteroventral, not emarginated, about half (49%) of the body width, and bordered at its lateral and posterior margin by a row of short and round papillae. Few submarginal papillae are present laterally and below the third lower tooth row. The LTRF is 1/3(1) with a narrow median gap in P1. The first two tooth rows are about equal in length, occupying nearly the entire width of the oral disc, the third tooth row is slightly shorter, and the shortest is the most posterior one. Jaw sheaths are finely serrated. The upper jaw sheath is inversely U-shaped and the lower V-shaped and narrower.

The variation in external morphology of the larval series is limited to size (Table 3) and LTRF. Fourteen tadpoles differ from the above described LTRF: Seven tadpoles had a LTRF of 1/3(1, 3), three of 1/3(1, 2), two of 1/3(1, 2, 3), one of 1(1)/3, and

Table 3. Measurements (mm) of 57 larvae of *Hyperolius castaneus*. Mean followed by one standard deviation, and range in parentheses for sample sizes larger than 2.

<i>Hyperolius castaneus</i>				
Stage	N	Total length	Body length	Tail length
25	14	11.3–16.0 (13.4 ± 1.4)	3.7–5.1 (4.5 ± 0.4)	7.6–10.9 (9.0 ± 1.0)
26	6	18.6–20.7 (20.2 ± 0.8)	6.5–7.2 (7.0 ± 0.3)	12.1–13.5 (13.2 ± 0.5)
27	4	19.4–25.5 (22.3 ± 2.5)	7.2–8.6 (7.8 ± 0.6)	12.2–16.9 (14.5 ± 1.9)
28	2	23.7, 23.9	8.5, 8.6	15.2, 15.3
29	2	24.0, 27.2	9.0, 9.6	15.0, 17.6
31	4	25.0–28.9 (25.7 ± 3.0)	9.1–9.3 (9.2 ± 0.1)	12.7–19.6 (16.5 ± 2.9)
34	5	27.8–32.7 (29.6 ± 2.0)	9.4–11.0 (10.1 ± 0.6)	18.4–20.3 (19.6 ± 1.4)
35	6	29.3–33.0 (31.1 ± 1.5)	10.0–10.9 (10.6 ± 0.4)	19.1–22.0 (20.6 ± 1.3)
36	5	30.8–33.0 (31.9 ± 0.9)	9.7–11.7 (10.8 ± 0.7)	20.1–23.0 (21.2 ± 1.2)
37	4	32.9–34.9 (33.9 ± 0.8)	10.0–11.5 (11.1 ± 0.7)	22.3–22.9 (22.8 ± 0.5)
38	2	32.1, 33.1	10.6, 11.1	21.5, 22.0
39	2	33.3, 34.2	10.5, 10.6	22.8, 23.6
41	1	31.0	10.7	20.3

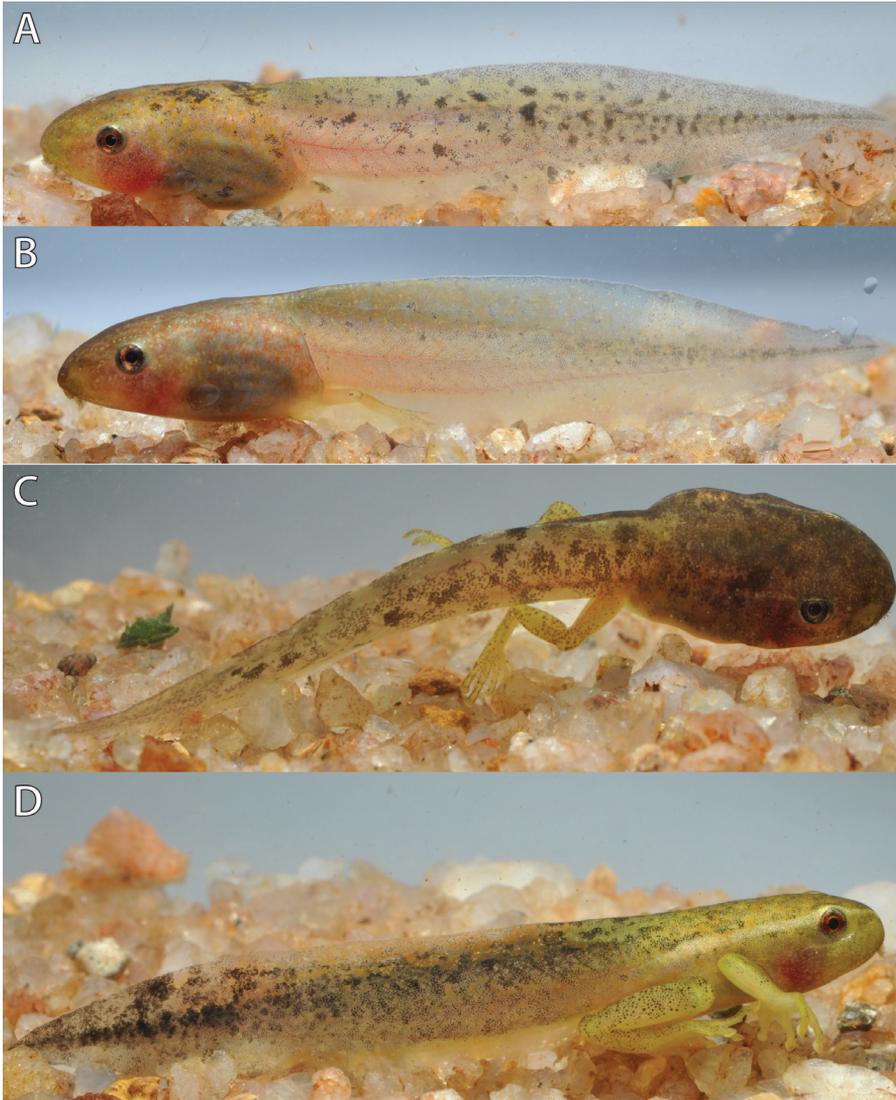


Figure 9. Color variation in life of *H. castaneus* from Karamba (ZFMK 97192) at different Gosner stages. **A** Stage 35 **B** Stage 37 **C** Stage 38 **D** Stage 44. Photos by M. Dehling.

one of 1/3. *Hyperolius castaneus* tadpoles from outside Rwanda (see Appendix I) correspond well with the description. One tadpole from Uganda (UTEP 21179) had a LTRF of 1(1)/3(1).

In preservative the larvae are entirely pale grayish brown to tan. The body is darker dorsally compared to the translucent venter. Tail musculature is tan and the fins are translucent, both bearing dark gray melanophores in various degrees.

The coloration in life (Figs 9, 10) of the body was dorsally tan with minute brownish-orange spots and translucent whitish on the venter. The tail musculature was green-

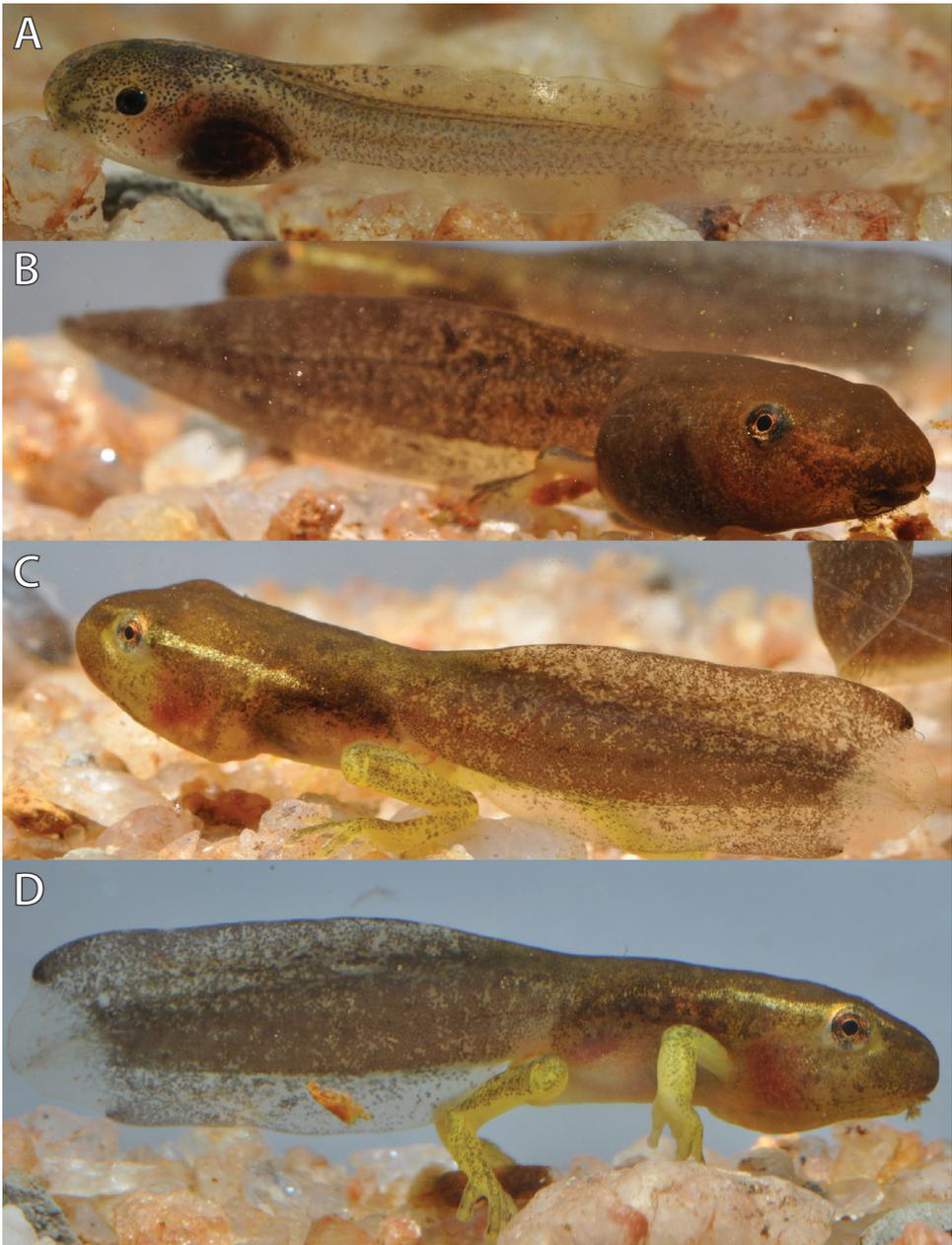


Figure 10. Color variation in life of *H. castaneus* from Uwasenkoko (ZFMK 97191) at different Gosner stages. **A** Stage 25 **B** Stage 38 **C** Stage 41 **D** Stage 44. Photos by M. Dehling.

ish tan and the fins were translucent tan with irregular dark marbling. Black spots and flecks were scattered dorsally and laterally on the body, tail musculature and dorsal fin. The ventral fin has fewer black spots and flecks or none at all. Younger stages (e.g.,

Gosner Stage 25, Fig. 10A) are paler compared to older stages (e.g., Gosner Stage 38, Fig. 10B). The series from Uwasenkoko was overall darker (e.g., Gosner Stage 38, Fig. 10B) compared to the series from Karamba (e.g., Gosner Stage 37, Fig. 9), possibly reflecting phenotypic plasticity. From stages 38 on in both series, distinct tan or whitish yellow dorsolateral stripes are present on each side extending from the snout to the end of the body. The iris was brownish orange with a few dark gray reticulations.

Tadpole of *Hyperolius jackie* Dehling, 2012

The following description is based on a Gosner Stage 32 individual from the Karamba swamp (Fig. 11, ZFMK 97193, from a series of 43 tadpoles, Gosner stages 25–41, ZFMK 97194, Figs 12–14). Exotrophic lentic benthic Type IV tadpole with the following measurements (mm): total length 31.5, body length 9.5, tail length 22.0, body width 5.2, body height 3.4, eye diameter 1.2, interorbital distance 4.8, internarial distance 3.0, distance–snout–naris 1.5, distance–naris–eye 1.6, spiracle length 1.9, spiracle width 0.6, distance–snout–spiracle 7.2, tail muscle height at its beginning 3.3, tail muscle height at tail mid-length 2.8, greatest tail height 6.8, oral disc width 1.6. In dorsal view the body is elongated and ovoid and is widest just posterior to the eye. The snout is rounded both in lateral and dorsal views. The interorbital distance is about three times the snout–naris–distance, and the internarial distance is 62.5% of the interorbital distance. The eyes are positioned laterally, directed dorsolaterally, and are slightly visible in ventral view. The external nares are ovoid and round (elongated horizontally), very small, and positioned laterally. They are nearly positioned in the middle between the eyes and snout (naris–eye–distance to snout–naris–distance 106.6%). In lateral view the body is highest at the mid-body length (approximately at the level of the spiracle opening). The body height is 36% of the body length, the body width is about half (55%) the length of the body, and the body height is 65% of the body width. The spiracle is single, sinistral, and attached to the body wall. Its shape is cylindrical and its length is 158% of the eye diameter. The spiracle opening is rounded, directed posteriorly, and located at mid-body with its upper margin reaching the level of the lower margin of the eye in lateral view. The length of the tail represents 70% of the total length. The tail is highest at about mid-tail and represents 31% of the tail length. The greatest tail height is 72% of the body length, and twice the body height. The dorsal fin does not extend onto the body. The dorsal fin is slightly higher than the ventral fin for about two thirds of the anterior tail length. The dorsal and ventral fins are of equal height for the posterior third of the tail. The tip of the tail is pointed and rounded. The height of the tail musculature at mid-body is slightly less than half (41%) of the maximum tail height. The vent tube is dextral, short, posteriorly directed, and linked to the tail musculature. The oral disc (Figs 11B, 12) is anteroventral, not emarginated, 31% of the body width, and bordered at its lateral and posterior margin by a row of short and round papillae. Few submarginal papillae are present laterally and below the third lower tooth row. The LTRF is 1/3(1) with a narrow median gap

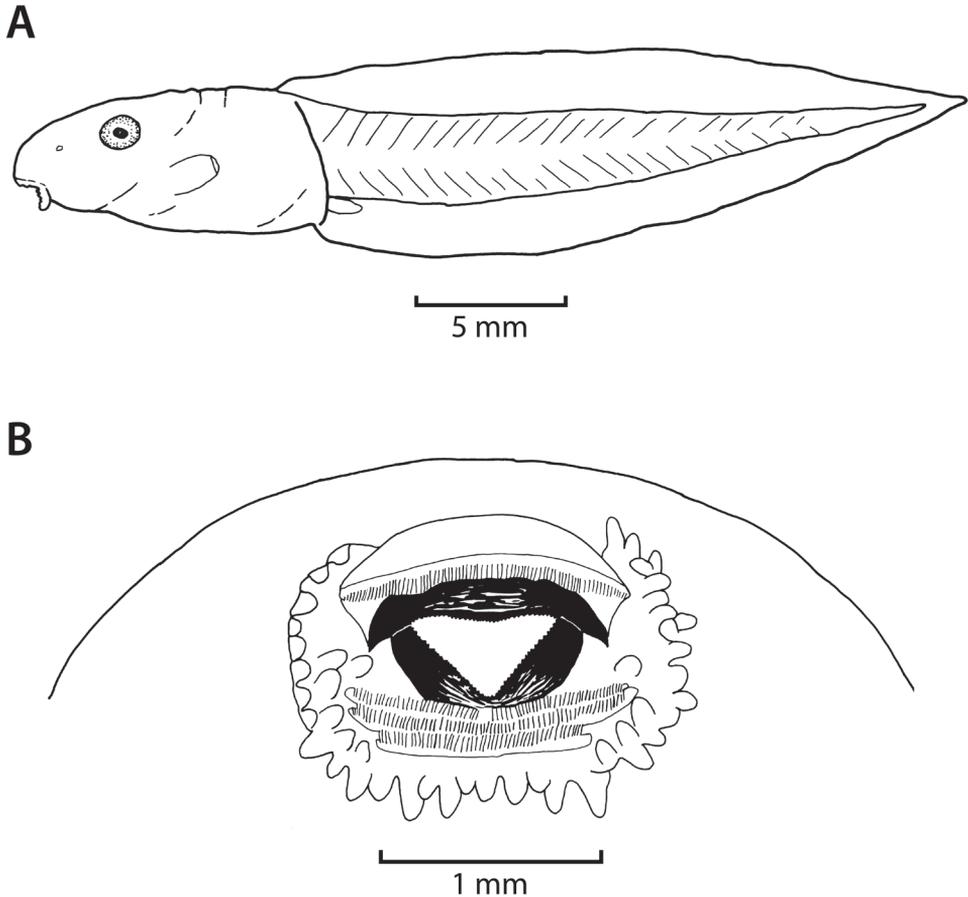


Figure 11. Tadpole of *H. jackie* (Stage 32, ZFMK 97193) in lateral view (A) and oral disc (B). Drawings by E. Lehr.

in P1. The first two tooth rows are about equal in length, occupying nearly the entire width of the oral disc, the third tooth row is slightly shorter, and the shortest is the most posterior one. Jaw sheaths are finely serrated. The upper jaw sheath is inversely U-shaped and the lower V-shaped and narrower.

The variation in external morphology of the larval series is limited to size (Table 4) and LTRF. Seven tadpoles differ from the above described LTRF: four had a LTRF of 1/3(1, 2), one of 1/3(1, 2, 3), one of 1/3, and one of 1/1.

In preservative the larvae are entirely pale grayish brown to tan. The body is darker dorsally compared to the translucent venter. The tail musculature is tan and the fins are translucent, both bearing dark gray melanophores in various degrees.

The coloration in life (Figs 13, 14) of the body was tan dorsally with minute brownish-orange and grayish-green spots and translucent whitish ventrally. The tail musculature was greenish tan and the fins were translucent tan with irregular dark marbling. Dark gray spots and flecks were scattered dorsally and laterally on the body,



Figure 12. Oral disc in life of *H. jackie* (Stage 35, ZFMK 97194). Photo by M. Dehling.

Table 4. Measurements (mm) of 43 larvae of *H. jackie*. Mean followed by one standard deviation, and range in parentheses for sample sizes larger than 2.

<i>Hyperolius jackie</i>				
Stage	N	Total length	Body length	Tail length
25	2	16.0, 16.1	5.5, 5.6	10.5, 11.5
26	1	19.6	6.5	13.1
28	2	20.4	7.1	13.3
30	1	25.0	7.3	17.7
31	2	26.4, 31.5	8.5, 9.3	17.9, 22.2
32	2	30.8, 31.5	9.5, 9.7	21.1, 22.0
34	4	31.4–37.6 (33.6 ± 2.9)	10.0–11.4 (10.6 ± 0.6)	20.8–26.2 (23.0 ± 2.5)
35	6	25.8–35.3 (31.2 ± 3.5)	9.4–11.0 (10.0 ± 0.7)	16.1–24.7 (21.2 ± 3.1)
36	4	30.7–36.7 (32.8 ± 2.8)	9.1–11.7 (10.6 ± 1.1)	19.1–25.5 (22.2 ± 2.7)
37	4	35.1–42.2 (38.2 ± 3.1)	10.8–12.1 (11.5 ± 0.7)	24.2–30.2 (26.8 ± 2.5)
38	4	39.5–41.4 (40.1 ± 0.9)	11.7–12.2 (11.9 ± 0.2)	27.4–29.6 (28.2 ± 1.0)
39	1	43.5	12.7	30.8
40	4	38.9–44.2 (41.9 ± 2.2)	11.2–13.5 (12.3 ± 0.9)	27.7–32.0 (29.6 ± 1.9)
41	2	38.1, 43.7	10.9, 12.1	27.2, 31.6

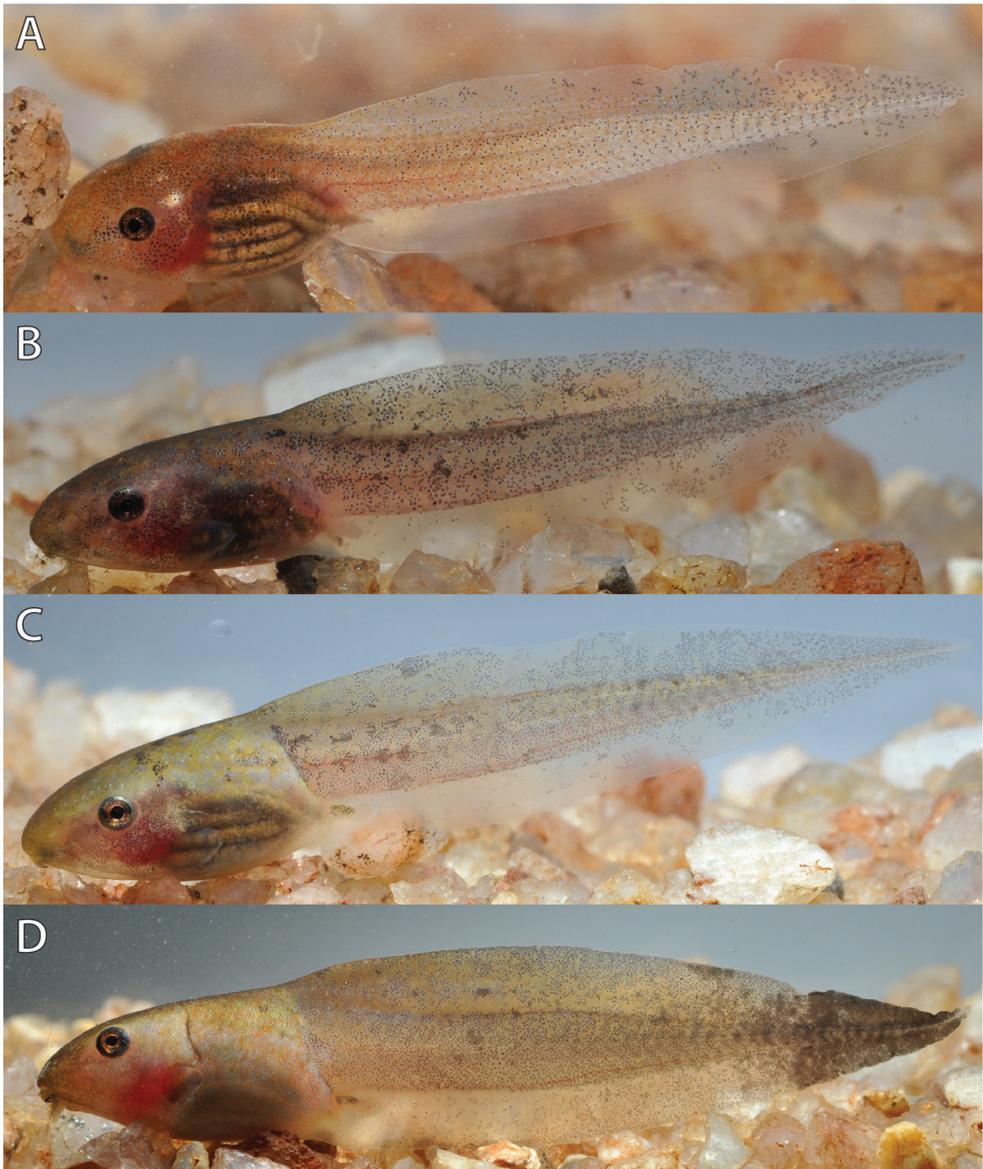


Figure 13. Color variation in life of *H. jackie* from Karamba (ZFMK 97194) at different Gosner stages. **A** Stage 25 **B** Stage 30 **C** Stage 34 **D** Stage 35. Photos by M. Dehling.

tail musculature and dorsal fin. The ventral fin has often fewer gray spots and flecks or is identical to the pattern of the dorsal fin (Figs 13A vs. 13C). Younger Gosner stages (e.g., Gosner Stage 25, Fig. 13A) are paler in overall coloration pattern compared to older Gosner stages (e.g., Gosner Stage 30, Fig. 13B). Individuals greatly differ in the amount of gray spots and flecks. Some have few gray spots and flecks scattered on the body and tail (Fig. 13C), whereas others have either numerous spots or flecks (Fig.

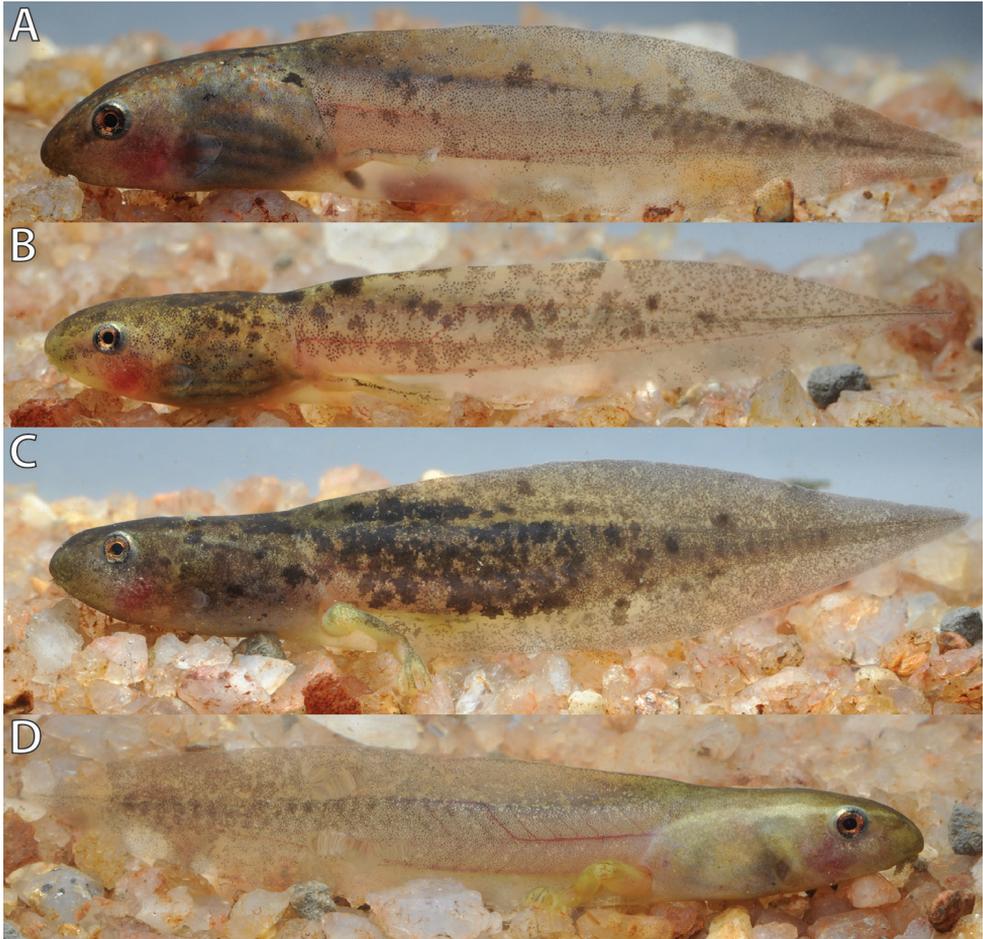


Figure 14. Color variation in life of *H. jackie* from Karamba (ZFMK 97194) at different Gosner stages. **A** Stage 36 **B** Stage 37 **C** Stage 40 **D** Stage 40. Photos by M. Dehling.

14C) or the tail tip can be nearly uniformly black (Fig. 13D). From Gosner stages 38 on, distinct tan or whitish yellow dorsolateral stripes are present on each side extending from the snout to the end of the body. The iris was brownish orange with a few dark gray reticulations.

Differential diagnosis of bog pool tadpoles

In the Nyungwe National Park *Hyperolius castaneus* and *H. jackie* tadpoles may co-occur and share the same pool with *Leptopelis karissimbensis* or *L. cf. kivuensis* 2. The tadpole of *L. karissimbensis* has been described in detail before (Roelke et al. 2009), and that of the morphologically similar *L. kivuensis* briefly in Channing et al. (2012).

At any stage the dark pigmented *Leptopelis* tadpoles are longer (e.g., total length for *L. karissimbensis* 51.4 mm at Gosner Stage 42 [Roelke et al. 2009], for *L. cf. kivuensis* 2 52.0 mm at Gosner Stage 39, for *Hyperolius castaneus* 31.0 mm at Gosner Stage 38, and *H. jackie* 43.7 mm at Gosner Stage 38) than *Hyperolius* tadpoles, mainly because of considerably longer tails. The tail fins are shorter in *L. karissimbensis* and *L. kivuensis*, and the LTRF in both species is $4(2-4)/3$ vs. $1/3(1)$ in *Hyperolius castaneus* and *H. jackie*. Applying morphometrics on tadpoles of Gosner range 30–39, Gosner stage-adjusted body length and tail length and consequently total length differ significantly among species (ANCOVA, $F_{3,81} = 21.0/67.9/62.3$, $P < 0.0001$: *H. castaneus* ($n = 56$; BL = 10.6 mm; TAL = 19.2 mm; TL = 29.8 mm; least square means) < *H. jackie* ($n = 34$; BL = 11.5 mm; TAL = 24.7 mm; TL = 36.2 mm) < *L. karissimbensis* ($n = 26$; BL = 13.6 mm; TAL = 27.6 mm; TL = 41.2 mm) < *L. cf. kivuensis* 2 ($n = 24$; BL = 12.4 mm; TAL = 30.3 mm; TL = 42.6 mm). We have not recorded any differences in external morphology or coloration to distinguish the tadpoles of *H. castaneus* and *H. jackie*.

Discussion

Eleven species of *Hyperolius* (*H. castaneus*, *H. cinnamomeoventris*, *H. discodactylus*, *H. frontalis* Laurent, 1950, *H. glandicolor* Peters, 1878, *H. kivuensis*, *H. jackie*, *H. lateralis*, *H. parallelus* Günther, 1858, *H. rwandae* Dehling, Sinsch, Rödel & Channing, 2013 in Channing et al. 2013, and *H. viridiflavus* Duméril & Bibron, 1841) are currently known to occur in Rwanda (Dehling 2012, unpubl. Data, Sinsch et al. 2011, 2012). Four of the Rwandan *Hyperolius* (*H. castaneus*, *H. discodactylus*, *H. frontalis*, and *H. jackie*) have been recorded in cloud forests of the Nyungwe National Park (Dehling 2012, unpubl. data), and three (*H. castaneus*, *H. cinnamomeoventris*, *H. glandicolor* [the latter recorded as *H. viridiflavus* by Roelke and Smith (2010), but species identification was corrected as *H. glandicolor* by Dehling, unpubl. data.]) in cloud forests of the Volcano National Park (Roelke and Smith 2010). The tadpoles of five species of Rwandan *Hyperolius* have been described: *H. castaneus* (this paper), *H. kivuensis* (Viertel et al. 2007), *H. jackie* (this paper), *H. lateralis* (Channing et al. 2012), and *H. viridiflavus* (Viertel et al. 2007), whereas the tadpole of *H. discodactylus* and *H. frontalis* will be described by Dehling and Sinsch in the near future. All five tadpoles share a LTRF of $1/3(1)$. At Gosner Stage 36 following total lengths (TL) have been reported (mean followed by range in parenthesis): *H. castaneus*: 31.9 ± 0.9 (30.8–33.0, $n = 5$); *H. jackie*: TL = 32.8 ± 2.8 (30.7–36.7, $n = 4$); *H. kivuensis*: TL = 34.9 (28.8–40.7, $n = 14$, Viertel et al. 2007); *H. lateralis*: unknown, 35 mm length given without stage assignment (Channing et al. 2012); *H. viridiflavus*: TL = 35.4 (30.0–39.6, $n = 38$, Viertel et al. 2007). Based on mean TL at this stage, the tadpole of *H. viridiflavus* is the largest, followed by *H. kivuensis*, *H. jackie*, and *H. castaneus* in descending TL, and unknown for *H. lateralis*. The external nares are positioned closer to the eyes than to the snout in *H. castaneus*, and positioned nearly

in the middle between the eyes and snout in *H. jackie* and *H. lateralis*, whereas the external nares are more closely positioned to the snout than to the eyes in *H. kivuensis* and *H. viridiflavus*. Dorsal and ventral fins are about equal in height throughout their length in *H. castaneus*, whereas the dorsal fin is slightly higher than the ventral fin for about two thirds of the anterior tail length and of equal height for the posterior third, the upper tail fin is larger in height than the lower in *H. kivuensis* and *H. viridiflavus*, and condition unknown for *H. lateralis*. From Gosner stages 38 on, both *H. castaneus* and *H. jackie* tadpoles can be differentiated from the other three tadpoles in having distinct tan or whitish yellow dorsolateral stripes on each side extending from the snout to the end of the body. In summary, the observable differences in *Hyperolius* tadpoles are subtle as expected for cryptic species and some tadpoles (*H. lateralis*) need further investigations.

Viertel et al. (2007) were the first ones to describe oral disc and buccal cavity morphology in *Hyperolius* tadpoles and their value for taxonomy. Applying scanning electron microscopy, Viertel et al (2007) noted inter- and intraspecific differences in the types of labial teeth as well as interspecific differences in the buccal cavity. However, such methodology is relatively expensive and time intensive. Regarding external morphology, proportions, coloration and LTRF, *Hyperolius* tadpoles are very similar with only minor differences, which make species identifications unreliable, especially in areas with high species diversity, syntopic distributions or areas that have not been surveyed. This is the case for both *H. castaneus* and *H. jackie* larva, which only differ externally by their size (*H. jackie* larva are larger). We therefore consider DNA barcoding the most reliable method for identifications of larval *Hyperolius*, which was already noted by Viertel et al. (2007).

Dipteran predation on arboreal frog eggs in Africa was first described by Vonesh and Ross (2000) for four species of *Hyperolius* from Uganda. An infestation rate of 40% was recorded within the 1261 observed clutches of *Hyperolius lateralis*, *H. cinnameoventris*, *H. platyceps* (Boulenger, 1900), and *H. kivuensis*. Larvae of ephydrid and phorid flies feed on frog ova and cause high embryonic mortality, and the surviving tadpoles hatch at a smaller size (Vonesh and Ross 2000, Vonesh 2005). Our observation of an infestation of egg mass by larval dipterid flies in *H. castaneus* is to our knowledge the first record for this species.

With continuing fieldwork in Rwanda and other African countries, we are confident that the knowledge on reproduction, embryogenesis and species diversity of *Hyperolius* will increase.

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Appendix I

Larval specimens examined

- Hyperolius castaneus*: DR CONGO: North Kivu: Mt. Tshiaberimu, Virunga National Park (S00.12605, E29.43284, 2767 m a.s.l.) collected on 7 July 2008 by E. Greenbaum, C. Kusamba, W. M. Moninga and M. M. Aristote: UTEP 20620 (one tadpole, Gosner Stage 35), UTEP 20621 (one tadpole, Gosner Stage 37); RWANDA: Nyungwe National Park, from a natural pond forming part of the Uwasenkoko swamp (2.529°S, 29.354°E, 2379 m a.s.l.), collected on 22 and 26 March 2012 by J. M. Dehling & U. Sinsch: ZFMK 97191 (series of 51 tadpoles, Gosner stages 25–38), ZFMK 97190 (single tadpole selected from ZFMK 97191, Gosner Stage 29); Nyungwe National Park, from a natural pond at Karamba (2.479°S, 29.112°E, 1936 m a.s.l.), collected on 24 March 2012 by J. M. Dehling & U. Sinsch: ZFMK 97192 (series of 5 tadpoles, Gosner stages 34–41); UGANDA: Muchuya Swamp, Stream (S01.25543, E29.79689, 2200 m a.s.l.) collected on 25 May 2014 by E. Greenbaum, D. F. Hughes and M. Behangana: UTEP 21179 (one tadpole, Gosner Stage 37).
- Hyperolius jackie*: RWANDA: Nyungwe National Park, from a natural pond at Karamba (2.479°S, 29.112°E, 1936 m a.s.l.), collected on 24 March 2012 by J. M. Dehling & U. Sinsch: ZFMK 97194 (series of 42 tadpoles, Gosner stages 25–41), ZFMK 97193 (single tadpole selected from ZFMK 97194, Gosner Stage 32).

Leptopelis karissimbensis: RWANDA: Nyungwe National Park, from a natural pond forming part of the Uwasenkoko swamp (2.529°S, 29.354°E, 2379 m a.s.l.), collected on 22 and 26 March 2012 by J. M. Dehling & U. Sinsch: ZFMK 97188 (series of 26 tadpoles, Gosner stages 25–39).

Leptopelis cf. *kivuensis* 2: RWANDA: Nyungwe National Park, from a natural pond at Karamba (2.479°S, 29.112°E, 1936 m a.s.l.), collected on 24 March 2012 by J. M. Dehling & U. Sinsch: ZFMK 97189 (series of 24 tadpoles, Gosner stages 28–39).

Description and phylogeny of three new species of *Synophis* (Colubridae, Dipsadinae) from the tropical Andes in Ecuador and Peru

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Abstract

The discovery of three new species of *Synophis* snakes from the eastern slopes of the tropical Andes in Ecuador and Peru is reported. All previous records of *S. bicolor* from eastern Ecuador correspond to *S. bogerti* **sp. n.**, which occurs between 1000–1750 m along a large part of the Amazonian slopes of the Ecuadorian Andes. In contrast, *Synophis zamora* **sp. n.** is restricted to southeastern Ecuador, including Cordillera del Cóndor, between 1543–1843 m. *Synophis insulomontanus* **sp. n.** is from the eastern slopes of the Andes in central and northern Peru, between 1122–1798 m, and represents the first record of *Synophis* from this country. All three new species share in common a large lateral spine at the base of the hemipenial body. A molecular phylogenetic tree based on three mitochondrial genes is presented, including samples of *Diaphorolepis wagneri*. Our tree strongly supports *Synophis* and *Diaphorolepis* as sister taxa, as well as monophyly of the three new species described here and *S. calamitus*. Inclusion of *Synophis* and *Diaphorolepis* within Dipsadinae as sister to a clade containing *Imantodes*, *Dipsas*, *Ninia*, *Hypsiglena* and *Pseudoleptodeira* is also supported.

Resumen

Se reporta el descubrimiento de tres especies nuevas de serpientes *Synophis* de las estribaciones orientales de los Andes tropicales en Ecuador y Perú. Todos los registros previos de *S. bogerti* del oriente ecuatoriano corresponden a *S. bogerti* **sp. n.**, la cual ocurre entre 1000–1750 m a lo largo de gran parte de las estribaciones amazónicas de los Andes ecuatorianos. En contraste, *Synophis zamora* **sp. n.** se restringe al suroriente de Ecuador, incluyendo la Cordillera del Cóndor, entre 1543–1843 m. *Synophis insulomontanus* **sp. n.** es de las estribaciones orientales de los Andes del centro y norte del Perú, entre 1122–1798 m, y representa el primer registro de *Synophis* para este país. Todas las tres especies nuevas comparten en común una espina lateral larga en la base del cuerpo del hemipene. Un árbol molecular filogenético, basado en tres genes mitocondriales es presentado, incluyendo muestras de *Diaphorolepis wagneri*. Nuestro árbol apoya fuertemente a *Synophis* y *Diaphorolepis* como taxa hermanos, así como la monofilia de las tres especies descritas y de *S. calamitus*. La inclusión de *Synophis* y *Diaphorolepis* dentro de Dipsadinae, como hermanas a un clado que contiene a *Imantodes*, *Dipsas*, *Ninia*, *Hypsiglena* y *Pseudoleptodeira* también es apoyada.

Keywords

Andes, Dipsadinae, Ecuador, new species, Peru, snakes, *Synophis*, systematics

Introduction

With only four recognized species, *Synophis* is among the least speciose snake groups formally recognized as genera in South America. Species of *Synophis* are known to occur in the Andes of Colombia and Ecuador between approximately 460–2200 m (Hillis 1990). Whereas *S. plectovertebralis* and *S. calamitus* are endemic to Colombia and Ecuador, respectively (Hillis 1990; Sheil and Grant 2001), *S. bicolor* and *S. lasallei* have been reported in both countries (Bogert 1964; Nicéforo-María 1970).

The taxonomic identity of specimens currently assigned to *Synophis bicolor* (Peracca 1896) has been problematic for two reasons. First, the type locality of this species is ambiguous ('America meridionale') preventing the collection of topotypes for comparison. Second, there is significant morphological variation between specimens of *S. bicolor* from Colombia and Ecuador. In his taxonomic review of *Synophis* and *Diaphorolepis* Bogert (1964) noted some differences between specimens from Ecuador and the holotype of *S. bicolor* (in parentheses): 10–11 infralabials (9), 160–166 ventrals (180), 100–118 subcaudals (136), 24–27 maxillary teeth (16), 14 palatine teeth (9–10), 32–34 pterygoid teeth (21–22). Based on this variation, Bogert (1964) recognized that "specimens tentatively referred to *S. bicolor* might not be conspecific". Subsequently, Nicéforo-María (1970) reported the first specimen of *S. bicolor* from Colombia and noted that the numbers of ventrals and subcaudals (184 and 127, respectively) are more similar to the holotype than the specimens from Ecuador. This suggests that at least some populations from Ecuador currently assigned to *S. bicolor* represent one or more similar undescribed species instead.

The study of *Synophis* has been hampered by the paucity of specimens in collections, possibly because of low densities or semifossorial habits (Sheil and Grant 2001). Recent collections in poorly explored areas of the Amazonian slopes of the Andes from

Ecuador and Peru yielded a few specimens of *Synophis* that are similar in morphology to specimens of *S. bicolor* previously reported from eastern Ecuador (Bogert 1964). Based on these recent collections, including the first specimens of *Synophis* from Peru, we combine evidence from morphology and phylogenetic analyses of DNA sequence data to describe three new species of *Synophis*.

Materials and methods

Morphological data

All type specimens of the new species are deposited at Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito (QCAZ); and Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Peru. Other specimens used for comparisons are listed in Appendix I. Sex was determined by observation of hemipenes from X-ray images or by noting the presence of everted hemipenes. Snout-vent length (SVL) and tail length were measured with a ruler and recorded to the nearest millimeter. Other measurements were taken with digital calipers (± 0.01 mm). We prepared partially everted hemipenes following Zaher and Prudente (2003), and immersed them for 6 h in an alcoholic solution of Alizarin Red to dye the calcareous ornaments (e.g., spines). Terminology for hemipenis description follows Dowling and Savage (1960), as augmented by Zaher (1999). Data on the hemipenes of *Synophis calamitus* and *S. lasallei* were taken from the literature (Zaher 1999).

Molecular data

Total genomic DNA was digested and extracted from liver or muscle tissue using a guanidinium isothiocyanate extraction protocol. Tissue samples were first mixed with Proteinase K and lysis buffer and digested overnight prior to extraction. DNA samples were quantified using a Nanodrop® ND-1000 (NanoDrop Technologies, Inc), re-suspended and diluted to 25 ng/ul in ddH₂O prior to amplification.

We amplified 2173 nucleotides (nt) encompassing three mitochondrial genes, NADH dehydrogenase subunit 4 (*ND4*, 567 nt), cytochrome b (*cyt-b*, 1069 nt) and the ribosomal large subunit (*16S*, 537 nt) from 10 individuals of the three new species described in this paper, five individuals of *Synophis calamitus*, and two of *Diaphorolepis wagneri*. *Cyt-b* was amplified using the primers GluDG, LGL765, L14910, H16064 (Bickham et al. 1995; Burbrink et al. 2000; Palumbi 1996; Parkinson et al. 2002), and primer *CytbV* 5'-GGCGAATAAGGAAGTATCATT-3' designed by A. Fouquet; *ND4* was amplified using the primers *ND4*, *LEU* and *ND412931L* (Arévalo et al. 1994; Blair et al. 2009); and *16S* was amplified with *16SF.0* and *16SR.0* (Pellegrino et al. 2001; Whiting et al. 2003). Amplification of genomic DNA consisted of an initial cycle at 94–96 °C for 3–5 min, followed by 35–40 cycles of a denaturation at 94 °C

for 30–40 s, annealing at 51–52 °C for 40–60 s, and extension at 72 °C for 40–60 s, as well as a final extension at 72 °C for 7–10 min. Genbank accession numbers of sequences generated in this study are shown in Table 1.

Additionally, we obtained from GenBank sequences of 12 Dipsadinae taxa and *Natrix natrix*, which was used to root the tree following the phylogenetic hypothesis presented by Pyron et al. (2013). We only selected those Dipsadinae species that had sequence data for all three genes included in our analyses. Gene regions of outgroup taxa included in phylogenetic analyses along with their GenBank accession numbers are shown in Table 2.

Phylogenetic analyses

Data were assembled and aligned in Geneious v7.1.7 (Kearse et al. 2012) under default settings for MAFFT (Katoh and Toh 2010). *ND4* and *cyt-b* sequences were translated into amino acids for confirmation of alignment. The best-fit nucleotide substitution models and partitioning scheme were chosen simultaneously using PartitionFinder v1.1.1 (Lanfear et al. 2012) under the Bayesian Information Criterion (BIC). The “greedy” algorithm was used with branch lengths of alternative partitions “linked” to search for the best-fit scheme, which consisted of three partitions: (i) 1^{6S}, 3rd codon positions of both *cyt-b* and *ND4* [GTR + I + G]; (ii) 2nd codon positions of both *cyt-b* and *ND4* [K81uf+G]; and (iii) 1st codon positions of both *cyt-b* and *ND4* [HKY + I + G]. Bayesian inference was used to obtain a phylogenetic tree of the combined dataset using the program MrBayes v3.2.1 (Ronquist et al. 2012). All parameters were unlinked between partitions (except topology and branch lengths), and rate variation (prset ratepr = variable) was invoked. Four independent runs, each with four MCMC chains, were run for five million generations, sampling every 1,000 generations. Results were analyzed in Tracer to assess convergence and effective sample sizes (ESS) for all parameters. Additionally, we verified that the average standard deviation of split frequencies between chains and the potential scale reduction factor (Gelman and Rubin 1992) of all the estimated parameters approached values of ≤ 0.01 and 1, respectively. Of the 5,000 trees resulting per run, 25% were arbitrarily discarded as “burn-in”. The remaining trees were used to calculate posterior probabilities (PP) for each bipartition in a 50% majority-rule consensus tree. The phylogenetic tree was visualized and edited using FigTree v1.4.2 (Rambaut 2014).

Results

The taxonomic conclusions of this study are based on the observation of morphological features and color patterns, as well as inferred phylogenetic relationships. We consider this information as species delimitation criteria following a general lineage or unified species concept (de Queiroz 1998; 2007).

Table 1. Vouchers, locality data, and GenBank accession numbers of new sequences obtained for this study.

Taxon	Voucher	Locality ^a	Genbank number			GenSeq Nomenclature
			<i>cyt-b</i>	<i>ND4</i>	<i>16S</i>	
<i>Diaphorolepis wagneri</i>	QCAZ11956	Ecuador: Imbabura: Reserva Manduriacu	KT345360	KT345377	KT345343	genseq-4
<i>Diaphorolepis wagneri</i>	QCAZ11961	Ecuador: Imbabura: Reserva Manduriacu	KT345361	KT345378	KT345344	genseq-4
<i>Synophis bogerti</i>	QCAZ5072	Ecuador: Napo: Wildsumaco Wildlife Sanctuary	KT345372	KT345389	KT345355	genseq-2
<i>Synophis bogerti</i>	QCAZ12791	Ecuador: Napo: Wildsumaco Wildlife Sanctuary	KT345365	KT345382	KT345348	genseq-1
<i>Synophis bogerti</i>	QCAZ13323	Ecuador: Morona Santiago: Sardinayacu, Parque Nacional Sangay	KT345368	KT345385	KT345351	genseq-2
<i>Synophis bogerti</i>	QCAZ13585	Ecuador: Pastaza: Zarentza, Parque Nacional Llanganates	KT345369	KT345386	KT345352	genseq-2
<i>Synophis bogerti</i>	QCAZ13586	Ecuador: Pastaza: Zarentza, Parque Nacional Llanganates	KT345370	KT345387	KT345353	genseq-2
<i>Synophis calanninus</i>	QCAZ3875	Ecuador: Cotopaxi: Naranjito, Bosque Integral Otonga	KT345371	KT345388	KT345354	genseq-4
<i>Synophis calanninus</i>	QCAZ5847	Ecuador: Carchi: 14 km El Chical-Gualchán	KT345373	KT345390	KT345356	genseq-4
<i>Synophis calanninus</i>	QCAZ8098	Ecuador: Pichincha: El Cedral	KT345374	KT345391	KT345357	genseq-4
<i>Synophis calanninus</i>	QCAZ10508	Ecuador: Pichincha: El Cedral	KT345362	KT345379	KT345345	genseq-4
<i>Synophis calanninus</i>	QCAZ11931	Ecuador: Pichincha: Reserva Ecológica Santa Lucía	KT345363	KT345380	KT345346	genseq-4
<i>Synophis insulomontanus</i>	CORBID19223	Perú: San Martín: Picoa: Puesto de Control 16 Chambirillo (Cordillera Azul)	KT345366	KT345383	KT345349	genseq-2
<i>Synophis insulomontanus</i>	CORBIDI13940	Perú: Huánuco: Pachitea: Cordillera El Sira	KT345367	KT345384	KT345350	genseq-1
<i>Synophis zamora</i>	QCAZ9174	Ecuador: Zamora Chinchipe: Las Orquídeas	KT345375	KT345392	KT345358	genseq-1
<i>Synophis zamora</i>	QCAZ9175	Ecuador: Zamora Chinchipe: Las Orquídeas	KT345376	KT345393	KT345359	genseq-2
<i>Synophis zamora</i>	QCAZ12773	Ecuador: Zamora Chinchipe: Numbami reserve, 18 km Zamora-Romerillos	KT345364	KT345381	KT345347	genseq-2

^aSee species accounts and Appendix I for geographic coordinates and altitude data.

Table 2. Outgroup taxa used in this study along with their GenBank accession numbers.

Taxon	Genbank number		
	<i>cyt-b</i>	<i>ND4</i>	<i>16S</i>
Natricinae			
<i>Natrix natrix</i>	AY487723	AY487799	KJ128951
Dipsadinae			
<i>Alsophis antillensis</i>	FJ416726	FJ416800	FJ416702
<i>Contia tenuis</i>	AF471095	AF402656	AY577030
<i>Diadophis punctatus</i>	AF471094	AF258889	AF544793
<i>Dipsas catesbyi</i>	EF078537	EF078585	JQ598868
<i>Farancia abacura</i>	U69832	DQ902307	Z46491
<i>Hypsiglena chlorophaea</i> ^a	KJ486459	KJ486459	KJ486459
<i>Imantodes cenchoa</i> ^a	EU728586	EU728586	EU728586
<i>Ninia atrata</i>	GQ334553	GQ334659	JQ598882
<i>Oxyrhopus</i>	GQ334554	GQ334660	GU018170
<i>Pseudoleptodeira latifasciata</i> ^a	NC013981	NC013981	NC013981
<i>Thermophis zhaoermi</i> ^a	GQ166168	GQ166168	GQ166168
<i>Uromacer catesbyi</i>	FJ416714	FJ416788	AF158523

^aSequences extracted from whole mitochondrial genomes.

Synophis bogerti sp. n.

<http://zoobank.org/05AC659D-BA2E-4953-B2EE-182ABFBF2324>

Proposed standard English name: Bogert's fishing snakes

Proposed standard Spanish name: Serpientes pescadoras de Bogert

Synophis bicolor (part)—Bogert (1964): 515.

Holotype. Ecuador: Provincia Napo: QCAZ 12791 (Figs 1, 2), adult male from Wildsumaco Wildlife Sanctuary, sendero Coatí (0°38'8.40"S, 77°31'19.20"W, 1000 m), collected on 18 July 2014 by J. D. Camper.

Paratypes. Ecuador: Provincia Morona Santiago: QCAZ 13323 adult male from Laguna Cormorán, Sardinayacu, Parque Nacional Sangay (2°4'17.51"S, 78°12'57.24"W, 1747 m), collected on 16 January 2015 by J. Pinto, D. Velalcázar and D. Nuñez. Provincia Napo: QCAZ 3511, adult female from Cordillera de los Guacamayos (0°37'40.16"S, 77°50'0.98"W, 1200 m), collected on 1 August 1995 by S. Burneo and M. Díaz; QCAZ 5072 adult male from Wildsumaco Wildlife Sanctuary (0°41'9.26"S, 77°35'54.93"W, 1250 m), collected on 26 July 2012 by J. D. Camper; QCAZ 11070 adult female from Reserva Ecológica Antisana, sector Cocodrilos, Cocodrilos-Tena road (0°39'42.50"S, 77°47'29.20"W, 1656 m), collected on 24 November 2010 by F. Velásquez-Alomoto. Provincia Pastaza: QCAZ 13585, adult male from Comunidad Zarentza, Parque Nacional Llanganates (1°21'45.47"S, 78°3'29.52"W, 1350 m), collected on 18 February 2015 by D. Rivadeneira, F. Mora, J. C. Sánchez, D. Velalcázar, D. Nuñez and J. Pinto; QCAZ 13586, adult female from Comunidad

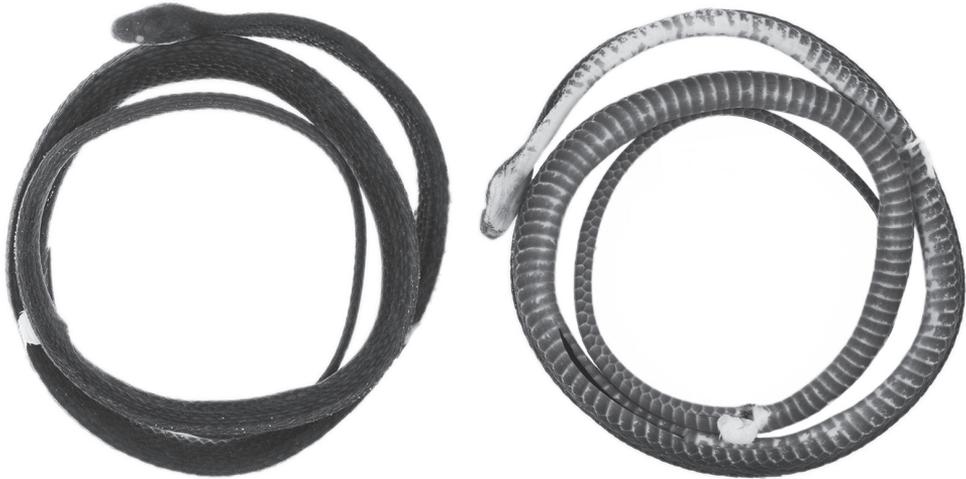


Figure 1. Holotype (QCAZ 12791, adult male, SVL = 367 mm) of *Synophis bogerti* sp. n. in dorsal (left) and ventral (right) views. Photographs by Omar Torres-Carvajal.

Zarentza, Parque Nacional Llanganates (1°21'45.25"S, 78°3'28.22"W, 1391 m), collected on 27 February 2015 by D. Rivadeneira, F. Mora, J. C. Sánchez, D. Velalcázar, D. Nuñez and J. Pinto.

Diagnosis. *Synophis bogerti* can be distinguished from other species of *Synophis* by having a semicapitate, bilobed hemipenis with a large lateral spine at the base of the hemipenial body (Fig. 3); 19 longitudinal rows of dorsals at midbody; strongly keeled dorsals except for first row, which is weakly keeled (at least posteriorly); and 154–163 ventrals in males, 161–168 in females. Scutellational characters of all recognized species of *Synophis* are presented in Table 3.

Description of the holotype. Adult male (Figs 1, 2), SVL 367 mm; tail length 184 mm; eye diameter 1.17 mm; pupil round; head width 6.32 mm at level of supralabial 6; and head length 11.7 mm from snout to posterior margin of jaw; width at midbody 5.19 mm; head distinct from neck.

Prefrontals fused in a rectangular scale, wider than long; frontal single, with an incomplete suture from anterior margin to the middle of the scale, heptagonal, slightly wider than long; parietals large, paired, longer than wide; loreal trapezoidal, almost two times longer than high; preocular single, bordering anterior margin of orbit; supraocular single, bordering dorsal margin of orbit; temporals 1+2; anterior temporal more than two times longer than high; posterior temporals two times longer than high, approximately one half the length of anterior temporal; internasals in contact medially, distinctly wider than long; nasals not in contact; rostral visible from above, concave, nearly two times wider than long, in contact with first supralabials, nasals, and internasals; mental triangular, in contact with first pair of infralabials; infralabials 10/11; supralabials 8/8 (fourth and fifth entering orbit on both sides); anterior genials three times longer than wide, bordered laterally by

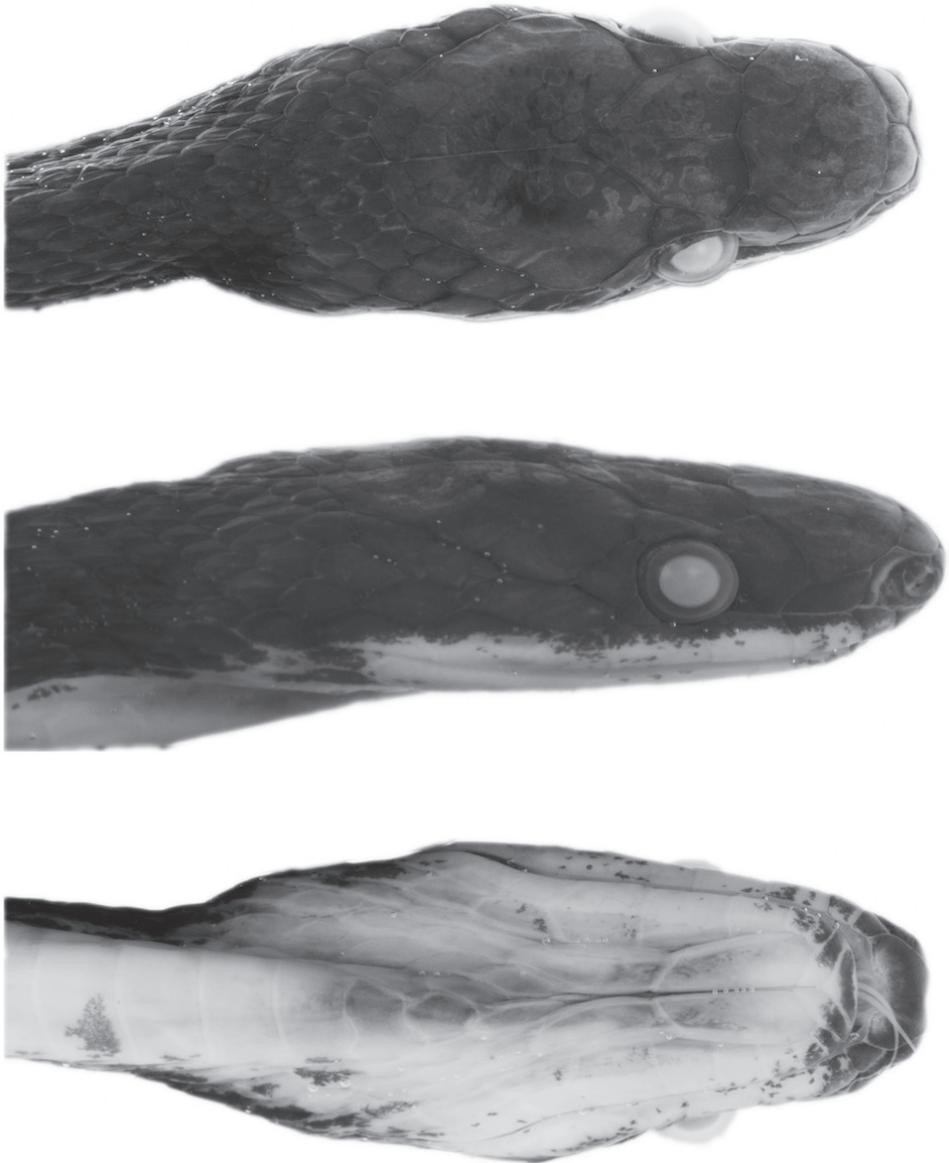


Figure 2. Head of holotype of *Synophis bogerti* sp. n. (QCAZ 12791) in dorsal (top), lateral (middle) and ventral (bottom) views. Photographs by Omar Torres-Carvajal.

infralabials 1-5 on right side, 1-6 on left side; posterior genials two times longer than wide, in contact anteromedially and separated by two gulars posteriorly, and bordered laterally by infralabials 5-6 on right side and 6-7 on left side; dorsal scale rows 19-19-17, first dorsal row weakly keeled from ventral 118, other rows strongly keeled; anal single; ventrals 163; subcaudals 115, paired.

Table 3. Summary of morphological characters and measurements (mm) of seven species of *Synophis*. Range (first line), and mean \pm standard deviation (second line) are given for quantitative characters if available.

Characters	<i>Synophis bicolor</i> N = 2 ¹	<i>Synophis bogerti</i> sp. n. N = 7	<i>Synophis calamitus</i> N = 10 ²	<i>Synophis insulomontanus</i> sp. n. N = 4	<i>Synophis lasallei</i> N = 16 ³	<i>Synophis plectrovertebralis</i> N = 2 ⁴	<i>Synophis zamora</i> sp. n. N = 4
Dorsal scales at midbody	19	19	19	19	21-23	19	19
Dorsal scales relief (except 1st row)	Weakly keeled	Strongly keeled	Weakly keeled	Strongly keeled	Strongly keeled	Smooth (rows 2-6) and weakly keeled	Strongly keeled
Relief of first row of dorsals	Smooth	Weakly keeled	Smooth	Keeled	Keeled	Smooth	Weakly keeled
Postoculars	2	2	1-2	2	2	-	2
Internasals	-	In contact	In contact/hot in contact	In contact	In contact	In contact	In contact
Supralabials	8	8	7-8	8-9	7-9	7-8	8-9
Infralabials	9-11	10-11	8-10	10-11	10-11	7-9	9-10
Ventrals in males	184	154-163 158.25 \pm 3.77	157-165 161.4 \pm 2.97	151-152 151.5 \pm 0.71	-	144	147-153 150.75 \pm 2.63
Ventrals in females	-	161-168 164 \pm 3.6	160-166 162.88 \pm 2.23	147-149 148 \pm 1.41	-	147	-
Ventrals (sex undetermined)	180	-	-	-	144-158	-	-
Subcaudals in males	127	101-115 109.75 \pm 6.4	107-120 113 \pm 6.06	108-109 108.5 \pm 0.71	-	91	103-111 108.25 \pm 3.59
Subcaudals in females	-	98-111 105 \pm 6.56	106-113 109.67 \pm 2.42	103	-	79	-
Subcaudals (sex undetermined)	136			-	101-125	-	
Maximum total length in males (SVL)	617 (407)	641 (422)	790 (507)	541.6 (349.8)	-	212 (100) ⁵	546 (359)
Maximum total length in females (SVL)	-	603 (419)	756 (496)	467.9 (379.7)	-	272 (195.5) ⁵	-

¹Data from Peracca (1896) and Nicéforo-María (1970); ²Type specimen data from Hillis (1990); ³Data from Hillis (1990); ⁴Data from Sheil and Grant (2001); ⁵Juvenile.



Figure 3. Right hemipenis of *Synphis bogerti* sp. n. (QCAZ 12791, holotype). Distal end in sulcal (upper left) and asulcal (upper right) views; body in sulcal (lower left) and asulcal (lower right) views. Scale bar = 1 mm. Photographs by Denisse Galarza.

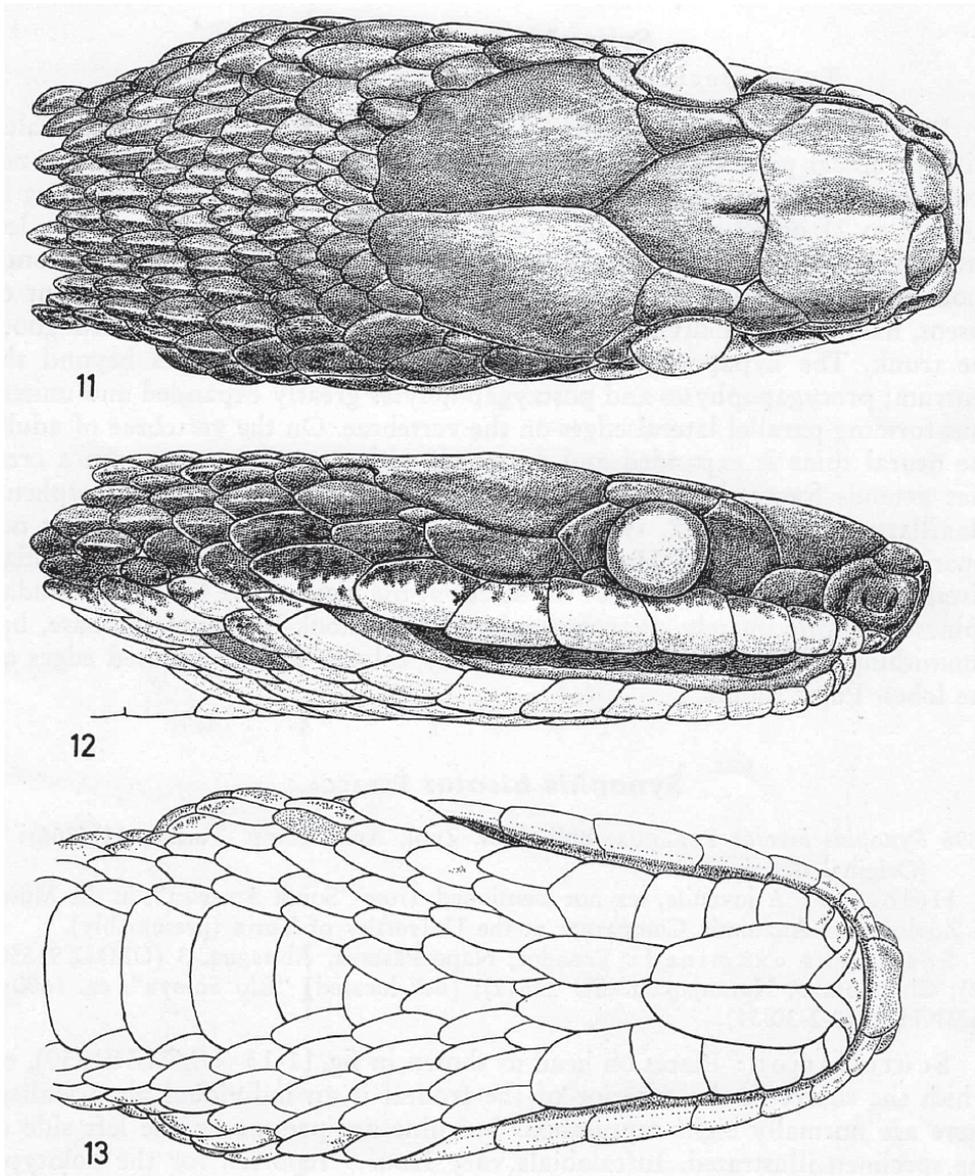


Figure 4. Head of specimen of *Synopsis bogerti* (UMMZ 91550) illustrated by Bogert (1964) as *S. bicolor* showing incomplete suture on frontal scale. Illustration taken from Bogert (1964).

Hemipenial morphology. The following description is based on the right hemipenis of the holotype (Fig. 3; QCAZ 12791). The fully everted and maximally expanded organ is bilobed, semicalyculate, semicapitate, and extends to the sixth subcaudal. Capitular grooves are on the asulcate side; capitula are ornamented with calcified papillae, larger on the asulcate side. Numerous larger papillae meet on the asulcate side of the lobular crotch.



Figure 5. Four species of *Synophis* from Ecuador and Peru: *S. calamitus* (QCAZ 11931, upper left); *S. bogerti* sp. n. (QCAZ 13586, upper right); *S. zamora* sp. n. (QCAZ 13854, lower left); *S. insulomontanus* sp. n. (CORBIDI 13940, lower right). Photographs by Diego Quirola, Omar Torres-Carvajal and Germán Chávez.

On the sulcate side, the capitula extend along the sides of the branches of the sulcus spermaticus, far down the hemipenial body. The sulcus spermaticus bifurcates on the proximal half of the body and its branches extend centrolineally to the tip of each lobe. The hemipenial body is ornamented with large calcified spines, except on the medial region of the asulcate side, where the spines are small. The spines increase in length towards the base of the hemipenial body, with one spine on the left side (sulcate view) being considerably longer than the others. The base of the hemipenial body bears much smaller and scattered spines.

Color in preservative of the holotype (Figs 1, 2). Dorsal surface of head, body and tail uniformly dark grey; skin among scales on flanks cream, visible on anterior half of body; ventrals mostly cream on anterior end of body (ventrals 1-10), becoming progressively pigmented with light grey posteriorly except on their margins; anal plate cream medially and grey laterally; subcaudals with cream margins and same tone of grey as posterior ventrals; sides of head same tone of grey as dorsal surface, except for labials, which are mostly cream ventrally; chin cream with light grey anterior margin (most of mental and first three pairs of infralabials).

Variation. Intraspecific variation in scale counts and measurements in *Synophis bogerti* is presented in Table 3. Keeling on the first row of dorsals starts on ventrals 5, 10, 87, 98 and 114 in paratypes QCAZ 5072, 13323, 3511, 13585 and 11070, respectively. Besides the holotype, male paratype QCAZ 5072 is the only specimen with

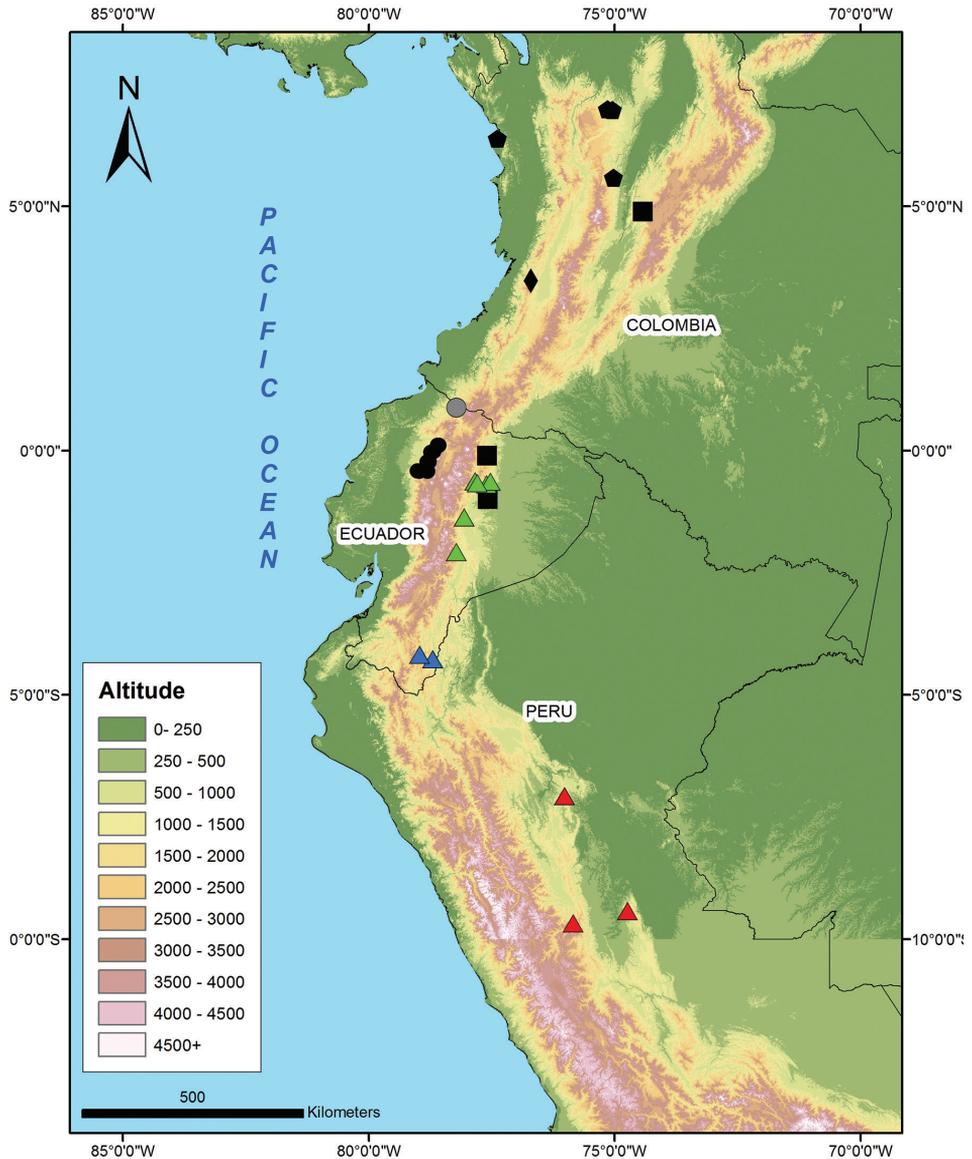


Figure 6. Distribution of seven species of *Synopsis* in South America. *S. bicolor* (pentagons), *S. bogerti* sp. n. (green triangles), *S. calamitus* (circles), *S. insulomontanus* sp. n. (red triangles), *S. lasallei* (squares), *S. plectovertebralis* (diamond), *S. zamora* sp. n. (blue triangles). Grey circle corresponds to specimen QCAZ 5847 from Carchi, Ecuador (see Discussion).

an incomplete medial suture on the frontal scale. This condition was also reported and illustrated by Bogert (1964) in a specimen (UMMZ 91550) from eastern Ecuador, referred by him as *S. bicolor* and recognized by us as *S. bogerti* (Fig. 4).

Distribution and natural history. *Synophis bogerti* occurs along the Amazonian slopes of the Andes in central Ecuador at elevations between 1000–1750 m (Fig. 6). The type locality is part of Wildsumaco Wildlife Sanctuary, a 400 ha reserve consisting of primary and secondary forests in a matrix of agricultural land. Most localities where *S. bogerti* was collected lie within protected areas including two large national parks (Llanganates and Sangay), indicating that at least some populations of this species are protected. All specimens were found active at night (20h45–00h00), mostly on the ground or on shrubs 0.5 m above ground.

Etymology. The specific epithet *bogerti* is a noun in the genitive case and is a patronym for Charles M. Bogert (1908–1992), an American herpetologist and former curator of the American Museum of Natural History. Among his many contributions, Bogert published a systematic revision of *Diaphorolepis* and *Synophis*, in which he recognized that “It is also possible, of course, that specimens tentatively referred to *S. bicolor* are not actually conspecific” (Bogert 1964: 517). Specimens of “*S. bicolor*” from eastern Ecuador examined by Bogert (1964) correspond to *S. bogerti* sp. n.

***Synophis zamora* sp. n.**

<http://zoobank.org/CAC93737-0629-4405-9E30-F1BDA841A39C>

Proposed standard English name: Zamoran fishing snakes

Proposed standard Spanish name: Serpientes pescadoras de Zamora

Holotype. Ecuador: Provincia Zamora Chinchipe: QCAZ 9174 (Figs 7, 8), adult male from Las Orquídeas, 4 km from río Nangaritza (4°15'47.52"S, 78°41'27.93"W, 1843 m), collected on 19 April 2009 by E. E. Tapia, J. Loe Deichmann and A. F. Jiménez.

Paratypes. Ecuador: Provincia Zamora Chinchipe: QCAZ 9175, adult male, same locality data as holotype; QCAZ 12773, adult male from Reserva Numbami, 18 km on road Zamora-Romerillos bajo (4°10'24.64"S, 78°57'29.63"W, 1552 m), collected on 09 July 2014 by S. R. Ron, D. A. Paucar, P.J. Venegas, D. Almeida, D. Velalcázar, M. J. Navarrete, S. Arroyo, N. Páez and Z. Lange; QCAZ 13854, adult male from Bombuscaro (4°6'42.98"S, 78°58'21.22"W, 1543 m), Podocarpus National Park, collected on 2 March 2015 by D. Rivadeneira, F. Mora, J. C. Sánchez, D. Velalcázar, D. Núñez, J. Pinto, K. Cruz and Luis T.

Diagnosis. *Synophis zamora* can be distinguished from other species of *Synophis* by having a noncapitate, bilobed hemipenis with a large lateral spine at the base of the hemipenial body (Fig. 9); 19 longitudinal rows of dorsals at midbody; strongly keeled dorsals except for first row, which is weakly keeled (at least posteriorly); and 147–153 ventrals in males. Scutellational characters of all recognized species of *Synophis* are presented in Table 3.

Description of the holotype. Adult male (Figs 7, 8); SVL 349 mm; tail length 185 mm; eye diameter 1.34 mm; pupil round; head width 5.15 mm at level of supralabials 6 and 7; head length 11.05 mm from snout to posterior margin of jaw; width at midbody 5.06 mm; head distinct from neck.

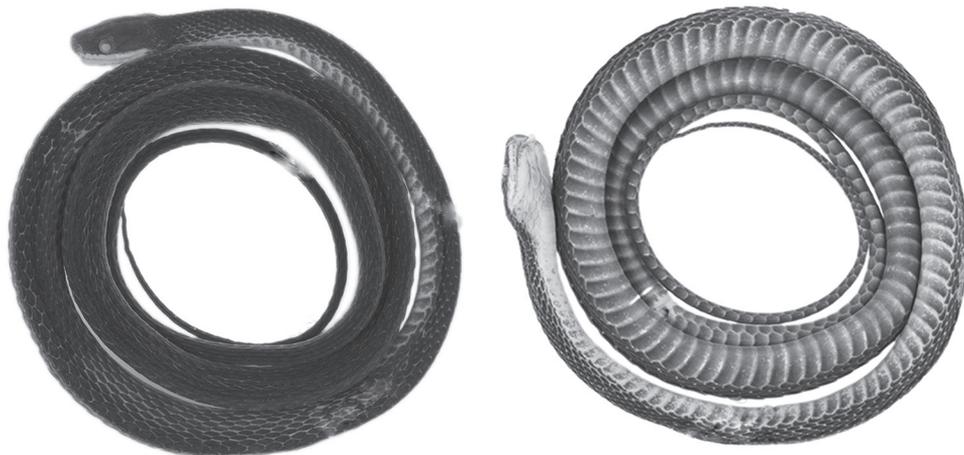


Figure 7. Holotype (QCAZ 9174, adult male, SVL = 349 mm) of *Synophis zamora* sp. n. in dorsal (left) and ventral (right) views. Photographs by Omar Torres-Carvajal.

Prefrontals fused in a rectangular scale, wider than long; frontal single, heptagonal, slightly wider than long; parietals large, paired, longer than wide; loreal trapezoidal, two times longer than high; preocular single, bordering anterior margin of orbit; supraocular single, bordering dorsal margin of orbit; temporals 1+2; anterior temporal more than two times longer than high; posterior temporals longer than high, approximately one half the length of anterior temporal; internasals in contact medially, distinctly wider than long; nasals not in contact; rostral visible from above, concave, two times wider than long, in contact with first supralabials, nasals, and internasals; mental triangular, in contact with first pair of infralabials; infralabials 10/10; supralabials 9/9 (fourth, fifth and sixth entering orbit on both sides); anterior genials almost three times longer than wide, bordered laterally by infralabials 1-5; posterior genials three times longer than wide, in contact anteromedially and separated by three gulars posteriorly, and bordered laterally by infralabials 5-6; dorsal scale rows 19-19-17, first row weakly keeled from 15th ventral, other rows strongly keeled; anal single; ventrals 147; subcaudals 103, paired.

Hemipenial morphology. The following description is based on the right hemipenis of the holotype (Fig. 9; QCAZ 9174). The fully everted and maximally expanded organ is bilobed, semicalyculate, noncapitate, and extends to the sixth subcaudal. Each lobe is ornamented with small calcified papillae, slightly larger on the asulcate and lateral sides and more scattered on the sulcate side. Some larger lobular papillae meet medially at the lobular crotch on the asulcate side. The sulcus spermaticus bifurcates on the proximal half of the body and its branches extend centrolineally to the tip of each lobe. The hemipenial body is ornamented with medium-sized calcified spines, except on the medial region of the asulcate side, where the spines are small. The spines increase in length towards the base of the



Figure 8. Head of holotype of *Synopsis zamora* sp. n. (QCAZ 9174) in dorsal (top), lateral (middle) and ventral (bottom) views. Photographs by Omar Torres-Carvajal.

hemipenial body, with one spine on the left side (sulcate view) being considerably longer than the others. The base of the hemipenial body bears much smaller and scattered spines.



Figure 9. Right hemipenis of *Synopsis zamora* sp. n. (QCAZ 9174, holotype). Distal end in sulcal (upper left) and asulcal (upper right) views; body in sulcal (lower left) and asulcal (lower right) views. Scale bar = 1 mm. Photographs by Denisse Galarza.

Color in preservative of the holotype (Figs 7, 8). Dorsal surface of head, body and tail uniformly dark grey; skin among dorsal scales cream, visible on anterior half of body; ventrals cream on anterior end of body (ventrals 1-5), becoming progressively pigmented with light grey posteriorly except on their margins; anal plate cream posteriorly and grey anteriorly; subcaudals with cream margins and same tone of grey as posterior ventrals; sides of head same tone of grey as dorsal surface, except for labials, which are mostly cream; chin cream with light grey anterior margin (most of mental and first two pairs of infralabials).

Variation. Intraspecific variation in scale counts and measurements in *Synophis zamora* is presented in Table 3. Keeling on the first row of dorsals starts on ventrals 9, 10, and 105 in paratypes 9175, 13854, and 12773, respectively. No major differences were found between the hemipenis of the holotype and those of paratypes QCAZ 12773 and 13854. Coloration in life (QCAZ 13854; Fig. 5) is the same as that described for the holotype above, except that the cream color has a light yellow tint.

Distribution and natural history. *Synophis zamora* occurs in the southeastern portion of the northern Andes in Cordillera del Cóndor and the Amazonian slopes of the Andes at elevations between 1543–1843 m (Fig. 6). It is known from localities close to the Bombuscaro and Nangaritzta rivers, which are tributaries of the Zamora river. These localities lie in Ecuador within protected areas, such as Podocarpus National Park and Numbami Ecological Reserve, indicating that at least some populations of *S. zamora* are protected. All specimens were found active at night (20h30-00h00), mostly on the ground or on shrubs 1-1.5 m above ground. One specimen was found on a boulder covered with moss.

Etymology. The epithet *zamora* is a noun in apposition and refers to both the Zamora river and the province of Zamora Chinchipe. All type specimens were collected in this province along the upper basin of Zamora river.

***Synophis insulomontanus* sp. n.**

<http://zoobank.org/0CDDA542-89E8-4DC8-B9A9-B39DF707F804>

Proposed standard English name: Mountain fishing snakes

Proposed standard Spanish name: Serpientes pescadoras monteses

Holotype. Peru: Departamento Huánuco: Provincia Puerto Inca: Distrito Lullapichis: CORBIDI 13940 (Figs 10, 11), adult male from Campamento Peligroso-Reserva Comunal El Sira (9°25'34.22"S, 74°44'6.60"W, 1507 m), collected on 1 December 2013 by G. Chavez.

Paratypes. Peru: Departamento San Martín: Provincia Picota: Distrito Shaboyacu: CORBIDI 9223 adult female from Parque Nacional Cordillera Azul, Puesto de Control 16 (Chambirillo) (7°4'8.90"S, 76°0'55.20"W, 1122 m), collected on 8 May 2011 by P. J. Venegas and V. Duran and CORBIDI 10418, from same locality, collected on 20 February 2012 by V. Duran. Departamento Huánuco: Provincia Huánuco: Distrito Chinchao: CORBIDI 13705 adult male from Miraflores



Figure 10. Holotype (CORBIDI 13940, adult male, SVL = 335.3 mm) of *Synophis insulomontanus* sp. n. in dorsal (left) and ventral (right) views. Photographs by Juan C. Chávez-Arribasplata.

(9°40'40.60"S, 75°50'11.09"W, 1798 m), collected 8 December 2013 by V. Duran and L. Lujan.

Diagnosis. *Synophis insulomontanus* can be distinguished from other species of *Synophis* by having a semicapitate, bilobed hemipenis with a large lateral spine at the base of the hemipenial body, and the sulcus spermaticus bifurcating on the center of the hemipenial body (Fig. 12); 19 longitudinal rows of dorsals at midbody; strongly keeled dorsals except for first row, which is keeled to a lesser extent; 151-152 ventrals in males, 147-149 in females; 108-109 subcaudals in males, 103 in females. Scutelational characters of all recognized species of *Synophis* are presented in Table 3.

Description of the holotype. Adult male (Figs 5, 10, 11), SVL 335.3 mm; tail length 180.9 mm; eye diameter 1.46 mm; pupil round; head width 6.7 mm at level of supralabial 6; head length 11.07 mm from snout to posterior margin of jaw; width at midbody 6.48 mm; head distinct from neck.

Prefrontals fused in a roughly pentagonal scale, wider than long; frontal single, pentagonal, posterior suture angular with apex directed posteriorly, wider than long; parietals large, paired, longer than wide; loreal trapezoidal, almost two times longer than high; preocular single, bordering anterior margin of orbit; supraocular single, bordering dorsal margin of orbit; temporals 1+3+3; anterior temporal more than two times longer than high; posterior temporals two times longer than high, approximately one half the length of anterior temporal; internasals in contact medially, wider than long; nasals not in contact; rostral visible from above, concave, nearly two times wider than long, in contact with first supralabials, nasals, and internasals; mental triangular, in contact with first pair of infralabials; infralabials 11/11; supralabials 8/8 (fourth and fifth entering orbit on both sides); anterior genials three times longer than wide, bordered laterally by infralabials 1-6 on both sides; posterior genials two times longer than wide, separated by gulars, and bordered laterally by infralabials 6-7 on both sides;



Figure 11. Head of holotype of *Synopsis insulomontanus* sp. n. (CORBIDI 13940) in dorsal (top), lateral (middle) and ventral (bottom) views. Photographs by Germán Chávez.

dorsal scale rows 20-19-19, first dorsal row moderately keeled from ventral 7, other rows strongly keeled; anal single; ventrals 151; subcaudals 108, paired.

Hemipenial morphology. The following description is based on the left hemipenis of the holotype (Fig. 12; CORBIDI 13940). The fully everted and maximally



Figure 12. Left hemipenis of *Synopsis insulomontanus* sp. n. (CORBIDI 13940, holotype) in sulcal (left), asulcal (center), and lateral (right) views. Scale bar = 1 mm. Photographs by Germán Chávez.

expanded organ is bilobed, semicalyculate, semicapitate, and extends to the fifth subcaudal. Capitular grooves are present on the asulcate side; capitula are ornamented with calcified papillae, remarkably larger on the asulcate side. A few papillae meet on the asulcate side of the lobular crotch. The sulcus spermaticus bifurcates on the center of the hemipenial body and its branches extend centrolineally to the tip of each lobe. Papillae are relatively small on the sulcate side of the hemipenial body between the bifurcating branches of the sulcus spermaticus. The hemipenial body is ornamented with large calcified spines, except on the medial region of the asulcate side and near the sulcus spermaticus, where the spines are very small. One spine on the left side (sulcate view) is considerably longer than the others. Very small spines cover the base of the hemipenial body.

Color in life of the holotype (Fig. 5). Dorsal surface of head, body and tail uniformly dark grey; skin among scales on flanks cream, visible on anterior half of body; first five ventrals cream, becoming progressively pigmented with grey, except on their posterior margin where cream pigmentation is always present; anal plate grey with cream posterior border; subcaudals grey, with the posterior borders weakly pigmented with cream in some scales; sides of head and 1st supralabial same tone of grey as dorsal surface, other supralabials mostly cream; first three infralabials mostly grey, others mostly cream; scales on throat with a pale blue tone.

Variation. Intraspecific variation in scale counts and measurements in *Synopsis insulomontanus* is presented in Table 3. Two or three scales can be present on second row of temporals, three in the holotype and CORBIDI 9223, and two in CORBIDI 10418 and CORBIDI 13705. Paratype CORBIDI 10418 has 21 dorsals at midbody. No major differences were found between the hemipenis of the holotype and that of paratype CORBIDI 13705, except that the latter has more papillae between the bifurcating branches of the sulcus spermaticus on the sulcate side of the hemipenial body.

Specimen CORBIDI 10418 has a dense cream pigmentation on ventrals from anterior end of body to midbody.

Distribution and natural history. *Synophis insulomontanus* is known to occur between 1122–1798 m on the Amazonian slopes of the Andes in northern and central Peru (Fig. 6). Two localities within Departamento Huánuco, Cordillera Azul and Cordillera El Sira, correspond to sub-Andean mountain ridges, whereas the locality of Miraflores lies on the Amazonian slopes next to the Huallaga River.

The holotype was found at night, coiling inside a bromeliad, 1 m above the ground in primary premontane forest. Other specimens were found active at night, moving through leaf litter. Specimens from Cordillera Azul (CORBIDI 9223 and 10418) were found in primary premontane forest, whereas specimen CORBIDI 13705 from Miraflores, Huánuco, was found in secondary montane forest.

Etymology. The epithet *insulomontanus* is a noun that derives from the Latin words *insulo* (= isolated) and *montanus* (= mountain). It refers to the isolated mountain ridges in Departamento Huánuco, where the new species was discovered.

Phylogenetic relationships

The phylogenetic tree inferred in this study (Fig. 13) supports strongly the monophyly of *Synophis* (PP = 1). Within Dipsadinae (sensu Pyron et al. 2013), *Synophis* is sister to *Diaphorolepis* (PP = 1), and together they form a clade sister (PP = 0.84) to the strongly supported (PP = 1) clade (*Imantodes*, ((*Dipsas*, *Ninia*), (*Hypsiglena*, *Pseudoleptodeira*))).

Within *Synophis* there is a basal split into two clades, one (PP = 1) containing the trans-Andean taxon *S. calamitus*, and the other (PP = 0.88) including the three cis-Andean species described in this paper (*S. bogerti*, *S. insulomontanus* and *S. zamora*). Within the cis-Andean clade, *S. bogerti* and *S. zamora* are recovered as sister species with maximum support (PP=1), forming a clade sister to *S. insulomontanus*.

Discussion

Phylogeny of *Synophis* and *Diaphorolepis*

In spite of recent efforts to resolve the phylogenetic relationships of dipsadid snakes using DNA sequence data (e.g., Grazziotin et al. 2012; Pyron et al. 2013; Zaher et al. 2009), *Synophis* and *Diaphorolepis* have remained unsampled. Consequently, they have been considered as Dipsadidae *incertae sedis* (Zaher et al. 2009). In order to have a general idea of the phylogenetic position of both *Synophis* and *Diaphorolepis*, we included in our analysis 12 additional dipsadinae taxa used in previous phylogenetic studies (e.g., Pyron et al. 2013). We did not attempt to perform a taxonomically extensive phylogenetic analysis of Dipsadidae or Dipsadinae (sensu Pyron et al. 2013); instead, we preferred to include in our analysis only those species of Dipsadinae, for

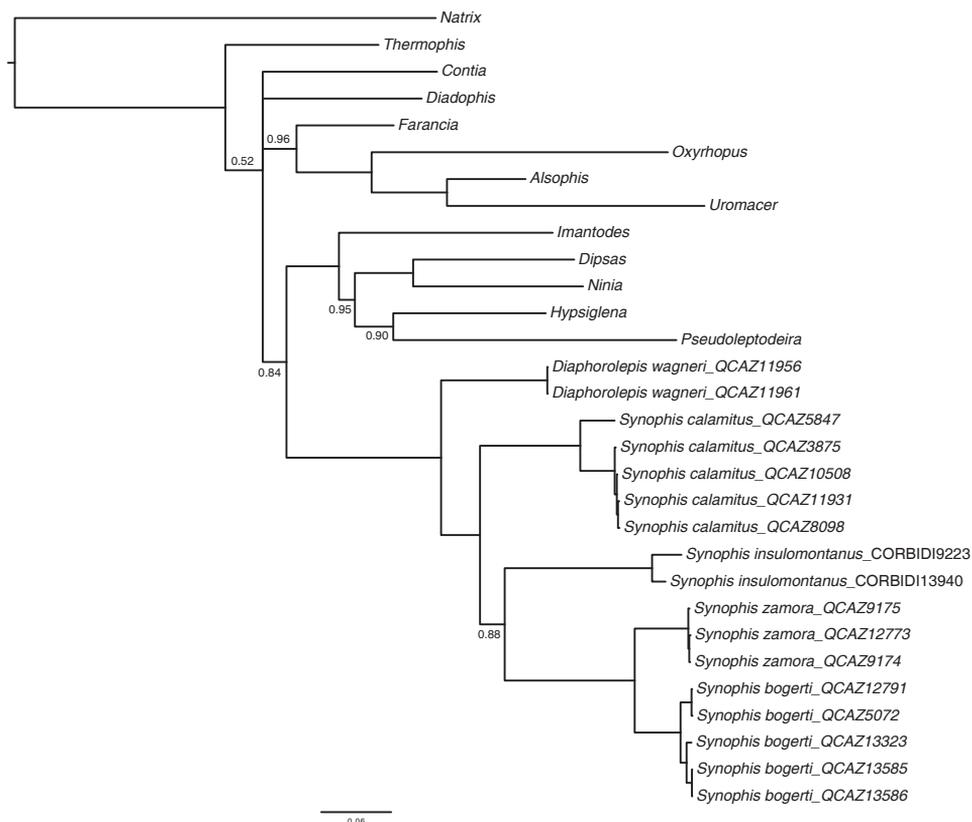


Figure 13. 50% Majority rule consensus tree of *Synopsis* snakes based on a Bayesian analysis of mtDNA sequences. Posterior probabilities are equal to 1, unless otherwise noted by numbers next to branches. Outgroup taxa are not shown.

which all gene regions used in this study were available in GenBank. Our phylogeny strongly supports (1) inclusion of *Diaphorolepis* and *Synopsis* within Dipsadinae; (2) a close relationship between these genera and a clade including *Imantodes*, *Dipsas*, *Ninia*, *Hypsiglena* and *Pseudoleptodeira*; and (3) a close relationship between *Diaphorolepis* and *Synopsis*, as has been hypothesized using morphological evidence (Hillis 1990).

All species of *Synopsis* are known to occur on Andean slopes in Colombia and Ecuador, with *S. insulomontanus* sp. n. representing the first record from Peru. Along with *S. lasallei*, the three species described in this paper are restricted to Amazonian slopes of the Andes, except for one record of *S. lasallei* from the western slopes of the eastern Cordillera in Colombia (Fig. 6). Thus, the Andes represent a major geographic barrier separating species of *Synopsis*. Recent studies on other reptile taxa (e.g., *Alopglossus*, *Enyalioides*) with similar distributions suggest that the uplift of the northern Andes represents a major vicariant event explaining their radiation and present distribution (Torres-Carvajal and de Queiroz 2009; Torres-Carvajal and Lobos 2014). We

could not test this hypothesis with *Synophis* because we had no access to tissue samples of *S. bicolor*, *S. plectovertebralis* and *S. lasallei*. Nonetheless, based on morphological similarity (e.g., strongly keeled dorsals, first row of dorsals keeled; Table 3), it is likely that *S. lasallei* is nested in the same clade with the three eastern-Andean species described in this paper. Vertebral morphology, not examined in most species of *Synophis*, seems to support this idea. Bogert (1964) noted that the vertebrae of *S. "bicolor"* (= *S. bogerti* sp. n.) and *S. lasallei* were similar in morphology, which is different from at least *S. plectovertebralis* (Sheil and Grant 2001). We examined superficially trunk vertebrae of *S. bogerti* and *S. zamora* using digital X-rays, and found that the vertebrae of both species are very similar and agree with the description presented by Bogert (1964) in that "vast expansions of the prezygapophyses and postzygapophyses are coalesced as projections with relatively straight margins parallel to the main axis of each vertebra". In addition, the zygapophyseal foramen is largely ossified as opposed to the same foramen in *S. plectovertebralis* (Sheil and Grant 2001). In conclusion, external and internal anatomy supports both the hypothesis presented above and the idea of a radiation of *Synophis* east of the Andes.

Postoculars and internasals as taxonomic characters

Hillis (1990) described *Synophis calamitus* based on two specimens. Among other characters, he proposed that the number of postoculars and whether the internasals are in contact or not were useful taxonomic characters. According to Hillis (1990), *S. calamitus* differed from other species of *Synophis* in having one postocular (two in other species) and internasals separated by rostral and prefrontal (internasals in contact medially in other species). Among 12 specimens of *S. calamitus* examined in this study (Appendix I), nine have two postoculars on each side, two have one postocular on one side and two on the opposite side, and only one specimen (QCAZ 11931) has one postocular on each side. Moreover, we were able to examine the paratype of *S. calamitus* (KU 164208), a juvenile, badly-crushed roadkill, and found out that this specimen has one postocular on the left side and two on the right side, the ventral one difficult to observe because of the condition of the specimen. Thus, the number of postoculars is variable in *S. calamitus* and, therefore, it is not a useful taxonomic character. Regarding the contact between internasals, all specimens examined except for one (QCAZ 5847) had internasals in contact medially, as opposed to the condition described for both the holotype and paratype (internasals separated; Hillis 1990). Specimen QCAZ 5847 is a roadkill collected in the northern province of Carchi, and does not seem to have other differences with the remaining specimens of *S. calamitus* examined in this study. However, given the large branch separating this specimen from all other specimens of *S. calamitus* in the phylogenetic tree (Fig. 13), as well as its disjunct distribution (Fig. 6), we believe that the taxonomic status of northern (Carchi) populations should be addressed in more detail.

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Appendix I

Specimens examined

- Diaphorolepis wagneri*.—ECUADOR: **Provincia Imbabura**: QCAZ 11956, 11961, Reserva Manduriacu, 0°18'36.94"S, 78°51'27.50"W, 1213 m.
- Synophis calamitus*.—ECUADOR: **Provincia Carchi**: QCAZ 5847, Km 14 vía El Chical-Gualchán, 0°52'51.65"S, 78°13'22.80"W, 1934 m. **Provincia Cotopaxi**: QCAZ 1688, 2807, 3875, 7264, Naranjito, Bosque Integral Otonga (BIO), 0°24'53.22"S, 79°0'2.64"W, 1700 m; QCAZ 10453, Naranjito, Bosque Integral Otonga (BIO), 0°24'57.48"S, 79°0'17.28"W, 2145 m. **Provincia Pichincha**: QCAZ 381, Tandapi, 0°25'6.74"S, 78°47'58.02"W; QCAZ 1136, Chiriboga, 0°13'28.31"S, 78°46'3.90"W, 1700 m; QCAZ 3386, Cerca a Chiriboga, Las Palmeras, La Soledad, Estación Científica Río Guajalito, 0°13'44.40"S, 78°48'21.60"W, 1830 m; QCAZ 8098, 10508, Cooperativa El Porvenir, finca El Cedral, 0°6'50.40"S, 78°34'11.75"W, 2297 m; QCAZ 11931, Reserva Ecológica Bosque Nublado Santa Lucía, 0°6'56.48"S, 78°35'36.74"W, 1727 m.

Complete mitochondrial DNA sequence of the endangered fish (*Bahaba taipingensis*): Mitogenome characterization and phylogenetic implications

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Abstract

To understand the systematic status of *Bahaba taipingensis* within Sciaenidae, the complete mitochondrial genome (mitogenome) sequence of Chinese bahaba has recently been determined by long PCR and primer walking methods. The complete mitochondrial genome is 16500 bp in length and contains 37 mitochondrial genes (13 protein-coding genes, 2 ribosomal RNA genes and 22 transfer RNA genes) as well as a control region (CR) as other bony fishes. Within the control region, we identified the extended termination associated sequence domain (ETAS), the central conserved sequence block domain (CSB-D, SCB-E and CSB-F) and the conserved sequence block domain (CSB-1, CSB-2 and CSB-3). Phylogenetic analyses revealed that *B. taipingensis* is more closely related to Pseudosciaeninae than Argyrosominae and Sciaeninae. Additionally, *B. taipingensis* is the sister taxon of *Miichthys miiuy*, and those two are sister to *Collichthys plus Larimichthys*.

Keywords

Bahaba taipingensis, Sciaenidae, mitochondrial genome, control region, phylogenetic analysis

Introduction

The complete mitochondrial DNA (mtDNA) sequence of vertebrates is a circular molecule with a length of 16–19 kb that includes 37 genes containing 13 protein-coding genes, 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a control region (CR) (Anderson et al. 1981; Boore et al. 1999). The mitochondrial genome is frequently used for phylogenetic studies and population genetic analyses, due to its compact gene organization, fast evolutionary rate, maternal inheritance and lack of genetic recombination (Miya et al. 2003; Inoue et al. 2009). In recent years, complete mitochondrial DNA sequences have been widely used to reconstruct the phylogeny of higher-level taxa (Jondeung et al. 2007; Wang et al. 2008; Yang et al. 2010).

The family Sciaenidae in the order Perciformes is widely distributed throughout the world with approximately 70 genera and 300 species (Nelson 2006). Fishes of this family are popularly known as croakers and drums because of the ability using muscles associated with gas bladder to produce sound. In China, the family comprises 13 genera with about 37 species, and can be divided into seven subfamilies: Johniinae, Megalonibinae, Bahabinae, Sciaeninae, Otolithinae, Argyrosominae, Pseudosciaeninae (Zhu et al. 1963; Cheng et al. 1987; Tetsuji et al. 2000). The Chinese bahaba, *Bahaba taipingensis*, is one of the largest croakers and has a limited geographical distribution from Zhoushan Island southwards to the Pearl River (Zhu et al. 1963; Lu et al. 2002). Over the past years, its stock has been declining due to heavy catch pressure and environmental degradation, therefore it is defined as National Class II Protected Animals of China and Critically Endangered by the IUCN. There have been a few reports on the general ecology of this species covering resources, biology, and otolith morphology (Lu et al. 2002; Ye et al. 2001; Ou et al. 2011). Additionally, the phylogenetic relationships of Sciaenidae have been investigated by means of molecular markers (Meng et al. 2004; Chen et al. 2007; Liu et al. 2010; Cheng et al. 2010), but only one study included *B. taipingensis* (He et al. 2012), which revealed that *B. taipingensis* is closely related to Pseudosciaeninae.

In this study, we sequenced the complete mtDNA sequence of *B. taipingensis* for the first time and analyzed its genomic structure. Additionally, we conducted phylogenetic analyses based on the mitochondrial sequence data with the purpose of investigating the phylogenetic position of *B. taipingensis* within the family Sciaenidae. The information reported in this article will facilitate further investigations of phylogenetic relationships of species in the Sciaenidae.

Materials and methods

Sample collection and DNA extraction

The sample of *B. taipingensis* was collected from Dongguan offshore water, Guangdong, China. A piece of muscle tissue excised from the individual was preserved in

95% ethanol for DNA extraction. Total genomic DNA from muscle tissue was extracted with a standard phenol/chloroform procedure followed by ethanol precipitation and kept at 4 °C for subsequent use.

Mitochondrial DNA amplification

The complete *B. taipingensis* mitogenome was amplified using a long-PCR technique (Miya et al. 1999). Six sets of primers (Table 1) were designed based on multiple alignments of the conserved region of the complete mitochondrial DNA sequences of other Sciaenidae fishes: *Larimichthys crocea* (EU339149), *Collichthys niveatus* (JN678726), *Collichthys lucida* (JN857362), *Larimichthys polyactis* (FJ618559), *Miichthys miiuy* (HM447240) and *Pennahia argentata* (HQ890946), as well as previously determined, partial sequences of the 16S rRNA, Cyt b, COI genes and control region. Subsequent sequencing was accomplished by primer walking method. After the sequencing of these fragments, 31 normal PCR primer sets were designed using Premier 5.0 (Primer Biosoft International) to obtain contiguous, overlapping segments of the entire mitogenome. It was necessary that every two contiguous segments overlapped by at least 50 bp to ascertain the accuracy of sequencing.

All PCRs were performed in a Takara thermal cycler. Takara Ex-Taq and LA-Taq polymerase (Takara Biomedical) were used for normal and long-PCR reactions, respectively. Long-PCR reactions were carried out in 25 µl reaction mixture containing 15.25 µl of sterile distilled H₂O, 2.5 µl of LA-Buffer, 4 µl of dNTP, 1 µl of each primer (5 µM), 0.25 µl of LA-Taq polymerase (1 unit/µl, Takara), and 1 µl of DNA template. The long-PCR reactions consisted of an initial denaturing step at 94 °C for 2 min, followed by 30 cycles of denaturing at 94 °C for 30 s, annealing at about 57 °C for 3 min and a final extension at 72 °C for 15 min. The normal PCR was performed following the standard procedure. Negative controls were included in all PCR amplifications to confirm the absence of contaminants. PCR products were cleaned by adding 0.45 µl of Shrimp Alkaline Phosphatase (Biotech Pharmakon), 0.9 µl of Exonuclease I (GE Healthcare) and 1.65 µl of sterile distilled H₂O to 9 µL of PCR product and incubating at 37 °C for 30 min and 80 °C for 20 min. The purified product was then sequenced on ABI Prism 3730 (Applied Biosystems) from both strands with the same primers as those used for PCRs.

Sequence editing and analysis

Sequence trace files were corrected and aligned with the DNASTar 5.0 software package (DNASTar, Inc., Wisconsin, USA). The locations of 13 protein-coding genes and 2 rRNA genes were determined by their similarity to published mitogenomes of other Sciaenidae species as shown in Table 2, whereas the tRNA genes were identified using the program tRNAscan-SE 1.21 (Lowe et al. 1997). Some tRNA genes, e.g. tRNA-Ser

Table 1. Primers used to amplify mtDNA of the *B. taipingensis*.

Segment	Primer code	Nucleotide sequence(5'-3')	Expected product length	Annealing temperature
A	H16396-F	TGAGATCACTAACACTCCTGTA	3064bp	57 °C
	H2080-R	GTGACCATGAGTTTAACGG		
B	H2004-F	CGCCTGTTTAACAAAAACAT	4174bp	58 °C
	H6194-R	TAGACTTCTGGGTGGCCAAAGAATCA		
C	H6108-F	CAATGCTTCTAACAGACCG	3388bp	57 °C
	H9516-R	CAAGACCCGGTGATTGGAA		
D	H9428-F	TTGGCTCTACATTCCCTAGC	3554bp	57 °C
	H12002-R	TAGGCTAGGAGGAAGAAGA		
E	H11932-F	CTCTTGGTGCAAATCCAAG	2471bp	56 °C
	H14423-R	AGTGCGTCGTTAGCGATTT		
F	H14326-F	AGGACTCTAACCCAGGACTA	2181bp	56 °C
	H27-R	CATCTAACATCTTCAGTGT		

Table 2. Fish species analyzed in this study.

Species	Length/bp	GenBank accession no.
Family Sciaenidae		
Subfamily Pseudosciaenidae		
<i>Larimichthys crocea</i>	16466	EU339149
<i>Larimichthys polyactis</i>	16470	FJ618559
<i>Collichthys lucida</i>	16451	JN857362
<i>Collichthys niveatus</i>	16450	JN678726
<i>Miichthys miui</i>	16493	HM447240
subfamily Argyrosominae		
<i>Pennahia argentata</i> (China)	16485	HQ890946
<i>Pennahia argentata</i> (Japan)	16486	KC545800
<i>Nibea albiflora</i>	16499	HQ890947
<i>Nibea coibor</i>	16509	KM373207
subfamily Sciaeninae		
<i>Dendrophysa russelii</i>	16626	JQ728562
family Haemulidae		
<i>Parapristipoma trilineatum</i>	16546	NC009857

(AGY) that could not be found by the tRNAscan-SE, were identified by their secondary structure and their position in the mitogenome (Zhang et al. 2009).

The structure of the control region and its conserved motifs were identified by making a comparison with homologous sequences of reported teleost (Lee et al. 1995; Cui et al. 2009; Cheng et al. 2010). The proposed secondary structure of the putative O_L was analyzed with the program Mfold v.3.2 with default setting (Zuker 2003) and visualized using RNAviz (De Rijk and De Wachter 1997).

Phylogenetic analyses

To clarify the phylogenetic position of *B. taipingensis* within the family Sciaenidae, the complete mitogenome sequences of 9 fish species with 10 complete mitogenome sequences in Sciaenidae (Table 2) were incorporated together with the presently obtained mitogenome sequence of *B. taipingensis* for phylogenetic analysis. In addition, possible close outgroups in Percoidei (Table 2) were chosen to root phylogenetic trees (Boger and Kritsky 2003). Sequences were aligned using Clustal W (Thompson et al. 1994), and adjustments were made manually. Phylogenetic analyses were based on the concatenated sequences of 12 protein-coding genes and 2 rRNA. The ND6 gene was excluded because of its heterogeneous base composition and consistently poor performance in phylogenetic analysis (Miya et al. 2003). For protein-coding genes, all stop codons were excluded from the analysis. The possible bias of substitution saturation at each codon position of protein-coding genes and 2 rRNA genes was investigated using DAMBE v.4.5.57 (Xia et al. 2001), and the results suggested that the third codons position were saturated both for transitions and transversions in the plot against with pairwise sequence divergence. Finally, unambiguously aligned sequences were 3630, 3630, 2728 nucleotide positions from first and second codon position of 12 protein-coding genes, 2 rRNA genes, respectively, and thus a total of 9988 bp positions were utilized for phylogenetic analysis.

Two different methods, Bayesian inference (BI) and maximum likelihood (ML), were used to construct the phylogenetic tree. Three partitions (first and second codon positions of protein-coding genes, 2 rRNA genes) were set in the combined data set for partitioned Bayesian analyses using MrBayes 3.1.2 (Ronquist et al. 2003), which allowed different substitution models in individual partitions. Markov Chain Monte Carlo (MCMC) Bayesian analyses were undertaken with MrBayes 3.1.2 setting for the best-fit model of nucleotide evolution selected by Hierarchical Likelihood Ratio Tests (hLRTs) in MrModeltest version 2.3 (Posada et al. 2004). Four Markov chains (one cold and three heated) were used in each of two simultaneous runs starting from different random trees. Analyses were run for 1,000,000 generations, sampled every 100 generations to assess convergence. The distribution of log-likelihood scores was examined to determine stationarity for each search and to determine if extra runs were required to achieve convergence in log likelihoods searches. We discarded initial trees with non-stationary log-likelihood values as part of a burn-in procedure, and combined the remaining trees that resulted in convergent log-likelihood scores from both independent searches. These trees were used to construct a 50% majority rule consensus tree.

Maximum likelihood analysis (ML) was performed in PAUP 4.0 (Swofford 2000), and the GTR+I+G (I=0.45, G=0.88) model of DNA substitution for the analysis was assessed by Modeltest version 3.7 (Posada and Crandall, 1998). The ML analysis was performed with random sequence addition replicates. Heuristic search was undertaken using 10 random addition sequence starting trees and tree bisection reconnection (TBR) branch swapping. The confidence level (Felsenstein, 1985) at each branch was evaluated by performing bootstrapping (BP) with 100 replicates in ML analysis.

Results and discussion

Mitochondrial genomic structure

The complete mitogenome of *B. taipingensis* was sequenced to be 16500 bp which consisted of 13 typical vertebrate protein-coding genes, 22 tRNA genes, 2 rRNA genes, and 1 putative control region (CR, Table 3). It had been submitted to GenBank with accession number JX232404. The mitogenome of *B. taipingensis* had substantially similar patterns on mitogenome structural organization with other vertebrates (Anderson et al. 1981; Miya et al. 1999; Cui et al. 2009). The encoding genes of mitogenome were located on H-strand with the exception of ND6 and 8 tRNA genes that were transcribed from L-strand (Table 3). All genes from *B. taipingensis* mitogenome were similar in size to most Perciformes species (Kim et al. 2004; Mabuchi et al. 2007; Cui et al. 2009; Cheng et al. 2011a; Cheng et al. 2012a) and the presence length of control region assumed variation in size, because they were prone to undergo the insertion/deletion events in the sequences (Sbisa et al. 1997).

The overall base composition of the *B. taipingensis* mitogenome was estimated to be 28.2% for A, 31.1% for C, 16.2% for G, and 24.6% for T (Table 4), respectively, indicating an obvious antiguanine bias. Furthermore, the G content of all protein-coding genes presents obviously lower just as found in other bony fishes (Miya et al. 2003; Mabuchi et al. 2007). The most remarkable character of metazoan mitogenomes is the strand-specific bias in nucleotide composition (Reyes et al. 1998; Hassanin et al. 2005), which can be measured as GC-skew $(G\%-C\%)/(G\%+C\%)$ and AT-skew $(A\%-T\%)/(A\%+T\%)$, respectively (Perna et al. 1995). The overall GC- and AT-skews of the H-strand of *B. taipingensis* mitogenome were -0.328 and 0.047, respectively, indicating a strand compositional bias characterized by a strong excess of C over G nucleotides and a slight excess of A over T nucleotides on the H-strand.

Protein-coding genes

The *B. taipingensis* genome contained 13 protein-coding genes encoded on the H-strand excluding ND6 gene that was oriented to L-strand. The 13 protein-coding genes were total 11,436 bp in size, accounting for 69.15% of the whole mitogenome. All protein-coding genes initiated with an ATG codon, just as in most vertebrates. Three open reading frames (ATP8, ND4L and ND6) of *B. taipingensis* ended with TAA, two open reading frames (ND1 and ND5) with TAG, and one open reading frames (COI) with AGA. The remainder used incomplete stop codons, either TA (ND2, ATP6 and COIII) or T (COII, ND3, ND4 and Cytb), probably completed by post-transcriptional polyadenylation (Ojala et al. 1981). It should be noted that these genes (ND4L with ND4, ATP8 with ATP6 and COI with tRNA^{Ser}(UUR)) could complete their stopped codons within the overlapping portion of the next genes.

Table 3. Characteristics of the mitochondrial genome of *B. taipingensis*.

Gene	Position		Size(bp)	Amino acid	Condon Initiation	Stop	Intergenic nucleotide	Stand
	From	To	Nucleotide					
tRN ^A _{Ph} e	1	68	68				0	H
12S rRNA	69	1017	949				0	H
tRN ^V _{al}	1018	1090	73				0	H
16S rRNA	1091	2792	1702				0	H
tRN ^L _{eu} (UUR)	2793	2866	74				0	H
ND1	2867	3841	975	324	ATG	TAG	4	H
tRN ^I _{le}	3846	3915	70				-1	H
tRN ^G _{ln}	3915	3985	71				-1	L
tRN ^A _{Me} t	3985	4054	69				0	H
ND2	4055	5099	1046	328	ATG	TA	0	H
tRN ^T _{rp}	5100	5170	71				1	L
tRN ^A _{la}	5172	5240	69				2	L
tRN ^A _{sn}	5243	5315	73				37	L
tRN ^C _{ys}	5353	5418	66				0	L
tRN ^T _{yr}	5419	5488	70				1	L
COI	5490	7046	1557	518	ATG	AGA	-5	H
tRN ^S _{er} (UCN)	7042	7112	71				3	L
tRN ^A _{sp}	7116	7184	69				8	H
COII	7193	7883	691	230	ATG	T	0	H
tRN ^L _{ys}	7884	7957	74				1	H
ATPase8	7959	8126	168	55	ATG	TAA	-10	H
ATPase6	8117	8799	683	227	ATG	TA	0	H
COIII	8800	9584	785	261	ATG	TA	0	H
tRN ^G _{ly}	9585	9655	71				0	H
ND3	9656	10005	349	118	ATG	T	0	H
tRN ^A _{rg}	10005	10073	69				0	H
ND4L	10074	10370	297	98	ATG	TAA	-7	H
ND4	10364	11744	1381	460	ATG	T	0	H
tRN ^H _{is}	11745	11813	69				0	H
tRN ^S _{er} (AGY)	11814	11880	67				5	H
tRN ^L _{eu} (CUN)	11886	11958	73				0	H
ND5	11959	13797	1839	612	ATG	TAG	4	H
ND6	13794	14315	522	173	ATG	TAA	0	L
tRN ^G _{lu}	14316	14384	69				4	L
Cytb	14389	15529	1141	380	ATG	T	0	H
tRN ^T _{hr}	15530	15601	72				3	H
tRN ^P _{ro}	15605	15674	70				0	L
Control Region	15675	16500	826					H

Nucleotide composition and codon using frequencies were calculated from a concatenated sequence of all protein-coding genes on the H-strand, except for ND6 on the L-strand. The base composition of protein-coding genes revealed weak bias against

Table 4. Base composition for protein-coding, tRNA, and rRNA genes of *B. taipingensis* mitogenome.

Gene/region	Base composition(%)				A+T	number
	T	C	A	G		
ND1	25.9	35.3	24.5	14.3	50.4	975
ND2	24.6	38.1	25.6	11.7	50.2	1046
ND3	26.4	38.1	20.9	14.6	47.3	349
ND4	24.6	35	26.1	14.3	50.7	1381
ND4L	25.6	38.7	21.9	13.8	47.5	297
ND5	26.2	33.4	28.3	12.1	54.5	1839
ND6	12.3	35.4	38.3	14	50.6	522
COI	29.2	28.7	23	19.1	52.2	1557
COII	27.1	28.9	28.5	15.5	55.6	691
COIII	28.3	31.2	23.6	16.9	51.9	785
ATP6	25.2	38.4	23.4	13	48.6	683
ATP8	23.2	33.3	32.8	10.7	56	168
Cytb	26.8	35	24	14.2	50.8	1141
Protein coding						
1st	29.1	30.6	24	16.3	53.1	3630
2nd	21.7	35.1	26.9	16.3	48.6	3630
3rd	28.3	36.2	24.7	10.8	53	3630
Total	26.4	34	25.2	14.4	51.6	10890
tRNA	27.1	22.6	27.4	23.9	54.5	1553
rRNA	20.8	26.7	32.2	20.3	53	2651
D-loop	30.4	22.8	31.6	15.2	62	826
Overall	25.1	31.4	27.6	15.9	52.7	16500

G (14.4%), especially at third codon positions (10.8%, Table 4). For all protein genes, C was the most frequent nucleotide at the first and third positions whereas T was most frequent at the second position as found in other bony fishes (Oh et al. 2007).

Ribosomal and transfer RNA genes

Like other mitochondrial genomes (Zardoya et al. 1995; Inoue et al. 2000), twenty-two tRNA genes were identified. The tRNA genes were interspersed among the mitochondrial genome and ranged in size from 66 to 74 bp (Table 3). They showed the typical gene arrangement as found in most vertebrates. Fourteen tRNA genes were transcribed on the H-strand, whereas the remaining eight tRNA genes were oriented on the L-strand (Table 3). These tRNA genes were predicted capable of folding into typical cloverleaf secondary structures with normal base pairing. The *B. taipingensis* mitogenome also contained a small subunit of rRNA (12S rRNA) and a large subunit of rRNA (16S rRNA) as in other bony fishes (Zardoya et al. 1995; Inoue et al. 2000), which were 947 bp and 1684 bp in length, respectively. As in other vertebrate ge-

nomes, these genes were located between the tRNA^{Phe} and tRNA^{Val} genes and between tRNA^{Val} and tRNA^{Leu}(UUR) genes, respectively.

Non-coding regions

As shown in Table 3, there were non-coding intergenic spacers from 1 to 8 bp observed in *B. taipingensis*, spanning the contiguous genes apart from O_L and control region. Furthermore, mitochondrial intergenic spacers were a total of 36 bp in eleven different locations.

As in most vertebrates, the major non-coding region in *B. taipingensis* mitochondrial genome was located between tRNA-Pro and tRNA-Phe. It was determined to be 826 bp in length, longer than other reported Sciaenidae species, and it had an overall base composition that was rich in A and T (A+T=62.0%). By comparing with the recognition sites in some reported fishes (Lee et al. 1995; Cui et al. 2009; Cheng et al. 2010; Cheng et al. 2011a; Cheng et al. 2012a), three domains were detected in *B. taipingensis*, namely, the termination associated sequence domain (ETAS), the central conserved sequence block domain (CSB-D, CSB-E and CSB-F) and the conserved sequence block domain (CSB-1, CSB-2 and CSB-3) (Figure 1). The ETAS was thought to act as a signal for the termination of H-strand elongation (Clayton 1991), and this domain was a hypervariable domain that might be useful for population genetic analyses. Furthermore, the motif sequence of ETAS was TACATAT with one palindromic sequence ATGTATA. The control region of mammals contained five blocks (CSB-B, CSB-C, CSB-D, CSB-E and CSB-F) in central conserved sequence blocks, however, only CSB-D, CSB-E and CSB-F were mostly detected in fishes (Brought et al. 1994; Lee et al. 1995). In this study, all these three motifs were identified in the central domain in accordance with *M. miiuy* (Cheng et al. 2010), and *Nibeia albiflora* (Cheng et al. 2011b) within Sciaenidae, which was not detected in four other species of Pseudosciaenidae (Cui et al. 2009; Cheng et al. 2011a; Cheng et al. 2012a; Cheng et al. 2012b). In addition, the consensus sequence of CSB-F was ATGTAATAAGAAC-CGACCAT, which distinguished the central conserved sequence block domain from the termination associated sequence domain. CSB-E was located downstream of CSB-F, whose consensus sequence was AGGGACAAGTATTGTGGGGG, characterized by the box GTGGGG. CSB-E was followed by CSB-D with its consensus sequence TATTCCTGGCATTGGT. Generally, these key sequences were highly conserved and easily recognized. Three conserved sequence blocks (CSB-1, CSB-2 and CSB-3) were determined in the conserved sequence block domain which was thought to be involved in positioning RNA polymerase both for transcription and for priming replication (Shadel et al. 1997). Moreover, the critical central conserved sequences of CSB-1, CSB-2, and CSB-3 were ATTTGGATATCAAGTGCATAAA, ACCCCCCCTACCCCCC, and AAACCCCCCGTAAA, respectively.

The additional non-coding region, the putative origin of L-strand replication (O_L), was located in a cluster of five tRNA genes (the WANCY region) between the tRNA-

ETAS
TACATATATGTATA TTCACCATAACAATTATATTAACCATATCAATAGCATTCAAGTA
 CATACATGTTTTATCAACATTTCTTGGTGTACACATTCATACACCACCATAAAA
 ACAAGACATACATAAACCATAAATAATTA AACCCAACAATCCTTTATATAATTGC
 AGGCGAAACTTAAGCTCCTAACAGTTCCGTCCATAAGTCTAGATATACCACGGA
 CTCAACATCCCGCCATACCTCACAATTT AATGTAATAAGAACCGACCATCA ^{CSB-F} GTT
 GATTTCTTAATGCATACGGTTATTGA AGGTGAGGGACAAGTATTGTGGGGG ^{CSB-E} TTT
 CACAAAATGAAC TATTCCTGGCATTGGT ^{CSB-D} TCCTATTT CAGGGCCATTTATTGGTA
 TCATTCCTCACACTTTCATCGACGCTTGCATAAGTTAATGGTGGTAATACATAAG
 CGGGAGCACCCCATGCCGAGCGTTCTTTCTAGAGGGTCACTGGTATTTTTTT
 TTTGGTTTCCTTTCGCCTTGCATTTACAGTGCATACAGAAATGAAATAATAAGG
 TTGAACATTTCTTGC GTTCAAAGTAAATGGTATTCAATGATATAAGTCATTACT
 CAAGAATCACAT ATTTGGATATCAAGTGCATAAA ^{CSB-1} CTATGGCTTATCACTTGGAAG
 ATATCTAAGTTATGCCCCCTGGGTTCCTGCGCGTTAA ACCCCCCTACCCCCC ^{CSB-2} A
 ATACTCCTGAGATCACTAACACTCCTGT AAACCCCCCGTAAA ^{CSB-3} CAGGAAAACCC
 CGGGTAGTATAATTTTTAGTCCAAAATGTATCTATTTACATTATTAATAATGACGCA
 CGC

Figure 1. The structure of control region about *B. taipingensis*.

Asn and tRNA-Cys genes, almost identical with other Sciaenidae fishes. The putative O_L could form a stable stem-loop secondary structure with 20 bp in the stem and 13 bp in the loop (Figure 2), which was 37 bp in length (CCTTTCCCCCGCCTACTATAGGACTAAAGGCGGGGA). Furthermore, the conserved stem-loop structures in mitochondrial genomes was thought to play an importance role in conjunction with the origin of mtDNA replication.

Phylogenetic analyses within family Sciaenidae

The phylogenetic trees (the 50% majority-rule consensus tree is shown in Figure 3) were highly coincident regardless of the analytic method used, and were statistically supported by high posterior probability and intermediate bootstrap values. This phylogenetic analysis represented the first investigation of relationships of *B. taipingensis* within the Sciaenidae based on the whole mitogenome. In our analysis, *B. taipingensis*

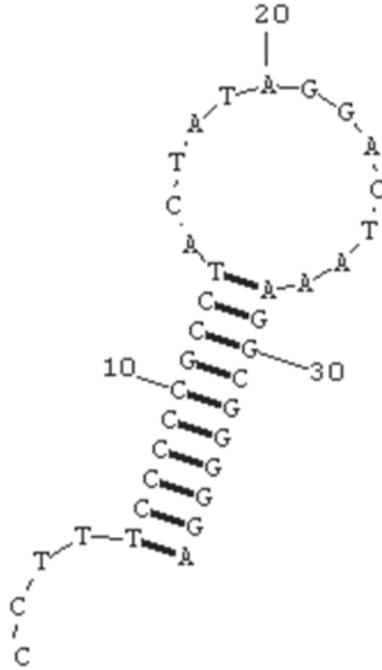


Figure 2. Potential secondary structure of the origin of L-strand replication (O_L) of *B. taipingensis* mtDNA.

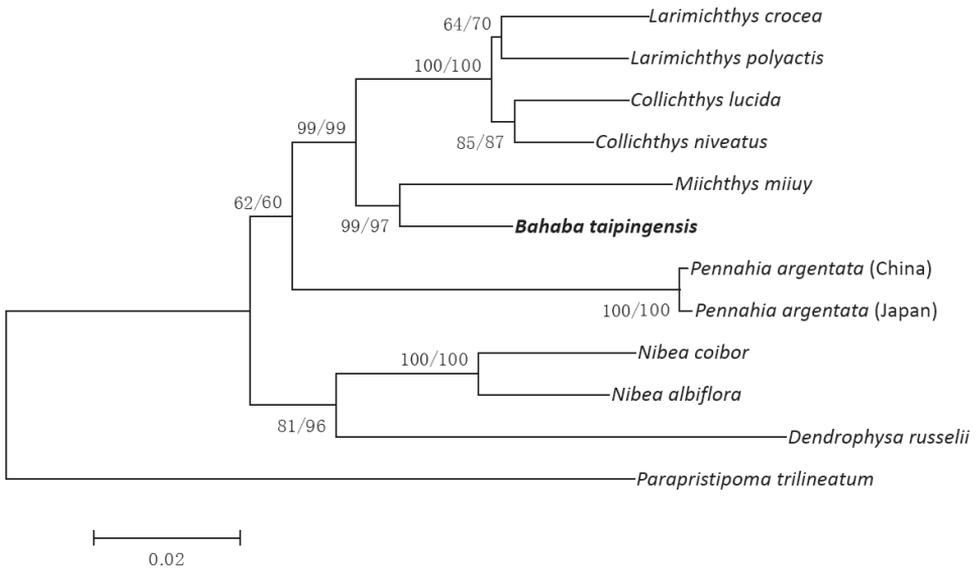


Figure 3. Phylogenetic relationships among Sciaenidae species based on the combined 9988 bp nucleotide positions. The posterior probability value of BI analyses and bootstrap support values of ML analyses (in the order: BI, ML) are indicated near the branches.

was found to be more closely related to Pseudosciaenidae (*Collichthys*, *Larimichthys* and *Miichthys*) than to *Pennahia* and *Nibea*, the latter of which was suggested by morphological topology (Zhu et al. 1963; Cheng et al. 1987) and previous molecular study (He et al. 2012). However, phylogenetic analyses showed that *Miichthys* could not be merged into the *Collichthys*–*Larimichthys* clade. On the contrary, *Miichthys* and *Bahaba* formed an independent clade well supported by high posterior probability value, and this clade formed the sister group of the *Collichthys*–*Larimichthys* clade. Therefore, the relationship between *Miichthys* and Pseudosciaenidae deserves to be further studied. The proposed phylogenetic position of *B. taipingensis* within the Sciaenidae based on the findings of the present study should be accepted with caution due to limited taxon sampling. However, the phylogenetic relationship within the Sciaenidae remains to be resolved, and it is necessary to make further analysis based on more molecular information and extensive taxon sampling.

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