

Description of a new species *Gyraulus* (Pulmonata: Planorbidae) from the land thermal spring Khakusy of Lake Baikal

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Abstract

A new species of the family Planorbidae is described from the land thermal spring Khakusy, on the north-eastern shore of Lake Baikal. The description of *Gyraulus takhteevi* **sp. n.** includes morphological characters and gene sequences (COI of mtDNA) for the species separation from sister taxon *Gyraulus acronicus* (A. Férussac, 1807) collected from the small Krestovka River in-flowing into the south-western part of the Lake. The new species differs from *G. acronicus* in small shell size of adults, having smaller number of prostate folds (maximal up to 26 in *G. takhteevi* n. sp. vs. 40 in *G. acronicus*), a short preputium (approximately twice shorter than the phallotheca), and an elongated bursa copulatrix. The population of *Gyraulus takhteevi* **sp. n.** consists of two co-existent morphs: one of them has a narrow shell spire and the second is characterized by wide spire similar to the shell of *G. acronicus*. One of the two revealed haplotypes of the new species includes both morphs, while the second consists of snails with wide spired shells.

Keywords

COI mtDNA, morphology, planorbid gastropods

Introduction

The thermal spring Khakusy is located one kilometer from the shore in the north-eastern part of Lake Baikal and flows into it. The chemical composition of the water is hydrocarbonate-sulphate-sodium with a salinity of 0.3 g/l; the water temperature of

the mainstream is + 47 °C (Volkov 2010). Bottom ground of this warm stream is sand and pebbles, covered with mats of blue-green algae. Gastropods (limnaeids and planorbids) live far from the main stream, where the water temperature varies from + 31 °C to 10 °C. Among limnaeids two species, *Radix khakusyensis* Kruglov & Starobogatov, 1989 and *Radix thermobaicalica* Kruglov & Starobogatov, 1989, were described, which are now proposed to be considered as ecotypes of *Radix auricularia* (Linnaeus, 1758) (Aksenova et al. 2017). Together with *Radix*, small specimens of *Gyraulus* (Planorbidae) were found, which differed from all other species of *Gyraulus* inhabiting the Baikal region including the thermal springs (Sitnikova and Takhteev 2006). Unique morphological characters of these snails support to their status as a new species.

Materials and methods

Two hundred fourteen specimens of a new species were collected in the thermal spring Khakusy (north-eastern shore of Lake Baikal (55°21'42"N, 109°49'41"E), from pebbles covered by vegetation, mainly filamentous cyanobacterial mats. The samples were collected on 30 March 1990 (13 specimens) from spring with a water temperature of +31 °C, and 39 specimens from slightly downstream with water temperature at +22 °C by V. Takhteev; 3 July 2003 (6 spec.) by T. Sitnikova; 7 October 2004 (23 specimens) by V. Takhteev; 9 August 2009 (14 specimens, 6 dissected) by T. Sitnikova, 20 March 2003 (61 specimens) by V. Takhteev; 8 June 2015 (53 spec., 8 dissected) by T. Peretolchina, and July 2017 (5 specimens, 1 dissected) by T. Sitnikova.

The 60 adult specimens of the new species were compared with seven individuals of the *Gyraulus acronicus* (A. Férussac, 1807) collected in a small inflow of the Krestovka River (51°51'44"N, 104°51'11"E) on 13 October 2015 and 2 October 2017 by T. Sitnikova; 4 of these specimens were dissected. The holotype and paratypes of the newly described species were deposited in the collection of the Zoological Institute RAS (St. Petersburg), registration numbers are 522-2015 (1) for the holotype and 522-2015 (2) for three paratypes. An additional 28 paratypes were deposited in the gastropod collection of the Limnological Institute SB RAS (Irkutsk, Russia) under Nos: 901, 902, 1101, and 1102.

Anatomical study and molecular analyses were performed on snails fixed in 80% ethanol that was changed for 70% ethanol after one day. Eight snails were photographed and the shells of 12 individuals were dissected. DNA was extracted from the feet; the teeth of radula were SEM-photographed and the soft tissues were dissected under a light stereomicroscope. Morphological study and descriptive terminology are based on the review of morphological characters of planorbid gastropods (Meier-Brook 1964, 1983; Brown 2001; Glöer 2002). Measurements of the shells were performed using the Image-Pro-Plus program for Windows XP.

Genomic DNA was extracted from muscle tissue using a modified method from Sokolov (2000). Gene fragments of mitochondrial cytochrome c oxidase subunit 1 (CO1) were amplified using primers L1490 (5' – GGTCAACAAATCATAAAGATATTGG – 3') and H2198 (TAAACTTCAGGGTGACCAAAAAATCA – 3') (Fol-

mer et al., 1994) and mitochondrial large ribosome subunit (16S) were amplified using primers ARL (5' – CGCCTGTTTATCAAAAACAT – 3') and BRH (5' – CCGGTCTGAACTCAGATCACGT – 3') (Palumbi et al., 1996). An average of 1–3 µL of extracted DNA was amplified in a 25 µL of PCR-mix using BioMaster HS-*Taq* PCR Kit (Biolabmix, Russia) following the manufacturer's recommendations. Conditions of 30 cycles of amplification for both gene fragments were pre-denaturation at 94 °C for 5 min, followed by denaturation at 94 °C for 40 s, annealing of primers at 50 °C for 40 s, elongation at 72 °C for 60 s, and a final elongation step at 72 °C for 8 min. The reaction products were analyzed in 1% agarose gel. After electrophoresis, visible bands of the expected size were excised and then amplicons were cleaned up according to Maniatis et al. (1982). Sequencing was carried out in an ABI 3130 automated sequencer. The DNA sequences obtained were aligned using default settings by ClustalW (Thompson et al. 2002) and edited using the BioEdit software package (Hall 1999). All sequences were deposited in GenBank under accession numbers (Table 1). Additional sequences of other representatives of *Gyraulus* retrieved from GenBank are also listed in Table 1.

Mean pairwise, inter-specific *p*-distances between COI and 16S sequences were calculated using MEGA 6 (Tamura et al. 2013) (Table 3).

Phylogenetic reconstructions for COI mtDNA was inferred following a Bayesian method of phylogenetic inference as implemented by MrBayes v. 3.2.2 (Ronquist and Huelsenbeck 2003). Posterior probabilities of phylogenetic trees were estimated by a 15,000,000 generation Metropolis-coupled Markov chain Monte Carlo simulation (two runs with four chains) under the GTR+I+G model of substitution, determined as a best fit model using jModelTest v.2.1 (Darriba et al. 2012). A 50 % majority-rule consensus tree was constructed following a 25% burn-in of all sampled trees to allow likelihood values to reach stationary equilibrium.

Taxonomy

Family Planorbidae Rafinesque, 1815

Genus *Gyraulus* Charpentier, 1837

Gyraulus takhteevi sp. n.

<http://zoobank.org/975FA23C-CF59-44E2-91CC-A68285601B06>

Gyraulus cf. *borealis*: Sitnikova and Takhteev 2006: 143 (record from thermal spring Khakusy)

Type locality. Thermal spring Khakusy (East Siberia).

Types. Holotype (dry) registration number in ZIN collection (St. Petersburg, Russia) is 522-2015 (1), 3 paratypes (dry) registration number is 522-2015 (2) with a label: 'East-northern shore of Lake Baikal, thermal spring Khakusy, pebbles, water temperature 23–25 °C, #0957, col. T. Sitnikova, 09.08.2009'. Collections of the

Table 1. Taxa used for phylogenetic analyses, with their GenBank Accession Numbers and references.

Species name	COI GB#	16S GB#	References
<i>G. albus</i>	KC495835	KC495952	Oheimb et al. (2013)
<i>G. crista</i>	KC495836	KC495953	
<i>G. rosmaessleri</i>	KC495714	KC495844	
<i>G. connollyi</i>	KC495776		
<i>Gyraulus</i> sp.	KC495834	KC495951	
<i>G. convexiusculus</i>	KF966542		Unpublished
<i>G. acronicus</i> , Krestovaya River			
a435	MG773536		Present study
a430	MG773535	MG800654	
<i>Gyraulus takhteevi</i> sp. n. (haplotype 1)			Present study
401_n	MG773534		
a437_w	MG773537		
442_w	MG773541		
443_w	MG773542		
439_n	MG773539		
438_n	MG773538		
444_w	MG773543		
441_n	MG773540		
445_w	MG773544		
<i>Gyraulus takhteevi</i> sp. n. (haplotype 2)			Present study
425_w	MG773531		
429_w	MG773533		
427_w	MG773532	MG800655	

Limnological Institute SB RAS (Irkutsk, Russia): 2 paratypes (dry) under numbers 901 and 902 with the label: ‘Khakusy, shallow springs at a depth down to 3 cm, water temperature 33 °C, col. V. Takhteev, #57, 07.10.2004’; 20 paratypes (in alcohol) under number 1101 with a label ‘Khakusy, #1526, 08.06.2015, col. T. Peretolchina’ and 4 paratypes (dry) and 2 paratypes (in alcohol) under number 1102 collected 3 July 2003, #0344, T. Sitnikova.

Etymology. The species name ‘*takhteevi*’ is in honor of the Russian zoologist and hydrobiologist Prof. V.V. Takhteev (Irkutsk State University) who investigates biota of thermal springs in East Siberia.

Description. *Shell* (Fig. 1A–E) brown or green-brown, discoidal, pseudodextral, small, up to 5.0 mm of diameter at 4.0 whorls, smooth with fine growth lines on rounded last whorl, spire convex with rounded whorls, two last whorls of an umbilicus almost flat. Index a/b 0.36 – 0.57. Height of last whorl (a) less than aperture width. Aperture oval rounded. Species occurs in two morphs differing in spire width, narrow (Fig. 1A–C) and wide (Fig. 1D–E); all small individuals (less than three whorls) have a narrow spire; at 3.25 – 4.0 whorls the portion of morph 2 (with wide spire) is approximately 1/3 or 1/2 of total number of examined adult specimens. The designated



Figure 1. Shells of type specimens of *Gyraulus takhteevi* sp. n. and *G. acronicus* Férussac, 1807). **A** Holotype *G. takhteevi* sp. n. **B–E** Paratypes *G. takhteevi* sp. n. **A–C** morph 1 with narrow spire **D, E** morph 2 with wide spire **F–G** *G. acronicus* from Krestovka River: **F** young individual **G** mature individual after 5 months of a cultivation. Abbreviations: a – aperture view; b – right side with umbilicus, c – left side with spire. Scale bar 1 mm.

holotype belongs to the dominant morph 1 (with a narrow spire). Sizes of the holotype and paratypes are presented in Table 2.

Radular teeth. The formula of the radula is 10 (9)–1–(9) 10. The central radular teeth are bicuspid with two equal-sized cusps. Two first lateral teeth usually bicuspid, sometimes with third small cusp. Other teeth have three rounded cusps, and only young (not working or worn) tricuspid teeth have three long sharp cusps. Mesocone is flanked (Fig. 2). Both morphs have identical radular morphologies and formulae.

Reproductive system. Seminal vesicles have thickened bend before joining with hermaphrodite duct, which is thin up to carrefour. Prostate consists of 22–26 diverticula, bursa copulatrix is oval in shape, its length including duct more than $\frac{1}{2}$ length of

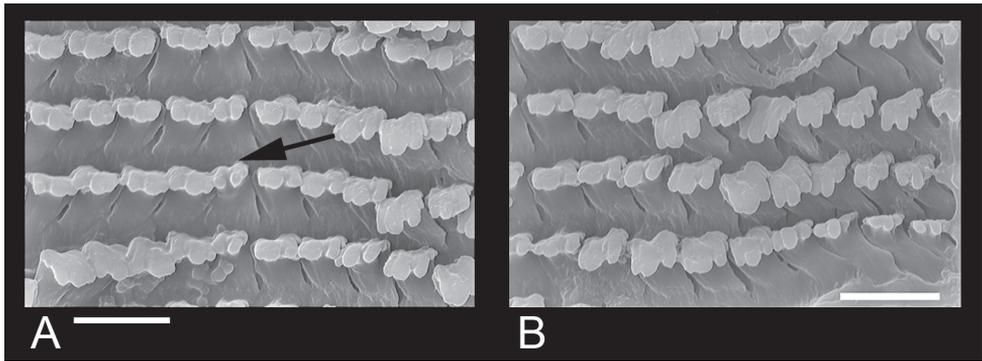


Figure 2. Radular teeth of *Gyraulus takhteevi* sp. n. **A** Central and lateral teeth (arrow shows a central tooth) **B** Marginal teeth. Scale bar 10 μ m.

Table 2. Shell dimensions and whorl counts of type specimens of *Gyraulus takhteevi* sp. n. Abbreviations: SH – height of shell; SW – width of shell; b – width of last whorl without aperture; a – height of this whorl; SpW – width of spire; AW – width of aperture; AH – height of aperture; n – number of whorls. Measurements are in mm except for number of whorls.

Specimens/Character	SW	SH	SpW	b	a	AW	AH	n
Holotype	4.31	1.68	1.23	3.01	1.27	1.67	1.20	3.75
Paratypes (narrow spire)	4.03	1.42	1.25	2.78	1.21	1.64	1.25	3.75
	3.57	1.5	1.10	2.4	1.17	1.67	1.42	3.5
	3.45	1.5	1.0	2.37	1.12	1.26	1.19	3.25
(wide spire)	5.2	1.6	1.93	3.8	1.3	1.5	1.45	4.0
	4.45	1.7	1.54	3.24	1.18	1.5	1.31	3.75
	4.1	1.65	1.3	2.9	1.1	1.5	1.4	3.5

oviduct. Length of copulatory organ almost equal to length of prostate (Fig. 3). Phalotheca twice as long as preputium length in morph with narrow spire and 1.7–1.8 times in morph with wide spire (Fig. 4A, B). Preputium slightly turned-up. Seminal pore lies near the proximal end of the thickening. Length of the stylet (Fig. 5A) varies from 189 to 260 μ m (n = 5).

Egg mass (= cluster or syncapsula) is a round transparent sac less than 1 mm in size, consisting of 4–5 eggs (Fig. 4F).

Differential diagnosis. The new species (especially morph 2 with wide spire) is similar to the Palearctic species *G. acronicus* (Fig. 1F, G) in shell shape, and differs from it in the small size of adults, which do not reach 6–7 mm diameter. Additionally, the new species differs from *G. acronicus* in having shorter preputium, the elongated oval shape of the bursa copulatrix, smaller number of prostate folds, and the smallest stylet length. A preputium of *G. acronicus* is 1.3–1.7 times shorter than the phalotheca (Fig. 4C, D); the bursa copulatrix has an elongate club-shaped (Meier-Brook, 1964, 1983) or a wide rounded shape, and length of copulatory organ is less than prostate length (Glöer and Vinarski 2009; own data). The length of stylet of *G. acronicus* is

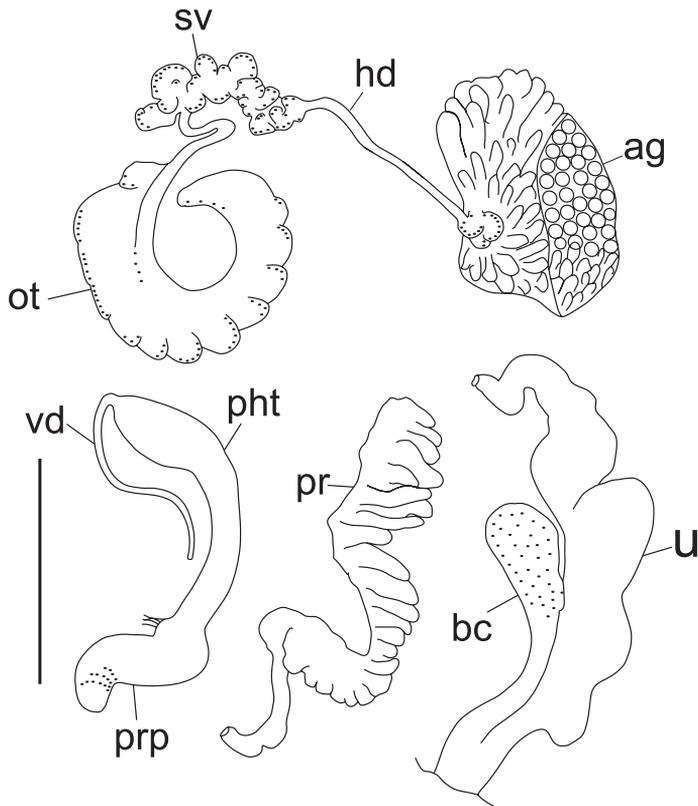


Figure 3. Drawing pictures of the reproductive organs of *Gyraulus takhteevi* sp. n. Abbreviations: **ag** albumen gland **bc** bursa copulatrix **hd** hermaphrodite duct **ot** ovotestis **pht** phallosome **pr** prostate **prp** preputium **sv** seminal vesicles **vd** vas deferens **u** uterus. Scale bar 1 mm.

Table 3. Pairwise p-distances (%) between COI sequences of different species of the genus *Gyraulus*.

Taxa	1	2	3	4	5	6	7	8	9
1. <i>G. acronicus</i> Krestovaya									
2. <i>Gyraulus takhteevi</i> sp. n.(haplotype 1)	0.8								
3. <i>G. takhteevi</i> sp. n. (haplotype 2)	1.0	0.2							
4. <i>Gyraulus</i> sp. KC495834 (Lake Baikal)	0.7	1.5	1.3						
5. <i>G. albus</i>	6.9	7.0	6.9	6.9					
6. <i>G. rosmaessleri</i>	11.0	11.0	10.8	10.6	11.3				
7. <i>G. convexius</i>	10.5	10.8	10.8	10.6	7.4	12.6			
8. <i>G. crista</i>	12.3	12.6	12.6	12.4	12.8	11.6	12.4		
9. <i>G. connollyi</i>	11.5	11.5	11.3	11.5	8.8	12.6	9.0	14.7	

more than 300 μm (Fig. 5B). The size of the egg mass of *G. takhteevi* sp. n. is less than that of *G. acronicus* (its length up to 2.05 mm) and consists of fewer eggs (4–5 eggs vs. 5–7 eggs in *G. acronicus*) (Berezkina and Starobogatov, 1988).

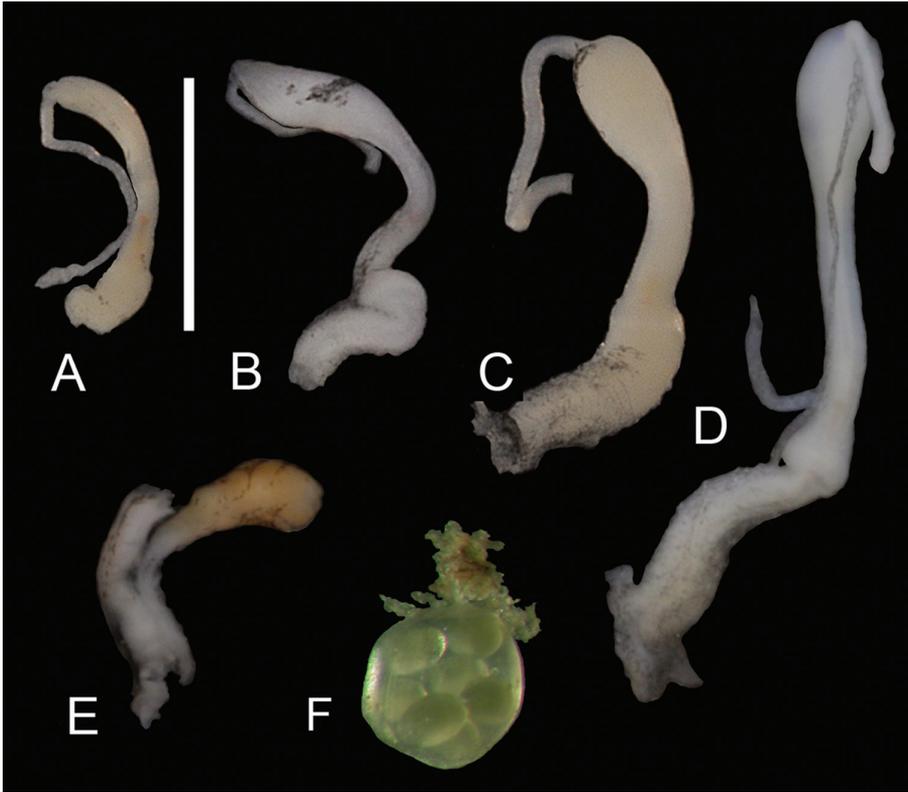


Figure 4. Copulatory organs (A–D), bursa copulatrix (E) and egg mass (F). A–B, E–F *Gyraulus takhteevi* sp. n.: A morph 1 B morph 2 C–D *G. acronicus* from Krestovka River: young (shell Fig. 1F), male stage of mature (shell Fig. 1G).

Distribution and ecology. Snails similar to morph 1 of the new species were also found in the thermal spring Frolikha located near to Khakusy (ca. 20 km north); all of them were young and were not examined in detail. The number of gastropods in the thermal spring Khakusy in March 2016 was 1,706 individuals/m² at water temperature +29 °C in a locality 2 m far from the main source; a minimal number of snails (59 indiv./m²) was registered at water temperature +10 °C in a small pond downstream of the main source (Epova et al. 2017). The proportions of *G. takhteevi* sp. n. and *Lymnaea* were about fifty-fifty.

The confinement of morphs to different sites of the thermal spring has not been confirmed. The population of *G. takhteevi* consists mainly of young snails, in which the morph with a narrow spire dominated: in July 2003 28 of the 39 collected snails were juvenile, with adults presented by six snails with shells of narrow spire (morph 1) and five individuals with wide spire (morph 2). In June 2015 there were 16 individuals of morph 1 and eleven specimens of morph 2, with more than 50 young snails; in March 2016 39 young specimens were collected and 16 adult snails of morph 1 and six individuals of morph 2.

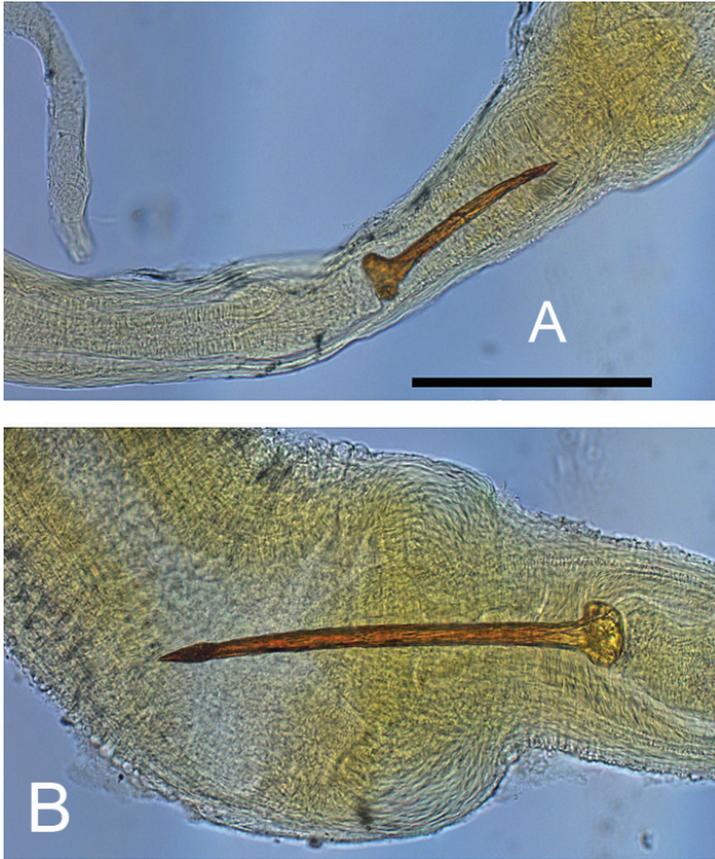


Figure 5. Stylets of *Gyraulus takhteevi* sp. n. (A) and *G. acronicus* from Krestovka River (B). Scale bar 200 μm .

The cultivation of the new species under summer conditions of ephemeral waterbodies (sand, pebbles, water temperature +20–24 °C, food items of vegetable fodder) was not successful, and all snails died in two weeks. It is worth noting that specimens of *G. acronicus* under the same conditions lived more than one year and reproduced, attached their egg masses to pebbles. According to the interruption lines on shells, the snails live up to 5 years.

Sequences analysis. A total 14 COI (620 bp long) and 14 16S (500 bp long) sequences were produced. Inspection of the sequences revealed the existence of two unique haplotypes for COI among *G. takhteevi* sp. n. These haplotypes weakly differ from each other (in 1–2 nucleotide substitutions). Both morphs (shells with narrow or wide spire) are part of the haplotype 1, while haplotype 2 consists of the morph with wide spire shell (Fig. 6). There are no genetic differences between 16S nucleotide sequences obtained.

The haplotype of *G. acronicus* from Krestovka River shares the same clade as *Gyraulus* sp., sequence of which is retrieved from GenBank (KC495834), which differs from that of *G. takhteevi* sp. n. with 5–6 nucleotide substitutions (~ 1%).

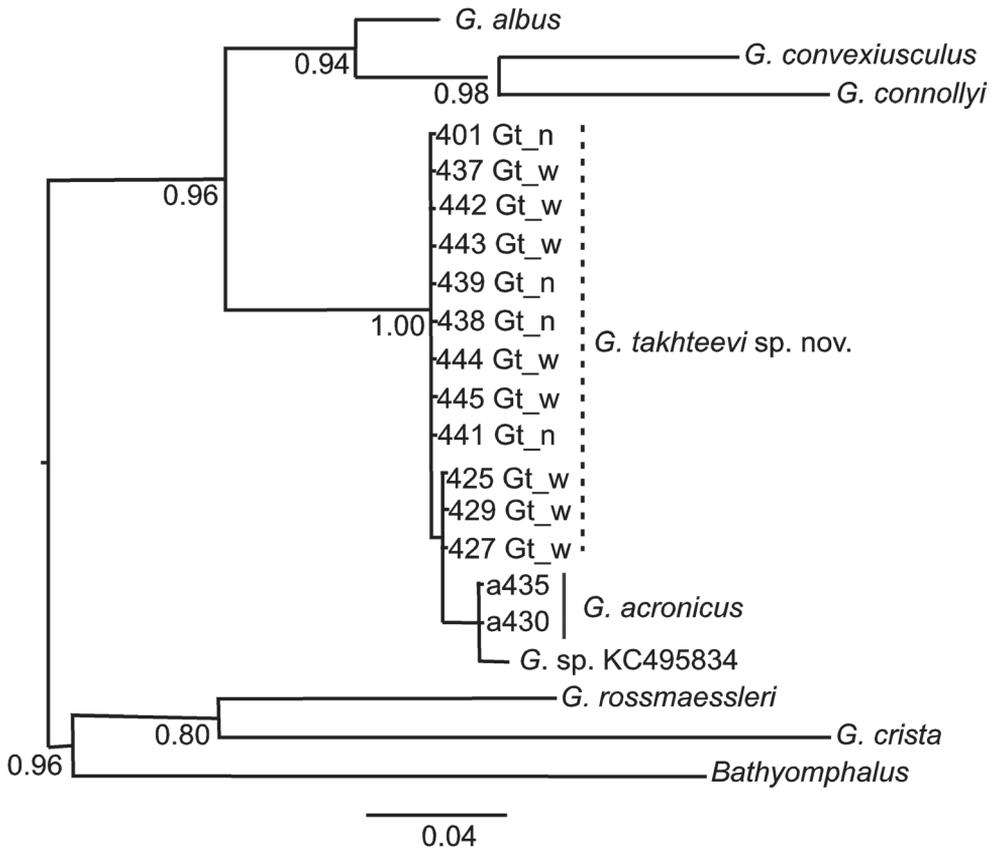


Figure 6. Phylogram derived from Bayesian analysis of CO1 mtDNA gene sequences. Values at nodes represent posterior probabilities. The scale bar represents the branch length as a measure of substitution per site.

Discussion

Despite the small genetic distances between *G. takhteevi* sp. n. and *G. acronicus*, morphological differences between them (shell size of mature individuals, maximal number of prostate folds, length of the phalloteka relative to length of the prepuceum, length of a stylet, shape of the bursa copulatrix) allow one to consider them as separate species. Moreover, the new species demonstrates significant adaptation to a specific habitat of the thermal spring Khakusy. The water in the spring does not freeze in winter, the temperature of the water changes very little, and the food items like microorganisms, bacterial mats, and vegetable detritus are present during all seasons of a year. However, the two co-existent morphs of *G. takhteevi* sp. n. are likely to correspond to two interbreeding generations, hatching from eggs, growth and reproduction of which are confined to different seasons. The co-existence of both morphs appears to be constantly maintained, since they have been found over several years of the investigation.

The low level of genetic distances between *G. takhteevi* sp. n. and *G. acronicus* from the Krestovka River indicates that a recent divergence of the species happened after the glaciers covering large areas of the northeast coast of Baikal during last glacial period started to melt about 18 kyr BP (Osipov and Khlystov 2010). The adaptation to the thermal conditions of ‘a closed habitat’ formed by island isolation under selective pressures led to an interruption of gene exchange between *G. takhteevi* sp. n. and *G. acronicus*, resulting in differences in their morphological and physiological characteristics, and ecological preferences.

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A new species of *Malletia* (Bivalvia, Malletiidae) and new records of deep-water bivalves from Pacific Southern Colombia

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Abstract

In order to enhance the understanding of Pacific Colombia's deep-water marine fauna, a benthic research cruise (2012 TUM Offshore 6 and 7) was conducted off the coast of the Department of Nariño, in southern Colombia. Biological, oceanographic and sediment samples from the continental shelf and slope were collected at depths between 350 and 941 m. A new species of *Malletia* obtained on that cruise is described and compared with other species from the eastern Pacific. Sixteen species of bivalve mollusks (belonging to 12 families and 15 genera) were identified. Five of them were the first records for Pacific Colombia (*Jupiteria lobula*, *Limatula saturna*, *Lucinoma heroica*, *Cuspidaria panamensis*, and *Dallicordia alaskana*). Four of them had geographic distributions that now extend to Tumaco at the southern end of Nariño.

Keywords

Bivalvia, benthos, Colombia, deep-water, *Malletia*, Malletiidae, Tumaco

Introduction

Throughout the past decade, the search for hydrocarbon and natural gas reserves in Colombia (Pacific and Caribbean coast) has sparked an interest in the country's remote deep-sea regions. This has resulted in intensified deep-sea baseline studies, primarily along the continental shelves and slopes. Nevertheless, deep-sea studies face logistical and cost limitations, including the availability of research vessels and proper equipment for collecting samples.

Despite the increase in knowledge during recent years, the presently known range of many invertebrates groups inhabiting soft sediments, including mollusks, is still fairly fragmentary in remote parts of the Colombian Pacific. There is a lack of published data on the biology, functional morphology, ecology, development and dispersal mechanisms for these invertebrates, as well as a lack of baseline faunal inventories. Thus, the true biodiversity of the Pacific Colombian deep-sea must be vastly underestimated.

As a result of recent Colombian expeditions, a rich benthic fauna inhabiting of the deep-seas of Pacific Southern Colombia has been discovered, but few species of mollusks have been described when compared with the mollusks from the coasts of the Colombian Atlantic (e.g., Ardila-Espitia and Diaz 2002, Simone and Gracia 2006, Gracia and Ardila-Espitia 2009).

In the context of faunal inventories, the tropical west coast of America is well documented, with 890 species of bivalves presently recorded (Coan and Valentich-Scott 2012). For northwestern South America to Peru, a basic knowledge of deep-sea bivalve mollusks has been covered by a few recent publications (e.g., Gracia and Valentich-Scott 2014; Paredes et al. 2016). The investigations in Pacific Colombian waters have hitherto focused mainly on the coastal zones (e.g., Cantera et al. 1979, Cosel 1984, Díaz et al. 1997, Cantera 2010, López de Mesa and Cantera 2015) rather than zones farther offshore (e.g., Hertlein and Strong 1955). Gracia and Valentich-Scott (2014) documented the bivalves off the Department of Choco (Colombian North Pacific) where more than 38 species of bivalves were found, 34 of which were new records for the country.

The current work presents a systematic and annotated list of bivalve species collected in the southern Colombian Pacific region. Each entry includes the species' geographic and bathymetric distribution, plus additional remarks and observations. From the above, several species stand out as being first records for the country. We are also including the description of a new species uncovered in this survey. Our records represent a significant expansion in the knowledge of the Pacific Colombian bivalve fauna, but much more sampling and analysis is needed when one takes into account the large geographic extent of this region.

Materials and methods

Study area

The present study was carried out in the tropical eastern Pacific Ocean (Fig. 1). The study area (TUM Offshore Blocks 6 and 7) covered 7,308 km² and extended from Sanquianga

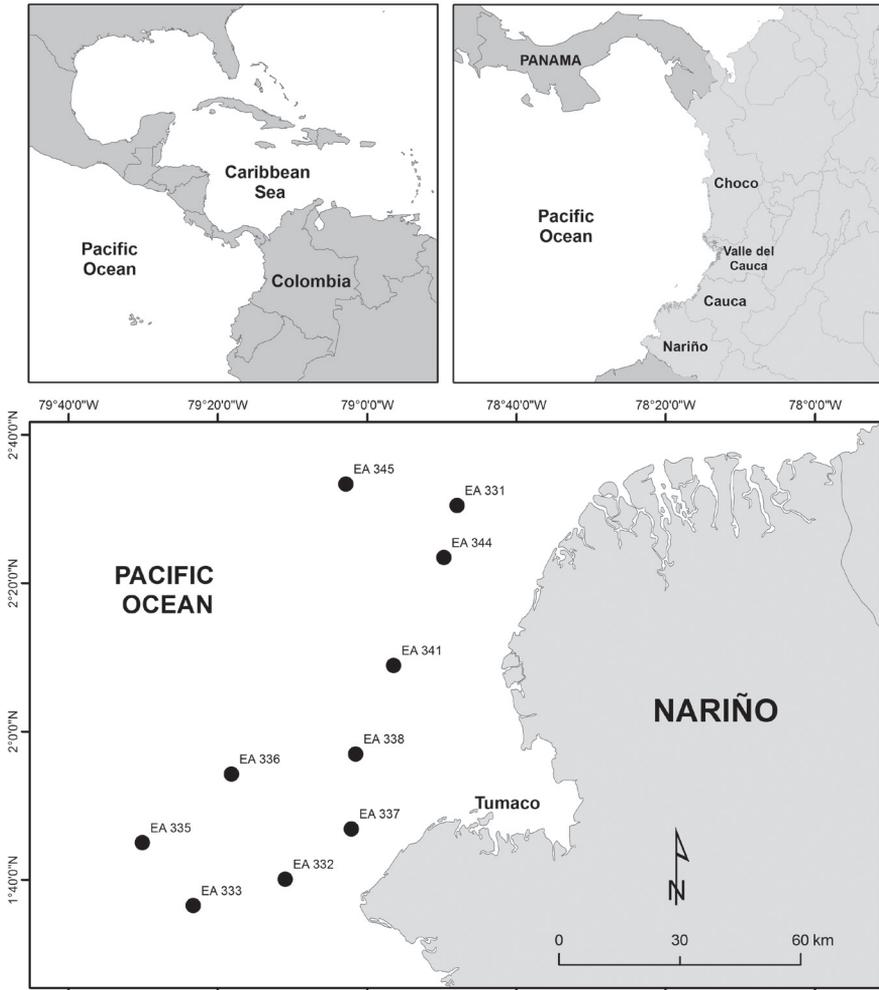


Figure 1. Locations along the Colombian Pacific Ocean where bivalves were collected.

National Nature Park in the Department of Nariño to the Colombia-Ecuador border. The region is influenced by continental contributions from Tumaco Bay, as well as by numerous rivers, including the Patia and Mira (IDEAM et al. 2007). This study area is part of the research project known as the “Biological and physical baseline survey of TUM Offshore Blocks 6 and 7 subject to hydrocarbon exploration” (ANH-Invermar 2013).

Sample collection

Samples were collected from 4–22 December 2012, on board the fishing vessel *Perla Verde*. Collection depths ranged from 350–941 m. All the 13 trawls made during the survey were taken in soft and homogenous sea beds. Ten of the 13 sampling locations included bivalves.

Each sample was collected with a benthic semi-balloon trawl net (9 × 1 m) for 10 minutes at a speed of 3 knots. Because the exact time at which the net opened was unknown, sampling was semi-quantitative. We acknowledge that this sampling technique could have missed small and microscopic species as would be taken by epibenthic sleds, but the equipment needed for this method was not available to us. Collected material was coarsely sorted on deck and later identified to lower levels at the Museo de Historia Natural Marina de Colombia (MHNMC) which is part of Instituto de Investigaciones Marinas y Costeras (INVEMAR). The empty valves were air-dried, while the soft-bodied organisms were preserved in 70% ethanol.

Specimen identification was based upon shell characters. Museum materials, bibliographic references and bivalve taxonomic experts were consulted to confirm the results (e.g., Dall 1896, 1908; Keen 1971; Coan and Valentich-Scott 2012). The identified material included many complete living organisms as well as empty shells of bivalves. The systematic order of this list corresponds to that proposed by Coan and Valentich-Scott (2012). Specimens from this study, including other mollusks not analyzed in this work (e.g., gastropods, chitons, and cephalopods), now reside at the MHNMC's mollusk collection in Santa Marta, Colombia.

Oceanographic data were collected with an Idronaut CTDO marine profiler (yielding data for conductivity, temperature, depth, and oxygen concentration) at sites S333 and S334, both of which contained bivalves (Table 1). Sediment core sets were collected five sites (S331, S333, S334, S341, and S345) with a Gomex II Box corer that had a 32 liter storage capacity. Sediment grain analysis revealed a predominance of silt (Table 1). Grain size classification was conducted according to Folk (1974). All samples were classified as silts; 57% of samples were purely silts, while the remaining 43% also contained sand and gravel fractions (INVEMAR-ANH 2013).

Abbreviations

EA	Trawl station; S sediment station
MHNMC	(Spanish acronym) Museo de Historia Natural Marina de Colombia
TUM OFF	Tumaco-Offshore

Table 1. Size distribution of analyzed sediment samples according to INVEMAR-ANH (2013).

Station	Depth (m)	% Gravel	% Sand	% Silt
331	320	0.1	23.2	76.7
333	833	0.0	1.0	99.0
334	864	0.0	0.7	99.3
341	894	0.2	1.1	98.7
345	570	0.0	49.7	50.3

Results

A total of 324 bivalve specimens was collected, including 247 empty or disjointed valves and 77 live-collected organisms. The specimens were sorted into 16 species, 15 genera, and 12 families; five species were new observations in the Colombian Pacific. The known geographic range of several species has now been expanded to the Department of Nariño.

Below is included a listing of the species collected, station data, live-dead status for each specimen, remarks on new verified localities, previously reported distributions for the species, plus general remarks. We have also included an illustration for all newly documented species in Colombia i.e., those other than *Nucula iphigenia*, *Orthoyoldia panamensis* and *Delectopecten zacae* which were previously reported for the Pacific of Colombia by Gracia and Valentich-Scott (2014).

Systematics

Class BIVALVIA Linnaeus, 1758

Subclass PROTOBRANCHIA Pelseneer, 1889

Order NUCULIDA Dall, 1889

Superfamily NUCULOIDEA J.E. Gray, 1824

Family NUCULIDAE J.E. Gray, 1824

Genus *Ennucula* Iredale, 1931

Ennucula panamina (Dall, 1908)

Fig. 2

Examined material. 1 valve plus 1 live specimen EA 336 (1.9045°N, 79.3030°W) at 612 m (INV MOL9797, INV MOL9796), 1 live specimen EA344 (2.3905°N, 78.8288°W) at 656 m (INV MOL9796), plus 1 live specimen EA 335 (1.7499°N, 79.50177°W) at 866 m (INV MOL9799).

New location. Off Nariño, Colombian Pacific.

Distribution. Panama to Peru (Coan and Valentich-Scott 2012).

Remarks. New species record for the Colombian Pacific.

Genus *Nucula* Lamarck, 1799

Subgenus *Lamellinucula* Schenck, 1944

Nucula (Lamellinucula) iphigenia Dall, 1896

Examined material. 1 valve plus 2 live specimens EA331 (2.5078°N, 78.7993°W) at 350 m (INV MOL9794, INV MOL9795).



Figure 2–12. 2 Exterior and interior views of *Ennucula panamina* 3 *Jupiteria lobula* (total length = 4.2 mm) 4 *Neilonella* cf. *atossa* (total length = 5 mm) 5 *Malletia goniura* 6 *Limatula* cf. *saturna* 7 *Lucinoma heroica* 8 *Calyptogena* cf. *gallardoii* 9 *Caryorbula* sp. 10 *Cuspidaria panamensis* 11 *Dallicordia alaskana* 12 *Lyonsiella* cf. *magnifica*. Scale bars: 2, 5, 6, 9, 11 5 mm. 7, 8, 10, 12 10 mm.

New location. Off Nariño, Colombian Pacific.

Distribution. Panama to Peru (Coan and Valentich-Scott 2012), Choco-Colombia (Gracia and Valentich-Scott 2014).

Remarks. Previously encountered in Colombia in the Department of Choco at a depth of 300 m (Gracia and Valentich-Scott 2014).

Order NUCULANOIDA D.C. Carter & M.R. Campbell, 2000**Superfamily NUCULANOIDEA H. & A. Adams, 1858****Family NUCULANIDAE H & A. Adams, 1858****Genus *Jupiteria* Bellardi, 1875*****Jupiteria lobula* (Dall, 1890)**

Fig. 3

Examined material. 2 valves EA337 (1.7811°N, 79.0351°W) at 530 m (INV MOL9791), 2 valves EA331 (2.5078°N, 78.7993°W) at 350 m (INV MOL9792).

New location. Colombian Pacific.

Distribution. Mexico to El Salvador (Coan and Valentich-Scott 2012).

Remarks. These records represent a new southern limit for this species. All the specimens were small (approx. 4 mm), but they were nearly identical to small specimens of *Jupiteria lobula* from Mexico and also the type specimens. The presence of dead valves at different stations and the distance from previous records suggest that this species is living in Colombia.

Family NEILONELLIDAE Schileyko, 1989**Genus *Neilonella* Dall, 1881*****Neilonella* cf. *atossa* (Dall, 1908)**

Fig. 4

Examined Material. 2 valves EA337 (1.7811°N, 79.0351°W) at 530 m (INV MOL9793).

New location. Off Nariño, Colombian Pacific.

Remarks. The identity of this species cannot be confirmed without a detailed comparative examination of additional material. It is potentially a new species.

Family MALLETIIDAE H. & A. Adams, 1858**Genus *Malletia* des Moulins, 1832*****Malletia goniura* Dall, 1890**

Fig. 5

Examined material. 8 valves EA341 (2.1484°N, 78.9409°W) at 934 m (INV MOL9774), 7 live specimens EA341 at 934 m (INV MOL9775), 3 valves EA335 (1.7499°N, 79.5017°W) at 855 m (INV MOL9776), 6 live specimens EA335 at 866 m (INV MOL9777), 2 valves EA333 (1.6087°N, 79.3883°W) at 836 m (INV M9778), 4 live specimens EA333 at 836 m (INV MOL9779), 3 valves EA338 (1.9490°N, 79.0257°W) at 941 m (INV MOL9780).

New location. Off Nariño, Colombian Pacific.

Distribution. Panama to Peru (Coan and Valentich-Scott 2012).

Remarks. These specimens represent the shallowest bathymetric records so far for *Malletia goniura* (836–941 m). It has previously been collected in deeper waters (1,500–3,300 m depth) (Coan and Valentich-Scott 2012).

***Malletia tumaquensis* sp. n.**

<http://zoobank.org/5DB5C232-C972-4E67-914D-AECB44A92CD8>

Figs 17–20, 22

Description. Shell shape: Shell equivalve, subquadrate, moderately inflated, thin, gaping at ends, longer than high (length to height ratio 1:0.5), inequilateral, much longer posteriorly. Umbones moderate in size, located about one-third of shell length from anterior end. Lunule broad, shallow, weakly outlined, raised medially. Escutcheon absent. Anterodorsal margin angled ventrally from umbo; posterodorsal margin straight from umbo. Anterior end narrowly rounded, posterior end truncate. Strong radial keel extending from umbo to posterior margin, with deep radial sulcus immediately dorsal to it. Left valve with low radial undulations extending from near umbone to posteroventral margins, right valve with little or no undulation. Anteroventral and posteroventral region slightly undulate. Inner ventral margin smooth. Interior of valves smooth and porcelaneous.

Adductor muscle scar and pallial scars: Pallial line weakly impressed; pallial sinus broad, shallow. Adductor muscle scars subequal, subovate and moderate in size.

Sculpture and periostracum: Exterior sculpture of fine commarginal striae. Periostracum thin, adherent, glossy, pale yellow to light brown, often with commarginal color bands.

Hinge: Hinge with 2 distinct series of teeth without any separation between them; approx. 12 anterior teeth, larger than posterior teeth; approx. 39–52 posterior teeth. Ligament external, sunken, opisthodontic, narrow, dark brown, extending nearly 3/4 the length of posterodorsal margin.

Anatomy: Foot large, deeply cleft medially, wide at neck; labial palp and palp proboscis anterior; labial palp large, with 2 distinct regions with finer and heavier lamellae; palp proboscis very long, coiled.

Material Type. Holotype: INV MOL9782; paired valves with soft body, length 33.2 mm, height 16.4 mm, width 11.8 mm.

Paratypes. See Table 2 for measurements and length/height dimensions.

Type locality. Colombia, Nariño, off Tumaco Bay. St. EA337 (1.7811°N, 79.0351°W); depth 530 m. Collected November 2012.

Habitat. Soft bottom.

Additional (non-type) material. 75 valves EA337 at 530 m (INV MOL9781) plus 19 live specimens EA337 at 530 m (INV MOL9782).

Distribution. The species is currently only known only from the type locality.

Table 2. Measurements of type specimens of *Malletia tumaquensis* sp. n.

Specimen	Length (mm)	Height (mm)	Width (mm)	Length/height
Holotype INV MOL9782	33.2	16.4	11.8	2.0
Paratype 1 INV MOL1161	30.5	15.7	11.4	1.9
Paratype 1 INV MOL1161	32.7	16.6	11.1	1.9
Paratype 1 INV MOL1161	30.4	15.1	10.3	2.0
Paratype 2 INV MOL1162	28.7	14.2	9.7	2.0
Paratype 2 INV MOL1162	28.4	14.7	9.8	1.9
Paratype 2 INV MOL1162	26.5	14.3	9.0	1.8
Paratype 3 INV MOL1163	26.1	13.4	9.0	1.9
Paratype 3 INV MOL1163	24.9	13.1	9.0	1.9
Paratype 3 INV MOL1163	27.2	13.3	9.6	2.0

Etymology. This species is named in honor of the municipality of Tumaco, Nariño, where this study was conducted.

Differential diagnosis. *Malletia tumaquensis* sp. n. is similar in shape to *M. alata* Bernard, 1989. However, consistent differences exist in conchological features (i.e., *M. tumaquensis* is more elongate, while *M. alata* has an alate process) and anatomical characteristics (i.e., very long, thin palp proboscis in *M. tumaquensis*) makes it a readily distinguishable new species. Ecologically, *M. tumaquensis* has a shallower depth distribution (530 m) than that of *M. alata* (740 m, Coan and Valentich-Scott 2012). Table 3 summarizes the shell characteristics of all the *Malletia* species recorded in the eastern Pacific Ocean.

Remarks. Members of the family Malletiidae occur throughout the Pacific and Atlantic Oceans with most records from deep-water (Coan and Valentich-Scott 2012, Kamenev 2015). *Malletia tumaquensis* is distinguished from the seven other species occurring in tropical west America by its more subquadrate and longer shell. Including our record, this represents the third species of the genus reported for the Colombian Pacific (i.e., *M. tumaquensis*, *M. truncata* and *M. goniura*).

Family YOLDIIDAE Dall, 1908

Subfamily YOLDIINAE Dall, 1908

Genus *Orthoyoldia* Verrill & Bush, 1897

Orthoyoldia panamensis (Dall, 1908)

Examined material. 10 valves EA344 (2.3905°N, 78.8288°W) at 656 m (INV MOL9812), 6 live specimens EA344 at 656 m (INV MOL9813), 5 valves EA337 (1.7811°N, 79.035139° W) at 530 m (INV MOL9814), 4 valves EA337 at 530 m (INV MOL9815), 4 live specimens EA332 (1.6677°N, 79.1826°W) at 730 m (INV

MOL9816), 2 live specimens EA345 (2.5557°N, 79.0476°W) at 668 m (INV MOL9817).

New location. Off Nariño, Colombian Pacific.

Distribution. Mexico to Peru (Coan and Valentich-Scott 2012), Choco-Colombia (Gracia and Valentich-Scott 2014).

Table 3. Summary of shell characters of *Malletia* species from the Pacific Ocean (after Coan and Valentich-Scott 2012).

Species	Shape	Type locality	Reported depth range (m)	Maximum Length (mm)	Posterior end	Hinge
<i>Malletia alata</i> F. R. Bernard, 1989		San Diego Trough, California, USA	1200	30	Straight, forming alate process	About 11–13 anterior teeth; about 45 posterior teeth
<i>Malletia arciformis</i> Dall, 1908		Off Acapulco, Guerrero, Mexico	902	11	Broadly flared, rounded	10–13 anterior teeth; 13–17 posterior teeth
<i>Malletia benthima</i> Dall, 1908		Off Acapulco, Guerrero, Mexico	902	10	Produced, broadly rounded	12–13 anterior teeth; 13–17 posterior teeth
<i>Malletia faba</i> Dall, 1897		Off Queen Charlotte Islands, British Columbia, Canada	200–1600	10	Broadly rounded	About 9 anterior teeth; about 32 posterior teeth
<i>Malletia goniura</i> Dall, 1890		Gulf of Panama, Panama	1500–3300	13	Flaring dorsally, truncate	14–19 anterior teeth; 27–30 posterior teeth
<i>Malletia peruviana</i> Dall, 1908		Off Punta Aguja, Piura, Peru	1900	28	Broadly rounded	10–11 anterior teeth; 33–36 posterior teeth
<i>Malletia truncata</i> Dall, 1908		Cascadia Plain, Oregon, USA	2700–4134	30	Flaring, compressed, truncate	About 18 anterior teeth; about 30 posterior teeth
<i>Malletia tumaquensis</i> sp. n.		Off Tumaco Bay, Nariño, Colombia	530	33	Truncate	12 anterior teeth; 39–52 posterior teeth

Remarks. *Orthoyoldia panamensis* has previously been collected in depths from 120 to 475 m in Colombia (Gracia and Valentich-Scott 2014). This study extends the bathymetric range to 730 m in the Colombian Pacific.

Order PECTINIDA J.E. Gray, 1854
Superfamily PECTINOIDEA Rafinesque, 1815
Family PECTINIDAE Rafinesque, 1815
Genus *Delectopecten* Stewart, 1930

Delectopecten zaca (Hertlein, 1935)

Examined material. 106 valves EA345 (2.5557°N, 79.0476°W) at 668 m (INV MOL9800).

New location. Off Nariño, Colombian Pacific.

Distribution. Mexico to Peru (Coan and Valentich-Scott 2012). Choco, Colombia (Gracia and Valentich-Scott 2014).

Remarks. No live *Delectopecten zaca* specimens were collected during the present study. In northern Colombia (Choco), both live specimens and empty valves were found. The present finding extends the bathymetric range of this species to 668 m in the Colombian Pacific.

Order LIMIDA Moore, 1952
Superfamily LIMOIDEA Rafinesque, 1815
Family LIMIDAE Rafinesque, 1815
Genus *Limatula* Wood, 1839
Subgenus *Limatula* s.s. Wood, 1839

Limatula saturna F.R. Bernard, 1978

Fig. 6

Examined material. 2 live specimens EA335 (1.7499°N, 79.5017°W) at 866 m (INV MOL9772).

New location. Colombian Pacific.

Distribution. U.S.A. to Mexico (Coan and Valentich-Scott 2012).

Remarks. *Limatula saturna* has been documented from Alaska to northern Mexico from 20–675 m (Coan and Valentich-Scott 2012). The Colombian specimens represent the first record for South America. Very recently (i.e., March 2018), this species has been observed in the region of Lambayeque, Peru (Valentich-Scott, pers. obs.).

Superorder HETEROCONCHIA J.E. Gray, 1854

Clade HETERODONTA Neumayr, 1884

Order LUCINIDA J.E. Gray, 1854

Superfamily LUCINOIDEA Fleming, 1828

Family LUCINIDAE Fleming, 1828

Genus *Lucinoma* Dall, 1901

Lucinoma heroica (Dall, 1901)

Fig. 7

Examined material. 3 valves EA345 (2.5557°N, 79.0476°W) at 668 m (INV MOL9773).

New location. Colombian Pacific.

Distribution. Mexico to Peru (Coan and Valentich-Scott 2012).

Remarks. *Lucinoma heroica* has previously been found in depths greater than 1,838 m (Coan and Valentich-Scott 2012). At 668 m, the Colombian specimens are the shallowest record for the species.

Order VENERIDA J.E. Gray, 1854

Superfamily GLOSSOIDEA J.E. Gray, 1847

Family VESICOMYIDAE Dall & Simpson, 1901

Genus *Calyptogena* Dall, 1891

Calyptogena cf. *gallardoii* Sellanes & Krylova, 2005

Fig. 8

Examined material. 1 valve EA345 (2.5557°N, 79.0476°W) at 668 m (INV MOL9805).

New location. Off Nariño, Colombian Pacific.

Distribution. South-central Chile, off Bahía de Concepción (Sellanes and Krylova 2005).

Remarks. The single valve collected is insufficient to allow a definitive identification to species. The shape and dentition place it closest to *Calyptogena gallardoii*.

Genus *Pliocardia* Woodring, 1925

“Pliocardia” cf. *donacia* (Dall, 1908)

Examined material. 1 valve EA337 (1.7811°N, 79.0351°W) at 530 m (INV MOL9768), 1 valve EA336 (1.9045°N, 79.3030°W) at 612 m (INV MOL9770), 1 live specimen EA344 (2.3905°N, 78.8288°W) at 656 m (INV MOL9771).

New location. Off Nariño, Colombian Pacific.

Distribution. Panama (Coan and Valentich-Scott 2012), Choco, Colombia (Gracia and Valentich-Scott 2014).

Remarks. Prior to this study, dead shells of *Pliocardia donacia* were identified in Pacific Colombia at depths between 272 and 295 m (Gracia and Valentich-Scott 2014). The present collection in southern Colombia yielded one live specimen and two empty valves, suggesting that the species inhabits both the northern and southern Colombian Pacific. Further, the bathymetric limit of the species is extended to 656 m in the Colombian Pacific. Many generic uncertainties exist within the family Vesicomidae. Thus, we follow Coan and Valentich-Scott (2012) in their tentative placement of *P. donacia* within the genus *Pliocardia*.

Order MYOIDA Goldfuss, 1820

Suborder MYINA Goldfuss, 1820

Superfamily MYOIDEA Lamarck, 1809

Family CORBULIDAE Lamarck, 1818

Genus *Caryocorbula* J. Gardner, 1926

***Caryocorbula* sp.**

Fig. 9

Examined material. 1 valve EA331 (2.5078°N, 78.7993°W) at 350 m (INV MOL9763).

New location. Off Nariño, Colombian Pacific.

Remarks. This single valve from station EA331 is similar to several Panamic and Peru-Chile Province species of *Caryocorbula*, but it is insufficient to allow a definitive identification to species.

Suborder SEPTIBRANCHIA Pelseneer, 1988

Superfamily CUSPIDARIOIDEA Dall, 1886

Family CUSPIDARIIDAE Dall, 1886

Genus *Cuspidaria* Nardo, 1840

***Cuspidaria panamensis* Dall, 1908**

Fig. 10

Examined material. 1 valve EA332 (1.6677°N, 79.1826°W) at 730 m (INV MOL9764), 7 valves EA345 (2.5557°N, 79.0476°W) at 668 m (INV MOL9765), 1 live specimen EA345 at 668 m (INV MOL9766), 4 live specimens A336 (1.9045°N, 79.3030°W) at 612 m (INV MOL9767).

New location. Off Nariño, Colombian Pacific.

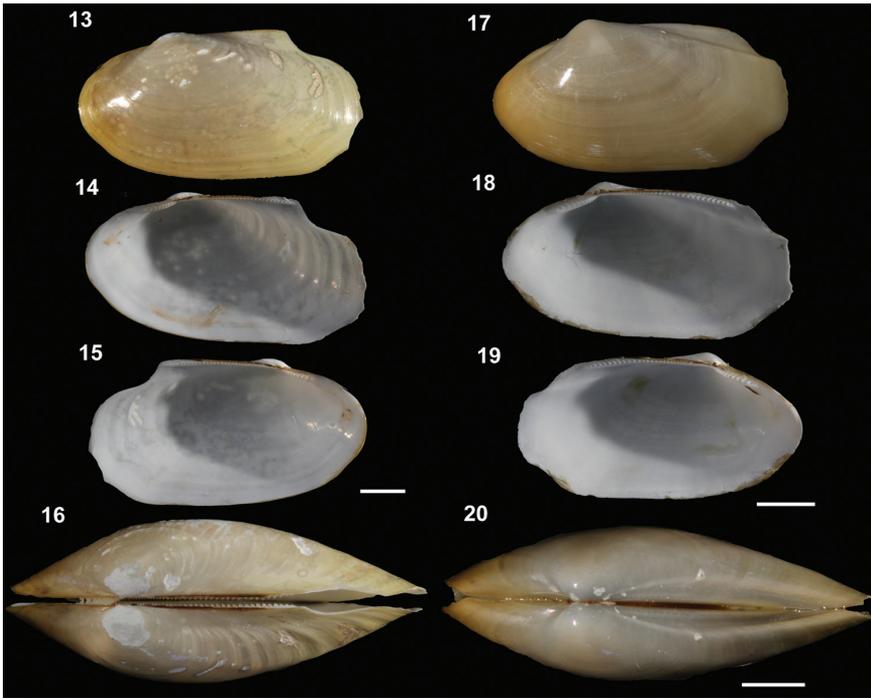


Figure 13–20. 13–16 *Malletia alata* (Holotype LACM 2343) 13 External view 14–15 Internal view of the shell, right and left respectively 16 Dorsal view (total length = 30 mm) 17–20 *Malletia tumaquensis* sp. n. 17 External view 18–19 Internal view of the shell, right and left valves, respectively 20 Dorsal view. Scale bars: 0.5 mm.

Distribution. Panama (Coan & Valentich-Scott 2012).

Remarks. *Cuspidaria panamensis* was previously known only been known from the type locality in the Gulf of Panama (Coan and Valentich-Scott 2012). Our records extend the distribution over 600 km to the south. Coan and Valentich-Scott (2012) indicate a maximum size of 41 mm for *Cuspidaria panamensis*. However, our material from station EA345 increases the maximum length to 44.2 mm.

Superfamily VERTICORDIOIDEA Stoliczka, 1870

Family VERTICORDIIDAE Stoliczka, 1870

Subfamily LYONSIELLINAE Dall, 1895

Genus *Dallicordia* Scarlato & Starobogatov, 1983

Dallicordia alaskana (Dall, 1895)

Fig. 11

Examined material. 10 valves EA337 (1.7811°N, 79.0351°W) at 530 m (INV MOL9802), 14 live specimens EA337 at 530 m (INV MOL9803).

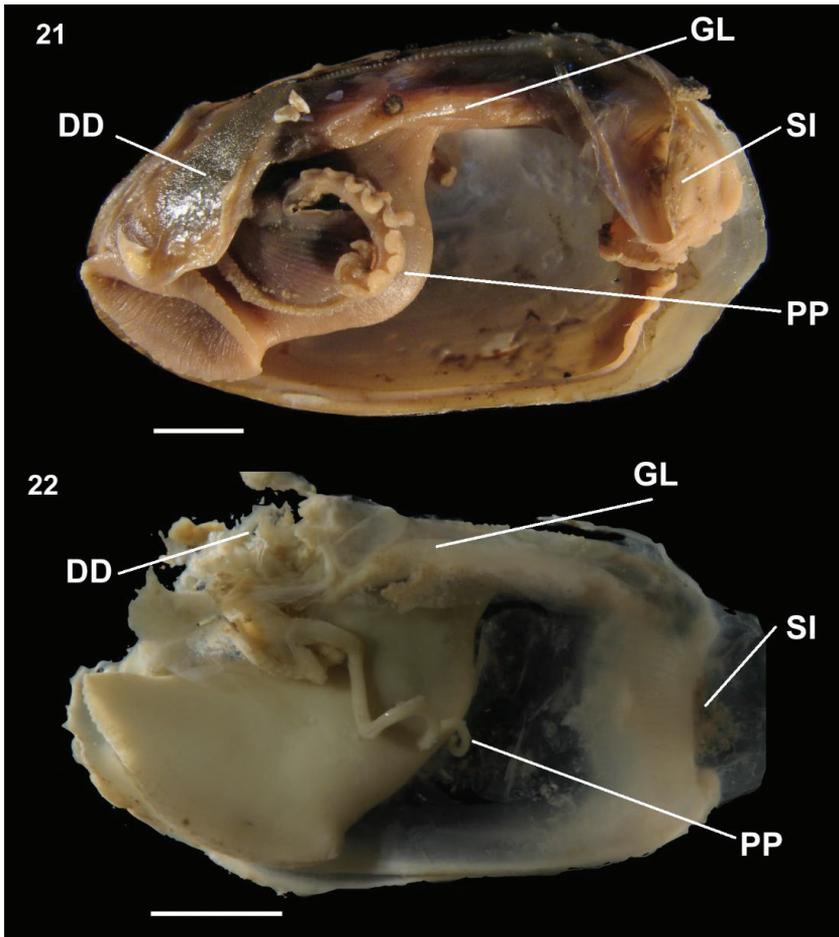


Figure 21–22. 21 *Malletia alata* (EMU7054) Gulf of California, Mexico, lateral view of anatomy 22 *Malletia tumaquensis* sp. n. Lateral view of anatomy. Scale bars: 0.5 mm. Abbreviations: **DD** Digestive diverticula, **FT** Foot, **GL** Gill, **SI** Siphon, **PP** Palp proboscis.

New location. Off Nariño, Colombian Pacific.

Distribution. Sitka, Alaska, to Tumbes, Peru (Coan and Valentich-Scott 2012).

Remarks. This material represents a new record for the Colombian Pacific.

Genus *Lyonsiella* G. O. Sars, 1872

Lyonsiella cf. *magnifica* Dall, 1913

Fig. 12

Examined material. 1 live specimen EA345 (2.5557°N, 79.0476°W) at 668 m (INV MOL9804).

New location. Off Nariño, Colombian Pacific.

Distribution. Mexico to Panama (Coan and Valentich-Scott 2012).

Remarks. Colombian material resembles the type material of *Lyonsiella magnifica*. However, our specimens have more prominent umbones, a more truncate anterior end, and a more obliquely truncate posterior end when compared to the type material. Many additional specimens would be necessary to determine if our single specimen falls within the range of intraspecific variation for *Lyonsiella magnifica* or it represents a new species.

Discussion

The new species of Mallettiidae herein described brings to eight the number of known species for this family in the eastern Pacific Ocean (Table 3). *Malletia* is a widely distributed genus that is associated mainly with deep water and soft sediments (Coan and Valentich-Scott 2012). Previously reported from Colombia were *M. goniura* Dall, 1890 and *M. truncata* Dall, 1908 (type locality, Malpelo Island, at 3,334 m).

As was true of the previous study in the northern Colombian Pacific (Gracia and Valentich-Scott, 2014), these recent collections not only expand the geographic distributions of many species on the Colombian continental margin, but they also represent new collection locations. This serves as potential evidence for the species actually living in the area, rather than the transport of dead shells into the region. Further, our findings have significantly expanded the bathymetric limits of several species. One new species has been described, indicating that this region of Colombia is still relatively unexplored. Further surveys are necessary to complement this malacological inventory and to clarify the taxonomic identity of several species. These are important preliminary steps for to assist in investigating the impacts of anthropogenic practices and changes (e.g., deep-sea trawling, pollution).

Deep-sea baseline surveys seek to expand bivalve records for the Colombian Pacific Ocean. In 2014, Gracia and Valentich-Scott reported on specimens collected in the northern Colombian Pacific; 89.5% of the identified species represented new records for the region. The present survey used a similar methodology but was conducted in the southern Colombian Pacific. The number of bivalve species we encountered in the southern Colombian Pacific (16) was far lower than that for the northern Colombian Pacific. This could possibly be due to the different depths sampled in either survey, or possibly the decreasing diversity associated with increasing depth.

The transport of sediment caused by river discharge, marine currents, and other factors stimulate the resuspension of material on soft sediments (Segall et al. 1989). In the northern Colombian Pacific there is a greater influence of the equatorial counter-current and the Panama Current, and the discharge from the Baudó River, while the southern Colombia Pacific (where Tumaco Bay is located) sees the influence of cold continental waters (CCCP 2002). All of these processes in Pacific Colombian result in a dense mixture of water and sediment that moves along the bottom of the sea and transports plant waste material. In both the northern (Choco) and the southern (Nari-

ño) zones, a great abundance of sunken wood was encountered, indicating similar conditions influenced by terrestrial deposits.

Characteristics of sediments, currents, organic matter, availability of oxygen and many others factors could influence the composition, abundance, and occurrence of the benthic fauna. It should be noted that collections made in deep water in both northern and southern Pacific Colombia have yielded only a limited number of living bivalves and those that were numerically dominant were empty shells.

In conclusion, this paper serves as a contribution to our understanding of marine bivalves in deep waters of the southern Colombian Pacific. Our results reveal the importance of continued deep-sea research cruises in Colombia and subsequent taxonomic analysis of the specimens collected.

Acknowledgments

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Four species of spider genus *Cheiracanthium* C. L. Koch, 1839 (Araneae, Eutichuridae) from Jinggang Mountains, Jiangxi Province, China

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Abstract

Four species of spider genus *Cheiracanthium* C. L. Koch, 1839 are reported from Jinggang Mountains, Jiangxi Province, China. Two of them are described as new to science: *C. auriculatum* **sp. n.** (♀♂) and *C. echinulatum* **sp. n.** (♂). *Cheiracanthium taiwanicum* Chen, Huang, Chen & Wang, 2006 is recorded from Mainland China for the first time. *Cheiracanthium zhejiangense* Hu & Song, 1982, the most similar species to *C. auriculatum* **sp. n.**, is a newly recorded species of Jiangxi Province. Detailed descriptions, diagnoses, and photographs of the two new species are given. *Cheiracanthium taiwanicum* and *C. zhejiangense* are also illustrated.

Keywords

Taxonomy, morphology, description, new species

Introduction

Cheiracanthium C. L. Koch, 1839 contains 210 catalogued species and is mainly distributed in the Old World (Marusik and Fomichev 2016; World Spider Catalogue 2018). Although this genus is relatively large and well known, its taxonomy is rather poorly studied. Almost half of its species are known from single sex or juveniles: 36 by males, 60 by females, 2 by juveniles (World Spider Catalogue 2018). Additionally, 16 species were never illustrated and many species were described based on poor illustrations. So far, the genus has not been the subject of any global or regional revisions (Marusik and Fomichev, 2016).

The *Cheiracanthium* fauna of China is relatively rare and poorly represented, with only 38 described species (Li and Lin 2016; World Spider Catalogue 2018), of which 14 species are known based on a single sex: for 11, only females are known, and for three, only males are known (World Spider Catalogue 2018). Additionally, illustrations of the internal structure of the epigyne are not provided in five species (World Spider Catalogue 2018). Moreover, the diversity of this genus in China is still insufficiently known and several new species have been described in the last few years (Chen and Huang 2012; Barrion et al. 2013; Wang and Zhang 2013).

Field collection in Jinggang Mountains of Jiangxi province, China, was carried out in April 2011. During this field exploration, four *Cheiracanthium* species were found: *C. auriculatum* sp. n., *C. echinulatum* sp. n., *C. taiwanicum* Chen, Huang, Chen & Wang, 2006 and *C. zhejiangense* Hu & Song, 1982. Descriptions and photographs of the new species, as well as supplementary micrographs of the known species, are provided.

Materials and methods

Spiders were fixed and preserved in 80% ethanol. Specimens were examined with an Olympus SZX7 stereomicroscope; details were studied with an Olympus BX51 compound microscope. Male palps and female epigynes were examined and illustrated after being dissected. Epigynes were cleared in boiling KOH solution to dissolve soft tissues. Photographs were made with a Leica DFC450 digital camera mounted on an Olympus BX51 compound microscope. The digital images were taken and assembled using Helicon Focus 3.10 software package.

All measurements were obtained using an Olympus SZX7 stereomicroscope and given in millimetres. Eye diameters are taken at the widest point. The total body length does not include chelicerae or spinnerets length. Leg lengths are given as total length (femur, patella, tibia, metatarsus, tarsus). The type specimens of the new species are deposited in College of Chemistry and Life Sciences, Guizhou Education University, Guiyang, Guizhou, China.

Abbreviations used are:

A	atrium;	FD	fertilisation duct;
AER	anterior eye row;	LL	total length of leg I;
AL	abdomen length	LL:CL	leg I / carapace length;
ALE	anterior lateral eyes;	MOQ	median ocular quadrangle;
AME	anterior median eyes;	MOQA	MOQ anterior width;
AME–AME	distance between AMEs;	MOQP	MOQ posterior width;
AME–ALE	distance between AME and ALE;	OAL	ocular area length;
AW	abdomen width;	OAW	ocular area width;
C	conductor;	PER	posterior eye row;
CD	copulatory duct;	PLE	posterior lateral eyes;
CF	cymbial fold;	PME	posterior median eyes;
CI	carapace index;	PME–PME	distance between PMEs;
CL	carapace length;	PME–PLE	distance between PME and PLE;
CLL	clypeal length;	PTA	prolateral tibial apophysis;
CO	copulatory opening;	RTA	retrolateral tibial apophy- sis;
CS	cymbial spur;	R	receptacle;
CW	carapace width;	STL	sternum length;
DTA	dorsal tibial apophysis;	STW	sternum width;
E	embolus;	TA	tegular apophysis;
EB	embolic base;		
TL	total body length.		

Leg setae: v, ventral; p, prolateral; r, retrolateral. Most of the terminologies used in text and figure legends followed Lotz (2015), while a few others followed Marusik and Fomichev (2016) and Morano and Bonal (2016).

Taxonomy

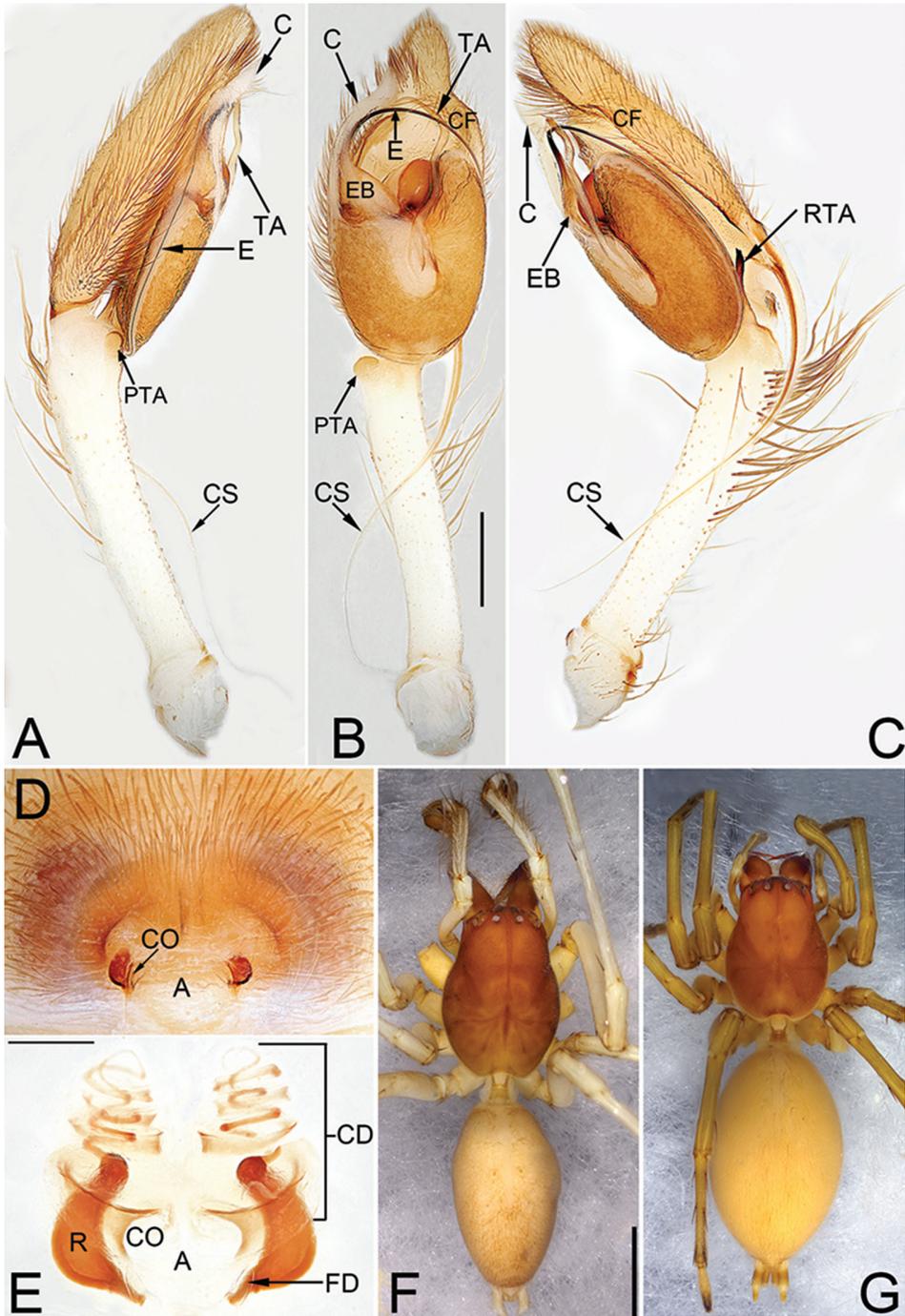
Genus *Cheiracanthium* C. L. Koch, 1839

***Cheiracanthium auriculatum* sp. n.**

<http://zoobank.org/45045AEA-20E9-4C6E-8BBD-F104775A8E6B>

Figs 1, 5

Type material. Holotype ♂ (SYSU-JX-11-177): China, Jiangxi Province, Jinggang Mountains Nature Reserve, Xiangzhou village (380 m; 26°35'30.23"N,



Figures 1. *Cheiracanthium auriculatum* sp. n., male holotype and female allotype. **A** left palp, prolateral view **B** same, ventral view **C** same, retrolateral view **D** epigyne, ventral view **E** vulva, dorsal view **F** male habitus, dorsal view **G** female habitus, dorsal view. Scale bars: 0.5 mm (**A–C**); 0.2 mm (**D–E**); 2 mm (**F–G**).

114°15'59.93"E), 26 April 2011, Hao Yu and Zhenyu Jin leg. Paratypes: 1♂ and 3♀, same data as holotype.

Etymology. The specific epithet is an adjective and is derived from a Latin word “*auriculatus*” (ear-like), referring to the tegular apophysis which is like the contour of an ear in ventral view.

Diagnosis. *Cheiracanthium auriculatum* sp. n. is distinguished from all other *Cheiracanthium* species, except *C. zhejiangense* Hu & Song, 1982 (Fig. 4A–E), by having a distally filiform cymbial spur in the male, and by the general shape of the vulva in the female. From *C. zhejiangense*, the male can be distinguished by the ear contour-shaped tegular apophysis and the uncoiling tip of cymbial spur (*vs* the falciform tegular apophysis and the coiled tip of the cymbial spur in *C. zhejiangense*) (Figs 1A–C; 4A–C), the female can be differentiated by the indistinct atrium and copulatory ducts (*vs* the distinct atrium and copulatory ducts in *C. zhejiangense*), the more or less lengthwise receptacles (*vs* the nearly horizontal receptacles in *C. zhejiangense*) (Figs 1D–E; 4D–E), and by the different coil number of copulatory ducts (7 coils in *C. auriculatum* sp. n., instead of 8 coils in *C. zhejiangense*) (Figs 1E; 4E). In addition, the two species can be separated by their habitus: abdomen without distinct colour pattern in *C. auriculatum* sp. n. (Fig. 1F–G), but with a median heart-shaped mark which reaches half of the opisthosoma length in *C. zhejiangense* (Fig. 4F–G).

Description. Male. Total length 8.58–9.15. Holotype (Fig. 1A–C, F): TL 9.15; CL 3.73, CW 2.41, CI (CL/CW) 1.55; AL 4.05, AW 2.42. *Carapace* (Fig. 1F) brown, uniformly coloured, without distinct pattern. Eye sizes and inter-distances: OAL 0.39, OAW 1.45; AME 0.14, ALE 0.16, PME 0.16, PLE 0.17; AME–AME 0.27, AME–ALE 0.27, PME–PME 0.36, PME–PLE 0.35; MOQA 0.56, MOQP 0.67, CLL 0.10. *Chelicerae* protruding and reddish brown, with 3 teeth on promargin and 3 on retromargin respectively. *Sternum* dark brown, STL 1.69, STW 1.44. Labium and endites brown. *Legs* yellowish-white, without distinct colour markings. Leg measurements: I 18.84 (4.65, 1.22, 5.10, 5.60, 2.26), II 12.09 (3.30, 1.14, 3.24, 3.23, 1.19), III 8.61 (2.38, 0.86, 1.92, 2.40, 1.05), IV 12.47 (3.72, 0.95, 3.17, 4.00, 1.07); LL:CL 5.03. Leg spines: I 0-0-1p, 2v-1v-1p, 2v1p-1p1v-1v; II 0-0-1p, 1v-2v-1p, 2v1p-1v1p-1v; III 0-0-1p1r, 0-1p1r-0, 2v1p-1p1r-1v2p2r; IV 0-0-1p1r, 1v-1v1p-0, 2v1p-1v1p1r-1v2p2r. *Abdomen* (Fig. 1F) elongate-oval, dorsally grey, dorsum with indistinct heart-shaped mark and two pairs of not obvious muscle depressions; venter brownish without distinct pattern.

Palp (Fig. 1A–C). Tibia extremely long, about as long as cymbium, with two apophyses; retrolateral tibial apophysis (RTA) about 20% of tibia length, with a more or less bifurcate apex and hiding behind tegulum; prolateral tibial apophysis (PTA) small and round; cymbial spur (CS) is approximately equal in length to tibia, tapering off into a filiform; cymbial fold (CF) poorly developed, for approximately 4/5 the length of cymbium; tip of cymbium short, about 1/4 of cymbium length. Tegulum oblong, 1.3 times longer than wide; tegular apophysis (TA) long and sinuate, more than 4/5 of tegulum length, filamentous and like an ear’s contour in ventral view; embolus (E) arising at approximately 10 o’clock position, terminating at approximately 11 o’clock position, it’s tip covered by conductor (C); conductor large, falciform.

Female. Total length 8.66–9.30. Slightly larger in size and lighter in colour. Allotype (Fig. 1D–E, G) measured: TL 9.30; CL = 3.03, CW = 2.22, CI (CL/CW) = 1.36; AL = 4.95, AW = 2.92. Eye diameters and inter-distances: OAL 0.37, OAW 1.23; AME 0.14, ALE 0.19, PME 0.13, PLE 0.14; AME–AME 0.23, AME–ALE 0.11, PME–PME 0.31, PME–PLE 0.23; MOQA 0.46, MOQP 0.58, CLL 0.24. PMT: RMT = 6:6.STL 1.47, STW 1.23. Leg measurements: I 12.70 (3.30, 1.06, 3.42, 3.39, 1.54), II 8.51 (2.42, 0.86, 2.23, 2.05, 0.96), III 6.42 (1.92, 0.69, 1.35, 1.65, 0.80), IV 9.67 (2.75, 0.89, 2.39, 2.68, 0.96); LL:CL 4.19. Leg spines: I 0-1p-1p, 2v-2v-0, 2v-1p1r-1v; II 0-0-1p, 1v-2v-1p, 2v1p-1p1r-1v; III 0-1p-1p1r, 1v-1p1r-0, 2v1p1r-1p1r-1v2p2r; IV 0-0-1p1r, 1v-1v1p1r-0, 2v1p1r-1v1p1r-1v2p2r.

Epigyne (Fig. 1D–E). Atrium (A) indistinct, without delimited margin, about four times wider than long; receptacles (R) are faintly visible through epigynal plate in ventral view; two copulatory openings (CO) located at lateral borders of atrium; the transparent copulatory ducts (CD) running spirally (length of spira about 1.4 times longer than receptacles), forming 7 entwined loops (including 4 ascending coils and 3 descending coils); receptacle sickle-shaped, separated by three diameters.

Distribution. Presently known only from the type locality, Jinggang Mountains, Jiangxi, China (Fig. 5).

***Cheiracanthium echinulatum* sp. n.**

<http://zoobank.org/A1935AC9-A0A9-45F2-8BFA-22E0F3172401>

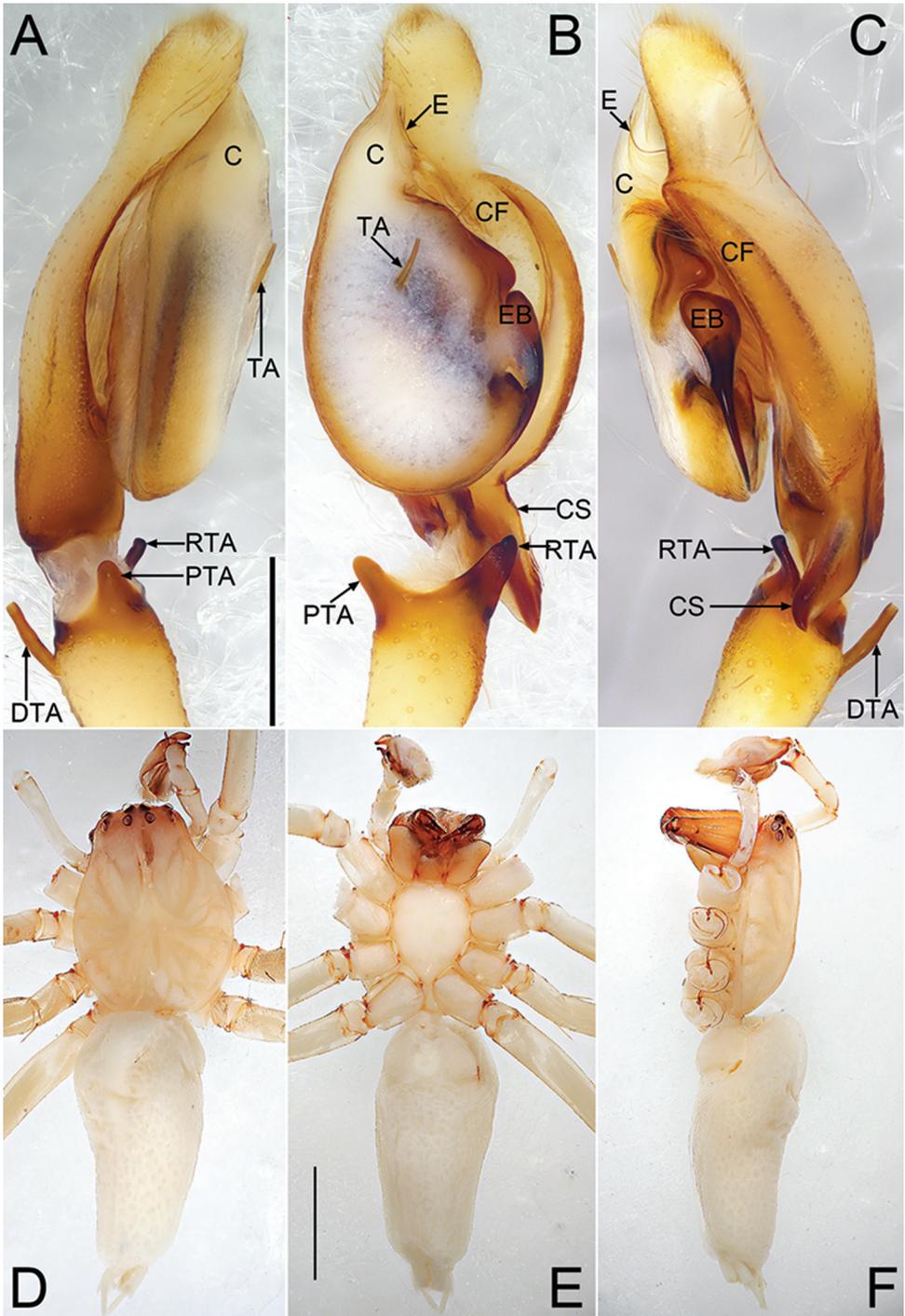
Figs 2, 5

Type material. Holotype ♂ (SYSU-JX-11-182): China, Jiangxi Province, Jinggang Mountains Nature Reserve, Xiangzhou village (380 m; 26°35'30.23"N, 114°15'59.93"E), 26 April 2011, Hao Yu and Zhenyu Jin leg. Paratypes: 1♂, same data as holotype.

Etymology. The species epithet is taken from the Latin adjective *echinulatus* and refers to the spinule-shaped tegular apophysis.

Diagnosis. This new species resembles *C. taegense* Paik, 1990 (Paik, 1990: 11, f. 39–47; Baba & Yoshitake, 2016: 39, f. 1–4) in having the similar beak-shaped cymbial spur, and stalk-like dorsal tibial apophysis, but can be distinguished by: (1) the embolus originated at 3 o'clock position (Fig. 2B–C), *vs.* originated at 1–2 o'clock position in *C. taegense* (Paik, 1990: 11, f. 41; Baba & Yoshitake, 2016: 39, f. 3–4); (2) tegular apophysis smaller, straight and acicular (Fig. 2A–C), instead of bigger and slightly curved in *C. taegense* (Paik, 1990: 11, f. 41, 43; Baba & Yoshitake, 2016: 39, f. 3–4); (3) RTA straight and digitiform (Fig. 2A–C), but with a curved and hook-shaped apex in *C. taegense* (Baba & Yoshitake, 2016: 39, f. 3–4).

Description. Male. Total length 9.06–9.12. Holotype (Fig. 2): TL 9.06; CL 3.58, CW 2.34, CI (CL/CW) 1.53; AL 4.99, AW 2.27. *Carapace* (Fig. 2D, F) yellow except reddish ocular area, without distinct colour pattern. Eye sizes and inter-distances: OAL 0.34, OAW 1.26; AME 0.15, ALE 0.15, PME 0.14, PLE 0.13; AME–AME 0.46,



Figures 2. *Cheiracanthium echinulatum* sp. n., male holotype. **A** left palp, prolateral view **B** same, ventral view **C** same, retrolateral view **D** male habitus, dorsal view **E** same, ventral view **F** same, lateral view. Scale bars: 0.5 mm (**A-C**); 2 mm (**D-F**).

AME–ALE 0.25, PME–PME 0.54, PME–PLE 0.22; MOQA 0.43, MOQP 0.55, CLL 0.13. *Chelicerae* light brown and robust, with long and wine-coloured fangs, with 3 teeth on promargin and 3 on retromargin respectively. *Sternum* (Fig. 2E) yellowish, STL 1.79, STW 1.32. Labium and endites brown. *Legs* yellowish, without distinct colour markings. Leg measurements: I 23.70 (5.90, 1.05, 7.31, 7.99, 1.46), II 14.92 (3.41, 0.82, 4.14, 5.26, 1.29), III 11.10 (2.53, 1.43, 2.16, 3.94, 1.03), IV 16.42 (4.18, 1.21, 4.13, 5.66, 1.24); LL:CL 6.62. Leg spines: I 0-1p1r-1p1r, 3v-3v-1v1p, 2v-0-1v; II 0-1p1r-1p1r, 3v-2v-1v1p, 2v1p-2v1p-1v; III 0-1p1r-1p1r, 2v1p1r-1p1r-0, 2v1p1r-2v1p1r-2v1p2r; IV 0-1p1r-1p1r, 1v1p1r-1v2r-1v1r, 2v1p1r-2v1p1r-1v1p3r. *Abdomen* (Fig. 2D–F) lanceolate, dorsally yellowish white, scattered numerous indistinct pigmented spots; venter yellowish without distinct pattern.

Palp (Fig. 2A–C). Tibia twice shorter than cymbium, with three apophyses; retrolateral tibial apophysis (RTA) about 50% of tibia length, heavily sclerotised and with a fingerlike apex; prolateral tibial apophysis (PTA) distinctly elevated and relatively short, about 30% of tibia length, coniform in prolateral view and digitiform in ventral view; dorsal tibial apophysis (DTA) thin and stalk-shaped, about as long as RTA; cymbial spur (CS) beak-shaped, twice shorter than tibia; cymbial fold (CF) strongly developed and well visible in ventral and retrolateral view, for approximately 2/3 the length of cymbium; tip of cymbium long, about 1/3 of cymbium length. Tegulum 1.3 longer than wide, membranous and semitransparent except its margin in ventral view; tegular apophysis (TA) short and thin, spiculate; embolus (E) starts on the retrolateral flank (approximately 3 o'clock of tegulum), surrounds the base and ends at conductor (C) apex, its tip filiform and curved behind conductor; conductor large and membranous.

Female. Unknown.

Comments. According to the World Spider Catalogue 2018, a total of 11 *Cheiracanthium* species are known from females only in China: *C. approximatum* O. P. Cambridge, 1885, *C. escaladae* Barrion et al., 2013, *C. fujianense* Gong, 1983, *C. hypocyrtum* Zhang & Zhu, 1993, *C. liuyangense* Xie et al., 1996, *C. olliforme* Zhang & Zhu, 1993, *C. potanini* Schenkel, 1963, *C. rupicola* (Thorell, 1897), *C. solidum* Zhang et al., 1993, *C. sphaericum* Zhang et al., 1993, *C. longtailen* Xu, 1993. Among them, *C. escaladae* is supposedly a *Clubiona* species based on epigyne morphology, while *C. potanini* is supposedly doubtful because of the poor original illustrations and description. The other nine can be considered tentatively as valid *Cheiracanthium* species. However, none of them could be matched with *C. echinulatum* sp. n. due to their different habitus (abdomen without distinct colour pattern in *C. echinulatum* sp. n., but with a median heart-shaped mark in *C. approximatum*, *C. fujianense* and *C. rupicola*, with two pairs of muscular depressions in *C. hypocyrtum*, *C. liuyangense* and *C. sphaericum*) and different number of cheliceral teeth (chelicerae with 3 promarginal and 3 retromarginal teeth in *C. echinulatum* sp. n., but with 3 promarginal and 2 retromarginal teeth in *C. hypocyrtum*, *C. olliforme* and *C. sphaericum*, with 3 promarginal and 1 retromarginal teeth in *C. liuyangense*, with 2 promarginal and 1 retromarginal teeth in *C. solidum*, with 2 promarginal and 3 retromarginal teeth in *C. longtailen*).

Distribution. Presently known only from the type locality, Jinggang Mountains, Jiangxi, China (Fig. 5).

***Cheiracanthium taiwanicum* Chen, Huang, Chen & Wang, 2006**

Figs 3, 5

Cheiracanthium taiwanicum Chen et al., 2006: 10, fig. 1A–E; Chen and Huang 2012: 25, fig. 7A–G, pl. 2C–D, 3A–B.

Examined material. 1♂ and 1♀, China, Jiangxi Province, Jinggang Mountains Nature Reserve, Hexilong village (680 m; 26°31'51.54"N, 114°8'46.02"E), 30 April 2011, Hao Yu leg.

Description. *Male* and *female* (Fig. 3). For details see Chen and Huang (2012).

Distribution. Jinggang Mountains in Jiangxi and Nantou County in Taiwan, China.

***Cheiracanthium zhejiangense* Hu & Song, 1982**

Figs 4–5

Cheiracanthium zhejiangense Hu & Song, 1982: 56, fig. 4A–D.

Cheiracanthium zhejiangense Paik 1990: 9, fig. 26–38.

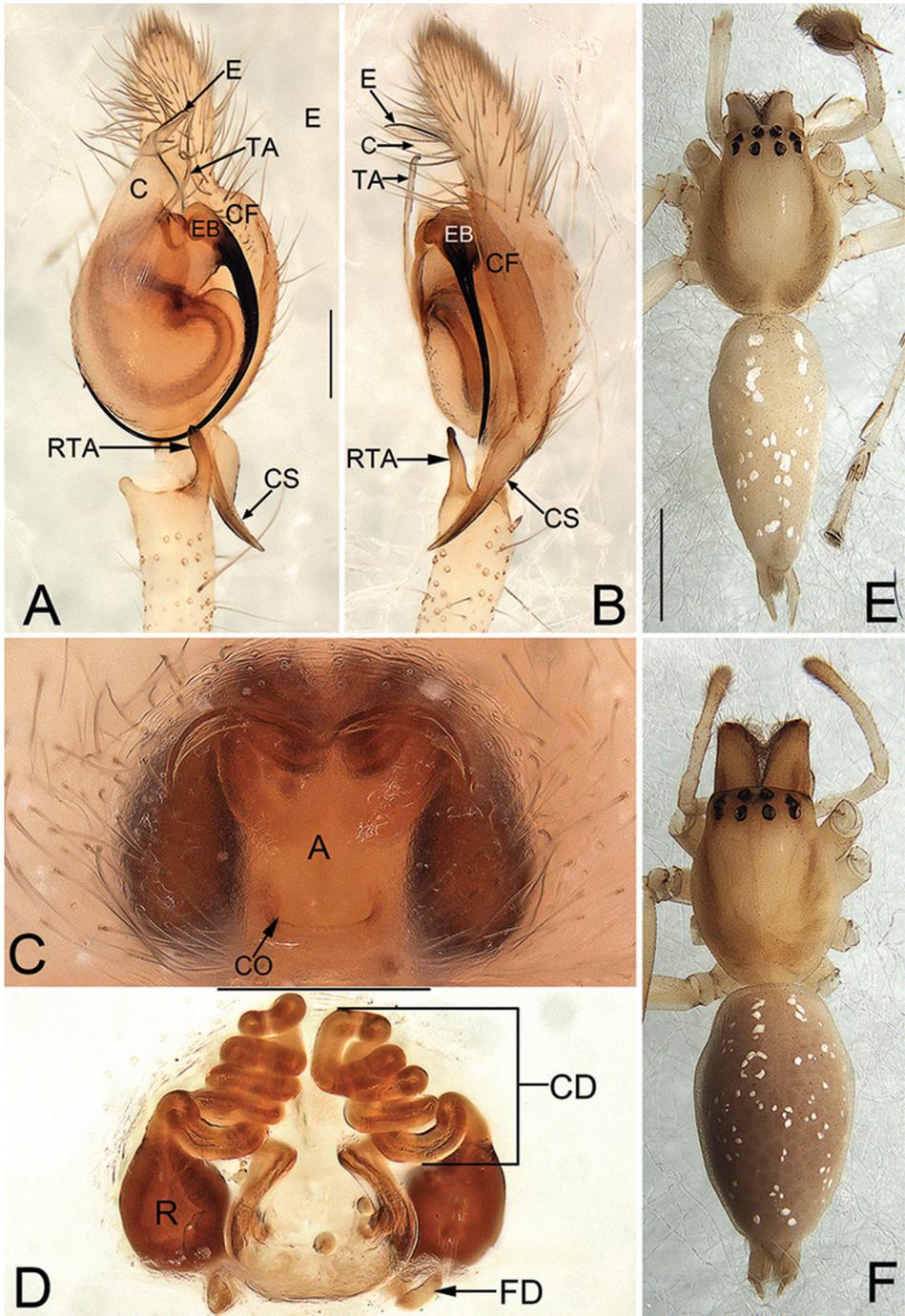
Remarks. See the World Spider Catalogue for the full list of references.

Examined material. 1♂ and 2♀, China, Jiangxi Province, Jinggang Mountains Nature Reserve, Xiangzhou village (380 m; 26°35'30.23"N, 114°15'59.93"E), 26 April 2011, Hao Yu leg.

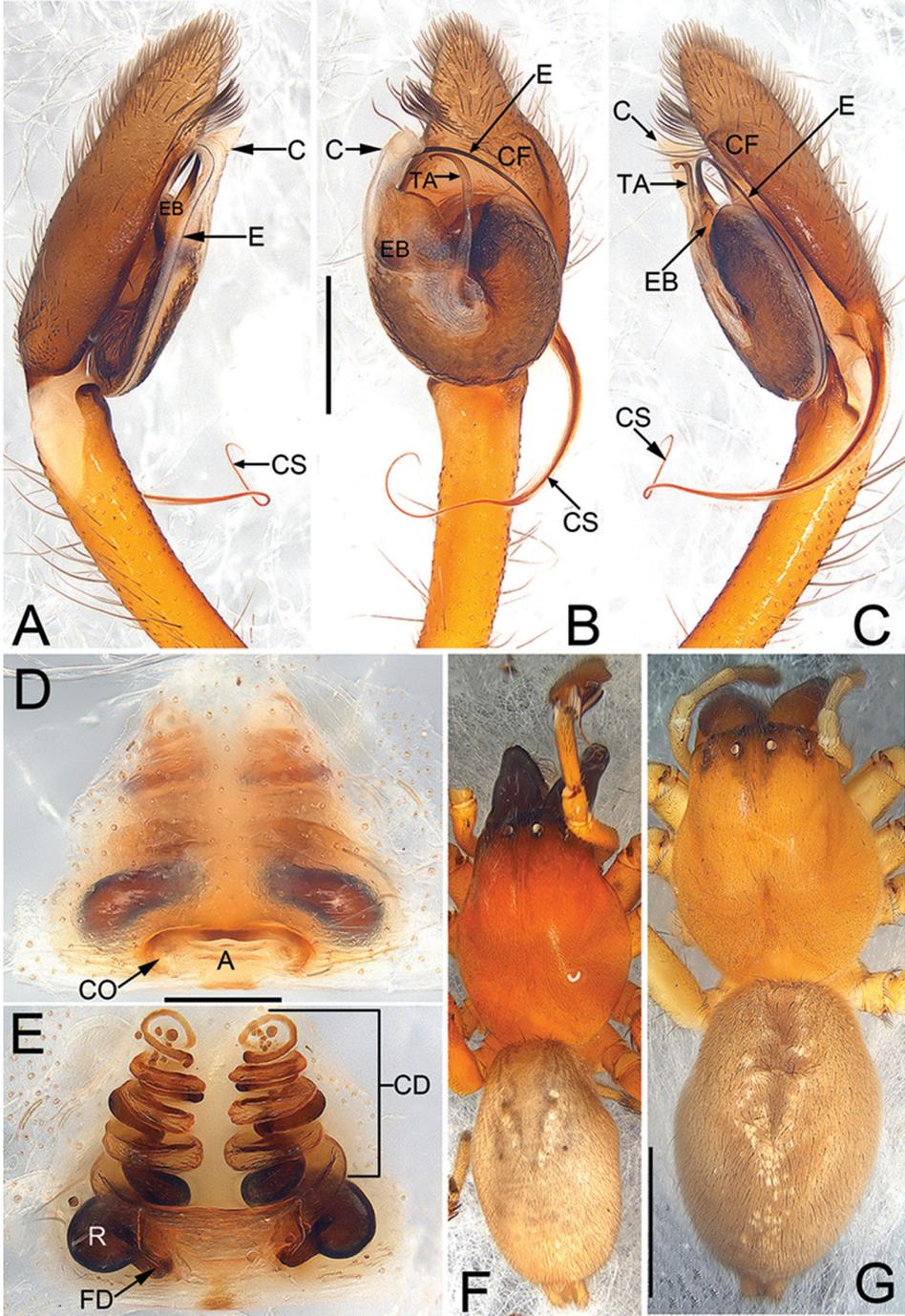
Description. *Male* and *female* (Fig. 4). Description of habitus, see Paik (1990). Since previous descriptions are rather brief, redescription of genitalia is provided as below.

Palp (Fig. 4A–C). Tibia about as long as cymbium, with only retrolateral apophysis; apophysis about 20% of tibia length, with a sharp apex and hiding behind tegulum; cymbial spur slightly shorter than tibia, tapering off into a thread and terminally coiled; cymbial fold distinct, for approximately 2/3 the length of cymbium; tip of cymbium about 1/3 of cymbium length. Tegulum egg-shaped, 1.2 longer than wide; regular apophysis long, more than 4/5 of tegulum length, thin hook-shaped; embolus originates at about 10 o'clock position, terminating at approximately 11 o'clock position, its tip covered by conductor; conductor large, membranous.

Epigyne (Fig. 4D–E). Apron-like atrium distinct, about four times wider than long; receptacles are faintly visible through epigynal plate in ventral view; two copulatory openings located at lateral borders of atrium; the transparent copulatory ducts running spirally (length of spira about 2.6 times longer than receptacles), forming 8 entwined loops (including 4 ascending coils and 4 descending coils); receptacle long and tubular, separated by two diameters.



Figures 3. *Cheiracanthium taiwanicum* Chen, Huang, Chen & Wang, 2006, male and female from Jinggang Mountains, Jiangxi, China. **A** left male palp, ventral view **B** same, retrolateral view **C** epigyne, ventral view **D** vulva, dorsal view **E** male habitus, dorsal view **F** female habitus, dorsal view. Scale bars: 0.2 mm (**A-B, C-D**); 1 mm (**E-F**).



Figures 4. *Cheiracanthium zhejiangense* Hu & Song, 1982, male and female from Jinggang Mountains, Jiangxi, China. **A** left male palp, prolateral view **B** same, ventral view **C** same, retrolateral view **D** epigyne, ventral view **E** vulva, dorsal view **F** male habitus, dorsal view **G** female habitus, dorsal view. Scale bars: 0.5 mm (**A-C**); 0.2 mm (**D-E**); 2 mm (**F-G**).

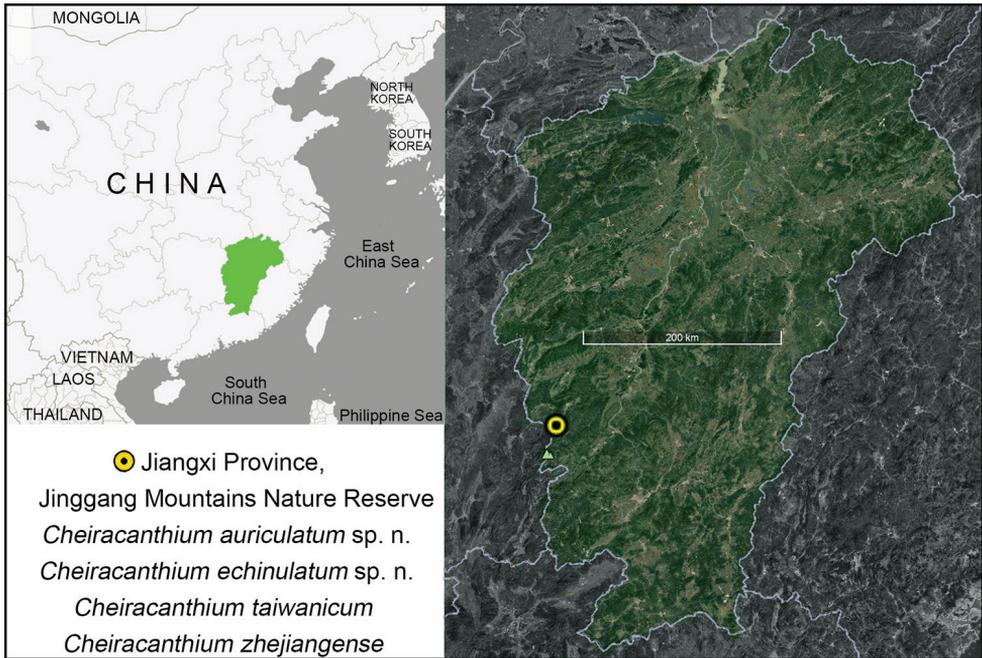


Figure 5. Locality of Jinggang Mountains in China.

Distribution. China (Guizhou, Hunan, Jiangxi, Zhejiang) and Korea.

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Two new species of the purse-web spider genus *Atypus* Latreille, 1804 from Hainan Island, China (Araneae, Atypidae)

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Abstract

Two species of the purse-web spider genus *Atypus* Latreille, 1804 collected from Hainan Island, China, are diagnosed and described as new to science based on genital morphology, *A. baotingensis* sp. n. (♂♀) and *A. jianfengensis* sp. n. (♀). The DNA barcodes of the two species are also provided for future use.

Keywords

Atypidae, *Atypus*, DNA barcode, East Asia, Mygalomorphae, taxonomy

Introduction

The purse-web spider family Atypidae is an ancient branch of the infraorder Mygalomorphae. Atypidae is one of the burrowing mygalomorph families. However, unlike other burrowing mygalomorph spiders, atypids form a tough web with silk from the end of their burrows to the upper ground section, which is expanded and camouflaged as a trap

for wandering arthropods (Fig. 1) (Jocque and Dippenaar-Schoeman 2006; Fourie et al. 2011). The family contains 52 species in three genera (*Atypus* Latreille, 1804, *Calommata* Lucas, 1837, and *Sphodros* Walckenaer, 1835) around the world. *Atypus* includes 32 species worldwide, of which, 13 are known from China (Zhang 1985; Schwendinger 1990; Zhu et al. 2006; Yin et al. 2012; Li and Lin 2016; World Spider Catalog 2018). *Atypus* can be distinguished from the other two genera as follows: male with marginal ridges in sternum; palp with short, straight, and spike-like embolus, as well as distally enlarged and straightforward conductor; female genitalia with bulbous or pyriform receptacula and with two lateral patches of pores on genital atrium (Fig. 2) (Schwendinger 1990; Zhu et al. 2006). Historically, Kraus and Baur (1974) gave detailed discussions on the taxonomic problems in *Atypus* species of Europe, and pointed out that the female genitalia are more reliable than the male bulb for atypid taxonomy. Schwendinger (1989) revised *Atypus* species in northern Thailand and also revised the whole genus in 1990 (Schwendinger 1990). Zhu et al. (2006) revised the genus *Atypus* of China. No new species has been described since the genus *Atypus* was revised a decade ago.

In this study, we diagnose and describe two new *Atypus* species collected from Hainan Island, China, using male and female genital morphology. To support our identifications in the future, here we provide COI barcode evidence. In addition, the female genitalia and male palp of the genus are illustrated for the first time with clear digital photographs.

Materials and methods

All specimens were excavated from their underground silk tubes by the roadside (Fig. 1A). They were collected alive and fixed in absolute alcohol, their right four legs were removed for molecular work, and the remains were preserved in 75% ethanol for morphological work. Male palp and female genitalia were dissected using a stereomicroscope SZM 45-B2 (Ningbo Sunny Instruments Co., Ltd.). After being cleared with Proteinase K by being incubated at 56° C for 3 hours, female genitalia were photographed with the Olympus BX51 compound microscope using a MicroPublisher 3.3 RTV camera. The others were photographed with a Leica M205C digital microscope. All measurements were given in millimetres. All the specimens were examined and deposited in the Centre for Behavioural Ecology & Evolution (CBEE), College of Life sciences, Hubei University, Wuhan, China.

Abbreviations used:

AL	abdomen length;	CW	carapace width;
ALE	anterior lateral eye;	MOA	median ocular area;
ALS	anterior lateral spinneret;	PLE	posterior lateral eye;
AME	anterior median eye;	PME	posterior median eye;
AW	abdomen width;	TL	total length.
CL	carapace length;		

DNA barcodes were obtained for future use: a fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) was amplified and sequenced using the primer pairs: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al. 1994) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). All molecular procedures on extraction, amplification and sequencing followed standard protocols (see Xu et al. 2015).

The genetic distance of the COI gene was calculated using MEGA version 6 (Tamura et al. 2013).

Taxonomy

Family Atypidae Thorell, 1870

Genus *Atypus* Latreille, 1804

Atypus baotingensis sp. n.

<http://zoobank.org/B8795BAD-EF8F-4E4E-B032-908E91AFFBFA>

Figs 1, 2

Type material. Holotype male (HN-2017-037A): CHINA: Hainan Province: Baoting County, 2 km to Qixianling National Forest Park along y044 Road, 18.71°N, 109.68°E, 205 m elevation, collected on 21 August 2017 by X. Xu, F. Liu, Z. Zhang, and D. Li (CBEE).

Paratypes. 6 females (HN-2017-032-HN-2017-037) and 1 male (HN-2017-037B), collected at the same locality as the holotype, 21 August 2017 by X. Xu, F. Liu, Z. Zhang, and D. Li (CBEE).

Etymology. The specific name refers to the type locality.

Diagnosis. The male palp of this new species resembles that of *A. suiningensis* Zhang, 1985, but can be diagnosed from the latter by 1) the wide triangular space between its embolus and conductor in lateral views (Fig. 2H–I); 2) the relatively long flat upper margin of its conductor in retrolateral view, with a triangular folded part of the upper corner of its conductor (Fig. 2H); and 3) the first pair of sigilla are connected by arched wrinkles across the anterior sternal margin (Fig. 2C). The female genitalia of the new species resembles that of *A. ledongensis* (Fig. 3D–F), but can be distinguished from the latter by the basal stalks of median pair of receptacula almost being as long as those of the lateral pair (Fig. 2J–L). In *A. ledongensis*, the basal stalks of the median pair of receptacula are obviously short, whereas the basal stalks of lateral pair of receptacula are as long as their diameter (Fig. 3D–F).

Description. Male (holotype). TL (including chelicerae) 11.44. CL 3.34, CW 3.55, AL 4.56, AW 2.78. Carapace black brown. Fovea placed back 2/3 of carapace length with some radiative grooves. Eye diameter: AME 0.24, ALE 0.10, PME 0.10. Distances: AME–AME 0.46, AME–ALE 0.31, PME–PME 0.83, PME–PLE 0.13. MOA 0.34, front width 0.94, back width 1.03. Labium wider than long. Sternum reddish brown,

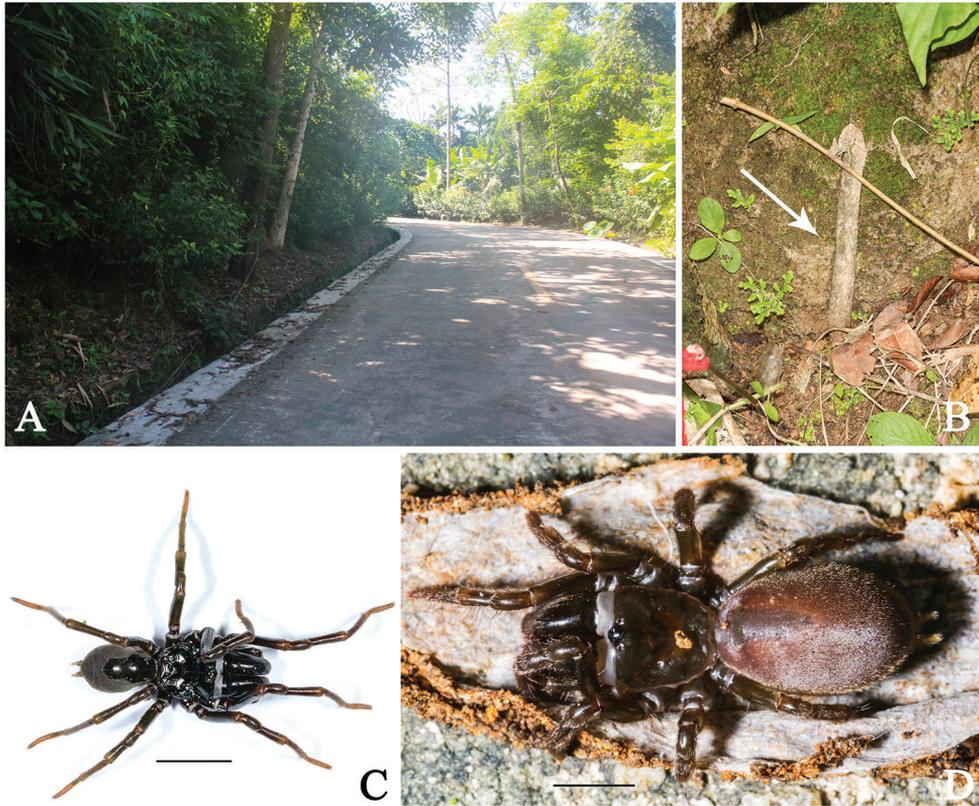


Figure 1. Microhabitat and general somatic morphology of *Atypus baotingensis* sp. n. **A–B** microhabitat **B** the purse-web, see white arrow point **C** male (HN-2017-037A) **D** female (HN-2017-032). Scale bars: 2 mm.

3.11 long, 2.26 wide, moderately roughened clothed with fine black hairs. Sigilla deeply imprinted; first pair anteriorly pointed, close to the margin of the sternum; posterior pair oval bigger than other pairs; second pair small (Fig. 2C). Chelicerae black brown, with 13 teeth on promargin in a single row, basal three fairly small (Fig. 2F).

Abdomen grey black, oval, with dorsal scutum gloss black. Spinnerets six: ALS 0.41 long, PMS 0.72 long, four-segmented PLS with lengths as follows: basal 0.41, median 0.52, subapical 0.41, apical 0.33, total 1.67.

Palpal femur with furrow. Legs slender in red grey. Granular texture only on femur I present. Spines on all metatarsus; metatarsus IV with 17 dorsal spines. Leg formula: 1243.

Male palp (Fig. 2G–I): long conductor with a triangular folded part of its upper corner in retro-lateral view; embolus long, thin spike with a wide triangular space between embolus and conductor in lateral views.

Female. TL (including chelicerae) 15.91. CL 4.19, CW 4.02, AL 7.35, AW 5.34. Carapace black-brown. Eye region black. Eye diameters: AME 0.24, ALE 0.14, PME 0.18, PLE 0.17. Distances: AME–AME 0.29, AME–ALE 0.20, PME–PME 0.81, PME–PLE 0.09. MOA 0.48, front width 0.77, back width 1.17. Fovea transverse, oc-

Table 1. Leg measurements of *Atypus baotingensis* sp. n., male.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	4.34	1.71	2.53	2.09	1.05	11.72
II	3.26	1.66	1.96	2.62	1.29	10.79
III	1.82	1.17	1.65	2.55	2.13	9.32
IV	1.83	1.39	2.39	2.66	1.70	9.97

Table 2. Leg measurements of *Atypus baotingensis* sp. n., female.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.46	1.23	1.51	1.66	1.12	7.98
II	1.62	1.30	1.17	1.52	0.87	6.48
III	2.81	1.52	0.92	1.14	0.52	6.91
IV	2.71	1.61	1.36	1.89	1.21	8.78

cupying about 1/5 of carapace width at that point. Chelicerae orange brown with 13 teeth on the promargin in a single row, basal three fairly small. Sternum (Fig. 2B) light orange brown, 3.51 long, 2.30 width, smooth, with scattered black hairs; sigilla relatively light impressions, first pair anteriorly pointed; oval posterior pair much larger.

Abdomen, oval and medium brown (Fig. 1D), with indistinct oval dorsal scutum on anterior half. ALS 0.56, PMS 0.83, four-segmented PLS with lengths as follows: basal 0.66, median 0.70, subapical 0.59, apical 0.75, total 2.7.

Spines on all metatarsus; metatarsus IV with 13 dorsal spines. Leg formula: 4132.

Vulva (Fig. 2J–L): Genital atrium very short, pore patches small, receptacula attached to anterior edge of atrium; median pair with upper incrassate basal stalks and the basal stalks of the median pair almost being as long as the lateral pair; lateral pair attached to patches of pores.

Variation. Size range of females: carapace length 4.19–5.12, carapace width 3.51–5.02, total length 14.13–16.91, $n = 6$; the basal stalks of left side pairs of receptacula connected in two specimens (Fig. 2L). Size range of males: carapace length 3.34–3.39, carapace width 3.23–3.55, total length 10.64–11.44, $n = 2$.

Habitat. Purse webs were found attached to the soil slope along roadside (Fig. 1A–B).

Distribution. Hainan Island (Baoting), China.

GenBank accession numbers. HN-2017-032: MH279555; HN-2017-033: MH279556; HN-2017-036: MH279557; HN-2017-037: MH279558; HN-2017-037A: MH279559.

Remarks. We examined the holotype and two paratypes of *A. ledongensis* (Museum of Hebei University, Baoding, Hebei, China), and also successfully sequenced the COI barcode of the holotype specimen (LD-001), which is available on GenBank (GenBank accession number MH279560). The lowest pairwise distance between the holotype of *A. ledongensis* and the specimens of *A. baotingensis* sp. n. is 7.2% in mean Kimura 2-parameter distance (K2P) and 6.8% in p-distance. The previous study revealed that the interspecific COI barcode for North American tarantulas is at 5%

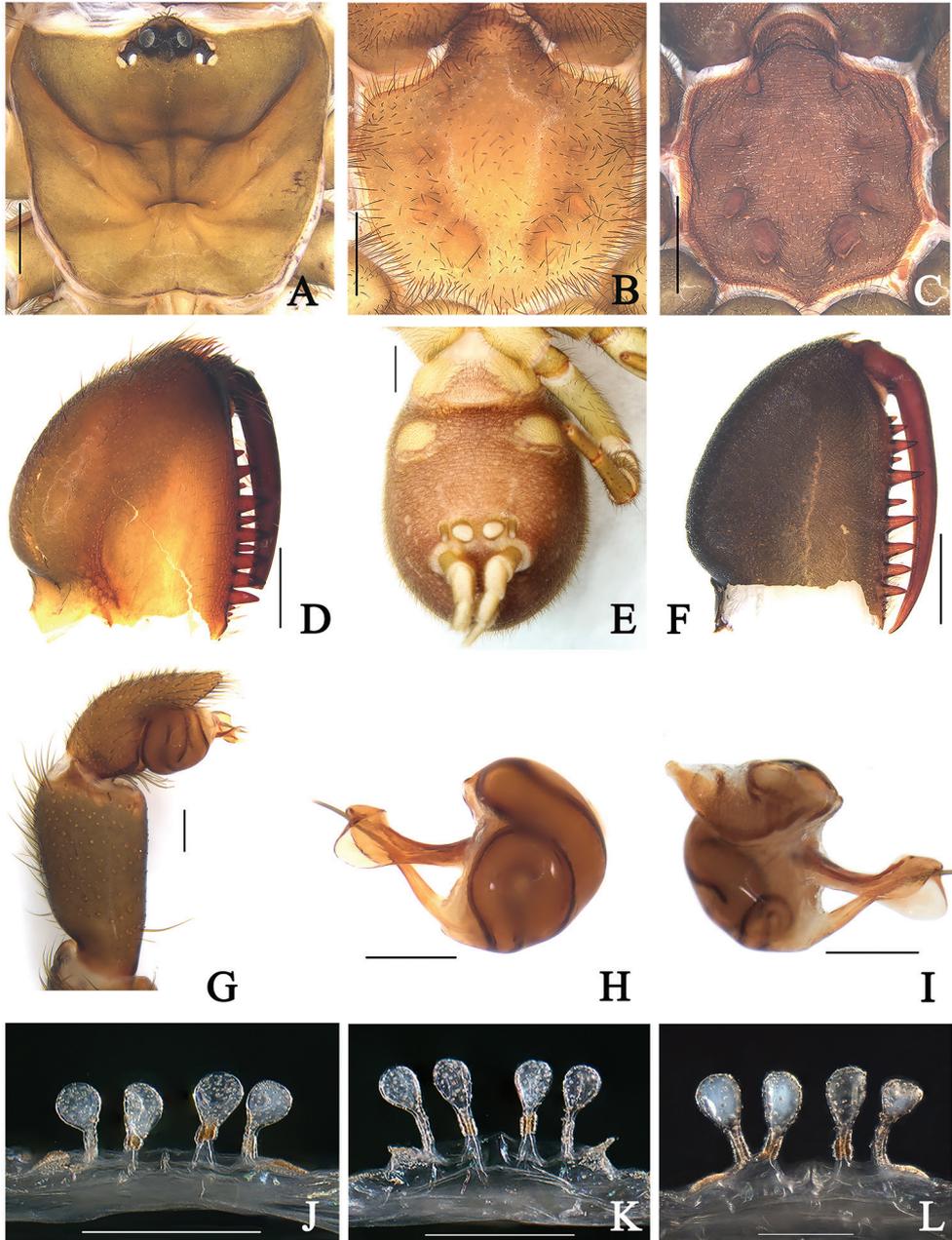


Figure 2. General somatic morphology and genital anatomy of *Atypus baotingensis* sp. n. **A–B, D–E, J** female (HN-2017-032) **C, F–I** male holotype (HN-2017-037A) **K** (HN-2017-033) **L** (HN-2017-036) **A** female carapace, dorsal view **B** female labium and sternum, ventral view **D** female left chelicera, inner-lateral view **E** epigyne and spinnerets, ventral view **J–L** vulva, dorsal view **C** labium and sternum, ventral view **F** left chelicera, inner-lateral view **G** left palpal, proteral view **H** left palpal bulb, retrolateral view **I** same, proteral view. Scale bars: 2 mm (**E**); 1 mm (**A–D, F**); 0.2 mm (**G–L**).

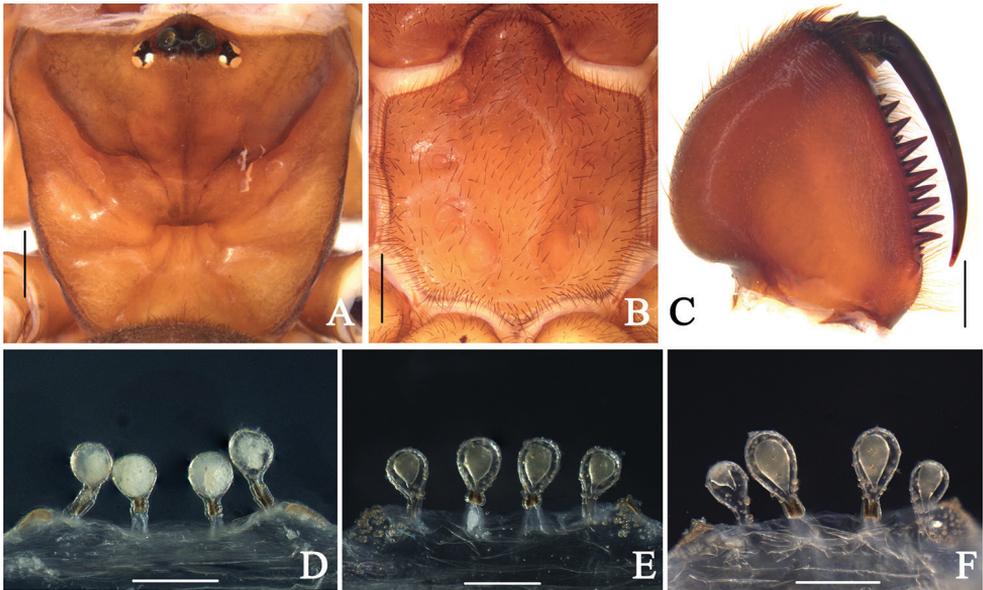


Figure 3. Genital anatomy of holotype and paratypes of *Atypus ledongensis*, see description details in Zhu et al. (2006). **A–D** female holotype (LD-001) **E–F** female paratypes **E** (LD-002) **F** (LD-003) **A** female carapace, dorsal view **B** female labium and sternum, ventral view **C** female left chelicera, inner-lateral view **D–F** vulva, dorsal view. Scale bars: 1 mm (**A–C**); 0.2 mm (**D–E**).

(Hamilton et al. 2014), thus it can guide us to diagnose our specimens as a new species, *A. baotingensis* sp. n. In addition, we provide COI barcode for identification in the future. The intraspecific genetic distance for *A. baotingensis* sp. n. based on the mean Kimura 2-parameter distance (K2P) and *p*-distance is 1.4% and 1.0%, respectively.

***Atypus jianfengensis* sp. n.**

<http://zoobank.org/82E29097-6474-4FC8-B3E7-22A31CE381FC>

Figs 4, 5

Type material. Holotype female (HN-2017-010): CHINA: Hainan Province: Ledong County, Jianfengling, Nantianchi, 18.74°N, 108.86°E, 823 m elevation, collected on 2 August 2017 by X. Xu, F. Liu, Z. Zhang, and D. Li (CBEE).

Paratypes. 4 females (HN-2017-003, HN-2017-005, HN-2017-007, HN-2017-008), collected at the same locality as the holotype, 2 August 2017 by X. Xu, F. Liu, Z. Zhang, and D. Li (CBEE). Male unknown.

Etymology. The specific name refers to the type locality.

Diagnosis. The female genitalia of the new species is similar to that of *A. karschi* Dönitz, 1887, but can be distinguished from the latter by the basal stalks of median pair of receptacula almost being as long as the diameter of their receptacula and

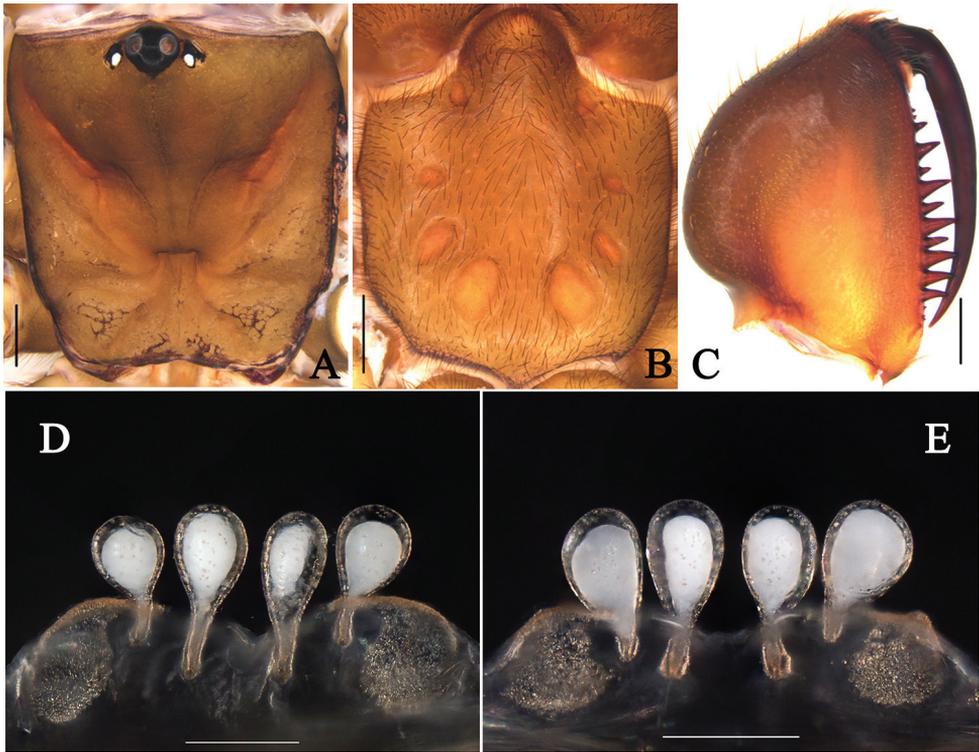


Figure 4. General somatic morphology and genital anatomy of *Atypus jianfengensis* sp. n. **A–D** female holotype (HN-2017-010) **E** female paratype (HN-2017-007) **A** carapace, dorsal view **B** labium and sternum, ventral view **C** left chelicera, inner-lateral view **D, E** vulva, dorsal view. Scale bars: 1 mm (**A–C**); 0.2 mm (**D–E**).

much longer than the basal stalks of lateral pair (Fig. 4D–E); first pair of sigilla irregular shape (Fig. 4B).

Description. Female (holotype). TL (including chelicerae) 18.74. CL 5.50, CW 4.97, AL 8.58, AW 5.98. Carapace red-brown. Eye region black. Eye diameters: AME 0.30, ALE 0.15, PME 0.16, PLE 0.14. Distances: AME–AME 0.27, AME–ALE 0.20, PME–PME 1.06, PME–PLE 0.12. MOA 0.29, front width 0.87, back width 1.38. Fovea transverse, occupying about 1/7 of carapace width at that point (Fig. 4A). Chelicerae orange red with 15 teeth on the promargin in a single row, two from apex small, basal three smallest (Fig. 4C). Labium wider than long, with a curved line marked in the middle. Sternum (Fig. 4B) light orange-brown, 4.80 long, 3.76 width, smooth, with scattered black hairs; sigilla deeply imprinted, first pair anteriorly pointed and irregular; fourth pair sub-oval, separated by nearly their width.

Abdomen, oval and medium brown (Fig. 5B), with a yellow dark dorsal tergite on anterior half. ALS 0.61, PMS 1.12, four-segmented PLS with lengths as follows: basal 0.77, median 0.69, subapical 0.62, apical 0.42, total 2.5.

Spines on all metatarsus; metatarsus IV with eleven dorsal spines. Leg formula: 1423.

Table 3. Leg measurements of *Atypus jianfengensis* sp. n., female.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	3.71	1.94	2.07	2.08	1.55	11.35
II	3.24	1.55	1.25	1.40	1.33	8.77
III	2.71	1.72	0.67	0.64	0.60	6.34
IV	2.96	1.71	1.93	2.13	1.36	10.09

**Figure 5.** Microhabitat and general somatic morphology of *Atypus jianfengensis* sp. n. **A** microhabitat **B** female (HN-2017-010) **C** purse-web.

Vulva (Fig. 4D–E): Genital atrium very short, pore patches large and rounded, the median pair of receptacula attached to the atrium more basally; the basal stalks of median pair of receptacula almost being as long as the diameter of their receptacula and much longer than the basal stalks of lateral pair.

Variation. Size range of females: carapace length 4.47–5.68, carapace width 4.02–5.15, total length 17.52–18.74, $n = 5$. Basal stalks of lateral pair of receptacula are much shorter in some specimens (Fig. 4E).

Habitat. Purse webs were found attached to the base of pine trees.

Distribution. Hainan Island (Jianfeng Mountain), China

GenBank accession numbers. HN-2017-003: MH279550; HN-2017-005: MH279551; HN-2017-007: MH279552; HN-2017-008: MH279553; HN-2017-010: MH279554.

Remarks. Although *A. jianfengensis* sp. n. is collected from Ledong County, Hainan Island, it can be diagnosed from *A. ledongensis* found at the same area, Jianfeng mountains, by the latter having very short basal stalks of median pair of receptacula. In addition, it can be distinguished from *A. baotingensis* sp. n. by the latter having upper incrassate basal stalks of the median pair of receptacula. The intraspecific genetic distance for *A. jianfengensis* sp. n. based on both the mean Kimura 2-parameter distance (K2P) and p-distance is 1.4% and 1.0%, respectively. The molecular data also provide the evidence that *A. jianfengensis* sp. n. can be distinguished from *A. baotingensis* sp. n. as well as from *A. ledongensis*: the interspecific genetic distance based on K2P and p-distance for the two new species are 15.4% and 13.8%, respectively, and between *A. jianfengensis* sp. n. and *A. ledongensis* are 17.2% and 15.3%, respectively.

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A new species of *Dicranocentrus* Schött from Hainan (China) with a key to the Chinese species of the genus (Collembola, Entomobryidae)

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Abstract

A new species, *Dicranocentrus hainanicus* Ren & Zhang, **sp. n.**, is described from Hainan Province, China. Complete tergal chaetotaxy including microchaetae is illustrated and discussed. It is characterized by having the dental spines arranged in 2–3 rows, two inner teeth on unguis, 5, 2, 2 central macrochaetae on Abd. I–III, two inner S-chaetae on Abd. V displaced anteriorly, and the additional microchaetae associated with the S-chaeta acc.p6 on Th. II–Abd. II. It is most similar to *D. chenae* Ma, Chen & Soto-Adames but differs from the latter in the number and arrangement of dental spines and the absence of macrochaeta Pa1 on dorsal head. A key to the Chinese species of the genus is provided.

Keywords

chaetotaxy, *Dicranocentrus hainanicus* sp. n., Heteromurinae

Introduction

The genus *Dicranocentrus* Schött, 1893 is widespread in pantropical regions. It is characterized by having the first and the second antennal segments subdivided, the third and the fourth antennal segments annulated, eyes 8+8, prelabral chaetae not bifurcated, postantennal organ absent, dental lobe without compound spines, dental spines

present or absent, and the mucro bidentate with a basal spine (Cipola et al. 2016). Mari-Mutt (1979) made great contribution to its biology, character assessments, and phylogeny, and further divided it into three groups based on dorsal cephalic chaetotaxy. Posterior macrochaetae on dorsal head are absent in *marias*-group, and macrochaeta S2 is present in *sundanensis*-group but absent in *gracilis*-group. *Dicranocentrus* belongs to Heteromurini (Heteromurinae) due to the presence of body scales (Zhang and Deharveng 2015) but its relationships to other genera of Heteromurini are still unclear (Mari-Mutt 1980; Cipola et al. 2016). Dorsal cephalic and tergal macrochaetotaxy, differentiated chaetae on tibiotarsi, tenent hairs, dental spines are the main diagnostic characters widely used by Mari-Mutt and subsequent authors. Soto-Adames and Anderson (2017) attempted to explore the complete idiochaetotaxy (including macrochaetae, microchaetae, partial S-chaetae) for the first time in *Dicranocentrus*; homology of some elements were uncertain due to the absence of chaetotaxic modification during postembryonic development. These characteristics are usually unknown in four species from China: *D. indicus* Bonet, 1930 from Taiwan, *D. chenae* Ma, Chen & Soto-Adames, 2006 from Guangxi, *D. wangi* Ma & Chen, 2007 from Guangdong and *D. liuae* Xu & Zhang, 2014 from Anhui. Here a new species is described from the southernmost province of China, Hainan.

Materials and methods

Specimens were cleared in Nesbitt's fluid, mounted under a coverslip in Hoyer's solution, and studied using a ZEISS AXIO Scope.A1 microscope and AxioCam camera. Dorsal body chaetae nomenclature follows Szeptycki (1979), dorsal cephalic chaetae follow Mari-Mutt (1979) and Soto-Adames (2008), inter-ocular chaetae follow Mari-Mutt (1986), tergal S-chaetae follow Zhang and Deharveng (2015), labial palp follows Fjellberg (1999), clypeal chaetae follow Yoshii and Suhardjono (1992) and Zhang et al. (2016), and labial chaetae follow Gisin (1967). The number of macrochaetae is given by half-tergite in the descriptions. Symbols representing chaetal elements used in the figures are as follows: circle, chaeta; cross, bothriotrichum; circle with a slash, pseudopore; "v", scales. All materials are deposited in the collections of the Department of Entomology, College of Plant Protection, Nanjing Agricultural University (NJAU), P. R. China.

Abbreviations

Th.	thoracic segment;	mes	mesochaeta/ae;
Abd.	abdominal segment;	mic	microchaeta/ae;
Ant.	antennal segment;	ms	S-microchaeta/ae;
mac	macrochaeta/ae;	sens	ordinary tergal S-chaeta/ae.

Taxonomy

Dicranocentrus hainanicus Ren & Zhang, sp. n.

<http://zoobank.org/85C9388D-C78D-4D2E-8E3B-6A4C8AA50375>

Figs 1–28

Material. Holotype: ♂ on slide, China, Hainan Province, Wuzhi Mountain, 18.903°N, 109.688°E, altitude ca. 901 m, 29 Dec 2015, DY Yu leg. (#15HN5). Paratypes: four ♀♀ on slides and three juveniles and one adult in alcohol, same data as holotype. All deposited in NJAU.

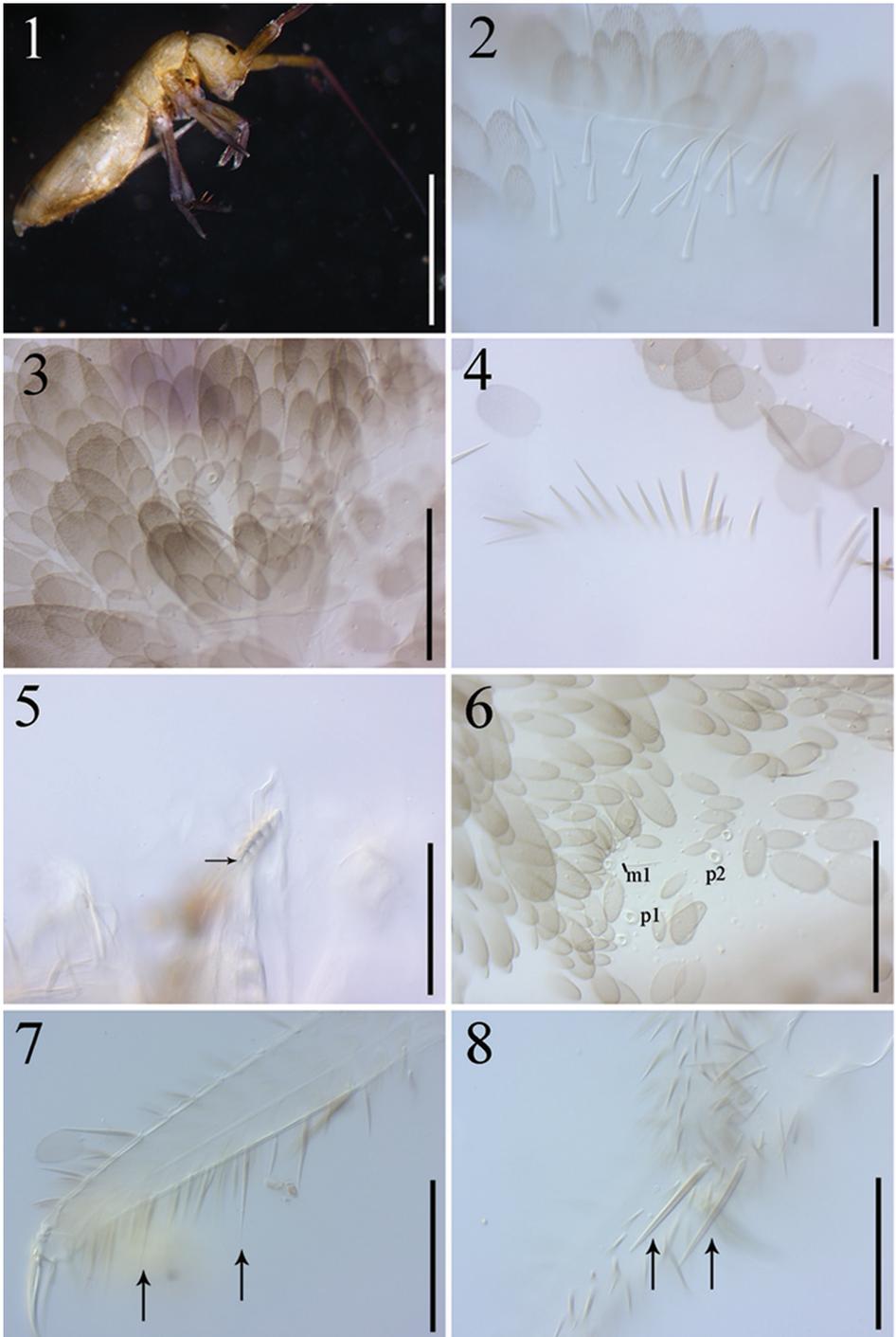
Etymology. Named after the type locality where the new species was collected.

Diagnosis. No obvious color pattern. Mac S2 present and mac Pa1 absent on dorsal head. Labial chaetae l_1 and l_2 smooth. Inner tibiotarsi and manubrium dorsally with smooth chaetae. Unguis with two inner teeth and without unpaired tooth. Tenent hairs acuminate. Den with 30–41 inner spines arranged in 2–3 rows. Th. II with two medio-medial, two medio-sublateral and eight posterior mac, and mac p5 present. Abd. I–IV with 5, 2, 2, 5 central mac. An additional mic associated with acc.p6 present on Th. II–Abd. II. Tergal sens as 2, 2|1, 3, 3, (3+≈35), 4; on Abd. V two inner sens anterior to lateral two.

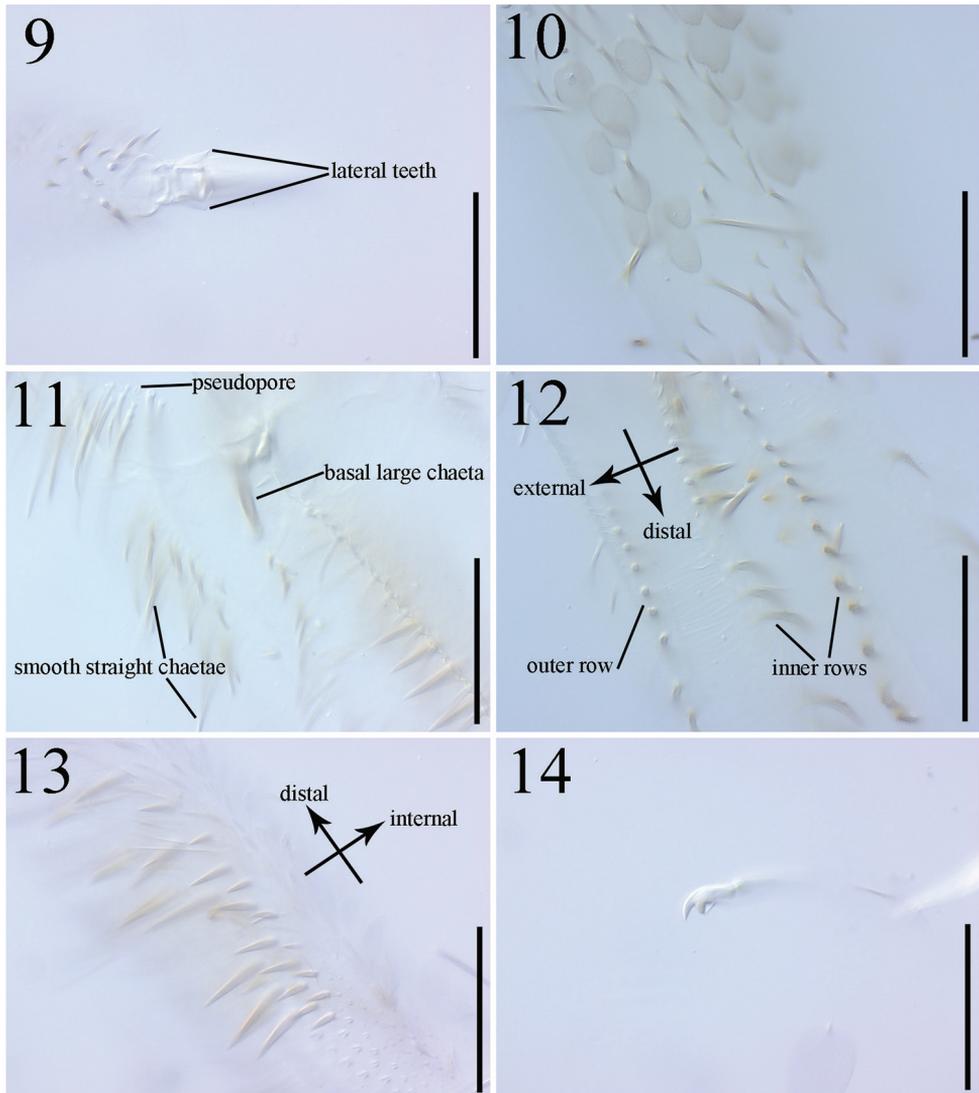
Description. Body length (head + thorax + abdomen) up to 3.96 mm. Ground color yellow. Antennae gradually dark purple towards tip. Eye patches dark (Fig. 1). Scales rounded, truncate, or pointed with numerous short striations; scales present on Ant. I–II, body, legs, both sides of head, ventral tube and manubrium, and ventral side of dens (Figs 2–4, 6, 8, 10). Scales in the posterior row along tergal margin much larger than anterior ones. Scales on dens much narrower.

Antenna approx. 3.5 times as long as cephalic diagonal. Antennal segment ratio as Ia: Ib: IIa: IIb: III: IV = 1: 4.04–5.29: 1.19–1.57: 4.57–6.29: 13.04–18.08: 4.71; ratio in holotype as 1: 4.04: 1.26: 4.57: 13.04 (Ant. IV lost). Ant. III distally and IV annulated and often fused. Smooth spiny mic at base of antennae indistinctly separated from ordinary mic and thus their number unclear (Fig. 2). Swollen S-chaetae of antennal organ apico-laterally 3–4, 3, 4 on Ant. Ib, IIb and III, respectively. Subcylindrical, thick S-chaetae 6–7, 3–4, 7 ventro-laterally on Ant Ib, IIb and III, respectively (Figs 15, 16). Pseudopores 2, 2, 2 ventro-apically on Ant. I, II, III, respectively. Ant. IV without apical bulb but its apex with a pin chaeta.

Eyes 8+8. Labral papillae four, all with a pointed tip and outer two slightly larger. Prelabral and labral chaetae 4/5, 5, 4, all smooth. Clypeal chaetae ciliate on prefrontal and frontal areas but their number unclear; lateral L_1 and L_2 smooth. Dorsal cephalic chaetotaxy with 13–16 antennal (An), four anterior (A), three median (M), eight sutural (S), one postocular (Po=Pa5) mac, and seven (Pa2–3, Pm3, Pp3, Pp5, Pe3–4) posterior (P) mac; mac Pa1 absent; inter-ocular chaetae as p, s, t (Fig. 17). A transverse of small ciliate chaetae present along posterior margin (Fig. 4). Mandibles with 4+6 apical teeth; five apical teeth much larger than distal one on right mandible (Fig. 5).

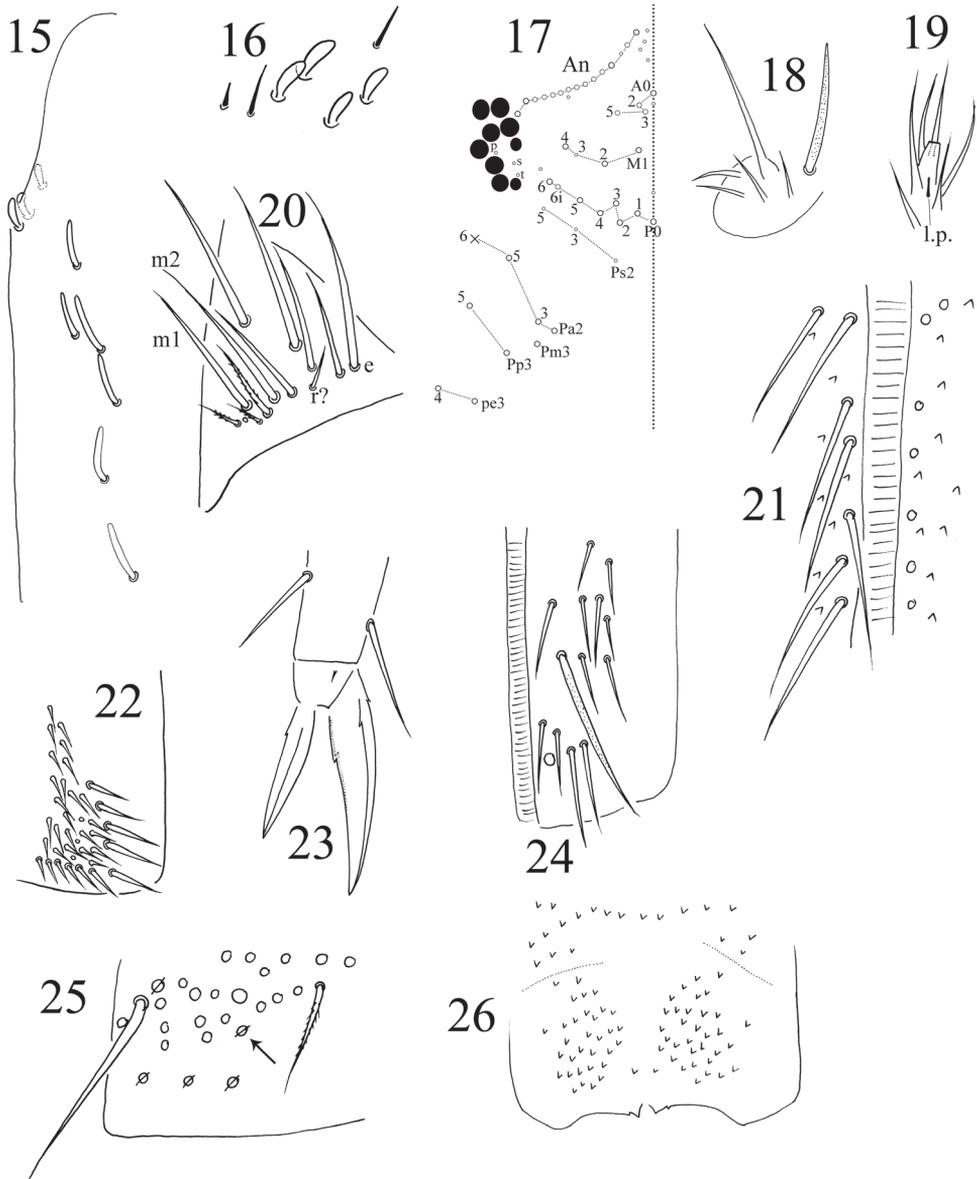


Figures 1–8. *Dicranocentrus hainanicus* sp. n. **1** habitus **2** left Ant. Ia (dorsal side) **3** dorsal head **4** distal chaetae along posterior margin of head **5** right mandible **6** Th. III **7** tibiotarsus I **8** external side of tibiotarsus II. Scale bars: 1.5 mm (**1**); 50 μ m (**2**); 125 μ m (**3–8**).



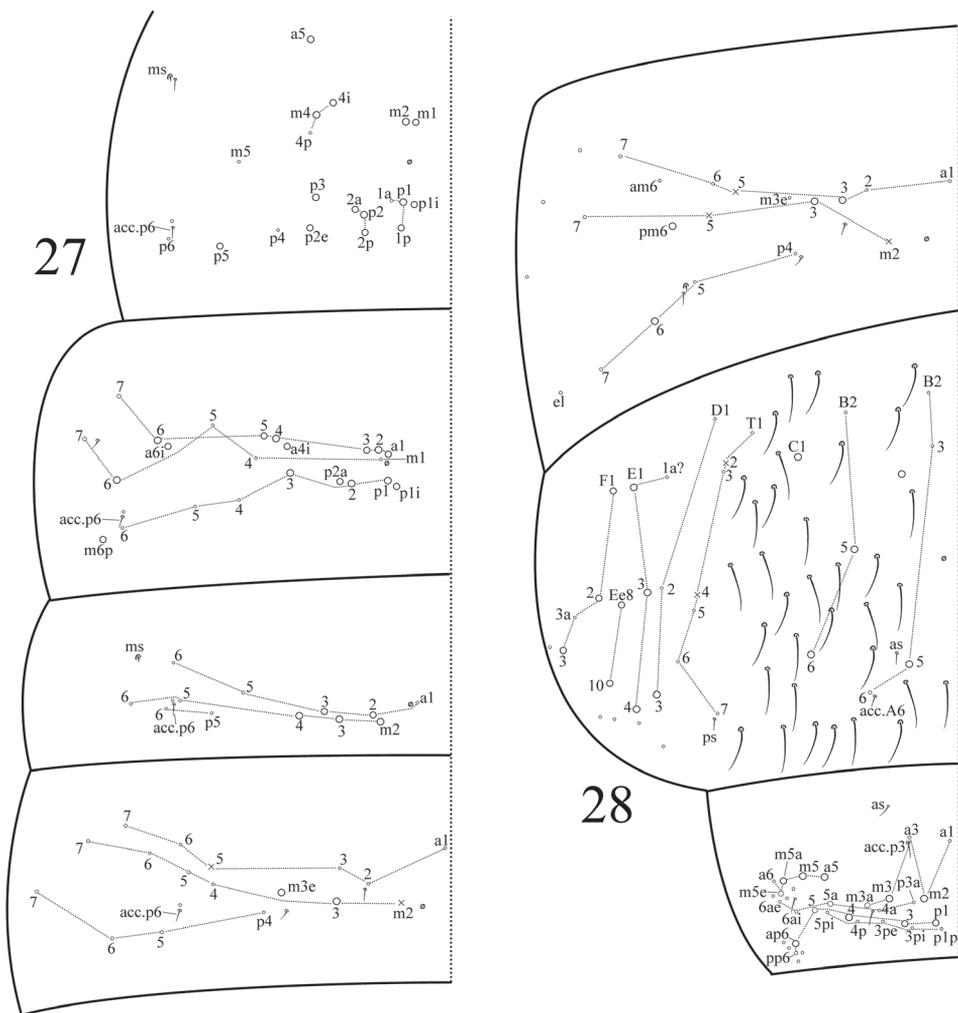
Figures 9–14. *Dicranocentrus hainanicus* sp. n. **9** unguis II external view **10** dorsal side of manubrium **11** manubrial plaque and base of dens dorsal view **12** dens dorsal view **13** dental spines **14** mucro. Scale bar: 125 μ m.

Maxillary outer lobe with four smooth sublobal hairs (Fig. 18). Labial papillae A–E with 0, 5, 0, 4, 5 guard chaetae, respectively; lateral process of papilla E thin, with tip not reaching apex of labial papilla (Fig. 19). Labium with five smooth proximal and five smooth anterior (a1–5) chaetae; submentum chaetae 8–13, 0–3 of them ciliate (Fig. 20), chaetae l_1 and l_2 smooth. Postlabial chaetae 6–7 smooth chaetae along cephalic groove (Fig. 21).



Figures 15–26. *Dicranocentrus hainanicus* sp. n. **15** Ant. I organ **16** left Ant. III organ **17** dorsal cephalic chaetotaxy **18** maxillary outer lobe (right side) **19** lateral process of labial palp (left side) **20** labial triangle (right side) **21** postlabial chaetae along groove **22** trochanteral organ **23** hind claw (posterior side) **24** anterior face of ventral tube **25** manubrial plaque (right side), arrow indicating that it is often absent **26** distal part of manubrium ventrally.

Trochanteral organ with 29–44 smooth spine-like chaetae; 12–13 in L-shaped arms and 17–31 between arms (Fig. 22). Tibiotarsi with smooth inner chaetae (Fig. 7) and few large, ciliate outer chaetae, but their number unclear because partial chaetae



Figures 27–28. *Dicranocentrus hainanicus* sp. n. Tergal chaetotaxy. **27** Th. II–Abd. II **28** Abd. III–V.

lost with only large sockets seen (Fig. 8). Unguis with two inner and two lateral teeth; unpaired inner and outer teeth not seen. Unguiculus lanceolate with a tiny outer tooth. Tenent hairs acuminate (Figs 9, 23). Abd. IV 1.24–1.53 times as long as Abd. III along dorsal midline. Ventral tube anteriorly with 14–17 weakly ciliate chaetae, two of them much larger (Fig. 24); posteriorly with more than 50 chaetae; each lateral flap with more than 40 chaetae and some of them ciliate. Tenaculum with 4+4 teeth, corpus with approx. six ciliate chaetae. Manubrium dorsally with 1+1 lateral rows of smooth straight chaetae, which are also present manubrial plaque (Fig. 25) and dental base (Fig. 11). Manubrial plaque with 4–5 pseudopores and 14–17 ciliate chaetae on each side (Fig. 25). Distal manubrium ventrally with 33–41 scales (Fig. 26). Dens dorsally with two rows of ciliate chaetae; inner ones stronger than outer ones (Fig. 12); the most

basal chaeta of inner row extremely thicker and longer (Fig. 11). Den internally with 30–41 (33 in holotype) basal spines arranged roughly in 2–3 rows; basal ones more dense than distal ones; outer spines larger than inner ones (Fig. 13). Smooth distal part of dens 4.70–6.25 times as long as mucro; apical tooth slightly longer than subapical one; mucronal spine just reaching the apex of subapical tooth (Fig. 14).

Th. II with two (m1, m2) medio-medial, two (m4, m4i) medio-sublateral and eight (p1, p1i, p1p, p2, p2a, p2p, p2e, p3, p5) posterior mac; m4p, m5, p1a, p4, p6 as mic. Th. III with 15 (a1–6, a4i, a6i, m6, p1–3, p1i, p2a, m6p) mac; a7 and m7 as mes; m1, m4–5 and p4–6 as mic. Abd. I with five (a2–3, m2–4) mac; a1, a5–6, m5–6, p5–6 as mic. Abd. II with two (m3, m3e) mac; a2–3, a5–6, m4–7 and p4–7 as mic (Fig. 27). Abd. III with two (a3, m3) central and two (pm6, p6) lateral mac; a1–2, a6–7, am6, m7, p4–5, and p7 as mic; m4 absent. Abd. IV with five (A5, B5–6, C1, a mac of unclear homology) central and nine (D3, E1, E3–4, F1–3, Ee8, Ee10) lateral mac. Abd. V with 13 (m2–3, m3a, a5, m5, m5a, m5e, p1, p3–5, ap6, p5a) mac (Fig. 28). Abd. VI without smooth chaetae. Tergal ms as 1, 0|1, 0, 1, 0, 0. Sens as 2, 2|1, 3, 3, (3+≈35), 4; Abd. IV with three ordinary sens (as, ps, acc.A6) and approx. 35 elongated sens; inner two sens anterior to lateral two on Abd. V. An additional mic associated with acc.p6 and of unclear homology present on Th. II–Abd. II.

Ecology. In litter of coniferous and broad-leaved mixed forests.

Remarks. As a member of *sundanensis*-group (cephalic mac S2 present), *D. hainanicus* sp. n. is similar to *Dicranocentrus chenae* Ma, Chen & Soto-Adames in most features including two inner teeth on unguis and tergal macrochaetae, but differs from it in the absence of mac Pa1 on dorsal head, mac p5 present on Th. II and the abundant (30–41) dental spines arranged in 2–3 rows (single row in *D. chenae*). The absence of outer tooth on unguis is also interesting but the detail is often lacking or overlooked in descriptions of known species.

Nearly complete dorsal chaetotaxy is again illustrated and compared with Soto-Adames and Anderson (2017). On dorsal head, a mic between the outer two mac of series M is homologous to m3. For the two mac external to S5, the outer mac, having a larger socket than inner one, is supposed to be the primary chaeta S6. On Th. II, the most internal four posterior chaetae labeled as “p1, p2, p2a, p2p” in *D. icelosmaris* are named here as p1i, p1, p1a, p1p in the light of the presence of the set p2+ (p2, p2a, p2p) between p1 and p3. On Th. III, “p1, p2” in are possibly homologous to p2 and p2a in *D. hainanicus* sp. n. Mic p5 on Abd. I and mic m4 on Abd. II are absent in *D. icelosmaris* and *Heteromurus nitidus* but present in new species. On Abd. III, mic m4 is present in *D. icelosmaris* and *H. nitidus* but absent in *D. hainanicus* sp. n. On Abd. IV homologies of many chaetae are difficult to determine in the absence of sufficient evidence across Entomobryoidea; three ordinary sens are observed as those on Abd. II–III and named as as, acc.A6 and ps, respectively. On Abd. V, the number of sens is identical to those reported in Zhang and Deharveng (2015), therefore the inner two are supposed to as and acc.p3 here although their positions displace more anteriorly compared to *D. wangi* and *D. liuae*.

Key to the species of *Dicranocentrus* from China

- 1 Abd. I with 5+5 mac (Fig. 27) **2**
 – Abd. I with 3+3 mac **3**
 2 Mac Pa1 present on dorsal head; Th. II without p5 mac; dens with 15–19 inner spines arranged in a row..... ***chenae* Ma, Chen & Soto-Adames, 2006**
 – Mac Pa1 absent on dorsal head (Fig. 17); Th. II with p5 mac; dens with 30–41 inner spines arranged 2–3 rows ***bainanicus* sp. n.**
 3 Abd. III with 1+1 central mac ***liuae* Xu & Zhang, 2014**
 – Abd. III with 2+2 central mac (Fig. 28) **4**
 4 Abd. II with 3+3 mac; Abd. III with 3+3 lateral mac ... ***indicus* Bonet, 1930**
 – Abd. II with 2+2 mac (Fig. 27); Abd. III with 2+2 lateral mac (Fig. 28)
 ***wangi* Ma & Chen, 2007**

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A comparative study of external female genitalia (including the 8th and 9th abdominal segments) in the family Megalopodidae and other related families of Chrysomeloidea

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Abstract

The external female genitalia of 29 species belonging to three genera of Megalopodidae and 80 species belonging to 61 genera of another four families in Chrysomeloidea were studied. The external female genitalia within the superfamily Chrysomeloidea can be divided into a cerambycid type and a chrysomelid type. The comparative study of external female genitalia shows Megalopodidae is more closely related to the family Cerambycidae than to the family Chrysomelidae *s.l.* Among five subfamilies of Cerambycidae we studied, the subfamily Lamiinae is most closely allied to Megalopodidae. An evolutionary path is proposed for the spiculum gastrale in Chrysomeloidea: the characteristic state of the spiculum gastrale without a joint is primary, and that with a joint is secondary. The family Orsodacnidae has probably evolved in isolation from the early chrysomelids, due to their shared external female genitalia (cerambycid type). In the family Chrysomelidae, the structure of external female genitalia and ovipositing behavior show that the subfamily Synetinae is closer to the Camptosomata than the subfamily Eumolpinae. In general, the shape of the terminal ovipositor is palp-like in the Chrysomeloidea. Terminal ovipositors are generally palp-shaped in Chrysomeloidea except for those that are lamellate in the genus *Callispa* and the subfamily Cassidinae who produce egg-sheaths.

Keywords

Chrysomeloidea, female genitalia, Megalopodidae, morphology, 8th abdominal segment

Introduction

Adults of the family Megalopodidae commonly feed on the juices in stems and leaf tissues of plants in the families of Rosaceae, Oleaceae, Salicaceae, and Celastraceae, or consume the pollen of the *Araucaria* species (Tan et al. 1980, Reid 1989, Kuschel and May 1990, Li et al. 2013). They are distributed in Neotropical, Afrotropical, Palaearctic, Oriental, Nearctic and Australian regions (Tan et al. 1980). In China the Megalopodidae is represented by two subfamilies Megalopodinae and Zeugophorinae (Yu and Liang 2002, Medvedev 2010, Silfverberg 2010). The subfamily Megalopodinae includes two genera (*Temnaspis* Lacordaire, 1845 and *Poecilomorpha* Hope, 1840). The subfamily Zeugophorinae includes only a single genus (*Zeugophora* Kunze, 1818) which is divided into two subgenera, *Zeugophora* Kunze, 1818 and *Pedrillia* Westwood, 1864 (Monrós 1959, Gressitt and Kimoto 1961, Chen and Pu 1962, Kimoto and Gressitt 1979, Medvedev 1985, 1997, Reid 1989, 1992, 1995, Schöller 2009, Warchałowski 2010). There are about 450 species of Megalopodidae in the world with 31 species of Megalopodinae and 24 species of Zeugophorinae in China (Gressitt and Kimoto 1961, Chen and Pu 1962, Chen 1974, Kimoto and Gressitt 1979, Tan et al. 1980, Medvedev and Sprecher-Ubersax 1997, Medvedev 2002, 2010, Yu and Liang 2002, Medvedev 2010, Silfverberg 2010, Lawrence and Ślipiński 2013, Li et al. 2013). Latreille (1802) established the subfamily Megalopodinae. Chapuis (1874) constructed the early taxonomic system for the Chrysomelidae. The subfamily Megalopodinae was included in the Camptosomes for some time until Chen (1940) transferred this subfamily from the Camptosomes to the Eupodes, which was regarded as the most primitive group. The subfamily Megalopodinae was then included in the family Donaciidae. This included the subfamilies Sagrinae, Donaciinae, Orsodacninae, Megascelinae, and Megalopodinae. The subfamily Zeugophorinae was established by Böving and Craighead (1931) and was included in the family Orsodacnidae based on characteristics of larvae. Following research on the external male genitalia within the subfamily Zeugophorinae, Chûjô (1952) pointed out that subfamily Zeugophorinae was more closely related to the Megalopodinae than other taxa in the Chrysomeloidea. Chen (1964, 1973) proposed that both subfamilies Megalopodinae and Zeugophorinae belonged to the criocerid line. The criocerid line also included the six following subfamilies: Sagrinae, Donaciinae, Criocerinae, Bruchidae, Orsodacninae and Synetinae. Crowson (1981) included the subfamily Zeugophorinae as a member of Megalopodidae. Therefore the Megalopodidae now included two subfamilies the Megalopodinae and the Zeugophorinae. Kuschel and May (1990) added a new subfamily, the Palophaginae. Some researchers continued to regard the Megalopodi-

dae (subfamilies Megalopodinae, Zeugophorinae, Palophaginae) as an independent family or subfamily (Lawrence and Britton 1991, Suzuki 1994). In addition, Reid (1995) also supported the Megalopodidae as an independent family and gradually more researchers accepted this treatment (Hunt et al. 2007, Gómez-Zurita et al. 2007, 2008; Marvaldi et al. 2009, Löbl and Smetana 2010, McKenna et al. 2015). However, the phylogenetic relationships were not well resolved among the Chrysomeloidea (Reid 1995, 2000, Hunt et al. 2007, Gómez-Zurita et al. 2007, 2008, Marvaldi et al. 2009, McKenna et al. 2015). Chen (1985) examined several characteristics including head orientation, male genitalia, larval feeding habits, thorax, and wing-venation, and proposed that the Megalopodidae was related to the family of Cerambycidae. Suzuki (1988, 1994) considered that the Megalopodidae probably descended from lamiid-type ancestors after studying their internal reproductive systems and hind wing venation. Schmitt (1992) also considered the Megalopodidae to be closer to the Cerambycidae than to the Chrysomelidae. Reid (1995) revealed Megalopodidae as a monophyletic group, with Orsodacnidae and Chrysomelidae as its sister groups, but the evidence supporting a close relationship between Megalopodidae and Cerambycidae was insufficient. In recent years, molecular evidence has shown that the Megalopodidae is closest to the Cerambycidae or Oxypeltidae (Hunt et al. 2007, Gómez-Zurita et al. 2007, 2008, Marvaldi et al. 2009, McKenna et al. 2015).

Male and female genitalia are among the most important identification characteristics in the Megalopodidae and receive much attention from taxonomists. Chûjô (1952, 1953) studied the morphology of external male genitalia. Following his extensive analysis of the external male genitalia of the Megalopodinae and Zeugophorinae, Chûjô found similarities between the two subfamilies. Subsequently, taxonomists began to describe the male genitalia of the Megalopodidae when publishing descriptions of new species and when they constructed or analyzed molecular phylogenetic relationships within the Chrysomeloidea (Reid 1989, Kuschel and May 1990, Mann and Crowson 1996, Verma 1996, Medvedev 1997).

Research on external female genitalia in the Megalopodidae is rare. There were only a few descriptions of male or female genitalia for this family when new species were described or revisions were made (Reid 1989, 1992, Li et al. 2013, Rodríguez-Mirón and Zaragoza-Caballero 2017). Suzuki (1994) reported comprehensively on the internal reproductive system of Chrysomeloidea *s.l.*, including the Megalopodinae and Zeugophorinae. Kasap and Crowson (1985) described briefly the 8th abdominal segment of the external female genitalia of *Sphondylia afra* (Megalopodinae) and *Zeugophora fulvicollis* (Zeugophorinae). Reid (1989, 1992) described the structure of the ovipositor and spermathecae of the Zeugophorinae when he published new species. Recently Rodríguez-Mirón et al. (2017) studied the spermathecae morphology of the Megalopodidae. In the present study, we dissected and describe the 8th abdominal segments and the external genitalia in females, then compared them with those of other related families in the Chrysomeloidea.

Material and methods

Preparation of specimens

All measurements were made with the aid of an ocular micrometer in the Nikon SMZ1500 stereoscopic microscope. Dry specimens were soaked in boiled water for 1–2 hours. For larger specimens the lateral margin of the abdomen was opened at its apex and the genitalia were pulled out of the abdomen with fine forceps. For smaller specimens the whole abdomen was removed from the body. The genitalia and/or whole abdomen were soaked in a warm solution of 10% KOH for 10–20 minutes as a pretreatment. The treatment time depended upon the degree of sclerotization found in different species. After pretreatment these organs were washed in water several times. Genitalia were then detached and some were dyed with Chlorazol Black E to stain membranous tissue a blue color. They were then transferred to glycerin for observation, photography, and preservation.

Photographs of female genitalia were taken using a Nikon SMZ-1500 stereoscopic dissecting microscope fitted with a Cannon 450D digital camera. Photographs of habitus were captured by a Cannon Macro 100 mm lens fitted to the Cannon 450D camera. For each final image, several photographs were taken at different focal planes, combined with Helicon Focus software to obtain one synthesized photograph, and finally edited with Adobe Photoshop software.

Terminology

Morphological terminology for the female genitalia of the Megalopodidae follows Snodgrass (1935), Chûjô (1952, 1953), Kasap and Crowson (1985) and Lawrence et al. (2010). In females of the Chrysomeloidea, the genitalia segment is located on the 9th segment of the abdomen. The ovipositor consists of the proctiger, paraproct, median plate, valvifer, coxite, and stylus (Kasap and Crowson, 1985). In contrast, the ovipositor of the Cerambycidae consists of the proctiger and vaginal palpi with baculi. The dorsal side of the baculi of the vaginal palpi is called the dorsal baculi. The ventral side of the baculi includes the paraproct baculi, valvifer baculi, and coxite baculi (Saito 1989). The ovipositor of the Megalopodidae includes two long vaginal palpi, which have one or two pair of baculi. Therefore, the ovipositor consists of the paraproct, valvifer, coxite, and stylus without a proctiger or a median plate.

Outside the ovipositor of *Nupserha bicolor* Thomson (Cerambycidae) there is a membranous bag-like capsule known as the capsular sheath (Dutt 1958). Saito (1989) suggested that this structure was the inter-segmental membrane found between the ovipositor and the 8th segment. The ovipositor of Megalopodidae is surrounded by a pocket-like membrane. Kuschel and May (1990) named this membrane as a genital pocket. The base of the pocket is connected with the apical margin of the 8th segment and the apex of the pocket is connected with the base of the ovipositor. The surface of the genital pocket is thickened by many sclerotized rings (Figs 9, 10).

Specimens studied

Twenty-nine species belonging to three genera of Megalopodidae (including two subfamilies Megalopodinae and Zeugophorinae) were examined in this study (Table 1). Only one species *Orsodacne cerasi* (Linnaeus) is native to China. Twenty species belonging to 17 genera of five subfamilies (Prioninae, Spondylidinae, Lepturinae, Cerambycinae, and Lamiinae) were selected in the family Cerambycidae. Fifty-nine Chinese species in the Chrysomelidae belonging to 43 genera of 12 subfamilies (Table 1) were chosen including the Sagrinae, Bruchinae, Donaciinae, Criocerinae, Eumolpinae, Lamprosomatinae, Cryptocephalinae, Syntetinae, Chrysomelinae, Galerucinae, Alticinae, and Cassidinae.

Table 1. Species of Chrysomeloidea studied.

Family	Subfamily	Species	Locality
Megalopodidae	Megalopodinae	<i>Poecilomorpha cyanipennis</i> (Kraatz)	Zhejiang (Hangzhou)
		<i>Poecilomorpha discolineata</i> (Pic)	Yunnan
		<i>Poecilomorpha downesi</i> (Baly)	Sichuan
		<i>Poecilomorpha laosensis</i> (Pic)	Indochina
		<i>Poecilomorpha maculata</i> (Pic)	Guangxi, Yunnan; Vietnam
		<i>Poecilomorpha mouhoti</i> (Baly)	Yunnan
		<i>Poecilomorpha pretiosa</i> Reineck	Guangxi, Zhejiang
		<i>Temnaspis bonneuili</i> Pic	Northeast of China
		<i>Temnaspis femorata</i> (Gressitt)	Fujian, Guangxi
		<i>Temnaspis fraxini</i> (Komiya)	Taiwan
		<i>Temnaspis humeralis</i> Jacoby	Hubei, Chongqing
		<i>Temnaspis nigriceps</i> Baly	Yunnan
		<i>Temnaspis japonica</i> Baly	Japan
		<i>Temnaspis nankinea</i> (Pic)	Henan
		<i>Temnaspis omeiensis</i> (Gressitt)	Sichuan
		<i>Temnaspis pulchra</i> Baly	Jiangxi
		<i>Temnaspis septemmaculata</i> (Hope)	Yunnan
		<i>Temnaspis syringa</i> Li and Liang	Beijing
	<i>Temnaspis vitalisi</i> (Pic)	Yunnan	
	Zeugophorinae	<i>Zeugophora (Pedrillia) annulata</i> Baly	Heilongjiang
		<i>Zeugophora (Pedrillia) bicolor</i> Kraatz	Liaoning
		<i>Zeugophora (Pedrillia) longicornis</i> Westwood	Yunnan
		<i>Zeugophora (Pedrillia) dimorpha</i> (Gressitt)	Hunan
		<i>Zeugophora (Pedrillia) yunnanica</i> Chen and Pu	Yunnan
		<i>Zeugophora ancora</i> Reitter	Ningxia
		<i>Zeugophora cribrata</i> Chen	Qinghai

Family	Subfamily	Species	Locality
Megalopodidae	Zeugophorinae	<i>Zeugophora cyanea</i> Chen	Qinghai
		<i>Zeugophora scutellaris</i> Suffrian	Heilongjiang
		<i>Zeugophora turneri</i> Power	Beijing
Cerambycidae	Prioninae	<i>Aegolipton marginalis</i> (Fabricius)	China
		<i>Megopis (Aegosoma) sinica sinica</i> (White)	China
	Spondylidinae	<i>Asemum amurense</i> Kraatz	China
		<i>Spondylis buprestoides</i> (Linnaeus)	China
	Lepturinae	<i>Leptura annularis annularis</i> Fabricius	Heilongjiang
		<i>Leptura</i> sp.	Guangxi
		<i>Gaurotes virginea aemula</i> (Mannerheim)	China
	Cerambycinae	<i>Anoplistes halodendri</i> (Pallas)	Inner Mongolia
		<i>Aromia bungii</i> (Faldermann)	China
		<i>Purpuricenus temminckii</i> (Guérin-Ménéville)	China
	Lamiinae	<i>Bacchisa comata</i> (Gahan)	Hainan
		<i>Eodorcadion brandti</i> (Gebler)	China
		<i>Glenea centroguttata</i> Fairmaire	Xizang
		<i>Glenea pulchra</i> Aurivillius	China
		<i>Monochamus alternatus alternatus</i> Hope	Hubei
<i>Moechotypa diphysis</i> (Pascoe)		China	
<i>Oberea formosana</i> Pic		China	
<i>Paraglenea fortunei</i> (Saunders)		China	
<i>Phytoecia rufiventris</i> Gautier des Cottes		China	
	<i>Thyestilla gebleri</i> (Faldermann)	China	
Orsodacnidae	Orsodacninae	<i>Orsodacne cerasi</i> (Linnaeus)	Yunnan
Chrysomelidae	Sagrinae	<i>Sagra (Sagra) femorata</i> Drury	China
		<i>Sagra (Sagrinola) mouhoti</i> Baly	Yunnan
	Bruchinae	<i>Callosobruchus chinensis</i> (Linnaeus)	China
	Donaciinae	<i>Donacia clavipes clavipes</i> Fabricius	Beijing
		<i>Donacia longicornis</i> Jacoby	China
		<i>Donacia provostii</i> Fairmaire	Hebei
		<i>Donacia vulgaris vulgaris</i> Zschach	Hebei
		<i>Macrolepa mutica</i> (Fabricius)	Mongolia
		<i>Plateumaris weisei</i> (Duvivier)	China
		<i>Plateumaris sericea</i> (Linnaeus)	China
		<i>Plateumaris socia</i> (Chen)	Northeast of China
		<i>Sominella longicornis</i> (Jacobson)	China
		<i>Sominella macrocnemia</i> (Fischer Von Waldheim)	Heilongjiang
	Criocerinae	<i>Crioceris quatuordecimpunctata</i> (Scopoli)	China
		<i>Lema coromandeliana</i> (Fabricius)	Yunnan
<i>Lema nigricollis</i> Jacoby		Yunnan	
<i>Lilioceris cheni</i> Gressitt & Kimoto		Guangxi	

Family	Subfamily	Species	Locality
Chrysomelidae	Criocerinae	<i>Lilioceric consentanea</i> Lacordaire	China
		<i>Lilioceric gibba</i> (Baly)	Jiangxi, Fujian
		<i>Oulema oryzae</i> (Kuwayama)	Heilongjiang
	Eumolpinae	<i>Abiromorphus anceyi</i> Pic	Hebei
		<i>Basilepta</i> sp.	China
		<i>Chrysochus chinensis</i> Baly	Jilin, Heilongjiang
		<i>Colasposoma dauricum</i> Mannerheim	Yunnan
		<i>Platycorynus parryi</i> Baly	Guangxi
		<i>Platycorynus</i> sp.	Yunnan
	Lamprosomatinae	<i>Oomorhoides yaosanicus</i> (Chen)	Fujian
	Cryptocephalinae	<i>Cryptocephalus altaicus</i> Harold	Inner Mongolia
		<i>Cryptocephalus limbellus semenovi</i> Weise	Inner Mongolia
		<i>Aspidolopha bisignata</i> Pic	Jilin, Yunnan
		<i>Clytra laeviuscula</i> Ratzeburg	Jilin
		<i>Physosmaragdina nigrifrons</i> (Hope)	Guangxi
		<i>Chlamisus sterconalis</i> (Jacoby)	Guangxi
	Synetinae	<i>Syneta adamsi</i> Baly	Shanxi
	Chrysomelinae	<i>Ambrostoma quadriimpressum</i> (Motschulsky)	Heilongjiang
		<i>Agasta formosa</i> Hope	Yunnan
		<i>Chrysolina aurichalcea</i> (Mannerheim)	Guangxi
		<i>Chrysomela populi</i> Linnaeus	Harbin, Jilin
		<i>Gastrophysa atrocyanea</i> (Motschulsky)	Sichuan
		<i>Phratora bicolor</i> Gressitt & Kimoto	Sichuan
	Galerucinae	<i>Gallerucida</i> sp.	Yunnan
		<i>Medythia nigrobilineata</i> (Motschulsky)	Heilongjiang
		<i>Mimastra limbata</i> Baly	Yunnan
		<i>Morphosphaera cavaleriei</i> Laboissière	China
		<i>Oides tarsatus</i> (Baly)	Guangxi
		<i>Paleosephraria</i> sp.	Yunnan
	Alticinae	<i>Alica viridicyanea</i> (Baly)	Beijing
<i>Podontia affinis</i> (Gröndal)		Yunnan	
<i>Podontia dalmani</i> Baly		Yunnan	
<i>Podontia lutea</i> (Olivier)		Guangxi	
<i>Hemipyxis</i> sp.		China	
Cassidinae	<i>Octodonta nipae</i> (Maulik)	Hainan	
	<i>Lasiobila cylindrica</i> (Hope)	Yunnan	
	<i>Dactylispa</i> sp.	Yunnan	
	<i>Callispa bretteinghami</i> Baly	Yunnan	
	<i>Callispa fortunei</i> Baly	Yunnan	
	<i>Callispa nigricollis</i> Chen & Yu	Yunnan	
	<i>Leptispa longipennis</i> (Gestro)	China	
<i>Basiprionota bisignata</i> (Boheman)	Guangxi		

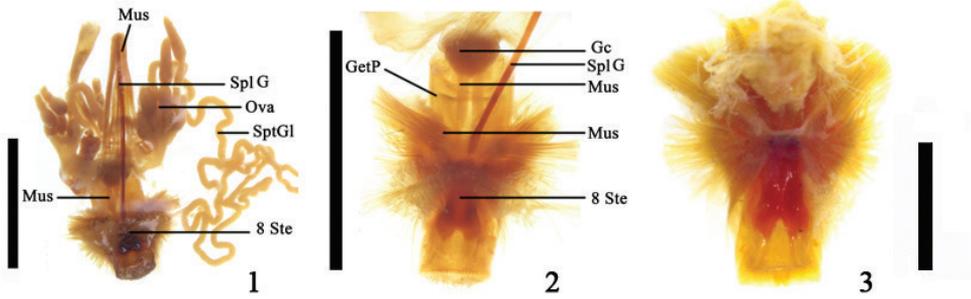
Results

Megalopodidae

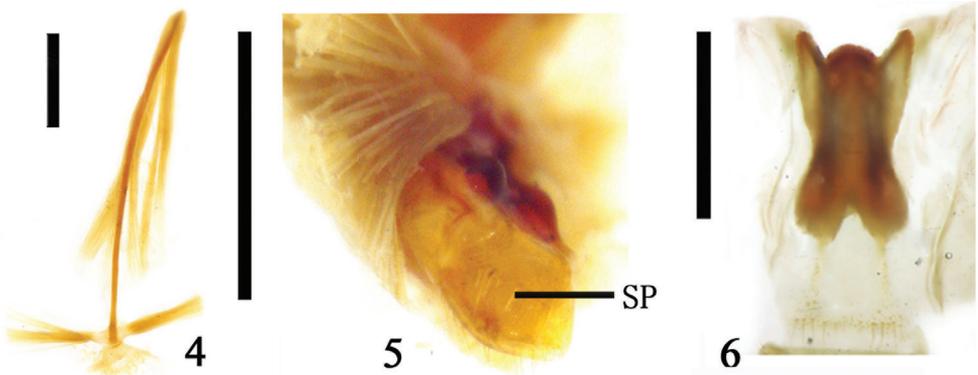
The 8th and 9th abdominal segments (Figs 1, 2) of females in the Megalopodinae are hidden within the abdominal cavity. These are covered by the large pygidium (the tergite of the 7th abdominal segment). The 8th abdominal segment (Fig. 2) is long. Most of the surface of tergite 8 is membranous and slightly sclerotized. Sternite 8 (the genital plate) is shorter than tergite 8. It is an X-shaped, strongly sclerotized apodeme with an irregular surface (Fig. 6). Its middle is partly depressed with a convex lateral side. The anteromedian margin is distinctly prominent (Figs 5–10). The spiculum gastrale is thin, long, and slightly curved (Figs 1, 4), with a slightly thickened tip. The anterior portion reaches to the 1st abdominal cavity or is found slightly beyond the apical portion of the first abdominal segment. Along the apical median of the spiculum gastrale, there is a small groove attached to the longitudinal muscle. The base of the spiculum gastrale is connected to sternite 8 by a loose, slightly sclerotized membrane connecting it with the base of the spiculum gastrale (Figs 2, 4, 7, 8).

The lateral side of the tergite and sternite is connected tightly by a membrane that extends from the apex of the abdominal segment to form a flattened tube that is slightly curved on its ventral side (Figs 8, 10). This apical flattened tube extends inward to the body cavity to form a loosely membranous genital pocket. The membrane of the genital pocket is thick and has many sclerotized rings (Fig. 10). Its anterior portion is broad but the posterior portion is narrow (Figs 9–10), the anterior is located in the 4th abdominal segment cavity. The anterior opening of the flattened tube is mainly occupied by the bursa copulatrix, median oviduct, and the apical rectum. The posterior (i.e., the apex of the flattened tube) is the opening of the genital pocket and vagina to the outside. Apical margins of tergite 8 and sternite 8 rarely contained a row of setae (Figs 7–12). The margin of sternite 8 is slightly shorter than that of tergite 8. There is a transverse, oblique, sclerotized plate, which is connected with the apical margin of sternite 8. This plate usually covers the opening of the genital pocket (Figs 5, 8, 10, 12).

The 9th abdominal segment is long and all of its components are wrapped in a genital pocket. The ovipositor is a long scissor-like, strongly sclerotized, basal half portion that is broad and flattened. Its two oblique palpi are separated on the posterior portion. Their apices are generally close to each other. The outer margin of the ovipositor is slightly flattened (Figs 13–16). A majority of the components associated with the ovipositor fuse but do not have a clear boundary, lacking a proctiger and median plate (Figs 13–16). The paraproct and coxite are fused to form a flattened sclerite at the base. The base of the ovipositor is connected by a membrane to the genital pocket (Fig. 17). The coxite is rather long, with sensory setae on the inner side. The apex of the coxite is cylindrical. The stylus is rather small and connected to the outer margin of the coxite. There are rather long sensory setae on the tip of the stylus. The valvifer is fused with the coxite (Figs 13, 15, 16). The ovipositor has one or two pairs of baculi and one pair extends from the base of the ovipositor backward to the coxite. The bases



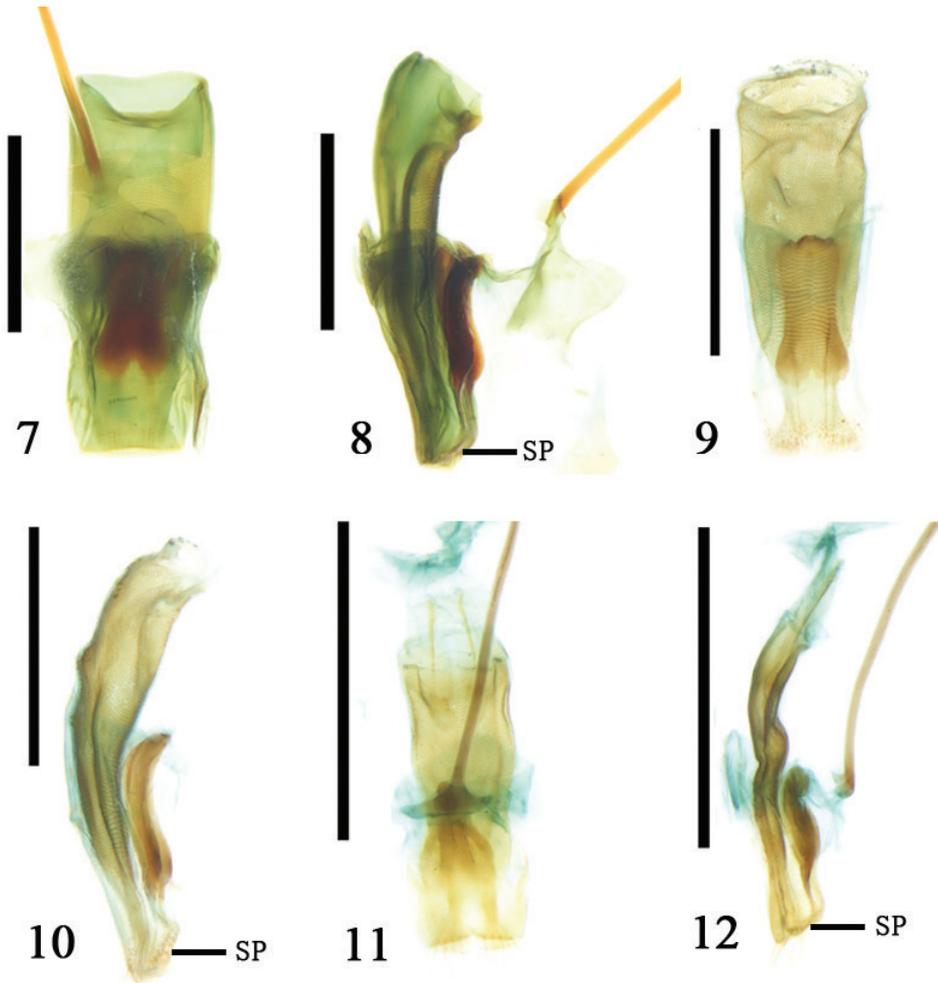
Figures 1–3. 1 Female internal reproductive system of *Temnaspis nankinea* (Pic), ventral view 2 8th and 9th abdominal segment of *T. nankinea*, ventral view 3 Muscles of abdominal segment of *T. nankinea*, ventral view; Abbreviations: genital chamber (Gc); genital pocket (GetP); muscle (Mus); ovary (Ova); spermathecal gland (SptGl); spiculum gastrale (SplG); sternite 8 (8 Ste); scale line = 2.0 mm (Figs 1–2); scale line = 0.5mm (Fig. 3).



Figures 4–6. 4 Muscles of the spiculum gastrale of *T. nankinea*, ventral view 5 The apical sclerite of sternite 8 of *T. nankinea*, ventrolateral view 6 The genital plate of *T. nankinea*; Abbreviations: sclerotized plate (SP); scale line = 0.5 mm.

of the two baculi are attached or alternatively can be found proximal to each other. The ovipositor has one or two pairs of baculi. One pair extends from the base of the ovipositor backward to the coxite; the bases of these two baculi are connected or close to each other. Another pair (if there are two pairs) of baculi are rather short and extend from the coxite to the middle of the ovipositor, the apex of the baculus is free or fused to that of the first pair on each side (Figs 13, 15, 16).

The main muscles of the 8th abdominal segment in *Temnaspis nankinea* (Pic) were examined. It was found that, on the genital plate, there is a large paired bundle of fan-shaped muscles between the median and the lateral apodeme of the anterior margin of sternite 8 (Figs 2, 3, 5), which arises from the lateral side of the longitudinal section of the pygidium. The muscles of the spiculum gastrale are comprised of longitudinal muscles and transverse muscles. The longitudinal muscles consist of left and right bun-



Figures 7–12. 7–8. 8th abdominal segment of *Temnaspis nankinea* (Pic). **7** ventral view **8** lateral view **9–10** Part of 8th abdominal segment of *Poecilomorpha discolineata* (Pic) **9** ventral view **10** lateral view **10–12** 8th abdominal segment of *Zeugophora (Pedrillia) bicolor* Kraatz **11** ventral view **12** lateral view; Abbreviations: sclerotized plate (SP); scale line = 1.0 mm.

dled and rather large muscles (Fig. 4), which extend inclining backward and connect to the dorsal side of the bursa copulatrix. On the lateral tendon of the basal spiculum gastrale, there are two short bands of transversal muscles (Fig. 4), the other end of which connects to the basal lateral angle of the pygidium. The ventral side of the bursa copulatrix has a pair of longitudinal muscle fibers along the inside of the genital plate, extends to the apical margin of sternite 8, and connects to the lateral margin. The egg passes through the median oviduct to the genital chamber and can be discharged through the vulva. The genital plate is located on the ventral side of the genital pocket. In the inner part of the genital plate, there is a concave area which perhaps guides the ovipositor palpi and ovulation.



Figures 13–17. 13–16. Ovipositor **13** *Temnaspis fraxini* (Komiya), ventral view **14** *Poecilomorpha cyanipennis* (Kraatz), ventral view **15** *Zeugophora (Pedrillia) dimorpha* (Gressitt), ventral view **16** *Zeugophora cyanea* Chen, ventral view **17** the connecting of ovipositor and 8th abdominal segment, *Zeugophora (Pedrillia) bicolor* Kraatz, ventral view; Abbreviations: membrane (Mem); ovipositor (Ovi); 8th abdominal segment (8 AS); scale line = 0.5 mm.

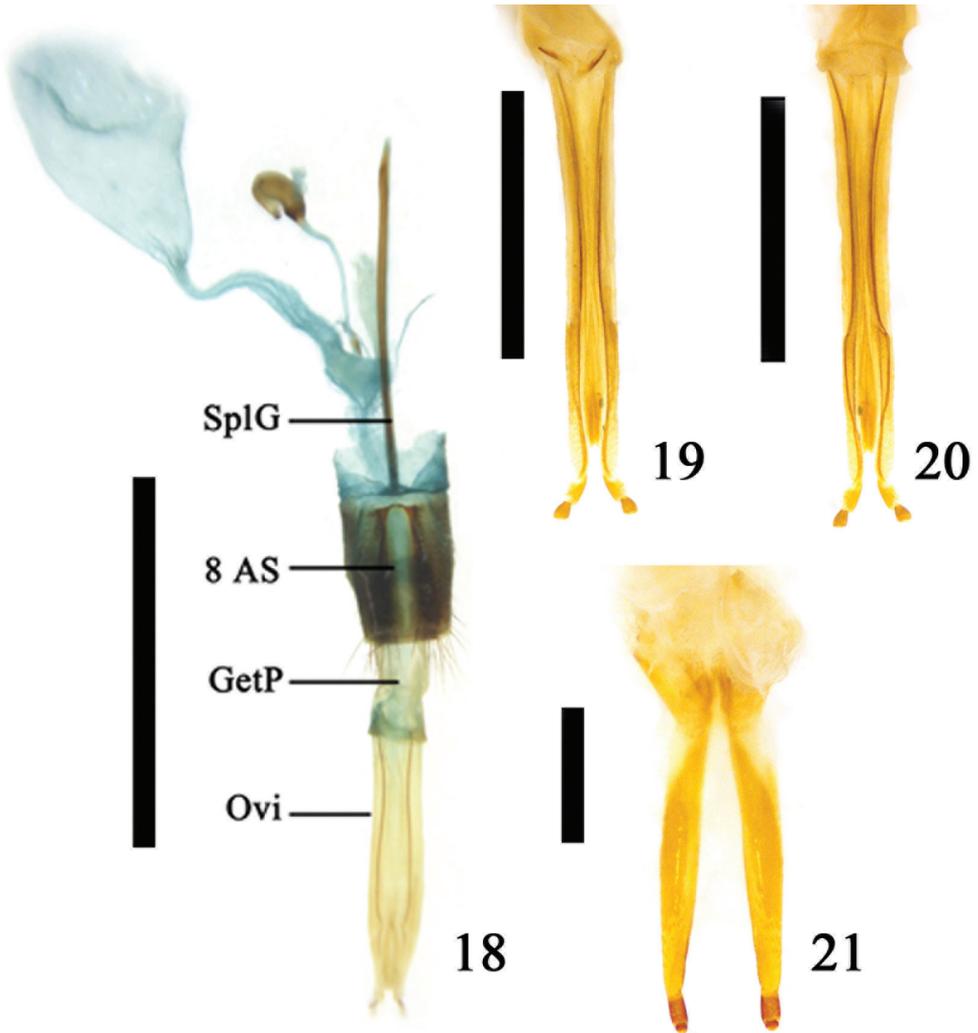
The structure and morphology of female genitalia of the subfamilies Zeugophorinae and Megalopodinae are very similar. However, their body sizes and external morphologies differ significantly. The genital pocket is approximately cylindrical, its anterior end is narrow, and its posterior portion is flat and generally broadly extended beyond the genital plate. Apical margins of tergite 8 and sternite 8 are nearly equal in length, and rarely contained a row of setae. The ventral apical margin is slightly sclerotized, and the opening of the genital pocket does not close tightly. The curved portion of the flattened tube is located far from the posterior opening. The coxite generally does not exceed the curved portion. The shape of the genital plate is different from that of the Megalopodinae. The genital plate in the Zeugophorinae is cordate in some species (Figs 11, 12), but nearly X-shaped in other species, similar to those in Megalopodinae, such as *Zeugophora scutellaris* Suffrian, *Zeugophora (Pedrillia) yunnanica* Chen and Pu, *Zeugophora (Pedrillia) dimorpha* (Gressitt), etc.

Cerambycidae

In the majority of members of the family Cerambycidae the tergite and sternite of the 8th abdominal segment are combined to form a flattened segment (Fig. 18). The tergite and sternite are generally equal in length and the apical margin generally contains setae along each side, or alternatively can be found on the lateral angle near the apical margin of the setae plexus (Fig. 18). Inside the apical margin of the 8th abdominal segment there is an inward fold, which extends to the body cavity to form a genital pocket (Fig. 18). The apical opening of the 8th sternite is the posterior opening of the genital pocket, which generally is not closed tightly. Although the lateroapical margin near the posterior opening is slightly dark or slightly sclerotized it is in fact not a sclerite. The genital pocket is stiff and membranous, and its surface has many sclerotized rings. The thickness of the membrane differs among different species. It generally stretches from the apex of the 8th abdominal segment to the basal ovipositor and connects with the ovipositor by a membrane. Morphological variation of the 8th abdominal segment is extremely variable in different species of cerambycids (Figs 18, 20, 23, 24).

In general, the genital pocket is cylindrical in shape, but variable among different species. The variation of genital pocket is correlated with the change of the 8th abdominal segment. The spiculum gastrale is thin and long, almost four times the length of sternite 8 and it also has a rod-like or slightly thick apex (Fig. 18). In some species the spiculum gastrale, which is tightly connected to sternite 8 by a tendon, moves freely (Figs 23–26), but a few species lack this joint between the spiculum gastrale and sternite 8 and do not move freely (Figs 22, 27). The species that have this joint are listed as follows (Figs 23–26): *Aegosoma sinica sinica* (White) (Prioninae), *Gaurotes virginea aemula* (Mannerheim), *Leptura annularis annularis* Fabricius (Lepturinae), *Aromia bungii* (Faldermann) (Cerambycinae), *Monochamus alternates* (Hope), *Bacchisa comata* (Gahan), *Oberea formosana* Pic, *Glenea centroguttata* Fairmaire, *Phytoecia rufiventris* Gautier des Cottés, *Paraglenea fortunei* (Saunders), *Thyestilla gebleri* (Faldermann), *Eodorcadion brandti* (Gebler) (Lamiinae); The species that have no joint are as follows: *Aseum amurense* Kraatz (Spondylidinae), *Anoplistes halodendri* (Pallas) (Figs 22, 27), *Purpuricenus temminckii* (Guérin-Méneville) (Cerambycinae). No species were lacking a spiculum gastrale in the Cerambycidae.

The 9th abdominal segment is generally elongated and membranous (Fig. 18). In a stationary state, all components of the 9th abdominal segment are located within the genital pocket. The ovipositor is also elongated and gradually narrows down to a posterior portion (Figs 18–21). Two palpi are usually located near the apex. The components of the ovipositor are generally complete, with all sections containing baculi (Figs 19–20). The proctiger is rather short or absent and the pair of baculi are located on each side (Fig. 19) (only the proctiger in the subfamily Philinae is elongated, Saito 1993). The paraproct is generally elongated, and on its basolateral side its baculus is long and straight. The valvifer is generally fused with the coxite. The apex of the coxite is cylindrical and its outer part is generally swollen (Figs 19–20). It is generally



Figures 18–21. 18 Part of internal reproductive system and ovipositor of *Aromia bungii* (Faldermann), ventral view 19–20 ovipositor of *Leptura annularis annularis* Fabricius 19 dorsal view 20 ventral view 21 ovipositor of *Glenea pulchra* Aurivillius. Abbreviations: genital pocket (GetP); ovipositor (Ovi); spiculum gastrale (SplG); 8th abdominal segment (8 AS); scale line = 1.0 mm (Figs 19–20); scale line = 5.0 mm (Figs 18, 21).

longer than the baculi. The stylus is located on the tip or lateroapical margin of the coxite (Figs 19–21). The ventral side of the ovipositor generally has a median plate (Figs 19–20). In some species of Cerambycidae, the ovipositor is reduced, but can be recognized from the site of the reserved baculus. They generally contain a pair of long dorsal baculi on the dorsal side of the ovipositor in the Cerambycidae (except in the Lamiinae, Saito 1993) (Fig. 21). The dorsal baculi extends from the posterior of the coxite to the base of the ovipositor.

1. Prioninae

The 8th abdominal segment in the subfamily Prioninae (genus *Aegosoma*) is exposed to the outside with the posterior extending into a cone-like shape. The genital pocket folds inward from the apex of the 8th abdominal segment. The apical portion of the genital pocket is narrow and its membrane is rather thick and tightly wrapped in the center forming a thin pipe. The apex of the spiculum gastrale is slightly thickened and is connected to the sternite by a joint. The components of the ovipositor are sectioned clearly with an outward apex. The coxite is slightly swollen. The stylus is small and located on the lateral side of the coxite.

2. Spondylidinae

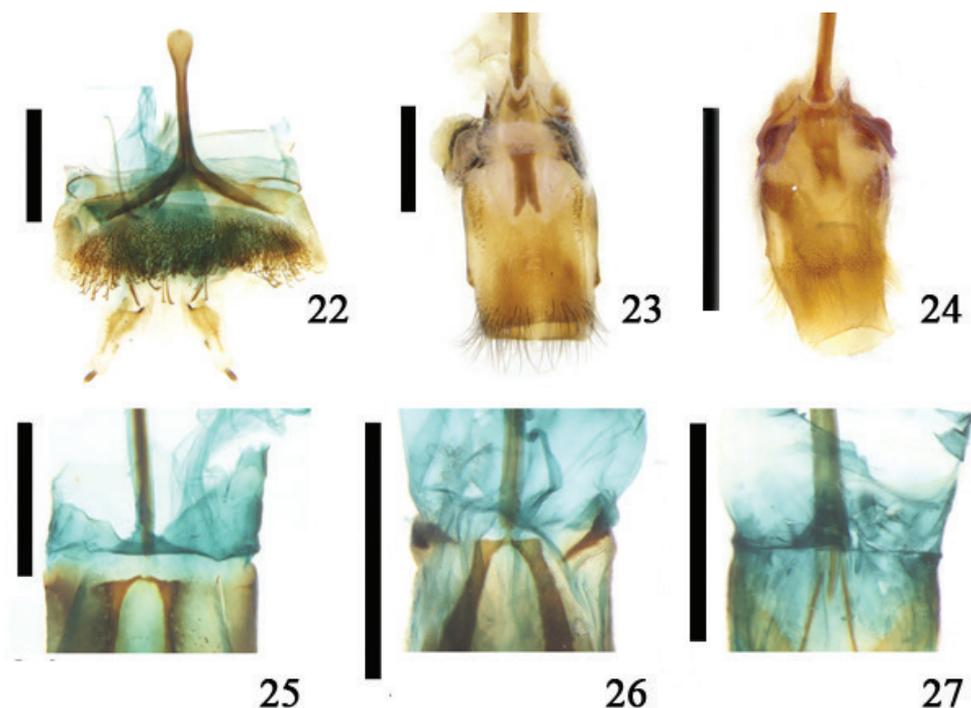
The tergite of the 8th abdominal segment in the subfamily Spondylidinae (genus *Spondylis*) is sclerotized and the ventrolateral side of the tergite is folded. Most portions of the sternite are membranous and a sclerite is found on either side. The apical margin of the 8th abdominal segment is folded inward to form a genital pocket. The spiculum gastrale is located between the sclerites of the sternite centroapical margin, which connects to the sternite without a joint. The components of the ovipositor are complete and easily viewed. The coxite is slightly swollen. The stylus is located on the lateral side of the coxite.

3. Cerambycinae

In the genus *Aromia*, the length of the 8th abdominal segment is longer than its width (Fig. 18). The tergite is sclerotized in its central portion and is membranous with the lateral margins folding towards the ventral side. Most portions of the sternite are slightly sclerotized. The apical margin of the 8th abdominal segment folds inward to form a genital pocket. There is a joint between the spiculum gastrale and the sternite. The components of the ovipositor are distinct with the stylus located on the apex of the coxite (Fig. 18). In the genera *Anoplistes* and *Purpuricenus*, the 8th abdominal segment is short, broad, and flattened. The anterior margin of the sternite is slightly sclerotized and the setae cluster contains a mixture of long and short setae. The lateral side of the tergite is slightly sclerotized. Species of both genera *Anoplistes* and *Purpuricenus* do not have genital pockets (Fig. 22). Their spiculum gastrale is short. There is no joint between the spiculum gastrale and the sternite. The ovipositor is short and lacks a proctiger. The ovipositors of the latter two genera are membranous. The remaining components of the ovipositor show clear boundaries. The coxite is swollen and its lateral side has setae. The stylus is located on the lateral side of the coxite.

4. Lepturinae

In the genus *Leptura*, the length of the 8th abdominal segment is longer than its width and its tergite is connected to the sternite via a membrane that forms a flattened segment. The tergite and the sternite are slightly sclerotized and the apical margin of the 8th abdominal segment folds inward to form a genital pocket. There is a joint between the spiculum gastrale and the sternite. The ovipositor is rather long, its posterior portion extends outward, making the components of the ovipositor clearly visible. The stylus is located on the apex of the swollen coxite (Figs 19–20).



Figures 22–27. Part of 8th abdominal sternite, ventral view **22** *Anoplistes halodendri* (Pallas) **23** *Glenea centroguttata* Fairmaire **24** *Thyestilla gebleri* (Faldermann) **25** *Aromia bungii* (Faldermann) **26** *Leptura annularis annularis* Fabricius **27** *Asemum amurense* Kraatz; scale line = 1.0 mm.

5. Lamiinae

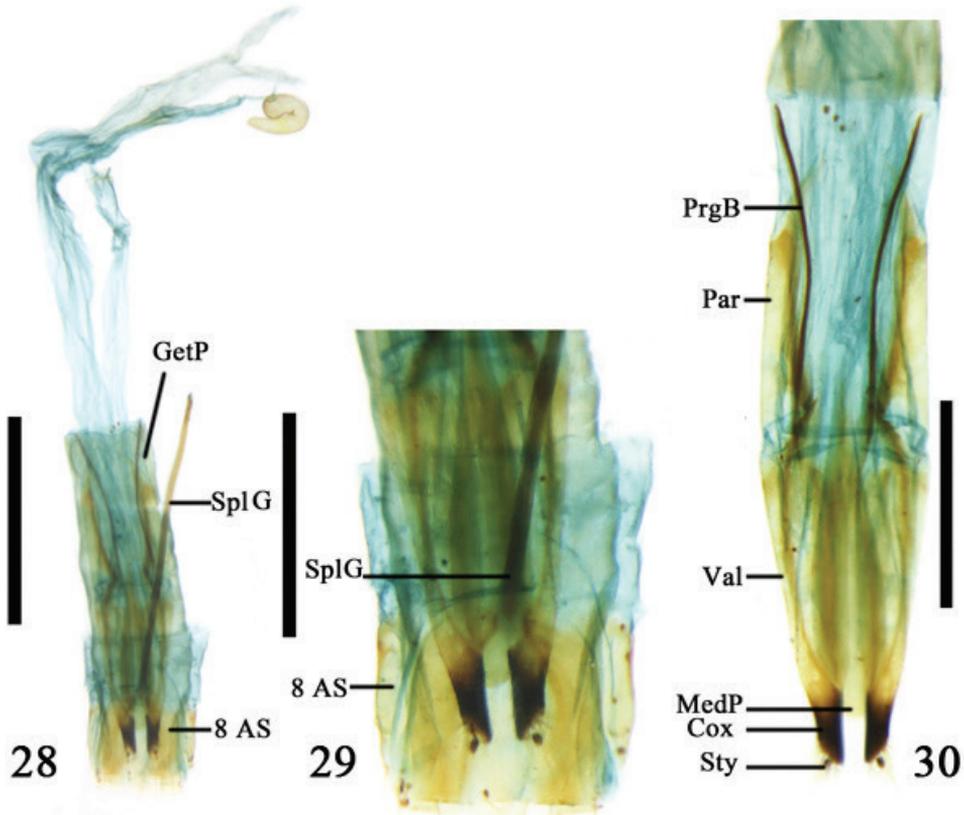
Lamiinae is a subfamily that includes a variety of taxa. We observed the genitalia of the following genera: *Monochamus*, *Oberea*, *Glenea* (Fig. 21), *Bacchisa*, *Paraglenea*, *Phytoecia* and *Thyestilla*. The tergite and sternite of the 8th abdominal segment is generally sclerotized and connect to form a flattened segment. The tergite and sternite are usually equal in length. The posterior opening of the 8th abdominal segment generally does not close tightly. Mostly, on the apical margin of the 8th abdominal segment, there are setae and a seta cluster is located at the near corners. The color of these setae is generally dark or slightly sclerotized but they do not form a sclerite. In other genera such as *Thyestilla*, *Phytoecia*, and *Paraglenea*, the apex of the 8th abdominal segment is membranous, forming the apex of the genital pocket. It is similar to the apex found in females of the Megalopodidae but their tergite and sternite margins are sub-equal in length and not tightly closed. The spiculum gastrale is thin, long and is connected to the sternite with a joint. We found that the apex of the spiculum gastrale in the genus *Eodorcadion* is not regular in shape and has an angled apodeme at its apex for muscle attachment. The 9th abdominal segment is long. The ovipositor is narrow from its base to apex. The apex of the ovipositor is not inclined towards the outer part. The coxite is not swollen and does not contract at

its base. The outer part of the coxite is comparatively straight (Fig. 21). Most of the components of the ovipositor are present. The proctiger is rather short or absent. The paraproct is shorter than in other taxa in the Cerambycidae but the structures are visible. The valvifer is generally fused to the coxite. The stylus is small and generally located on the apex of the coxite.

Morphological variation of female genitalia in the Cerambycidae is rather minimal compared to the Chrysomelidae. Females in the Cerambycidae usually lay their eggs in tree bark cracks, under tree bark or in soil (Saito 1993). We find that the genitalia of *Anoplistes halodendri* (Pallas) (Cerambycinae) and *Purpuricenus temminckii* (Guérin-Méneville) (Cerambycinae), are very different from those found in more common species of Cerambycidae but are closer to the chrysomelids. Their 8th abdominal segment does not have a genital pocket. Their spiculum gastrale is short and is connected to the sternite without a joint. Their ovipositors are rather short. In addition, two species *Phytoecia rufiventris* Gautier des Cottés, *Thyestilla gebleri* (Faldermann) consume herbs, and their life history and morphology require further investigation. We found that the apex of their abdominal segments were membranous differing from the Cerambycidae.

Orsodacnidae

Abdominal segment 8 is sub-quadrangle. The tergite and sternite are weakly sclerotized and are connected via a membrane on lateral sides forming a flattened cylindrical segment. The central portion of the tergite is membranous while the central portion of the sternite has one weakly sclerotized sclerite without an apodeme (Fig. 28). The spiculum gastrale is long and thin, connecting with sternite 8 via a ligament (Fig. 29). The inside of sternite 8 has a membranous genital pocket that is as thin as a common inter-segmental membrane, and covers the outside of the 9th abdominal segment. The 9th abdominal segment is long and slightly flattened. The components of the ovipositor are sclerotized with clear boundaries. The proctiger is rather long and membranous but slightly shorter than the ovipositor. The ovipositor has a pair of baculi on its lateral side. The proctiger connects with the paraproct at its base. The paraproct is long and narrow and it has one long baculus whose anterior connects with the baculus of the valvifer. The coxite is long with a cylindrical apex and is strongly sclerotized. The coxites are parallel in the central part and the apices do not reach outward. The apical margin is surrounded by long setae and its tip is oblique and truncated. The stylus is rather thin but not long and does not protrude. Its apex has long setae (Fig. 30). The valvifer is long and narrow but can be distinguished from the coxite. The base of the valvifer connects with the paraproct. The median plate is located on the ventrocentral of the valvifer. The structure of the external genitalia in this family is rather primitive for Chrysomeloidea.



Figures 28–30. Ovipositor and 8th abdominal segment of *Orsodacne cerasi* (Linnaeus) **28–29** ventral view **30** dorsal view; Abbreviations: coxite (Cox); genital pocket (GetP); median plate (MedP); paraproct (Par); baculus of proctiger (PrgB); spiculum gastrale (SplG); stylus (Sty); valvifer (Val); 8th abdominal segment (8 AS); scale line =0.5mm.

Chrysomelidae

In this family, the inside of the 8th abdominal segment has no genital pocket. Sternite 8 is usually reduced and the spiculum gastrale has no joint. The length of the 9th abdominal segment is usually shorter than the width of its base, and there is less membrane compared to the Megalopodidae, Cerambycidae, and Orsodacnidae. Morphological of the ovipositor is variable and has all components although some portions are reduced or fused. The baculi of the ovipositor are poorly developed. This includes the early branched groups (the Sagrinae and the Eumolpinae) and the late appeared group Alticinae and Cassidinae.

1. Sagrinae

The genus *Sagra* has a well-developed sternite 8 and a moderate to long spiculum gastrale. The apex of the spiculum gastrale is slightly expanded and its ovipositor is short

and thick. The proctiger is membranous with a sclerite on each side of the proctiger. The apex of the proctiger inserts into tergite 8. The paraproct is strongly sclerotized and thick, while its apex is connected to the valvifer, coxite, and stylus. There is a median plate between the valvifer on its ventral side. This is representative of primitive types in the Chrysomelidae (Figs 31–32).

2. Bruchinae

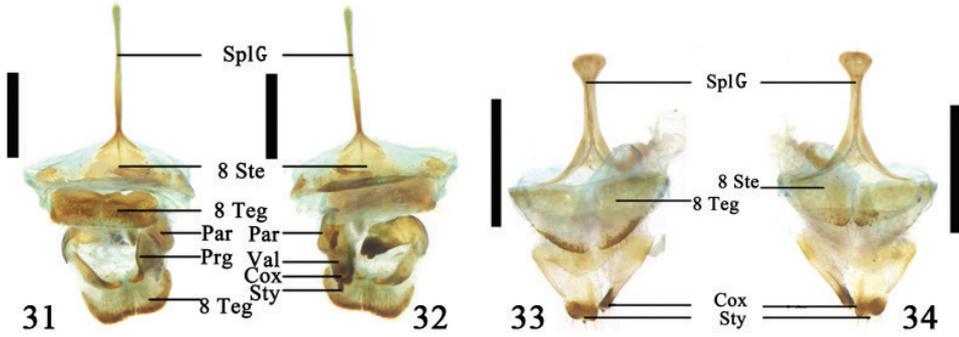
The 8th abdominal segment, associated with members of the genus *Callosobruchus*, is quadrate. The ventral side of the tergite is close to the proctiger. The central part of the tergite is membranous and its lateral side is sclerotized. The sternite is membranous. The tergite and sternite are connected to each other on the lateral side. The spiculum gastrale is short, about 1.5 times the length of the sternite, and lacks a broad apex. In *Callosobruchus chinensis* (Linnaeus) the spiculum gastrale extends forward turning backward at its base. The length of the 9th abdominal segment is almost equal to the length of the 8th abdominal segment. The proctiger is approximately triangular and membranous with sclerotized lateral parts. The base of the proctiger is connected to the paraproct. The ovipositor is small. The valvifer, coxite, and stylus lack clear boundaries.

3. Criocerinae

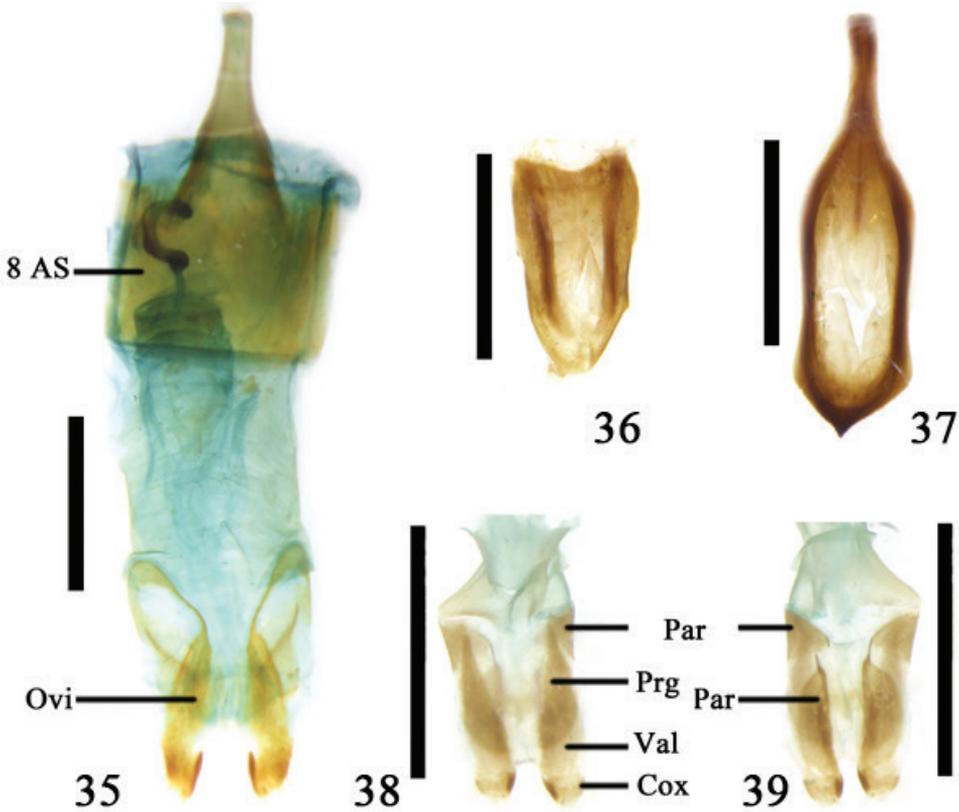
This subfamily is similar to the Sagrinae but the spiculum gastrale is relatively short (Figs 33–34). The components of the ovipositor show clear boundaries but the valvifer generally fuses with the coxite or the paraproct. In some cases the stylus may be either indistinct (genus *Oulema*) or distinct according to species (e.g. *Liliocercis* and *Lema*). The components of the ovipositor have clear boundaries in the genus *Mecoprosopus* although their valvifer is fused with the paraproct.

4. Donaciinae

Donaciinae is an aquatic subfamily. The 8th and 9th abdominal segments are slightly sclerotized. The spiculum gastrale of the 8th abdominal segment is rather long. The 9th abdominal segment is significantly long and membranous. The proctiger is usually long, triangular and its basolateral side is connected to the paraproct. The valvifer usually fuses with the coxite. The stylus is generally indistinct. The area of sensory setae is located on the surface of the tip of the coxite and may be interpreted as a stylus (Figs 35, 38, 39). It is only in the genus *Macrolea* that the ovipositor has all its components with clear boundaries. The female of *Donacia provostii* Fairmaire inserts her ovipositor in a small hole on the dorsal surface of a leaf and lays eggs on the leaf's ventral side. The inter-segmental membrane between the 8th and 9th abdominal segments is very long. In the genus *Plateumaris* the 8th abdominal segment is sclerotized and specialized forming a flattened sheath (Figs 35–36) with the ovipositor in this sheath. Members of this genus have no proctiger. We found that the inter-segmental membrane between the 8th and 9th abdominal segments in *Plateumaris socia* (Chen) was rather long.



Figures 31–34. Ovipositor and 8th abdominal segment **31–32** *Sagra (Sagrinola) mouhoti* Baly **31** dorsal view **32** ventral view **33–34** *Lilioceric cheni* Gressitt & Kimoto **33** dorsal view **34** ventral view; Abbreviations: coxite (Cox); paraproct (Par); proctiger (Prg); spiculum gastrale (SplG); stylus (Sty); sternite 8 (8 Ste); tergite 8 (8 Teg); valvifer (Val); Scale line = 1.0 mm.



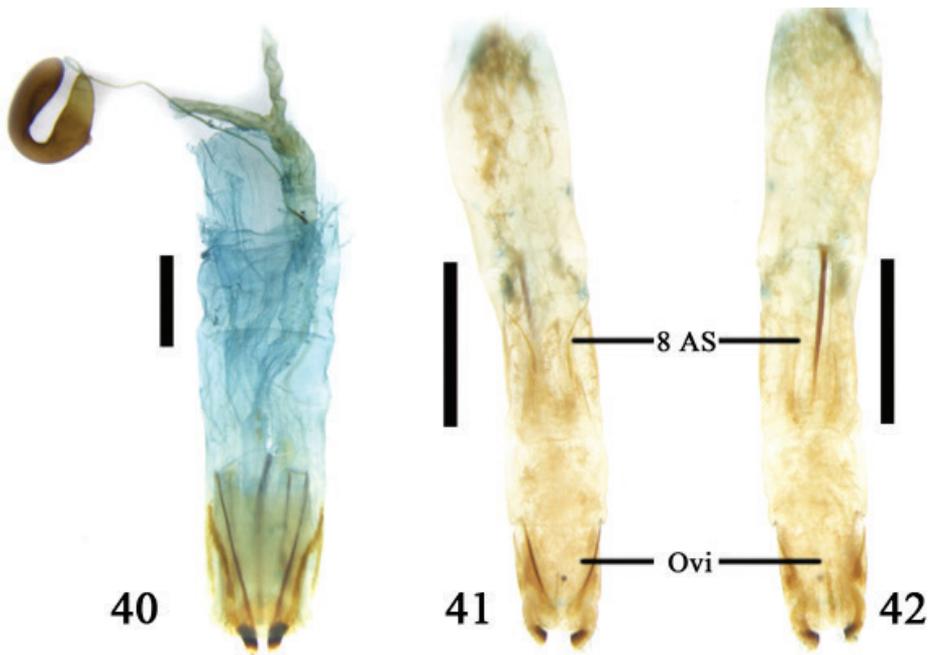
Figures 35–39. **35** Ovipositor and 8th abdominal segment of *Donacia longicornis* Jacoby **36–37** *Platteumaris weisei* (Duvivier) **36** tergite 8 **37** sternite 8 **38–39** ovipositor of *Macrolea mutica* (Fabricius) **38** dorsal view **39** ventral view; Abbreviations: coxite (Cox); ovipositor (Ovi); paraproct (Par); proctiger (Prg); valvifer (Val); 8th abdominal segment (8 AS); scale line = 1.0 mm.

5. Eumolpinae

The reproductive segment of this subfamily is distinctive in the Chrysomelidae. In the genera *Platycornus*, *Colasposoma* and *Chrysochus* the tergite and sternite of the 8th abdominal segments fuse laterally, forming a flattened cylinder. The lateral side of tergite 8 has a sclerotized area and sternite 8 is sclerotized (Figs 46–47) but the sclerite of sternite 8 is longer than the length of tergite 8. The inter-segmental membrane of the 8th and 9th abdominal segment is very long forming a membrane surrounding the outside of the 9th segment (Figs 40–42). The 9th abdominal segment is longer than broad at its base and its membrane is developed. The ovipositor has all components with clear boundaries and it has developed baculi. The proctiger is membranous, long, triangular, and its apical margin is emarginated with its lateral side connected to the paraproct (Fig. 43). The ventrocentral portion of the ovipositor has a long and narrow median plate (Fig. 44). We dissected one specimen of *Chrysochus chinensis* Baly. Its ovipositor was much elongated and 1/2 the length of the body (from the head to the apex of the elytra) (Fig. 45).

6. Lamprosomatinae

In the genus *Oomorhoides*, the lateral side of tergite 8 is sclerotized and sternite 8 is slightly sclerotized without a spiculum gastrale (Fig. 53). The middle of its proctiger is membranous with sclerotized lateral sides. The paraproct is fully developed connecting to the proctiger on its lateral side. The ovipositor has all components showing clear



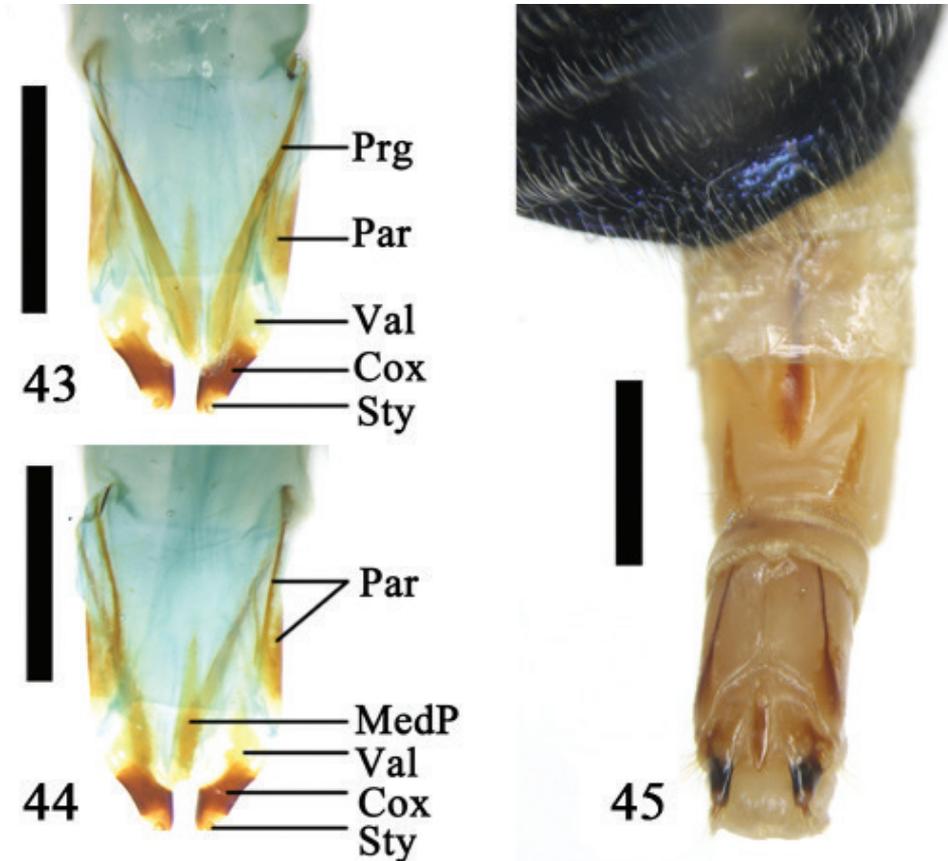
Figures 40–42. 40 ovipositor and 8th abdominal segment of *Platycornus* sp. 41–42 ovipositor and 8th abdominal segment of *Colasposoma dauricum dauricum* Mannerheim; Abbreviations: ovipositor (Ovi); 8th abdominal segment (8 AS); scale line = 1.0 mm.

boundaries. The stylus is located on the tip of the coxite, which is fused with the valvifer at its base. The ventrocentral portion of the ovipositor has a median plate.

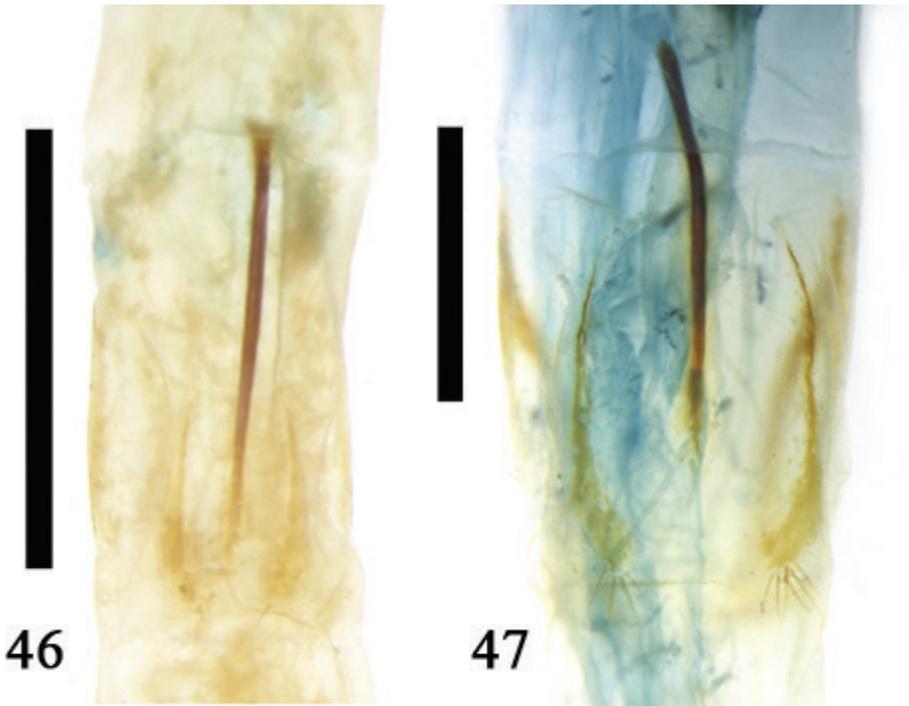
7. *Cryptocephalinae*

7.1. *Cryptocephalini*

In the genus *Cryptocephalus* the proctiger is membranous and the central portion of its apex is emarginated with the lateral side sclerotized with sharp protrusions. The paraproct is developed with most of this structure connected to the proctiger. The ventral portion of the paraproct is located on the lateral side of the 9th segment. The coxite is a wedge-shaped sclerite, extending from central region to the outside, which connects it with the valvifer. This valvifer connects to the paraproct. The inner side of the coxite protrudes backward while the outer part is an inclined plate with some setae. This inclined plate should be the stylus (Figs 51–53).



Figures 43–45. *Chrysochus chinensis* Baly 43–44 ovipositor 43 dorsal view 44 ventral view 45 ovipositor and 8th abdominal segment, ventral view; Abbreviations: coxite (Cox); median plate (MedP); paraproct (Par); proctiger (Prg); stylus (Sty); valvifer (Val); scale line = 1.0 mm.



Figures 46–47. 8th abdominal sternite, ventral **46** *Colasposoma dauricum dauricum* Mannerheim **47** *Platycorynus* sp.; scale line = 1.0 mm.

7.2. Clytrini

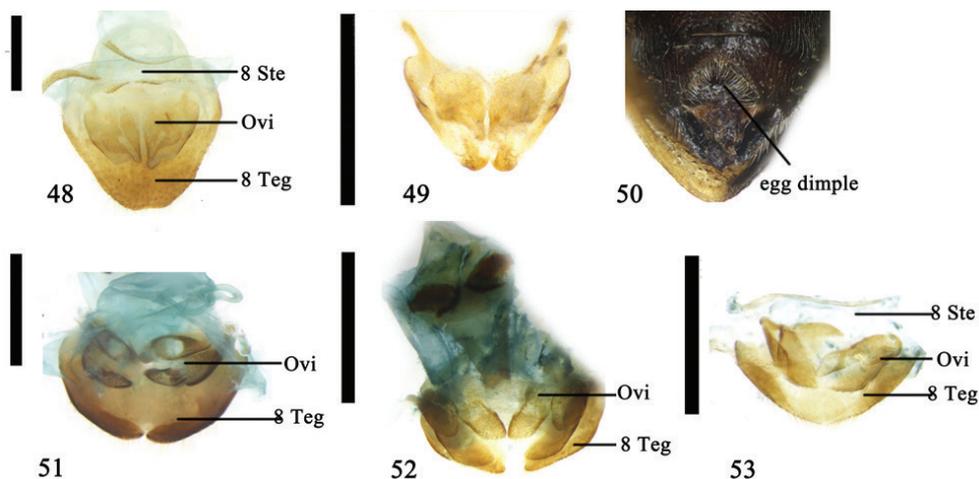
In genus *Aspidolopha*, the ovipositor has all components but the stylus is tiny. The coxite and valvifer have clear boundaries while the paraproct is strongly sclerotized.

7.3. Fulcidacini

In the genus *Chlamisus*, sternite 8 is membranous and the vaginal palpi are robust with no stylus. Most portions of the boundaries between the valvifer and coxite are invisible, with just a small piece located inside being visible. The valvifer is very long.

8. Synetinae

The last visible tergite (pygidium) extends backward (Fig. 48). Sternite 8 is membranous but its margin is sclerotized without a spiculum gastrale (Fig. 48). Segment 9 is broader than its length. The proctiger and apex of the ovipositor palpi are on the ventral side of the pygidium. The proctiger is a broad plate and weakly sclerotized with an emarginated and sclerotized apical margin. The paraproct is broad with its lateral margin closely connected with the proctiger. The coxite is sub-triangular instead of cylindrical. Because of this variation in the coxite there is no obvious or prominent stylus. However on the inclined plane of the outside of the coxite there is a long membranous surface that may be the stylus according to the structure of the ovipositor. The valvifer is located on the base of the coxite, with



Figures 48–53. 48–50 *Syneta adamsi* Baly 48 8th abdominal segment, ventral view 49 ovipositor, dorsal view 50 egg dimple of last abdominal sternite, ventral view 51–53 Ovipositor, ventral view 51 *Physosmargadina nigrifrons* (Hope) 52 species of Cryptocephalinae 53 *Oomorphoides yaosanicus* (Chen); Abbreviations: ovipositor (Ovi); sternite 8 (8 Ste); tergite 8 (8 Teg); scale line = 0.5 mm (Figs 48, 49, 51, 53); scale line = 1.0 mm (Figs 52).

the outer part of the valvifer connected to the paraproct (Fig. 48). There is a smooth inner wall with the egg dimple on the apical margin of the 7th abdominal segment (Fig. 50).

9. Chrysomelinae

The central portion of tergite 8 is membranous, and it is sclerotized laterally. The sternite is normal or reduced. The whole subfamily, except in the genus *Timarcha*, has no spiculum gastrale. The central section of proctiger is membranous, sometimes reduced. The basolateral margin of the proctiger is connected to the paraproct. The ovipositor is short, robust, and palp-like, its components are fused and it may have a median plate. Sternite 8 in the genus *Chrysomela* is membranous. Its apical margin is sclerotized and centrally emarginated while the ovipositor is rod-like, weakly sclerotized with a rather thin base. The components of the ovipositor are fully fused together, with the stylus circle-shaped at the apex (Fig. 55). In the genus *Agasta*, the ovipositor palpi are strongly sclerotized with visible components lacking a clear boundary (Fig. 54). Each side of the proctiger in the genus *Chrysolina* has a strongly sclerotized sclerite. The outer part of the base is connected to the paraproct. The palpi of the ovipositor are strongly sclerotized and the valvifer fuses with the coxite. The sensory setae area at the tip of the coxite should be the stylus. The stylus and coxite in such genera as *Colaphellus* and *Gastrophysa* have clear boundaries. The latter genus has no proctiger and the center of its ventral ovipositor has a median plate.

10. Galerucinae

While the morphology of the spiculum gastrale is variable (Figs 56–57) most are long and narrow as in the genera *Cneorane*, *Galeruca*, *Gallerucida*, and *Monolepta*. In contrast, the spiculum gastrale is rather short in the genus *Paleosepharia* and sternite

8 is reduced to a membrane. In the genus *Morphosphaera*, sternite 8 is normal but the spiculum gastrale is absent while the center of the proctiger is membranous and its lateral sides are sclerotized. Its paraproct is generally not well differentiated. The morphology of the ovipositor in this subfamily is also variable. Generally it lacks a median plate. The ovipositor palpi in the genus *Gallerucida* consist of a pair of parallel, thin, long sclerites. The coxite is cylindrical and the stylus projects long setae at its tip. The valvifer is thin and long and there is no boundary between the valvifer and coxite. The base of the sclerite of the ovipositor palpi may be the paraproct. The inter-segmental membrane of the 8th and 9th abdominal segments is very long. In the genus *Morphosphaera* rod-like components fuse with a rather broad median plate. The ovipositor palpi in genus *Oides* fuses to form a strongly sclerotized rhomboid with a narrow and thin base. Its apical portion is narrow and pointed. The ovipositor in genus *Cneorane* is parallel and lacks a distinct stylus but the sensory setae on its tip are clearly visible. The valvifer fuses with the coxite. The basal sub-rounded sclerites of the ovipositor palpi may be made by the paraproct because there is a clear boundary between the paraproct and the coxite.

11. Alticinae

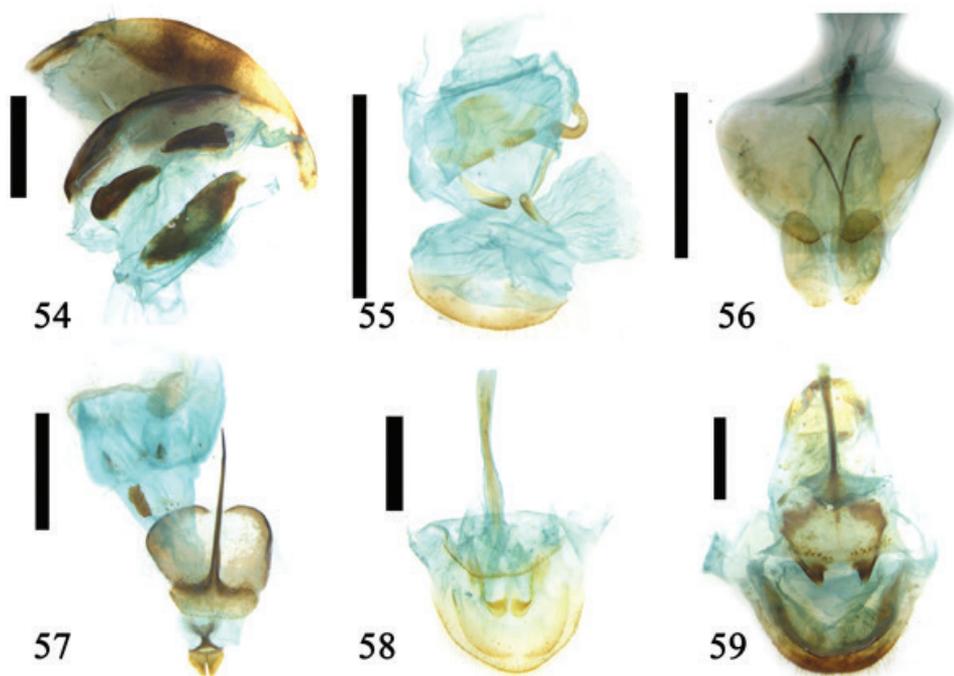
There is no obvious difference between the 8th and 9th abdominal segments in the subfamilies Alticinae and Galerucinae. Both subfamilies lack distinct paraproct. The coxite in the genus *Podontia* is cylindrical and has a small stylus (Figs 58–59). The base of the coxite of *Podontia lutea* (Olivier) has a small transverse sclerite. The base projecting the sclerite of *Podontia dalmani* Baly may be the paraproct (Fig. 59). The ovipositor in the genus *Hemipyxis* diverges at its base with a cylindrical coxite. The apex of this coxite lacks a stylus but bears a couple of sensory setae. The valvifer may be fused with the coxite. The sclerite is divided at the base of the ovipositor and may represent the paraproct. The ovipositor in the genus *Altica* is a simple cylinder. Its coxite is connected to a baculus and the stylus is on the tip of the coxite.

12. Cassidinae

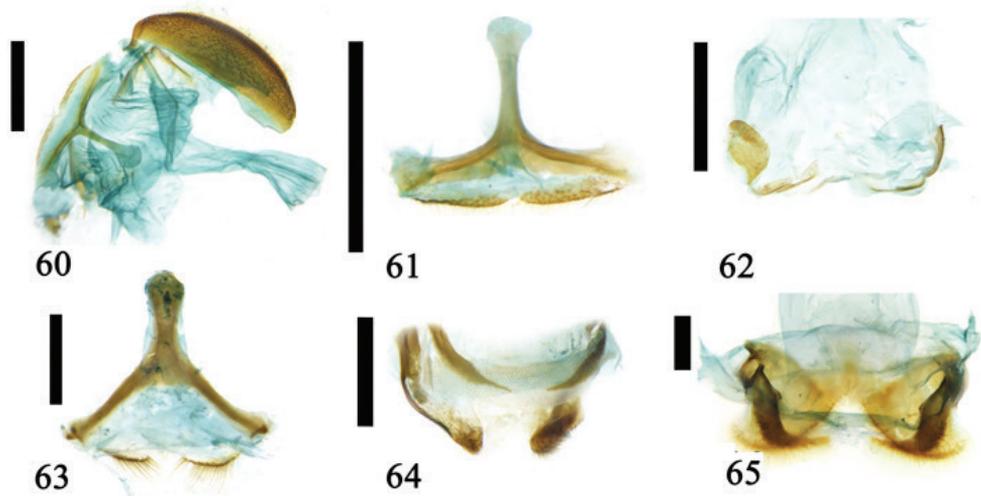
In this subfamily the pygidium is tergite 8, and in most species, sternite 8 is reduced to an arc-shaped piece. The spiculum gastrale is usually short and broad, but it is missing in a few species. The ovipositor palpi are rather short, with robust and plate-like apices. The coxite, valvifer, and paraproct are generally fused together. The stylus is usually indistinct. The proctiger is membranous, both sides have rectangular sclerites, base connected with the paraproct.

12.1. Hispini

The genus *Octodontia* has no spiculum gastrale and its ovipositor palpi are robust and broad, with apices bearing setae. The paraproct is transverse at the base. Sternite 8 in genus *Lasiochila* is arc-shaped with a short broad spiculum gastrale. Its ovipositor palpi are rather long, but are separated from each other at their bases becoming close to each other at their apices. (Figs 63–64).



Figures 54–59. Ovipositor and 8th abdominal segment **54** *Agasta formosa* Hope **55** *Chrysomela populi* Linnaeus **56** *Paleosephraria* **57** *Mimastra limbata* Baly **58** *Podontia affinis* (Gröndal) **59** *Podontia dalmani* Baly; scale line = 1.0 mm.



Figures 60–65. **60** 8th abdominal sternite of *Callispa bretteghami* Baly **61** 8th abdominal sternite of *Callispa nigricollis* Chen & Yu **62** ovipositor of *Callispa nigricollis* Chen & Yu **63** 8th abdominal sternite of *Lasiochila cylindrica* (Hope) **64** ovipositor of *Lasiochila cylindrica* (Hope) **65** ovipositor of *Basiprionota bisignata* (Boheman); scale line = 0.5 mm (Figs 63–65); scale line = 1.0 mm (Figs 60–62).

The central portion of sternite 8 in the genus *Dactylispa* is sclerotized and the spiculum gastrale is rather narrow. Ovipositor palpi are long with distinctly big, thick apices. The stylus is not distinct and the coxite and valvifer fuse. The paraproct is located at the base of the ovipositor.

In the genus *Callispa*, the proctiger is membranous and the anterior margin on the lateral side has a sclerite while the paraproct is reduced to a transverse sclerite at the base of the ovipositor. The ovipositor is plate-like and the stylus, coxite, and valvifer are fused without a clear boundary (Figs 60–62).

12. 2. Cassidini

In the genus *Basiprionota*, both proctiger and paraproct structure is similar to that of the genus *Callispa* but the paraproct is located at the base of the ovipositor and is reduced to form an erect sclerite. All parts of the ovipositor fuse into a plate-like structure (Fig. 65).

Discussion

After examining the external genitalia of females in the Megalopodidae and comparing them to families in Chrysomeloidea, we interpret the following.

I. We suggest dividing the female external genitalia of superfamily Chrysomeloidea into cerambycid type and chrysomelid type. In general, adult females with long ovipositors and a spiculum gastrale insert their egg into deeper substrate (plant tissue or soil). In contrast, females with shorter ovipositors and a spiculum gastrale lay eggs on substrate surfaces or shallow sites (Kasap and Crowson 1985). However, in the aquatic genus *Donacia* (*Cyphogaster*) and in some species in the genus *Colasposoma* (Chrysomeloidea; Figs 40–41), females with short ovipositors or a spiculum gastrale can elongate the inter-segmental membrane and insert eggs into deeper sites. Therefore the formation of a cerambycid type and a chrysomelid type of ovipositor is more reasonable than simply subdividing them into long and short type.

The main characteristics and representative group in these two types are discussed below.

1. The cerambycid type

The Megalopodidae, most species of Cerambycidae and the Orsodacnidae belong to this type. The 8th abdominal segment usually develops a genital pocket and the spiculum gastrale is thin and long. Indeed, it is four times longer than the sternite and is connected to the sternite via a joint. The length of its 9th abdominal segment exceeds the width of the base. The ovipositor is generally long with well-developed baculi.

2. The chrysomelid type

All chrysomelids, excluding members of the Orsodacnidae and a few species in the Cerambycidae, belong to this type. The 8th abdominal segment has no genital pocket

and the spiculum gastrale connects to the sternite without forming a joint. The sternite is up to four times the length of the spiculum gastrale. The length of the distinctive 9th abdominal segment is generally shorter than the width of its base. The components of the ovipositor are relatively short and its baculus is poorly developed or absent.

II. Megalopodidae is closely related to subfamily Lamiinae. The morphology of female genitalia in the family Megalopodidae (includes subfamilies Megalopodinae and Zeugophorinae) is significantly different from the chrysomelids. The female genitalia in this family belong to the cerambycid type. The distinctive characteristics are listed as follows: (1) sternite 8 has a strongly sclerotized apodeme, (2) the inner side of the 8th abdominal segment has a stiff genitalia pocket, (3) the spiculum gastrale is connected to the sternite by a joint, (4) the 9th abdominal segment has no proctiger, (5) the paraproct is rather short or absent, (6) the coxite is thin and long, (7) the stylus is much reduced. The family Cerambycidae includes many subfamilies but morphological variation among its subfamilies is rather low. The ovipositor of most species in the Cerambycidae belongs to the cerambycid type. After comparing the morphology of external female genitalia between the Megalopodidae and Cerambycidae we found that the Megalopodidae are most like the subfamily Lamiinae of Cerambycidae. Our research results are congruent with the research of McKenna et al. (2015). It shows that Megalopodidae was included in the clade with Cerambycidae and Orsodacnidae. And it also supports the researcher's results that Megalopodidae was close to Cerambycidae or within Cerambycidae (Hunt et al 2007, Gómez-Zurita et al. 2007, 2008; Marvaldi et al. 2009). Both Megalopodidae and Cerambycidae have a sternite 8 with strongly sclerotized apodemes. Their ovipositors have no proctiger or are much reduced. Their paraprocts are short or reduced and their coxites are long but their styluses are small. Dutt (1958) described the morphology of female genitalia in *Nupserha bicolor* (Lamiinae). Saito (1993) and Wang (1999) identified the main morphological characteristics of the Lamiinae ovipositor. Those results are similar to our observations of the Megalopodidae. Suzuki (1988, 1994) considered that Zeugophorinae and Megalopodinae derived from the same lamiid-type ancestor, based on the morphology of the internal reproductive systems of both sexes and the hindwing venation. In addition, Saito (1993) found that the females of Lamiinae used their mandibles to bite tree bark or a host plant's stem to prepare the substrate for oviposition. This habit is unique to all subfamilies of Cerambycidae. Females in the Megalopodidae generally use their mandibles to bite the stems of host plants before laying eggs. In all, these observations about the morphology of female genitalia in the Megalopodidae and Cerambycidae show that both families probably belong to the cerambycid lineage.

III. The evolution of the spiculum gastrale in the Chrysomeloidea. The spiculum gastrale is an apodeme projection extending from the anterior-central region of sternite 8. The muscles attached to the spiculum gastrale correlate the mode of oviposition (see I. Megalopodidae) (Kasap and Crowson 1985). The length of the spiculum gastrale or its absence also correlates with egg laying behavior (Kasap and Crowson 1985). The spiculum gastrale in the Megalopodidae, Cerambycidae, and Orsodacnidae is thin, long,

and generally exceeds four times the length of the sternite. It is connected to sternite 8 by a joint at the base. Thus far the spiculum gastrale is present in all three families. In contrast, the spiculum gastrale in the Chrysomelidae is much shorter than in the other families but its length varies from moderate to very short or absent depending on species. The spiculum gastrale is typically absent in such members of the Chrysomelidae as the Lamprosomatinae, Synetinae, and Chrysomelinae (excluding members of genus *Timarcha*). Sternite 8 in the first two taxa is completely reduced while sternite 8 in the latter subfamily has no spiculum gastrale even though sternite 8 is well developed. Sternite 8 in the Eupoda is normal and the development of its spiculum gastrale is also normal and the apical sternite is rather broad. However, in the genus *Sagra* morphological variation is more uniform. The spiculum gastrale in the subfamily Criocerinae is slightly shorter than it is in the genus *Sagra* and in the subfamily Donaciinae. This structure is more distinctive in the genus *Crioceris*. We have not found the sternite without a spiculum gastrale in these taxa. The species in the Donaciinae have a spiculum gastrale slightly shorter than in the genus *Sagra*. The spiculum gastrale in the genus *Sominella* has a hook-like anterior portion. In the genus *Plateumaris* the 8th abdominal segment is a specialized, sclerotized sheath and its apex has vanished. Therefore the spiculum gastrale forms a short and thick handle at the base of the sheath. The spiculum gastrale in subfamily Galerucinae and Alticinae varies considerably with its thin rod shape. It is generally thinner and smaller than in the Eupoda. However, this structure shows greater variation in some genera in the Galerucinae. For example the spiculum gastrale in the genus *Oides* (Galerucinae) is broad, ending in narrow sclerites. Sternite 8 is strongly sclerotized. Species belonging to the genus *Morphosphaera* lack a spiculum gastrale but sternite 8 is well developed. In hispid taxa, most of the spiculum gastrale is broad forming a short plate while most of sternite 8 is reduced to an arc-shaped structure. In contrast, the spiculum gastrale of *Leptispa longipennis* (Gestro) is rather narrow. In the genus *Ocotodonta* there is no spiculum gastrale, and the development of sternite 8 is normal. In subfamily Eumolpinae, the sternite in some genera (e.g., *Platycornus*, *Colasposoma*, and *Chrysochus*) is rather long while the spiculum gastrale is relatively short or not well developed. In *Colasposoma dauricum* Mannerheim, sternite 8 is centrally convex, with a longitudinal ridge and its lateral side is not completely separated and connects with the sternite. Its apex has no spiculum gastrale (Fig. 45). In genus *Platycornus*, sternite 8 also has a similar central longitudinal ridge. The anterior portion of this ridge exceeds the anterior of the sternite forming a short spiculum gastrale (Fig. 47). Based on the two above species, we suspect that the spiculum gastrale derives from the longitudinal ridge of the sternite. Based on our morphological analyses of the Chrysomeloidea, we suggest that the spiculum gastrale derived first from the center of sternite 8, then gradually exceeded the original sternite (Fig. 66). The early formation of the spiculum gastrale had no joint, and we regard this as a primary stage. The spiculum gastrale with its joint must have developed relatively late. Our observation is supported by Kasap and Crowson (1985) who also concluded that the spiculum gastrale with its joint was secondary. From an evolutionary point of view, researchers have treated the Cerambycidae as a primary group (Chen 1986, Suzuki 1994), due to the presence of a spiculum gastrale connected to sternite 8 via a joint but this earlier hypothesis is based only on one characteristic.

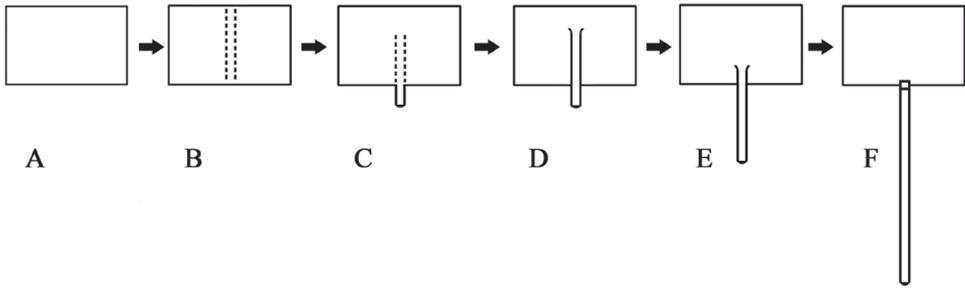


Figure 66. The development of spiculum gastrale **A** sternite 8 **B** sternite 8 central with convex longitudinal ridge **C, D, E** sternite 8 central with convex longitudinal ridge and lateral sides separated from sternite to form spiculum gastrale without joint **F** spiculum gastrale connected with sternite 8 with joint.

IV. The female genitalia in subfamily Eumolpinae is a typical representative of the primary type. In general, the female genitalia in Orsodacninae, Sagraeinae, *Timarcha* (Chrysomelidae) and Eumolpinae are interpreted as primary, because in these taxa female genitalia retain all the basic sclerites: proctiger, paraproct, valvifer, coxite, stylus, median plate (without in *Timarcha*) (Kasap and Crowson 1985). However, we suspect that in the 8th and 9th abdominal segments involved in reproductive behavior the ovipositor is controlled by the muscles of the 8th abdominal segment. Therefore, we believe it is more reasonable to include the 8th and 9th abdominal segment when we try to construct the evolutionary pathway of this taxon, especially when attempting to define ancestral states. We found in some species of Eumolpinae (e.g. *Colasposoma dauricum* Mannerheim) that its spiculum gastrale was not completely separated from sternite 8. This appears more basal compared to well-developed sternites. In addition, most genera in the Eumolpinae (e.g. *Chrysochus*, *Colasposoma*, and *Platycorynus*) with long 9th abdominal segments had ovipositors similar to the cerambycid type. Specifically, their ovipositors have all components, there is a clear boundary, and their long baculi are similar to the Cerambycidae differing from the Chrysomelidae. According to the morphology of the 8th and 9th abdominal segment, it is suggested that the female genitalia of the Eumolpinae represent the ancestral state in the Chrysomelidae.

V. Orsodacnidae

This is a controversial group. It was regarded previously as a subfamily in the Chrysomelidae. Mann and Crowson (1981) studied the morphology of adults and larvae in the genus *Orsodacne*; they believed that the ovipositor was very similar to most species in the Cerambycidae. Kuschel and May (1990) made a key to divide families using female genitalia characteristics when they published the new subfamily in the Megalopodidae called the Palophaginae. Their keys show that the Cerambycidae and the Orsodacnidae have common characteristic states (the 9th sternite long, paraprocts with distinct struts or bars and stylus) usually large in female genitalia. Suzuki (1988) thought that some inner reproductive characteristics in the Orsodacnidae were absent in the Chrysomelidae but they were present in some taxa in the Cerambycidae. Our observations on the female genitalia in genus *Orsodacne* are similar to earlier observations by Suzuki

(1988). We found that some structures associated with the ovipositor belong to the cerambycid type (including the Megalopodidae and Cerambycidae) and they differ distinctly from the chrysomelid type. The structure of the cerambycid type suggests it represents the ancestral structure. Although we found that the morphology of the reproductive segments in the genus *Orsodacne* differed from those in the Megalopodidae and Cerambycidae, for example sternite 8 has no strongly sclerotized genital plate, the genital pocket is an inter-segmental and long cylindrical membranous membrane, the posterior opening of the 8th abdominal segment has no sclerite plate, and the 9th abdominal segment has a rather long proctiger. These differences are not sufficient to indicate that *Orsodacne* is more closely allied to the chrysomelids.

Crowson (1955) thought that some morphological characteristics of the larvae of *Orsodacne* were similar to the Criocerinae, Donaciinae, *Sagra*, *Clytra*, and Eumolpinae. Cox (1981) also suggested that the Orsodacninae was closer to the Zeugophorinae and Donaciinae based on larva characteristics. There is no doubt that the Orsodacninae are closer to the Galerucinae. In our study, we found that the 9th abdominal segment of *Orsodacne* is very similar to the same structure in some genera (*Colasposoma*, *Platycorynus* and *Chrysochus*) placed in the Eumolpinae. They share the same following features: a long 9th abdominal segment, the ovipositor visibly retains all structures and has developed baculi. However, the 8th abdominal segment in these three genera differs from that in the genus *Orsodacne*. Based on our comparison of the external female genitalia in the Chrysomeloidea and observations by other researchers on the genus *Orsodacne*, we suggest that *Orsodacne* separated from the cerambycid lineage at an earlier period. It moved from bored stems of plants to leaf-eating in the early evolution of the ancestor of Chrysomeloidea, but it keeps some plesiomorphy. It was in the clade that became the earliest independent chrysomelid lineage. Chrysomelidae (include other chrysomelids) was in the clade that evolved later than *Orsodacne*. We agree that the Orsodacnidae was an independent family but a sister family of the Chrysomelidae and it was equal in status to the Megalopodidae and Cerambycidae (Kuschel and May 1990, Reid 1995). Specifically the Chrysomeloidea now includes four families: the Megalopodidae, Cerambycidae, Orsodacnidae, and Chrysomelidae.

VI. Synetinae

With a reduced sternite 8, the oviposition behavior in the Synetinae is very similar to the Camptosomata including the subfamily Cryptocephalinae (Cryptocephalini, Clytrini and *Chlamisus*). All of them place a protective layer outside their eggs. They hold each egg by their hind tarsi and keep turning in the egg dimple of the 7th abdominal segment when the egg is expelled from their bodies (Jiang 1983, Yu 1996). Their digestive tracts discharge evenly to cover each egg forming a protective layer and then the egg is kicked out of their bodies (Yu 1996, Jiang 1983). The protective layer is formed by the Kotpresse (German language, meaning fecal press), which is a special structure on the rectum (Lécaillon 1899). The Kotpresse was reported in the Camptosomata (Erber 1968) and Synetinae (Reid 1995). We also found some vertical arrangements of a special Kotpresse on the rectum of *Syneta adamsi* Baly.

The female genitalia of the Synetinae are different from the more common Chrysomelidae. The tergite 8 (pygidium) was exposed and backward, so one could see the exposed apices of the ovipositor from its ventral side. The coxite is triangular lacking a distinct stylus. But on the inclined plane outside the coxite there is a small protrusion that should be the stylus because the valvifer connects with the coxite and paraproct at the base. This structure is similar to the membrane of the ovipositor in the Camptosomata (especially in some species in the genera *Cryptocephalus* and *Clytra*). Both the Synetinae and Camptosomata have a well-developed proctiger and a broad, sclerotized paraproct. Based on similar oviposition structure and behaviors, we speculate that the Synetinae has the closest relationship to the Camptosomata in the Chrysomelidae. However, we cannot exclude the possibility of parallel or convergent evolution of oviposition behavior in both taxa. Schmitt (1996) suggested that the Camptosomata, Synetinae, and some species in the Eumolpinae belonged to a monophyletic group because their Kotpresses were homologous structures. Our observation supports Schmitt (1996), but we found that the ovipositor of the Eumolpinae is ancestral and differs from the ovipositor in the Camptosomata. We have not observed the egg-laying habits of the genus *Chlamisus*.

VII. Based on comprehensive observations of the female genitalia of the Chrysomeloidea, it was found that the apices of the ovipositor are usually palp-like and backward except in the subfamily Cassidinae and in the genus *Callispa* of Hispini, which has a wide, plate-like ovipositor. Muir and Sharp (1904) found that the plate-like ovipositor of *Aspidomorpha puncticosta* Boheman was the key structure required to form the diaphragm between the eggs in the egg sac. Chen et al. (1986) suggested that the trait of laying eggs in an egg sheath in the genus *Callispa* and subfamily Cassidinae was further evidence of their close relationship. Our studies show that the plate-like ovipositor is found only in species that make a sheath to protect an egg. In addition, we found that the ovipositor of the genus *Leptispa* belongs to the same tribe as the genus *Callispa* and it is palp-like, not plate-like. We also found that the adult and larvae in the genus *Leptispa* live in a leaf roll (Chen 1986). The adult lays its egg in a leaf roll but does not make a sheath. Therefore we suggest that the morphology of the ovipositor in these genera show a close relationship even though they differ in life-history. Based on this evidence, we conclude that the genus *Callispa* branched early and the genus *Leptispa* appeared later.

Conclusions

Female genitalia are diverse, complex, and very important for classification of the Chrysomeloidea. With a comparative analysis of the female genitalia we can reach a well-resolved classification system of this family. Egg-laying behavior and oviposition media cannot to be ignored when we explore the evolution of this huge taxon, but we still know very little about the reproductive biology of individual species. This research is particularly limited in Chinese lineages and future research is desperately needed to compare all the members of this lineage to resolve the systematics of the Chrysomeloidea.

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Plectranthias abiahiata, a new species of perchlet from a mesophotic ecosystem at Rapa Nui (Easter Island) (Teleostei, Serranidae, Anthiadae)

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Abstract

A new species of the perchlet genus *Plectranthias* is herein described from a single specimen found at Rapa Nui (Easter Island) in the South Pacific. *Plectranthias abiahiata* sp. n. was collected at a depth of 83 m in a mesophotic coral ecosystem at Rapa Nui. The main difference between *Plectranthias abiahiata* and other members of the genus is higher fin-ray counts (X, 18 dorsal; 18 pectoral) and its distinctive coloration. Compared to the three other known eastern South Pacific species, *P. abiahiata* has more dorsal-fin rays, more pectoral-fin rays, fewer tubed lateral-line scales, fewer gill rakers, a longer head relative to SL, a very short first dorsal spine relative to SL, and a short third anal spine relative to SL. *Plectranthias abiahiata* is distinguished from western Pacific species, by having more dorsal- and pectoral-fin rays. The closest relative based on genetic divergence (with 12.3% uncorrected divergence in the mitochondrial COI gene) is *Plectranthias winniensis*, a widely distributed species, suggesting important links between Rapa Nui and western Pacific islands. This new species adds to the high endemism of the Rapa Nui ichthyofauna, and is further evidence of the importance of mesophotic reefs as unique communities.

Keywords

Endemism, ichthyology, reef fish, South Pacific, taxonomy

Introduction

Rapa Nui (Easter Island) is the most isolated inhabited island in the Indo-Pacific, located approximately 3,700 km west of Chile and 2,000 km from the nearest inhabited island, Pitcairn (Randall and Cea 2010; Easton et al. 2017). Due to the isolation, the fish fauna exhibits the highest degree of local and regional endemism in both shallow-water and deep-sea fishes in the Indo-Pacific, and very low overall diversity (Friedlander et al. 2013; Easton et al. 2017). Nearly 22% of the shore fishes of Easter Island are endemic (Randall and Cea 2010; Friedlander et al. 2013). Although ichthyological studies since the 1980's have greatly expanded the knowledge of fish diversity in this ecoregion, there have been relatively few surveys of fishes at depths greater than 40 m (Friedlander et al. 2013; Wieters et al. 2014; Easton et al. 2017). Recent advances in technical mixed-gas rebreather diving are now allowing scientists to safely document small, active, cryptic, and demersal fishes in ways that have been previously impossible (Pyle 2000; Pyle et al. 2016; Rocha et al. 2017). Using these techniques, our team collected a distinctive new species of *Plectranthias* off Hanga Piko, Rapa Nui.

The anthiadine genus *Plectranthias* Bleeker 1873 currently comprises 56 valid species from tropical and temperate waters in the Atlantic, Pacific, and Indian oceans (Eschmeyer et al. 2017). Most of these fishes are found in relatively deep waters (90–420 m) on hard substrates such as mesophotic coral ecosystems and rocky formations (Allen and Walsh 2015; Gill et al. 2016). Due to their diminutive size and cryptic habits they are not often caught by hook and line or in trawls, so they are poorly represented in museum collections, and most species have been described based on a single or a small number of specimens (Randall 1980; Heemstra and Randall 2009; Bineesh et al. 2014; Allen and Walsh 2015; Gill et al. 2016). The three known eastern Pacific species within the genus are *P. exsul* Heemstra and Anderson 1983, *P. nazcae* Anderson 2008, and *P. parini* Anderson and Randall 1991. A fourth eastern Pacific species, *P. lamillai* Rojas and Pequeño 1998, was proposed, however it was later determined to be a junior synonym of *P. exsul* (Anderson and Baldwin 2002). *Plectranthias parini* is the only other congener known to occur at Rapa Nui, where it was recorded at depths of 150 m during drop-cam surveys (Friedlander et al. 2013). Here *Plectranthias abiahiata* sp. n. is described and illustrated from a single specimen collected at Rapa Nui.

Materials and methods

This species is described from a single specimen collected with hand nets while diving on mixed-gas, closed-circuit rebreather (Hollis Prism 2). Counts were performed with a microscope, and morphological characters were measured to the nearest 0.01 mm with digital calipers following the conventions described in Anderson and Heemstra (2012) and Williams et al. (2013). Comparative material included *Plectranthias japonicus* Steindachner 1883 (CAS33555), *P. sagamiensis* Katayama 1964 (CAS235596), and *P. winniensis* Tyler 1966 (CAS219169), along with recent publications, and keys

and revisions to the genus made by Randall (1980, 1996) and Heemstra and Randall (2008). Morphometric and meristic data for the holotype and comparisons with related species are presented in Table 1 and the Supplementary Online Material. The holotype was deposited at the California Academy of Sciences ichthyological collection.

Mitochondrial Cytochrome c oxidase subunit I (COI) DNA was sequenced and analyzed for the new species. DNA extraction and PCR amplification of the COI were performed following Weigt et al. (2012) protocols. DNA sequences were compared to the ten *Plectranthias* species available in GenBank (*P. bennetti* Allen and Walsh 2015: KT601636; *P. flammeus* Williams, Delrieu-Trottin and Planes 2013: KC565477–KC565480; *P. fourmanoiri* Randall 1980: KC567662, KC567663; *P. japonicus*: JQ681323, JQ681324; *P. kamii* Randall 1980: KU943548; *P. kelloggi* Jordan and Evermann 1903: KP267643; *P. longimanus* Weber 1913: JF494178; *P. nanus* Randall 1980: JQ432001–JQ432004, KC565481, KC567661; *P. randalli* Fourmanoir and Rivaton 1980: KP267613; *P. winniensis*: KC565482, KC565483). GenBank accession number for the new species is MH025944.

Taxonomy

Plectranthias abiahiata sp. n.

<http://zoobank.org/36C66D98-05BE-40F1-ABD7-9684B51D0E65>

Figure 1–3, Table 1

Sunset perchlet

Type locality. Hanga Piko, Rapa Nui (Easter Island), Chile.

Holotype. CAS244172 (Field number LAR 2644). 39.95 mm SL, GenBank accession number MH025944. Location: Hanga Piko, Rapa Nui, Chile (27°9'12"S 109°26'52"W). Collected by B. Shepherd using a hand-net, 7 March 2017 (Figure 1–3).

Comparative material. Published morphometric and meristic data from the known eastern Pacific species, *Plectranthias exsul* (Anderson and Baldwin 2002), *P. nazcae* (Anderson 2008) and *P. parini* (Anderson and Randall 1991); the Atlantic species *P. garrupellus* Anderson and Heemstra 2012; the Western Pacific species *P. anthiodes* Gunther 1872, *P. bennetti*, *P. elongatus* Wu, Randall and Chen 2011, *P. flammeus*, *P. fourmanoiri*, *P. jothyi* Randall 1996, *P. kamii*, *P. inermis* Randall 1980, *P. randalli*, *P. sheni* Chen and Shao 2002, *P. taylori* Randall 1980, and *P. xanthomaculatus* Wu, Randall and Chen 2011 (Yoshino 1972; Randall 1980; Randall 1996; Wu et al. 2011; Williams et al. 2013; Peristiwady et al. 2014; Allen and Walsh 2015; Tashiro and Motomura 2017); the Japanese species, *P. altipinnatus* Katayama and Masuda 1980, *P. takasei* Gill, Tea and Senou 2016, and *P. yamakawai* Yoshino, 1972; and the Arabian species, *P. alcocki* Bineesh, Akhilesh, Gopalakrishnan and Jena 2014. Specimens of *P. japonicus* (CAS33555), *P. sagamiensis* (CAS235596), and *P. winniensis* (CAS19169).

Diagnosis. *Plectranthias abiahiata* differs from all of its congeners by the following combination of characters: dorsal rays X, 18; pectoral rays 18; longest dorsal spine the

Table 1. Morphological counts and proportional measurements of the known *Plectranthias* species from the eastern South Pacific, the closest sequenced relative, *P. winniensis*, and comparative material from the CAS Ichthyology collection. All proportional measurements are given in percent SL.

	<i>P. abiabiata</i> sp. n.	<i>P. exsul</i>	<i>P. nazcae</i>	<i>P. parini</i>	<i>P. winniensis</i>	<i>P. sagamiensis</i>	<i>P. japonicus</i>
	CAS244172	Anderson and Baldwin (2002)	Anderson (2008)	Anderson and Randall (1991)	CAS219169	CAS235596	CAS33555
Standard length [mm]	39.95	37.6–158	115.0	84.7–163.0	25.5	41.6	78.1
Counts:							
Dorsal-fin rays	X, 18	X, 15–16	X, 16	X, 16	X, 16	X, 15	X, 15
Anal-fin rays	III, 7	III, 7	III, 7	III, 7	III, 7	III, 7	III, 7
Pectoral-fin rays	18	16–17	16	15–16	16	13	15
Pelvic-fin rays	I, 5	–	I, 5	–	I, 5	I, 5	I, 5
Tubed lateral line scales	31	36–46	40	37–40	18	30	33
Gill rakers (upper + lower)	6+11	7–10 + 18–22	9 + 19	8 + 18–20	6+10	8+14	7+13
Vertebrae (precaudal + caudal)	10+16	10+16	–	–	–	–	–
Circumpeduncular scales	16	20–22	17–18	16–17	12	14	13
Measurements:							
Body depth	30.8	30.1–39.1	38.4	39–41	31.7	35.3	36.3
Body width	16.7	14.9–18.6	17.5	–	17.4	19.6	19.3
Head length	43.3	37.0–39.1	38.4	39–41	39.9	36.5	40.7
Snout length	11.0	8.0–12.3	9.7	8–12	7.7	8.5	8.8
Orbit diameter	9.6	8.4–11.2	10.7	9–12	10.8	10.9	9.3
Postorbital of head length	12.9	17.2–20.0	18.6	–	13.7	18.5	22.3
Upper jaw length	16.8	16.0–19.6	18.7	18.9–20.6	20.1	21.0	19.7
Maxilla width	6.3	4.8–6.3	5.5	–	6.6	6.5	5.9
Caudal peduncle depth	12.5	9.8–13.1	10.9	11.7–11.9	12.0	13.1	13.4
Caudal peduncle length	10.4	18.6–22.7	20.7	–	12.8	12.3	13.5
Caudal fin length	24.6	–	damaged	–	26.8	31.3	broken
Pectoral fin length	33.8	30.1–34.3	30.5	33.9–36.7	30.7	32.7	33.9
Pelvic fin length	26.5	22.3–26.1	23.5	25.6–28.9	24.7	28.7	20.5
Dorsal-fin base length	51.4	49.9–52.8	–	–	48.9	51.8	50.8
Length of 1 st dorsal spine	4.5	6.2–7.9	6.2	–	4.4	7.0	4.0
Longest dorsal spine length (number)	15.64 (4 th)	16.3–19.9 (4 th or 5 th)	16.3 (5 th)	15.3–18.3 (5 th)	14.40 (4 th)	18.4 (5 th)	12.46 (6 th)
Length of 1 st anal-fin spine	7.2	7.6–11.3	7.6	–	6.0	7.4	5.4
Length of 2 nd anal-fin spine	19.0	16.0–20.7	16.0	–	15.8	16.9	13.5
Length of 3 rd anal-fin spine	13.7	14.7–15.9	14.8	15.1–17.9	12.3	14.5	10.6

fourth; LL continuous and complete with 31 tubed scales; circumpeduncular scales 16; head length 43.3% SL; first dorsal spine 4.5% SL; third anal spine 13.7% SL; gill rakers 6+11; and in coloration: overall orange-red in color, with predominantly yellow snout, dorsal, pelvic and anal fins, a brilliant red spot outlined in white on the caudal peduncle, and four white spots on each side, following the contour of the lateral line.

Description. Proportional measurements and morphological counts of the holotype are given in Table 1. Dorsal rays X, 18; last soft ray branched to base and counted as one; first dorsal spine very short, 4.5% SL; fourth dorsal spine longest, 15.6% SL; dorsal-fin base length 51.4% SL. Anal-fin rays III, 7; last soft ray branched to base and counted as one; anal-fin base 13.7% SL; second anal spine longest at 19.0% SL; anal-fin origin at vertical beneath fifth dorsal-fin ray; pectoral-fin rays 18; length 33.8% SL; pelvic fin I, 5; pelvic-fin length 26.5% SL; pelvic-spine length 16.5% SL; caudal fin with three dorsal and two ventral procurrent rays, two dorsal and one ventral unbranched rays, 7+7 branched rays; tubular lateral-line scales 31; vertical scale rows 30; scales above LL to origin of dorsal fin 3; scales above LL to base of middle dorsal spine 2; scales above LL to origin of anal fin 12; diagonal rows of scales on cheek 4; scales on top of head extending anteriorly to vertical from posterior margin of orbit, where two rows of mid-dorsal scales continue anteriorly to posterior margin of iris; area on top of head between eyes scaleless; no scales on chin, maxilla, or snout; circumpeduncular scales 16; caudal-peduncle length 10.4% SL; caudal-peduncle depth 12.5% SL; body moderately elongate, laterally compressed; body depth 30.8% SL; body width 16.7% SL; gill rakers 6+11; vertebrae 10+16; supraneurals 3.

Mouth large and terminal, slightly upturned; lower jaw protrudes slightly; maxilla expanded posteriorly, extending to below the posterior edge of eye; head long, length 43.3% SL; dorsal profile of head almost straight; post-orbital head length 12.9% SL; snout length 11.0% SL; orbit diameter 9.6% SL; upper jaw with a pair fixed, stout canines on either side of symphysis; inner canine larger of the pair; upper canines flanked internally by villiform band with four to eight rows of depressible, smaller, sharp-tipped teeth; inner rows become progressively longer, innermost row with largest teeth, some larger than upper canines; lower jaw has outer row of fixed, short stout canines at symphysis followed by smaller, depressible, sharp-tipped conical teeth in a villiform band of approx. four to six rows; lower teeth become progressively longer on inner rows, teeth of inner row approx. three times longer than teeth of middle rows, villiform band narrows to one row toward sides of lower jaw; two large fixed canines at midpoint on either side of lower jaw; vomer roughly V-shaped band of two rows of similarly-sized, sharp-tipped, conical teeth; palatines with two rows of small, sharp-tipped conical teeth; tongue small, narrow, pointed, and without teeth.

Scales ctenoid; lateral line complete and broadly arched over pectoral fin following body contour; 31 tubed scales, the last seven in a straight line. Opercle with three spines; preopercle with nine small spines along posterior margin and two antrorse spines on ventral margin; interopercle with one spine; subopercle smooth, with one spine; anterior nostrils positioned at middle of snout, each with a small rounded flap rising from anterior rim; posterior nostrils an elliptical opening at anterior border of orbit.



Figure 1. *Plectranthias abiahiata* sp. n., holotype shortly after death, 39.95 mm SL (photograph: LA Rocha).

Color in life. *Body:* overall yellow, orange, and red in color; chest and belly mostly yellow, with yellow extending dorsally to just behind origin of pectoral fin; ventral-most portion of belly white; dorsal portion of body orange-red; series of two to three indistinct orange bars alternating with light pink to white interspaces on the posterior half of the body, followed by a red bar originating below the eleventh dorsal ray and continuing to the base of caudal peduncle; brilliant red spot on the caudal peduncle, approximately same diameter as orbit, outlined in white; four irregularly-shaped white spots on each side of body: first on operculum outlined with dark pink to red border, second just behind the posterior edge of the operculum and beneath the anterior portion of the lateral line and the first and second dorsal spines, third below the eighth dorsal-fin spine, and fourth just below the lateral line beneath and between the fourth and fifth soft dorsal fin rays. These white spots resemble those of *P. winniensis* collected at Pitcairn Island (Randall, unpublished photograph) *Head:* snout, throat, and maxilla mostly yellow; anterior portion of lower lip orange; three indistinct yellow stripes radiating from the anterior portion of the snout through the eye and across the operculum with two light pink to white interspaces; upper interspace extending from lower third of iris to white spot on operculum, lower interspace originating beneath orbit and extending to origin of pectoral fin along upper edge of maxilla; orange stripe originating at tip of snout proceeding across dorsal third of eye and ending at the origin of the lateral line; iris alternating yellow and white lateral stripes, separated with faint, thin red borders; edge of iris outlined in dark-grey to black; *Fins:* spinous portion of dorsal fin translucent yellow; dorsal spines one to six outlined in orange-red along entire length; dorsal spines seven to ten mostly yellow, outlined with red primarily on spine tips; dorsal rays orange-red; lower third of soft dorsal fin mostly yellow, upper two-thirds translucent; caudal-fin membranes translucent with orange-red fin rays; pelvic and anal fins mostly yellow with white and translucent fin rays; pectoral fins translucent yellow with rays outlined in red; base of pectoral fins bright yellow.

Color in alcohol. Light tan overall, with no visible markings (Figure 2).

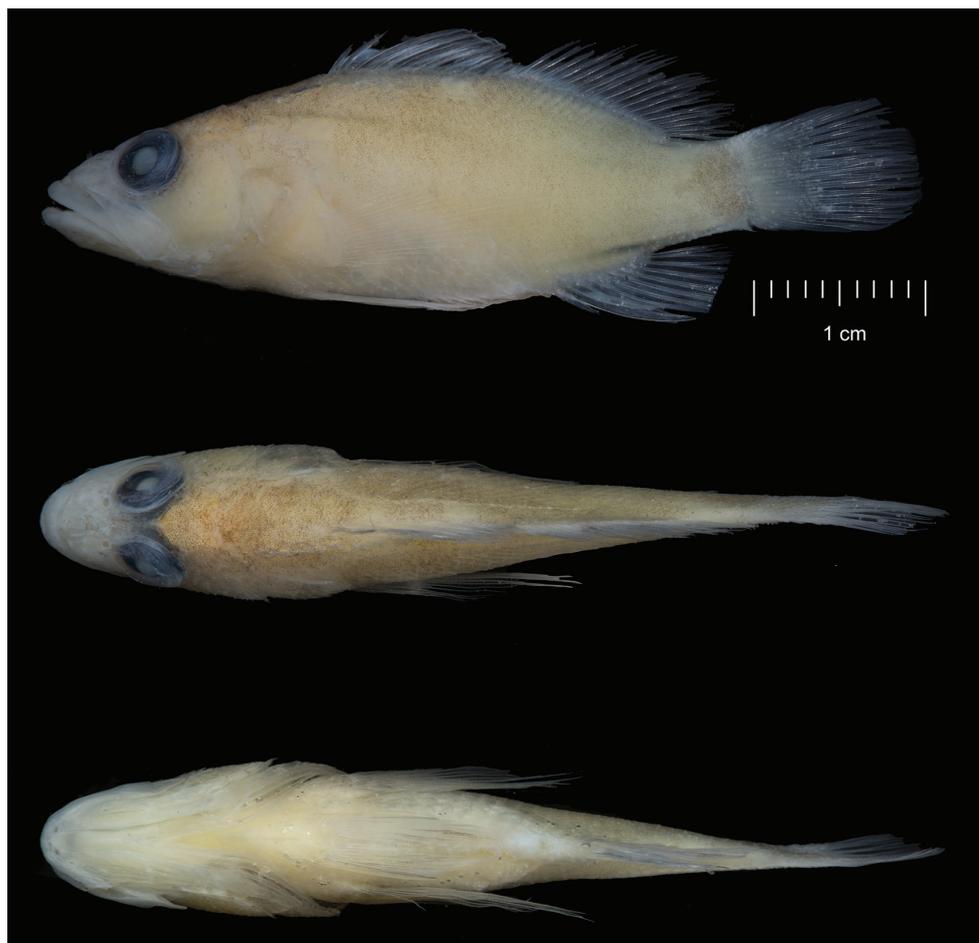


Figure 2. *Plectranthias abiahiata* sp. n., lateral, dorsal, and ventral views of preserved holotype (photographs: J Fong).

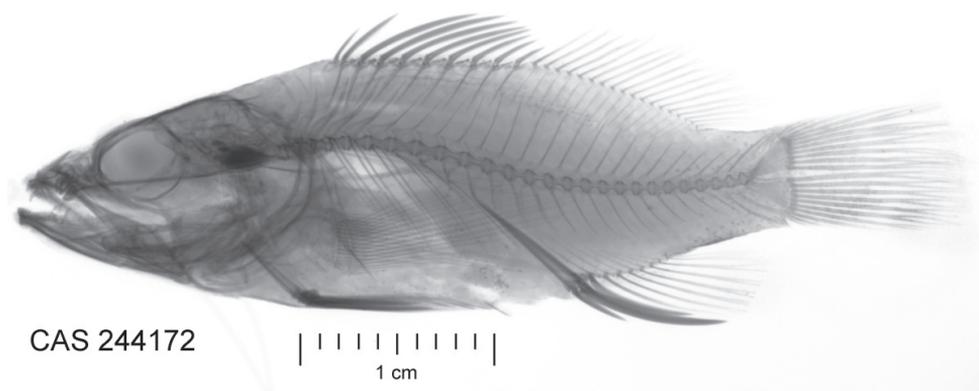


Figure 3. Radiograph of preserved holotype (photograph: J Fong).

Etymology. *Plectranthias abiahiata* is given a Rapa Nui name; the phrase *ahiahiata* means “the last moments of light before nightfall.” The species was given this name because the colors of the fish remind us of the beautiful Rapa Nui sunsets. To be treated as a noun in apposition.

Distribution and habitat. *Plectranthias abiahiata* is currently only known to occur at Rapa Nui (Easter Island). This fish was collected with hand nets at a depth of 83 m in a rocky patch reef surrounded by a large sandy area, and transported to the surface alive in a perforated plastic jar.

Discussion

The generic classification of the Anthiadiniinae is problematic, and the genus *Plectranthias* is especially in need of revision, as it is not currently defined on the basis of synapomorphies, and there is high variation within many of the defining characters (Anderson and Heemstra 2012; Gill et al. 2016). *Plectranthias abiahiata* is placed within the genus *Plectranthias* due to the combination of the following characters: dorsal fin X, 18; dorsal fin deeply notched before soft rayed part; anal fin III, 7; second anal spine the longest; pectoral fin rays 18; pelvic fin I, 5; body elongate, oval to oblong; body depth approximately 30–45% of SL; benthic, cryptic habits.

However, placement is provisional, as ongoing genetic and morphological studies indicate *Plectranthias* is not monophyletic, and it is likely that future work will split the genus (Anderson and Heemstra 2012; Gill, et al. 2016). The results of our molecular analysis indicate 12.3% uncorrected divergence in the mitochondrial COI gene from *P. winniensis*, its closest relative among the sequenced members of the genus (Table 2). *Plectranthias* species are minimally represented in GenBank, with COI sequences only available for ten species. Since there are 56 valid species in the genus, presenting a phylogenetic tree with just ten species (less than 20% taxon coverage) could be misleading (Zwickl and Hillis 2002); therefore, a phylogenetic analysis was not performed with the limited available data. As more discoveries are made and more species are sampled for phylogenetics, we can begin to unravel the taxonomic confusion within this genus.

Due to the high degree of endemism among shore fishes of Rapa Nui, as well as the large number of known *Plectranthias* species from the western Pacific, it is likely that *P. abiahiata* is also a locally or regionally endemic species. The fish was found at mesophotic depths on a rocky outcrop with a stand of the stony coral *Leptoseris scabra* (Figure 4). The ambient seawater temperature was approximately 19°C at the time of the dive (7 March 2017). Other fishes at this site included two unknown species of the family Serranidae and one from Pomacentridae, which our group will be describing. Although it is possible that the other species described from the Nazca and Sala y Gómez ridge region, *P. exsul* and *P. nazcae*, are geographically sympatric, the only member of the genus previously documented from Rapa Nui is *P. parini*. The affinity with *P. winniensis*, a widespread species ranging across the Indo-Pacific from the Red Sea to Pitcairn, suggests important links between the ichthyofauna of Rapa Nui and the western Pacific islands.

Table 2. Uncorrected percent pairwise genetic distances at the mtDNA COI gene between species of *Plectranthias* available in GenBank. *Plectranthias* sp. is an unidentified species from the Gambier Islands (KC567661).

	1	2	3	4	5	6	7	8	9	10	11
1. <i>P. abiahiata</i>	–										
2. <i>P. bennetti</i>	19.11	–									
3. <i>P. flammeus</i>	20.65	16.38	–								
4. <i>P. fourmanoiri</i>	16.89	15.36	17.41	–							
5. <i>P. japonicus</i>	17.92	14.68	15.87	13.48	–						
6. <i>P. kamii</i>	17.42	17.6	18.91	18.54	15.17	–					
7. <i>P. kelloggi</i>	17.75	14.51	15.7	13.31	0.17	14.98	–				
8. <i>P. longimanus</i>	17.75	18.09	14.51	18.77	18.94	19.29	18.77	–			
9. <i>P. nanus</i>	18.43	17.92	12.97	18.09	18.6	18.73	18.43	16.55	–		
10. <i>P. randalli</i>	16.04	18.26	19.62	17.41	16.21	17.79	16.04	19.45	19.97	–	
11. <i>Plectranthias</i> sp.	18.6	17.75	12.8	18.26	18.43	18.54	18.26	16.38	0.17	20.14	–
12. <i>P. winniensis</i>	12.29	18.09	17.06	16.38	18.09	18.16	17.92	17.58	17.58	17.92	17.41



Figure 4. Collection site of the holotype 83 m depth, Hanga Piko, Rapa Nui (photograph: LA Rocha).

Mesophotic ecosystems are known to shelter numerous specialist and exclusive species, which contribute to making this environment biologically unique in tropical and semitropical environments (Pinheiro et al. 2016). The few surveys of fishes at depths greater than 40 m at Rapa Nui (Easton et al. 2017) and the high degree of endemism at the island suggest that many other unknown species remain to be discov-

ered. Scientists' use of closed-circuit, mixed-gas rebreathers is allowing exploration and comparison between mesophotic and shallow coral ecosystems. The many new species descriptions resulting from the exploration of mesophotic reefs reveal the importance and uniqueness of these ecosystems (Pyle et al. 2016; Baldwin et al. 2016, 2018; Rocha et al. 2017).

Acknowledgements

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Cis pallidus Mellié, 1849: redescription, new synonym, geographic distribution, and host fungi records

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Abstract

Cis pallidus Mellié, 1849 is redescribed based on specimens from Northeast, Southeast, and South Brazil, and from Argentina. A lectotype is designated for *Cis semipallidus* Pic, 1916, and the species is synonymized with *C. pallidus*. The first host fungi records and a distribution map for the species are provided.

Keywords

Ciinae, Ciini, minute tree-fungus beetles, Neotropical

Introduction

Cis Latreille is the most diverse genus of Ciidae with approximately 400 described species and a worldwide distribution (Oliveira et al. 2013, Lawrence 2016). The genus contains more than half of all described Ciidae species (Lawrence 1971, Oliveira et al. 2013, Lawrence 2016). The Neotropical species of *Cis* (biogeographic regions *sensu* Morrone 2015) are represented by nearly 70 described species and at least half of them are organized into artificial species-groups, such as the *comptus*, *creberrimus*, *melliei*, *pallidus*, *taurus*, *tricornis*, and *vitulus* species-groups.

The *pallidus*-group comprises *C. corticinus* Gorham, 1883 from Totonicapán, Guatemala, *C. pallidus* Mellié, 1849 from the state of Bahia, Northeast Brazil, *C. semipallidus* Pic, 1916 from Buenos Aires, Argentina, and *C. tetracentrum* Gorham, 1886, which occurs from southern California and Arizona to southern Mexico (Mellié 1849, Lawrence 1971, Gorham 1883, 1886,). These four species share an elongate body with single and uniform elytral punctation, dorsal vestiture of short to long bristles, slightly tumid prosternum, and males with a very small sex patch on the first abdominal ventrite or none at all (Lawrence 1971; pers. obs.). These species are morphologically closely related to species in the *vitulus*-group, which are similar but usually have a comparatively less elongate body (Lawrence 1971; pers. obs.).

The aim of this paper is to redescribe *C. pallidus*, propose a new synonym, and provide new host fungi and geographic distribution records.

Materials and methods

The examined specimens are listed in the section on “type material” and “additional material” below. A total of 12 males from eight localities were dissected, as follows (number of specimens between parentheses): Buenos Aires (1; lectotype of *Cis semipallidus*) and Famaillá (1), in Argentina; Rio de Janeiro (1), São João del-Rei (1), Viçosa (5), Palotina (1), Nova Teutônia (1) and Urubici (1), in Brazil. Abdominal ventrites shown in Fig. 29 and genitalia in Figs 34–35 were extracted from the same male. Genitalia shown in Figs 30–31 were extracted from the male shown in Fig. 16; the same applies for Figs 43–44 and Fig. 20.

Museum abbreviations are as follows:

- ANIC** Australian National Insect Collection, CSIRO Entomology (Canberra, Australian Capital Territory, Australia)
- CELC** Coleção Entomológica do Laboratório de Sistemática e Biologia de Coleoptera da Universidade Federal de Viçosa (Viçosa, MG, Brazil)
- DZUP** Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná (Curitiba, PR, Brazil)
- FMNH** Field Museum of Natural History (Chicago, Illinois, USA)
- MACN** Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (Buenos Aires, Argentina)
- MCNZ** Fundação Zoobotânica do Rio Grande do Sul (Porto Alegre, RS, Brazil)
- MNHN** Muséum National d’Histoire Naturelle (Paris, France)
- MNRJ** Museu Nacional do Rio de Janeiro (Rio de Janeiro, RJ, Brazil)

Terms for external morphology and male terminalia of ciids follow Lopes-Andrade and Lawrence (2005, 2011), Lawrence et al. (2011), and Lawrence (2016), but see also Oliveira et al. (2013) for an explanation on the use of “tegmen”. The following abbreviations are used for measurements (in mm) and ratios:

- BW** (basal width of scutellar shield),
CL (length of antennal club measured from base of the eighth to apex of the tenth antennomere),
EL (elytral length along the midline),
EW (greatest width of both elytra),
FL (length of antennal funicle measured from base of the third to apex of the seventh antennomere),
GD (greatest depth of body measured in lateral view),
GW (greatest diameter of eye),
PL (pronotal length along midline),
PW (greatest pronotal width),
SL (length of scutellar shield),
TL (total length counted as EL+PL, *i.e.* excluding head).

The GD/EW and TL/EW ratios indicate the degree of body convexity and elongation, respectively.

A total of 21 males and 20 females were measured, with representative specimens from all examined localities. Measurements of antennomeres, GW, and BW provided in the description are the mean measurements of three males from three localities (Viçosa, Atilio Vivacqua, and Nova Teutônia); in these cases, standard deviations are not provided because they were 0.01 or less.

For scanning electron microscopy (SEM), specimens were dehydrated in a series of alcohol and acetone solutions, critical point dried (CPD 020, Balzers, Liechtenstein), mounted on aluminum stubs and sputter coated with gold (sputter module SCA 010, Balzers). Samples were then examined under a SEM (LEO VP 1430, Zeiss). Transcription of labels, dissection, photography under optical equipment, and measurement of specimens followed the methods provided by Araujo and Lopes-Andrade (2016). Names of host fungi extracted from labels were updated consulting the online database of Index Fungorum (<http://www.indexfungorum.org>) and are summarized in the section “Host fungi”. The criteria provided in Orledge and Reynolds (2005) are followed for determining breeding records. The distribution map (Fig. 45) was generated using the on-line SimpleMappr tool (Shorthouse 2010).

Taxonomy

Variation in pronotal and elytral color occurred between specimens from the same locality or even from the same basidiome. Also noticeable was variation in the length of dorsal bristles, mainly those on the pronotum (compare Figs 1, 9, 12, 15–21). The comparatively darker elytra and longer pronotal bristles of specimens of the type series of *C. semipallidus* were observed in several named specimens of *C. pallidus*.

There was little variation in size and morphology of tegmen and penis between dissected specimens from eight localities (Figs 6–7, 30–44). Length of tegmen varied

from 0.29 to 0.36 mm, and of penis from 0.26 to 0.33 mm. The very rounded apical lobes of tegmen observed in two cases (Figs 6, 32) were artifacts of preparation, and occurred when the tegmen was dorso-ventrally-flattened between slide and cover slip. To confirm this, we photographed a tegmen before (Fig. 40) and after distortion (Fig. 41). No conspicuous sex patch was observed externally on the first abdominal ventrite of males, neither under stereomicroscopy nor under SEM (Figs 22, 28), but a possibly vestigial small mark was observed in all dissected males (e.g. Fig. 29, arrow). No diagnostic features to sustain *C. semipallidus* as a separate species have been found; thus we propose it as a junior synonym of *C. pallidus*.

Cis pallidus Mellié, 1849

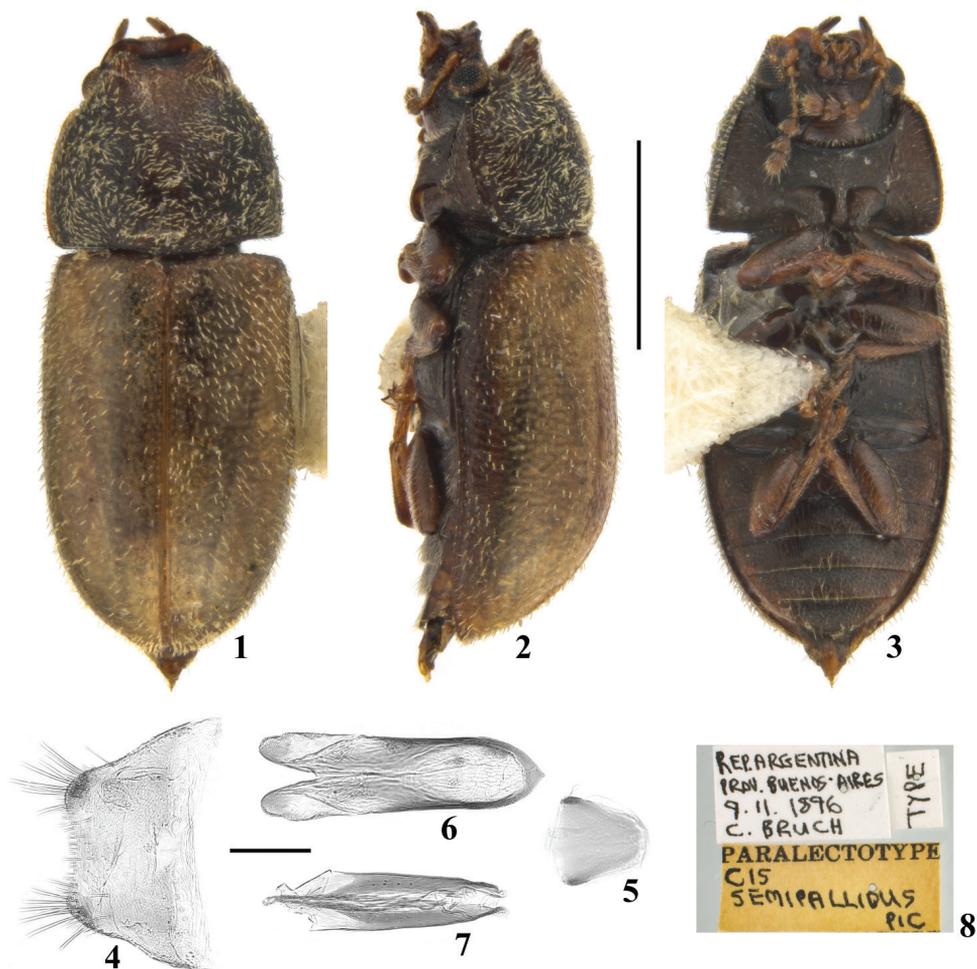
Figs 1–45

Cis pallidus Mellié, 1849: 246–247, pl. 10, fig. 10.

Cis semipallidus Pic, 1916: 5, **syn. n.**

Diagnosis. Distinguished from other South American ciids by the elongate body (TL/EW at least 2), reddish to dark brown head and pronotum, yellowish elytra with a black band on both sides, and pronotum projected forward, partially or completely covering head when seen from above. Among South American ciid species with light-colored elytra, *Orthocis platensis* Brèthes, 1922 differs in the pronotum being not projected over the head. *Cis bisbidens* Gorham, 1883 differs in lacking lateral elytral band, in the developed projections associated with the male head and pronotum, and the small but conspicuous sex patch on first abdominal ventrite of the male. *Cis granarius* Mellié, 1849 has a comparatively stouter body and the sides of pronotum are light-colored. In *C. grossus* Mellié, 1849 and *C. validithorax* Pic, 1916 only the apical portion of the elytra is light-colored. *Cis steinheili* Reitter, 1878 is devoid of conspicuous lateral dark longitudinal band on elytra and the male has an obvious sex patch on the first abdominal ventrite.

Description of adult males. *Body* elongate, subparallel-sided; head, pronotum and scutellar shield reddish-brown (Fig. 16) to dark brown (Fig. 20); pronotum sometimes lighter on disc than on sides (Fig. 17); elytral disc mostly pale yellowish (Figs 9, 17) to yellowish brown (Fig. 1), slightly translucent, gradually darkening to sides and forming a distinct dark longitudinal band on both sides easily seen in lateral view (Figs 2, 10); ventral surface (Figs 3, 11) dark brown, with appendages usually lighter; dorsal vestiture of short decumbent (Fig. 23), goldish bristles, as long as puncture-width (Fig. 20) and up to three puncture-widths (Fig. 19), the same length or longer on elytra than on pronotum, usually sparser on elytra than on pronotum; ventral vestiture of decumbent whitish, slender setae, longest on metaventrite and abdominal ventrites. **Head** partially or completely covered by anterior pronotal plate when seen from above (Figs 1, 9, 16–17, 19–20); dorsal surface with small shallow punctures and microreticulate interspaces; anterocephalic edge weakly projected forward and upward, sinuous, form-



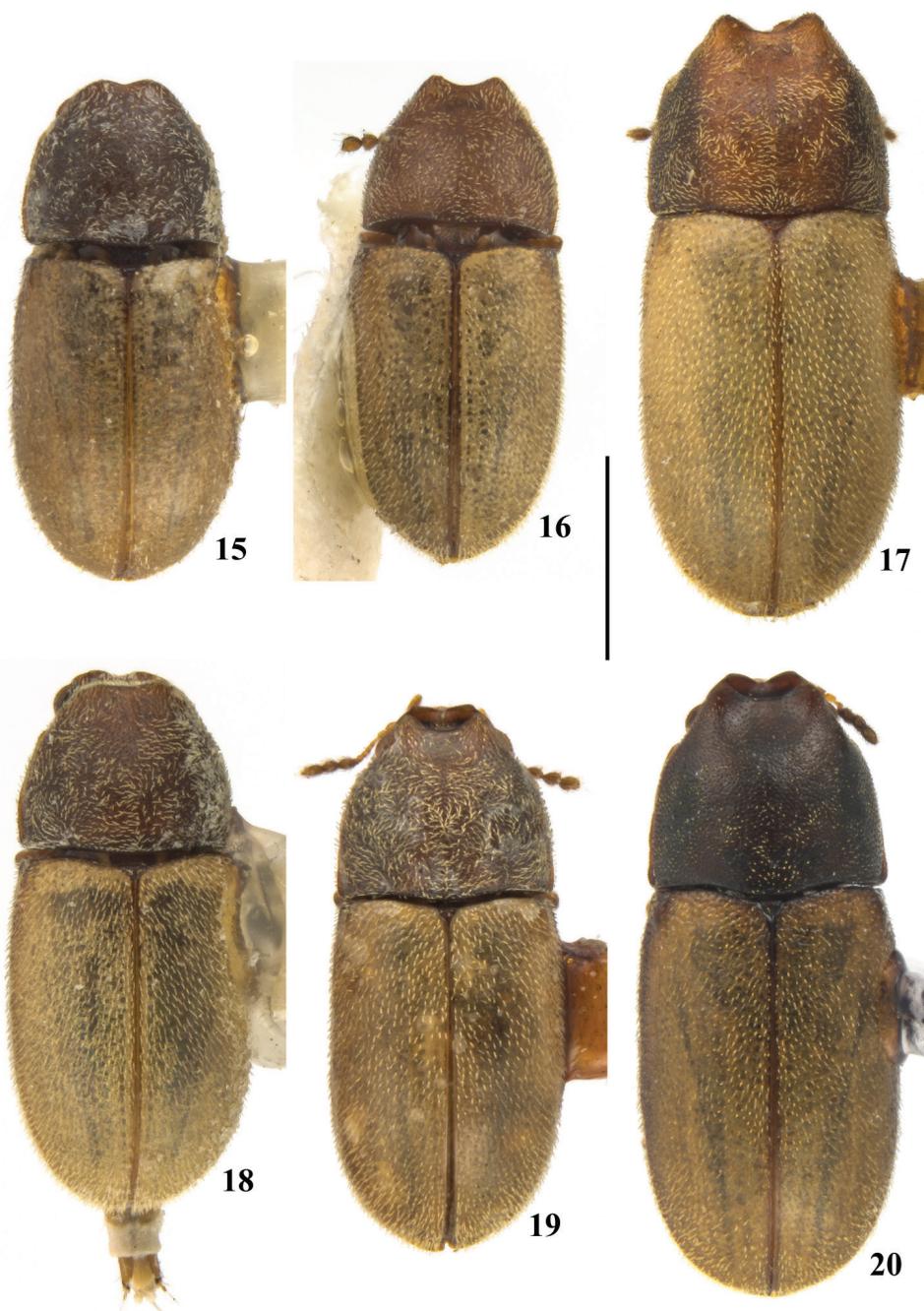
Figures 1–8. Male paralectotype of *Cis semipallidus* Pic, 1916. **1** Dorsal view **2** Lateral view **3** Ventral view **4** Sternite VIII **5** Basal piece **6** Tegmen **7** Penis **8** Labels. Scale bars: 1 mm (1–3); 0.1 mm (4–7).

ing two short, rounded projections (Fig. 11), separated from each other by about one-fifth the basal width of scutellar shield. **Antennae** (Fig. 24) bearing 10 antennomeres, with measurements as follow ($n = 3$, mean; in mm): 0.09, 0.05, 0.05, 0.03, 0.03, 0.03, 0.02, 0.08, 0.08, 0.09 (FL 0.16; CL 0.26; CL/FL 1.56). **Eyes** coarsely faceted, with approx. 100 ommatidia; GW ($n = 3$, mean; in mm) 0.17. **Pronotum** with coarse punctures, separated from each other by one puncture-width or less; interspaces of punctures, microreticulate; anterior edge produced forward overhead, broadly emarginate at apex, forming two short subtriangular plates (Figs 1, 9, 16–17, 19–20); anterior portion with concave impression before anterior plates; anterior angles produced forward and broadly rounded; lateral edges finely crenulate, barely visible when seen from above. **Scutellar shield** (Fig. 27) subpentagonal, with few punctures and bristles; BW



Figures 9–14. *Cis pallidus* Mellié, 1849, male (9–11) and female (12–14) from Viçosa, state of Minas Gerais, Southeast Brazil. **9, 12** Dorsal view **10, 13** Lateral view **11, 14** Ventral view. Scale bar: 1 mm.

($n = 3$, mean; in mm) 0.11. **Elytra** with single sized punctation, punctures shallower, finer and sparser than those on pronotum; interspaces of punctures irregular close to anterior portion of elytra and smooth at disc. **Metathoracic wings** developed, apparently functional. **Hypomera** with shallow, inconspicuous punctation; interspaces, finely shagreened. **Prosternum** (Fig. 25) slightly tumid at midline; surface similar to that of hypomera. **Prosternal process** (Fig. 25) about as long as prosternal disc, curved, slightly



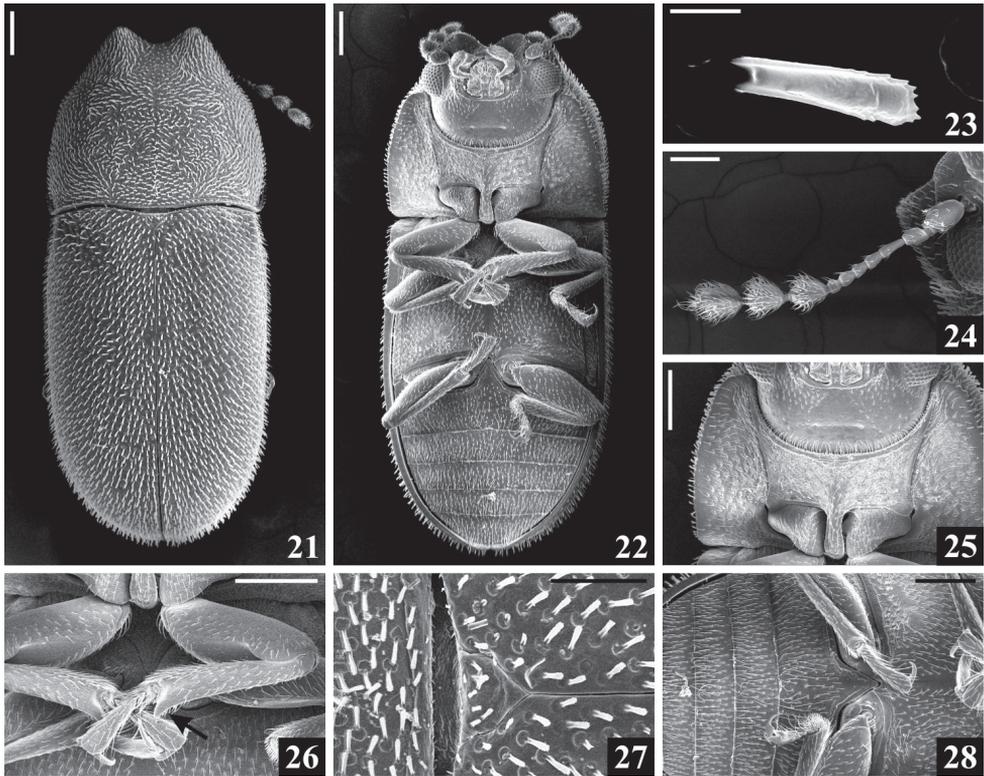
Figures 15–20. *Cis pallidus* Mellié, 1849, dorsal view from various localities, shown in the same scale. **15** Female from the state of Bahia, compared with the holotype deposited in the MNHN **16** Male from Corcovado, state of Rio de Janeiro **17** Male from Marechal Cândido Rondon, state of Paraná **18** Female from the province of Tucuman, Argentina **19** Male from Nova Teutônia, state of Santa Catarina **20** Male from Urubici, state of Santa Catarina. Scale bar: 1 mm.

enlarged close to rounded apex. *Protibiae* slightly expanded to apex; outer apical angle acute (Fig. 26, arrow); apical edge devoid of spines. *Meso- and metatibiae* comparatively less expanded than protibiae; outer apical angle rounded; apical edge with row of minute spines. *Metaventrite* with punctuation denser than those on hypomera, more conspicuous close to sides; interspaces shagreened; discripen shallow, about half the length of metaventrite at midline. *Abdominal ventrites* with punctuation and surface similar to that of metaventrite; length of ventrites (measured in male from Viçosa; in mm, from base to apex at longitudinal midline) as follows: 0.3, 0.12, 0.11, 0.11, 0.13; first ventrite apparently devoid of sex patch (Figs 22, 28), but with small barely discernible mark in slide preparation (Fig. 29, arrow). *Male abdominal terminalia* with *sternite VIII* (Fig. 4) subtrapezoidal, posterior edge slightly curved inwardly and with short setae, sides rounded and bearing long slender setae. *Tegmen* (Figs 6, 30, 32, 34, 36, 38, 40–41, 43) subcylindrical, 2.89–4.45× as long as wide, 1–1.13× as long as penis; apical portion with short emargination, forming two lateral lobes; apex narrow, preceded by lateral, broadly rounded membranous flaps. *Basal piece* (Fig. 5) subtriangular, about as long as wide, 1/3 to 1/4 length of tegmen. *Penis* (Figs 7, 31, 33, 35, 37, 39, 42, 44) subcylindrical, 3.6–4.35× as long as wide, gradually expanding from base to apex, apex subtriangular.

Females. Projections of head and pronotum more rounded and less prominent than in males. Otherwise like males, but first abdominal ventrite devoid of any discernible mark. *Female abdominal terminalia* (in specimen from Viçosa with everted terminalia) with *paraprocts* 1.25× as long as gonocoxites; each *gonocoxite* with three ventral lobes; *gonostyli* inserted at top of gonocoxites.

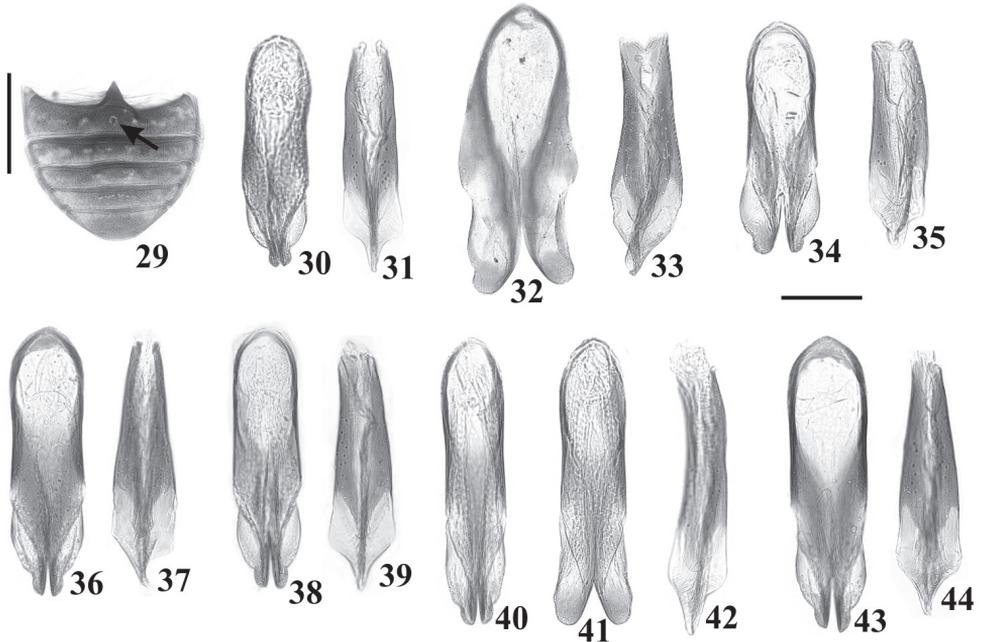
Measurements and ratios. Males, measurements in mm (n = 21): TL 1.95–3.00 (2.47 ± 0.32), PL 0.70–1.00 (0.86 ± 0.09), PW 0.80–1.20 (0.99 ± 0.12), EL 1.20–2 (1.60 ± 0.24), EW 0.85–1.30 (1.07 ± 0.13), GD 0.60–1.15 (0.83 ± 0.13), PL/PW 0.72–1.12 (0.87 ± 0.10), EL/EW 1.26–1.94 (1.49 ± 0.13), EL/PL 1.38–2.05 (1.86 ± 0.17), GD/EW 0.54–1.00 (0.77 ± 0.09), TL/EW 2.04–3.00 (2.30 ± 0.20). Females, measurements in mm (n = 20): TL 2.15–3.05 (2.53 ± 0.23), PL 0.75–1.00 (0.85 ± 0.07), PW 0.85–1.20 (1.02 ± 0.08), EL 1.40–2.05 (1.68 ± 0.17), EW 0.95–1.35 (1.11 ± 0.10), GD 0.75–1.05 (0.86 ± 0.09), PL/PW 0.75–0.89 (0.83 ± 0.03), EL/EW 1.42–1.56 (1.50 ± 0.03), EL/PL 1.76–2.20 (1.97 ± 0.11) GD/EW 0.68–0.87 (0.76 ± 0.04), TL/EW 2.16–2.36 (2.26 ± 0.04).

Type material. ♀ **Holotype** “Cis pallidus Mel. Bahia. T. Cast. 72 [green disc, handwritten] \ Bahia. Mocquerys [handwritten] \ Cis pallidus Reiche [handwritten] \ LECTOTYPE [printed] Cis pallidus Mellié [handwritten]”; ♂ **lectotype of *Cis semipallidus* Pic, 1916, here designated** (MNHN) “Rep Argentina. Prov. Buenos Aires 9.11.1896 C. Bruch \ Cis près Cis bisbidens Gorh. [handwritten] \ Cis [handwritten] \ Type [yellow paper; handwritten] \ C. semipallidus Pic [handwritten] \ Type [red paper] \ LECTOTYPE [printed] Cis semipallidus Pic [handwritten]”; 1 ♂ (MNHN, dissected) “Rep. Argentina Prov. Buenos Aires 9.11.1896 C. Bruch [handwritten] \ Type [handwritten] \ PARALECTOTYPE [printed] Cis semipallidus Pic [handwritten]”.



Figures 21–28. *Cis pallidus* Mellié, 1849, scanning electron microscopy. **21** Dorsal view **22** Ventral view **23** Elytral bristle **24** Antenna **25** Part of head and prothorax in ventral view **26** Protibiae, showing the outer apical angle (arrow) **27** Part of pronotum and elytra, with scutellar shield **28** Part of metaventrite and abdominal ventrites. Scale bars: 0.2 mm (**21–22**, **25–26**, **28**), 0.01 mm (**23**), 0.1 mm (**24**, **27**).

Additional material. BRASIL: 1 ♀ (MNHN) “Museum Paris Brésil Bahia (Tabacs) A. Grouvelle 1913 \ *Cis pallidus* Mellié 1969 J. F. Lawrence”; 1 ♂ and 1 ♀ (CELC); “BRASIL: ES, Atílio Vivacqua, Mata do Zé [sic], 16.vi.2007, Furieri, K. S. & Ana Paula”; 1 ♂ and 2 ♀ (MNRJ) “Saude 29.vi.[19]14 \ MNRJ 230”; 1 ♂ (DZUP, dissected) “Coleção M. Alvarenga \ CORCOVADO Guanabara BRASIL x.1968 Alvarenga & Seabra \ *Cis pallidus* Mellié, 1849 C. Lopes-Andrade det. \ DZUP 273653”; 22 ♂ (CELC, 5 dissected) and 13 ♀ (CELC) “BRASIL: MG, Viçosa, “Atrás do Insetário” 18.xi.2003 legs. D. J. Souza & C. Lopes-Andrade \ ex *Trametes hirsuta*”; 61 specimens (CELC, in alcohol) “Brasil: MG, Viçosa, Bom Jesus, 13.vii.2006 Oliveira, C. B.”; 19 specimens (CELC, in alcohol) “Brasil: MG, Viçosa, Apiário 09.vi.2010 leg. L. A. O. Campos”; 4 ♂ (CELC) and 3 ♀ (CELC) “BRASIL: MG, Viçosa, “Violeira” 17.xii.2004 leg. A. A. Zacaro”; 5 ♂ and 6 ♀ (CELC) “BRASIL: MG, Viçosa, Mata da Biologia, 03.v.2014, Lopes-Andrade et al. leg”; 2 ♀ (CELC) “BRASIL: MG, Jequeri; Piscamba 09.vi.2009 leg. D. M. da Silva”; 6 ♂ (CELC, 1 dissected) and 6 ♀ (CELC) “BRASIL: MG, São João del Rei [sic] Colônia 25.xii.2011 De Oliveira, G. A. & De



Figures 29–44. *Cis pallidus* Mellie, 1849. **29** Abdominal ventrites of a male from São João del-Rei, state of Minas Gerais, showing a small sex patch at the first ventrite (arrow), possibly vestigial **30–44** Tegmen (**30, 32, 34, 36, 38, 40–41, 43**) and penis (**31, 33, 35, 37, 39, 42, 44**) of males from various localities **30–31** Corcovado, state of Rio de Janeiro **32–33** Viçosa, state of Minas Gerais **34–35** São João del-Rei, state of Minas Gerais **36–37** Palotina, state of Paraná **38–39** Province of Tucuman, Argentina **40–42** Nova Teutônia, state of Santa Catarina, showing tegmen before (**40**) and after distortion (**41**) **43–44** Urubici, state of Santa Catarina. Scale bars: 0.5 mm (**29**); 0.1 mm (**30–44**).

Oliveira, E. H.”; 13 ♀ (CELC) “BRASIL: MG, Juiz de Fora Campus UFJF 11.vii.2012 Pecci-Maddalena leg.”; 2 ♂ and 2 ♀ (CELC) “BRASIL: MG, Ibitipoca, 03.iii.2014. leg. Pecci-Maddalena, I. \ *Trametes hirsuta*”; 2 ♂ (1 DZUP; 1 CELC, dissected) “BRASIL: PR, Palotina Parque Estadual de São Camilo (coleta manual) 02.iii.2011 G. A. Oliveira, col. \ Ciidae sp. 17 C. S. Santos, det. 2011 \ Coletado no fungo: *Trametes* sp. (Polyporaceae) V. G. Cortez det. 2011 \ C342 \ *Cis pallidus* Mellie, 1849 C. Lopes-Andrade det.”; 1 ♂ (ANIC, dissected) and 1 ♀ (ANIC) “Nova Teutonia Santa Catarina BRAZIL March F. Plaumann”; 1 ♂ (ANIC) “BRAZIL: Santa Catarina, Nova Teutonia Dec. F. Plaumann”; 1 ♂ (ANIC) “Brasilien [Marechal Cândido] Rondon 24° 38’ B. 54° 07’ L F. Plaumann”; 1 ♀ (ANIC) “Santa Catarina BRAZIL xi.64 Fritz Plaumann”; 1 ♀ (DZUP) “Brasilien Nova Teutonia 27° 11’ B 52° 23’ L 300-500m xii.1980 Fritz Plaumann \ *Cis pallidus* Mellie, 1849 C. Lopes-Andrade det. \ DZUP 273657”; 1 ♂ (FMNH) “Nova Teutonia, Sta. Catarina, BRAZ. Fritz Plaumann leg. \ *Cis pallidus* Mell. det. J. F. Lawrence \ FMNH 261”; 3 ♀ (FMNH) “Nova Teutonia, Sta. Catarina, BRAZ. 5.viii.44 Fritz Plaumann leg. \ *Cis pallidus* Mell. det. J. F. Lawrence \ FMNH 259; 260; 262”; 3 ♂ (CELC, 1 dissected) and 1 ♀ (CELC) “BRASIL: SC, Urubici (Es-

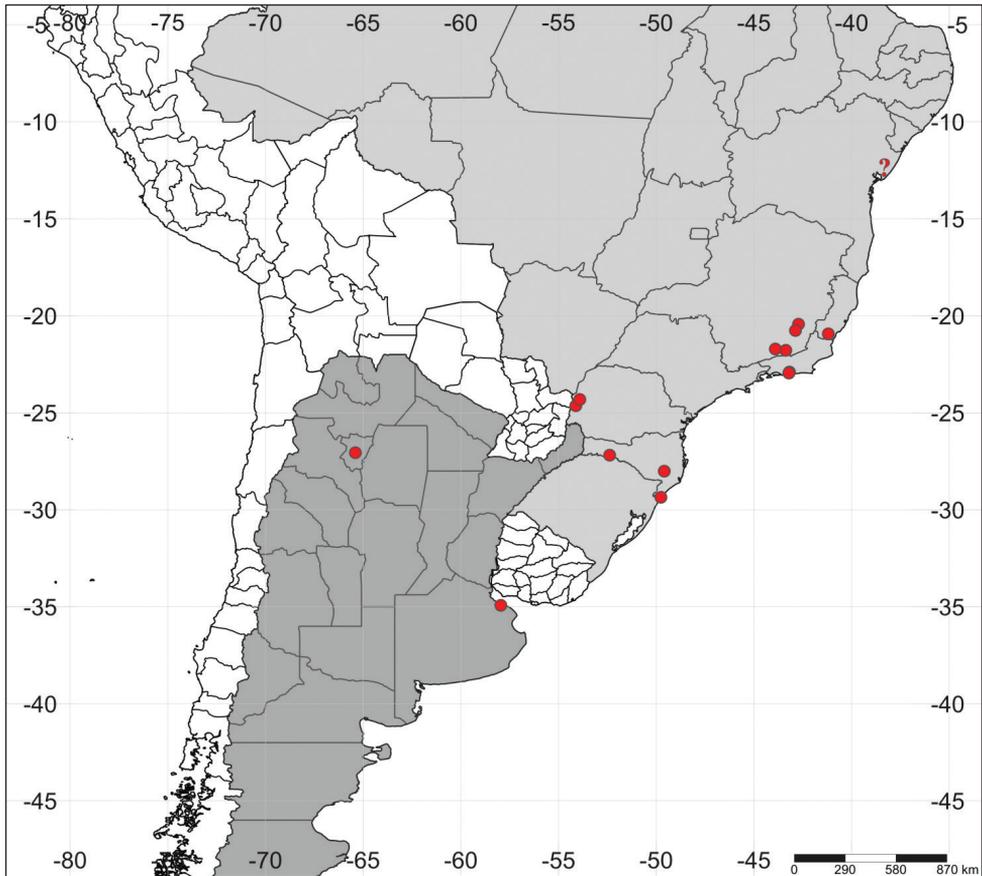


Figure 45. Distribution map of *Cis pallidus* Mellié, 1849. The question mark shows a presumed locality of the species in Recôncavo Baiano, an area of old tobacco farms in the state of Bahia. See text for more details.

trada para Serra do Corvo) 06.iii.2011 Grossi & Parizotto”; 1 ♂ (MCNZ) “Torres, RS 21/XI/1976 [handwritten] A. Lise leg. [p.] COL. NCN 28.253 [handwritten]”; 1 ♀ (ANIC) “Brazil J. Rick \ J. F. Lawrence Lot.1995 \ *Polyporus polyzonus* [= *Trametes polyzona*]”. **ARGENTINA:** 4 ♀ (FMNH) “R. Argentina La Plata Col. C. Bruch \ FMNH 263”; 1 ♂ (ANIC, dissected) and 1 ♀ (ANIC) “ARG: Tucuman Famailla vi.11.1971 \ L. A. Stange Lot 13 \ *Coriolus pinistus* [sic] [= *Trametes hirsuta*]”; 1 ♂ and 1 ♀ (MACN) on the same card “Rep Argentina. Prov. Buenos Aires 9.11.1896 C. Bruch \ *Cis præs bisbidens* Gorh. [handwritten]”.

Comments. There was no consistent difference in the morphology of tegmen and penis between specimens with different dorsal coloration and length of dorsal bristles, and such variation occurred within the populations throughout the geographic extension of the species. Therefore, we consider that the abovementioned specimens all belong to a single species. After the description of *C. pallidus*, Mellié (1849: 247) wrote “Provient de Bahia; a été donné à M. Reiche par M. Mocquerys

de Rouen”, a statement in the singular, suggesting that he had only one specimen at the time of the description. It is important to note that Mellié, in the same work, clearly used plural in case he had examined two or more specimens. Therefore, we consider the single type specimen located in the MNHN as the holotype, even though it has a lectotype label. Two specimens from MACN, pinned at the same card, have the same locality label of the type series of *C. semipallidus*, but these do not have any indication whether they were examined or not by Maurice Pic. Thus we think they do not belong to the type series but they were possibly collected together. The specimen from the MNHN labeled “(...) Bahia (Tabacs) A. Grouvelle (...)” is possibly from Recôncavo Baiano (Fig. 45, question mark), a name for the geographic area around Bay of All Saints, the biggest bay at the northeastern coast of Brazil. The collector Antoine Grouvelle was the director of the “Manufactures nationales des Tabacs” (national manufacturers of tobacco) and used to catch small insects in the tobacco leaves exported to France. These insects, mostly Coleoptera, were probably retained during their flight by the more or less abundant pubescence and viscosity which covers the tobacco leaves; it is also possible that some of them had been attracted by the rainwater which remains in the axils of the leaves, and that others came from the washing water in the country of production (Régimbart 1895). In the 19th century, most of the tobacco production from Bahia came from Recôncavo Baiano; therefore, we consider that it is plausible that this specimen of *C. pallidus* has come from this area.

Host fungi. *Trametes hirsuta* Lloyd (Polyporaceae), one record and two breeding records; *Trametes polyzona* Pers. (Polyporaceae), one record; and *Trametes* sp. (Polyporaceae), one record.

Acknowledgements

We would like to especially thank John F. Lawrence for sending a large number of named specimens from the *C. pallidus* and *C. vitulus* species-groups for comparison; and Vivian E. Sandoval-Gómez for examining historical and type material related to *C. pallidus* in MACN and MNHN. We are grateful to everyone who collected and sent us specimens (see section on Additional material for their names), and for museum curators for allowing examination and loan of specimens. We are also grateful to Glenda M. Orledge and Daniel K. Young for their corrections and suggestions to the manuscript, and Aaron D. Smith for his assistance during the revision process. P.V.B. thanks the Programa de Pós-Graduação em Ecologia (Universidade Federal de Viçosa, Brazil) for the academic support. C.L.A. thanks Núcleo de Microscopia e Microanálise (NMM) of Universidade Federal de Viçosa for SEM facilities in 2004. Financial support was provided by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; Edital 01/2016 – Demanda Universal, APQ-02675-16; Edital FORTIS; master grant to P.V.B.), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; research grant to C.L.A. n° 307116/2015-8).

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Anuran species composition of Cancão Municipal Natural Park, Municipality of Serra do Navio, Amapá state, Brazil

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Abstract

In this study, the first survey of anuran species in the Cancão Municipal Natural Park is presented, a protected area of approximately 370 hectares of Amazonian forest located in the northwest center region of the state of Amapá, Brazil. The work was performed during the dry and rainy season, through active visual and auditory survey, totaling 216 man hours of sampling effort. Forty-nine species of anuran amphibians were recorded in the Cancão Municipal Natural Park, including three new records: *Hyalinobatrachium iaspidiense*, *Pristimantis* cf. *ockendeni*, and *Scinax garbei*. Three species, *Hyalinobatrachium iaspidiense*, *Ameerega pulchripecta*, and *Anomaloglossus baebatrachus*, are listed as Data Deficient and one is listed as Vulnerable (*Atelopus hoogmoedi*) according red lists of IUCN. The rarefaction curve cumulative species did not reach an asymptote, indicating that site has potential for species that have not yet been recorded. Nine species were represented by only one individual and were considered rare in the studied environments, eight species were defined as common, and the 32 remaining species were classified as having intermediary abundance. Our data indicated that Cancão Municipal Natural Park contains a considerable portion of the anurans species richness of Amapá state, turn the area into a place of great importance for the conservation of the anurans of the Eastern Amazon.

Keywords

Amazonia, conservation, eastern Amazon, species list

Introduction

Most of the currently documented amphibian species in Brazil have been discovered during the last forty years (Campos et al. 2014). These new species descriptions, which have occurred at regular rates, are a strong indication that the Brazilian amphibian fauna is poorly known (Peloso 2010). Brazil has the highest diversity of amphibian species on the planet with 1080 species, 1039 of which are anurans, 36 caecilians, and five salamanders (Segalla et al. 2016). According to a recent publication of species list, 308 species of anurans (29.6 % of the species known in Brazil), 18 gymnophionans and five caudates (Hoogmoed and Galatti 2016) are known in the Brazilian Amazon, representing approximately one-third of the total of amphibians recorded for the country (Ávila-Pires et al. 2010, Neckel-Oliveira et al. 2013).

This amphibian species richness can be considered underestimated in number and complexity when considering enormous areas of Brazil which have yet to be inventoried, and there are many localities where surveys have been insufficient (Silvano and Segalla 2005). Additionally, the political limits and geographic distributions, the existence of cryptic species (Fouquet et al. 2007), sampling gaps due to the concentration of researches in a few areas (Azevedo-Ramos and Galatti 2002), sampling effort used appropriate methods for inventories of amphibians (Miranda et al. 2015) and problems in various taxonomic groups, frustrate attempts to obtain a comprehensive understanding of Brazil amphibians (Silvano and Segalla 2005).

Due to difficult to access, many Amazonian areas are still poorly known regarding their amphibian fauna and with insufficient sampling (Funk et al. 2012). In the Brazilian Amazonia, knowledge has increased in the last ten years based on studies on anurans composition conducted mostly in the state of Amazonas (França and Venâncio 2010, Ilha and Dixo 2010, Pantoja and Fraga 2012, Prudente et al. 2013, Waldez et al. 2013, Ferrão et al. 2016, Ferreira et al. 2017), state of Pará (Ávila-Pires et al. 2010, Mendes-Pinto and Souza 2011, Bernardo et al. 2012, Vaz-Silva et al. 2015), state of Rondônia (Ávila-Pires et al. 2010, Piatti et al. 2012) and state of Acre (Bernarde et al. 2011, Bernarde et al. 2013, Miranda et al. 2015, Venâncio and Souza 2016, França et al. 2017).

In the Amazonian biome, studies on anurans are concentrated in states of Amazonas, Pará, Rondônia and Acre, other localities in the Brazilian Amazon lack inventories (Azevedo-Ramos and Galatti 2002), a fact observed for the Amapá state. Although be inserted in a region of great interest for conservation and presents gaps knowledge on anurans (Azevedo-Ramos and Galatti 2002), Amapá state is little studied in relation to its anurans fauna (Lima 2008, Queiroz et al. 2011, Pereira-Júnior et al. 2013, Araújo and Costa-Campos 2014, Costa-Campos 2015, Costa-Campos et al. 2015, Lima et al. 2017, Benício and Lima 2017). In this context, the present study aims to provide the list of species of anuran amphibians that occur in the area of the Cancão Municipal Natural Park, municipality of Serra do Navio, state of Amapá, eastern Amazon.

Materials and methods

Study area. Fieldwork was conducted at the Cancão Municipal Natural Park (Figure 1), municipality of Serra do Navio, Amapá state (0.90263°N, 52.00505°W and 0.90858°N, 52.00422°W). The study area covers approximately 370 hectares of primary forest, including terra-firme rainforests, streams, open areas, and treefall gaps. The climate of the region is Equatorial (Am) according Köppen-Geiger classification and the average temperature is 27.6 °C, varying seasonally between 25.8 to 29.0 °C, with annual rainfall approximately 2,850 mm with monsoon period between February and May, when the monthly rainfall is nearly 400 mm (Alvares et al. 2013).

Sampling. Animals were registered during diurnal and nocturnal active visual search and auditory census in different microhabitats used by frogs (Heyer et al. 1994). These methods were conducted by three researchers for three consecutive days from January to December 2013, resulting in a sampling effort of 216 hours/man. A wide variety of environments were surveyed including ponds, brooks, forest interior, temporary ponds, and other water bodies. These environments were sampled in four mainly sites in the Cancão Municipal Natural Park (Figure 2): Terra firme trail at Cancão forest (0.90275°N, 52.00497°W); River Amapari trail (0.90083°N, 52.01347°W), Treefall gap at stream Cancão 01 (0.91183°N, 52.00205°W) and Treefall gap at Cancão forest 02 (0.91388°N, 51.99977°W).

The specimens were collected under permit SISBIO number 32651-1 issued by the Brazilian Ministry of Environment (MMA-ICMBio). Voucher adults collected were deposited at the Coleção Herpetológica da Universidade Federal do Amapá (UNIFAP) and Coleção Herpetológica do Museu Paraense Emílio Goeldi “Oswaldo Rodrigues da Cunha” (MPEG). The conservation status quoted follows IUCN (2017). The species taxonomy applied follows the Brazilian Society of Herpetology (SBH), according to Segalla et al. (2016) and Dubois (2017). *Adenomera andreae* and *A. hylaedactyla* were identified through morphology and vocalization (cf. Heyer 1973; Angulo et al. 2003).

Data analysis. To analyze the anurans species richness, rarefaction curves of species were constructed based on the number of individuals and number of samples (Gotelli and Colwell 2001) with 1000 randomizations. Species richness estimators Jackknife1 and Bootstrap were used for determine the expected richness of anurans (Colwell 2013). To determine similarities of species compositions amongst habitats sampled, cluster analyses were performed by the UPGMA method, using the modified index of similarity of Jaccard (Clarke 2003). This analysis was performed using ESTIMATES 9.1 (Gotelli and Colwell 2001).

The dominances were represented by Whittaker Diagram, obtained by ranking species, starting with the most abundant, along the x-axis and the logarithm abundances on the y-axis. Rare species were those represented by a single individual (singletons). The other species were classified as having intermediate abundance. The pattern of the species abundance distribution was fitted to the geometric, logarithmic, log-normal, and broken-stick models. Model fit was assessed by the chi square adherence test (Magurran 2011) using the software PAST version 2.17c (Hammer et al. 2001).

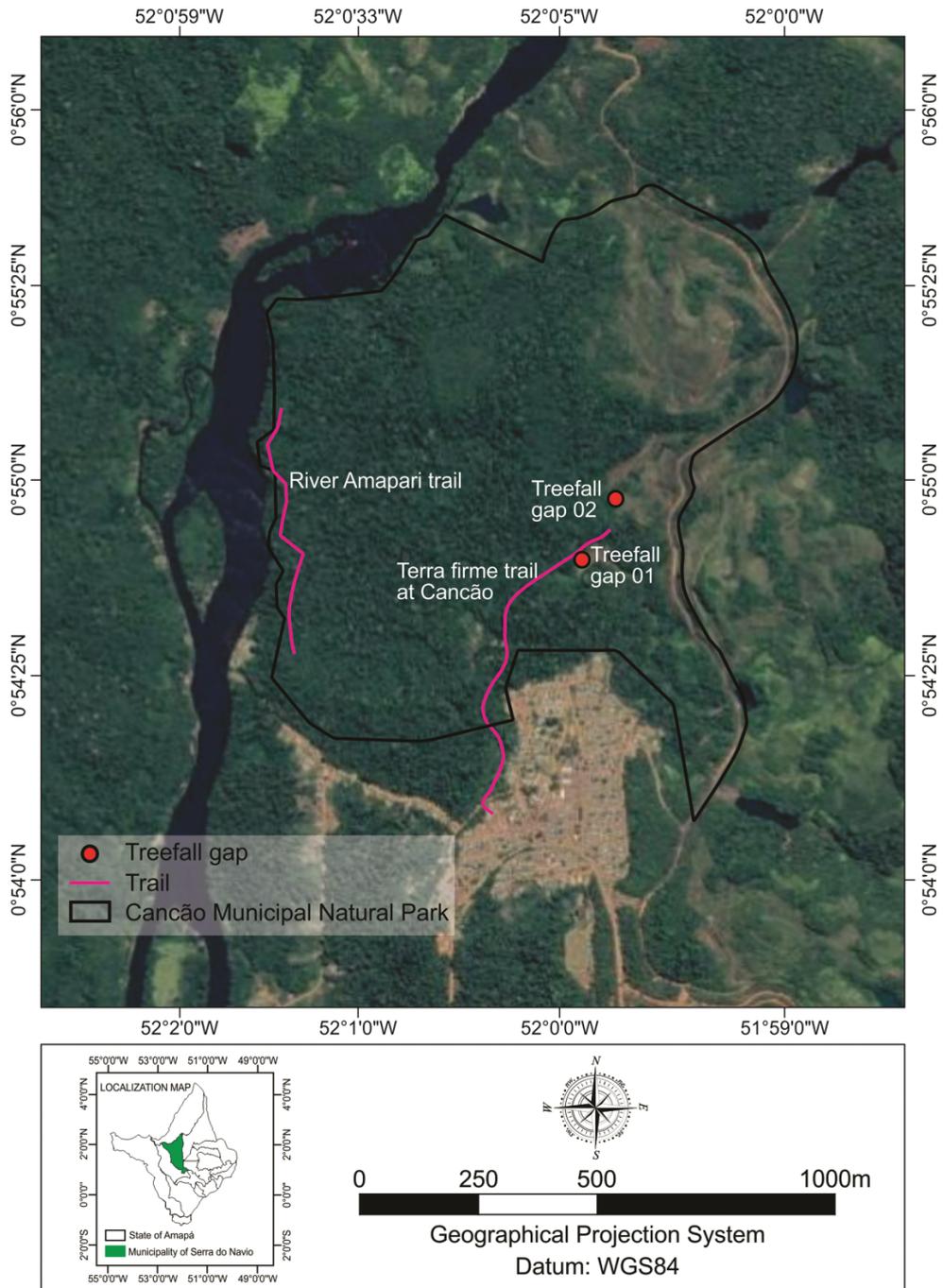


Figure 1. Maps showing the Amapá state and sampling sites in the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá State, northern Brazil.

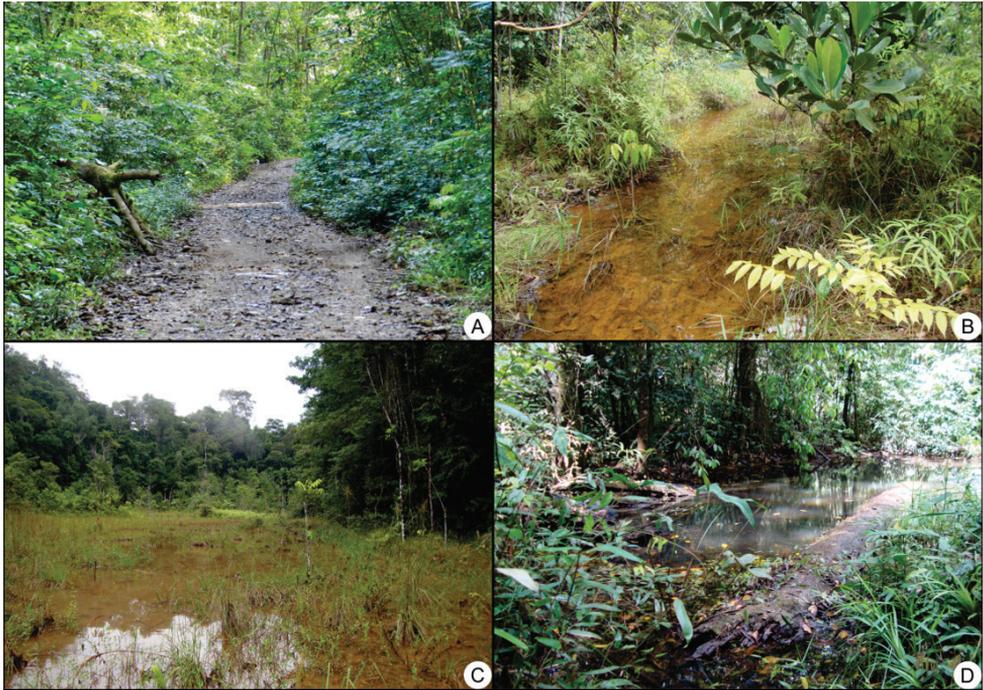


Figure 2. Habitats sampled at the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá state: **A** Terra firme trail at Cancão forest **B** Treefall gap at stream Cancão 01 **C** Treefall gap at Cancão forest 02 **D** River Amapari trail.

The Spearman correlation coefficient analysis was performed to compare climatic conditions (available from the NHMET database) during the sampling period with abundance. To check the influence of environmental data on amphibian abundance, multiple regression analyses were conducted, including data on rainfall, temperature, and humidity as independent variables. The normality of the data was tested with the KOLMOGOROV-SMIRNOV analysis (Zar 1999). Statistical analyses were performed with BIOESTAT 5.3 software (Ayres et al. 2007), using a significance index of $P < 0.05$ for all analyses.

Results

Forty-nine anuran species have been recorded in the Cancão Municipal Natural Park (Table 1, Figure 3) during the dry and rainy season, totaling 216 hours of sample effort. These species are distributed in 22 genera, belonging to five families: Allophrynidae (1 species); Aromobatidae (2 species), Bufonidae (5 species), Centrolenidae (1 species), Craugastoridae (5 species), Dendrobatidae (2 species), Eleutherodactylidae (1 species); Hylidae (18 species), Leptodactylidae (10 species), Phyllomedusidae (3 species); Pipidae (1 species).

Table 1. List of amphibian species recorded at Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá State. Sampled areas: Terra firme trail at Cancão forest (TC), Amapari trail (TA), treefall gap at stream Cancão (TS), and treefall gap at Cancão forest (TF). Red List species included in some category of IUCN (2017): LC – Least Concern; VU – Vulnerable; DD – Data Deficient.

Family/Species	Sampled areas				IUCN
	TC	TA	TS	TF	
Allophrynidae					
<i>Allophryne ruthveni</i> Gaige, 1926		X			LC
Aromobatidae (Allobatinae)					
<i>Allobates femoralis</i> (Boulenger, 1884)	X	X			LC
Aromobatidae (Aromobatinae)					
<i>Anomaloglossus baeobatrachus</i> (Boistel & de Massari, 1999)				X	DD
Bufonidae					
<i>Atelopus hoogmoedi</i> Lescure, 1974		X			VU
<i>Rhaebo guttatus</i> (Schneider, 1799)	X	X			LC
<i>Rhinella margaritifera</i> complex of species	X	X	X	X	LC
<i>Rhinella marina</i> (Linnaeus, 1758)	X	X	X	X	LC
<i>Rhinella martyi</i> Fouquet, Gaucher, Blanc & Vélez-Rodriguez, 2007		X			LC
Centrolenidae (Hyalinobatrachinae)					
<i>Hyalinobatrachium iaspidiense</i> (Ayarzaqüena, 1992) *		X			DD
Craugastoridae (Ceuthomantinae)					
<i>Pristimantis chiastonotus</i> (Lynch & Hoogmoed, 1977)	X	X		X	LC
<i>Pristimantis marmoratus</i> (Boulenger, 1900)		X			LC
<i>Pristimantis</i> cf. <i>ockendeni</i> (Boulenger, 1912) *	X	X		X	LC
<i>Pristimantis zeuctotylus</i> (Lynch & Hoogmoed, 1977)		X			LC
<i>Pristimantis zimmermanae</i> (Heyer & Hardy, 1991)		X			LC
Dendrobatidae (Colostethinae)					
<i>Ameerega pulchripecta</i> (Silverstone, 1976) **	X	X			DD
Dendrobatidae (Dendrobatinae)					
<i>Dendrobates tinctorius</i> (Cuvier, 1797)		X			LC
Eleutherodactylidae					
<i>Adelophryne gutturosa</i> Hoogmoed & Lescure, 1984	X				LC
Hylidae					
<i>Boana boans</i> (Linnaeus, 1758)	X	X	X	X	LC
<i>Boana calcarata</i> (Troschel in Schomburgk 1848)		X			LC
<i>Boana cinerascens</i> (Spix, 1824)	X		X	X	LC
<i>Boana dentei</i> (Bokermann, 1967) **		X			LC
<i>Boana fasciata</i> (Günther, 1859 “1858”)		X		X	LC
<i>Boana geographica</i> (Spix, 1824)		X			LC
<i>Boana multifasciata</i> (Günther, 1859 “1858”)	X		X	X	LC
<i>Dendropsophus counani</i> Fouquet, Souza, Nunes, Kok, Curcio, de Carvalho, Grant & Rodrigues, 2015	X	X			–
<i>Dendropsophus leucophyllatus</i> (Beireis, 1783)				X	LC
<i>Dendropsophus</i> cf. <i>microcephalus</i> (Cope, 1886)	X				LC
<i>Dendropsophus minutus</i> (Peters, 1872)	X				LC
<i>Osteocephalus oophagus</i> Jungfer & Schiesari, 1995		X			LC
<i>Osteocephalus taurinus</i> Steindachner, 1862	X	X			LC

Family/Species	Sampled areas				IUCN
	TC	TA	TS	TF	
<i>Scinax boesemani</i> (Goin, 1966)			X	X	LC
<i>Scinax garbei</i> (Miranda-Ribeiro, 1926) *				X	LC
<i>Scinax nebulosus</i> (Spix, 1824)			X	X	LC
<i>Scinax ruber</i> (Laurenti, 1768)	X	X	X	X	LC
<i>Trachycephalus resinifictrix</i> (Goeldi, 1907)	X	X			LC
Leptodactylidae (Leptodactylinae)					
<i>Adenomera andreae</i> (Müller, 1923)	X	X	X	X	LC
<i>Adenomera hylaedactyla</i> (Cope, 1868)	X	X	X	X	LC
<i>Leptodactylus knudseni</i> Heyer, 1972	X				LC
<i>Leptodactylus lineatus</i> (Schneider, 1799)	X				LC
<i>Leptodactylus longirostris</i> Boulenger, 1882		X	X	X	LC
<i>Leptodactylus mystaceus</i> (Spix, 1824)	X	X	X	X	LC
<i>Leptodactylus pentadactylus</i> (Laurenti, 1768)	X				LC
<i>Leptodactylus petersii</i> (Steindachner, 1864)	X				LC
<i>Leptodactylus rhodomystax</i> Boulenger, 1884 "1883"	X				LC
<i>Leptodactylus stenodema</i> Jiménez de la Espada, 1875	X				LC
Phyllomedusidae					
<i>Phyllomedusa bicolor</i> (Boddaert, 1772)		X			LC
<i>Phyllomedusa vaillantii</i> Boulenger, 1882	X	X			LC
<i>Pithecopus hypochondrialis</i> (Daudin, 1800)	X	X		X	LC
Pipidae					
<i>Pipa pipa</i> (Linnaeus, 1758)		X			LC

* First record for the state of Amapá.

** Species endemic to the municipality of Serra do Navio, Amapá state.

Three new records of anurans are presented for the Cancão Municipal Natural Park, namely *Hyalinobatrachium iaspidiense* (Centrolenidae), *Pristimantis* cf. *ockendeni* (Craugastoridae), and *Scinax garbei* (Hylidae). None of the frog species recorded at the Cancão Municipal Natural Park is classified as threatened in the red lists of IUCN (2017). However, three species (*Hyalinobatrachium iaspidiense*, *Ameerega pulchripecta*, and *Anomaloglossus baeobatrachus*) are listed as Data Deficient and one is listed as Vulnerable (*Atelopus hoogmoedi*).

Hyalinobatrachium iaspidiense is known from Brazil, Ecuador, French Guiana, Guyana, Peru, Suriname, Venezuela, and is expected to occur in the Amazonian areas between the Ecuadorian and Peruvian localities and the Guiana region (Castroviejo-Fisher et al. 2011). This record is the first for Amapá and extends the known distribution of the species 1,020 km east from the type locality Quebrada de Jaspe, San Ignacio de Yuraní, Bolívar state, Venezuela (Silva e Silva and Costa-Campos 2016).

Pristimantis cf. *ockendeni* is distributed throughout the Amazonian basin of Peru, Ecuador, southern Colombia, and Brazil in the states of Acre and Amazonas (Rodrigues et al. 2004). This is the first state record for Amapá, extending the range 986 km NW from the Manaus, Amazonas state (Silva e Silva et al. 2015).

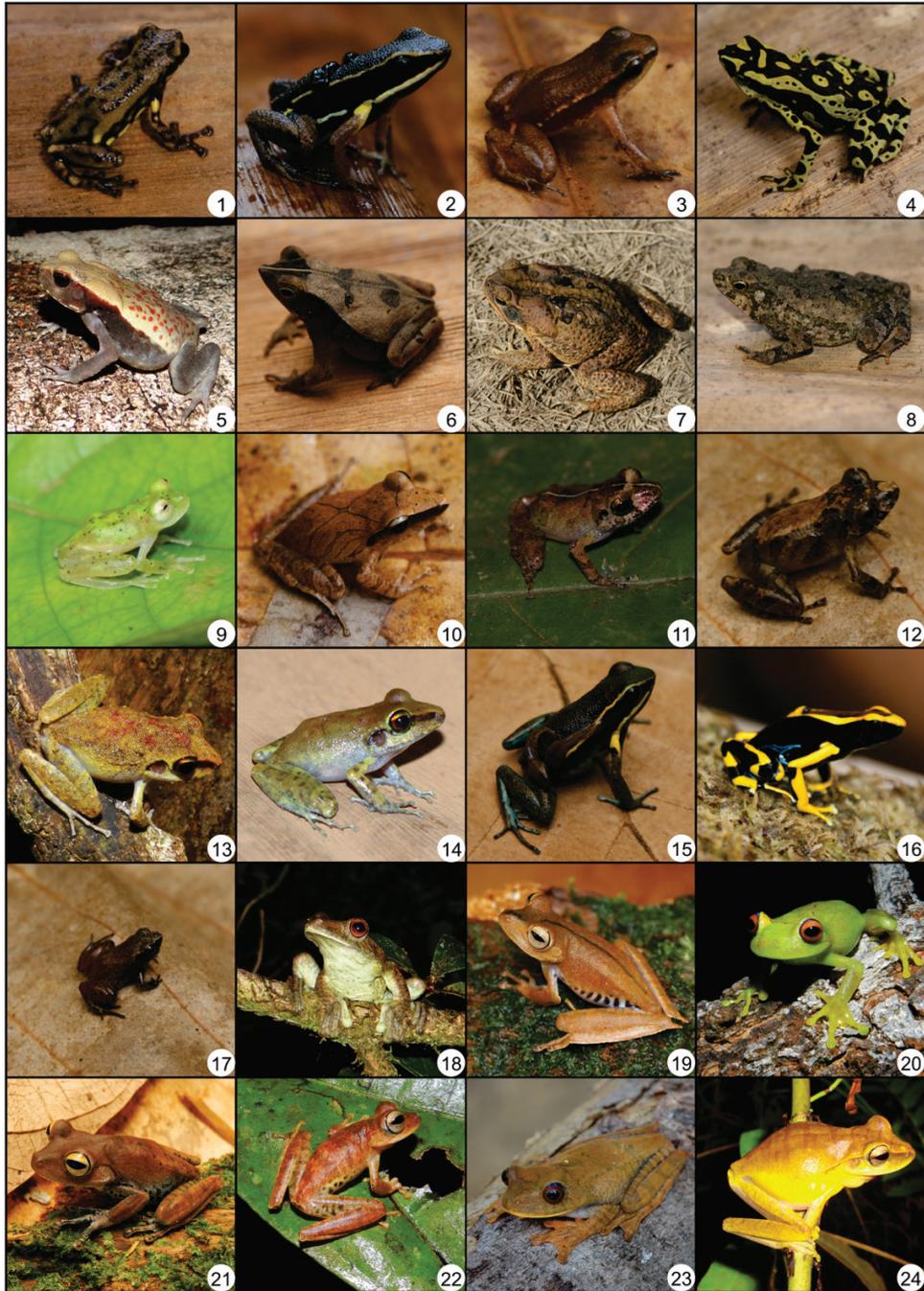


Figure 3. Species recorded in the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá state: **1** *Allophryne ruthveni* **2** *Allobates femoralis* **3** *Anomaloglossus baeobatrachus* **4** *Atelopus hoogmoedi* **5** *Rhaebo guttatus* **6** *Rhinella margaritifera* complex **7** *R. marina* **8** *R. martyi* **9** *Hyalinobatrachium iaspidiense* **10** *Pristimantis chiastonotus* **11** *P. marmoratus* **12** *P. cf. ockendeni* **13** *P. zeuctotylus* **14** *P. zimmermanae* **15** *Ameerega pulchripicta* **16** *Dendrobates tinctorius* **17** *Adelophryne gutturosa* **18** *Boana boans* **19** *B. calcarata* **20** *B. cinerascens* **21** *B. denti* **22** *B. fasciata* **23** *B. geographica* **24** *B. multifasciata*.



Figure 3. Continued. 25 *Dendropsophus counani* 26 *D. leucophyllatus* 27 *Dendropsophus* cf. *microcephalus* 28 *D. minutus* 29 *Osteocephalus oophagus* 30 *O. taurinus* 31 *Scinax boesemani* 32 *S. garbei* 33 *S. nebulosus* 34 *S. ruber* 35 *Adenomera andreae* 36 *A. hylaedactyla* 37 *Leptodactylus knudseni* 38 *L. lineatus* 39 *L. longirostris* 40 *L. mystaceus* 41 *L. pentadactylus* 42 *L. petersii* 43 *L. rhodomystax* 44 *L. stenodema* 45 *Phyllomedusa bicolor* 46 *P. vaillantii* 47 *Pithecopus hypochondrialis*, and 48 *Pipa pipa*.

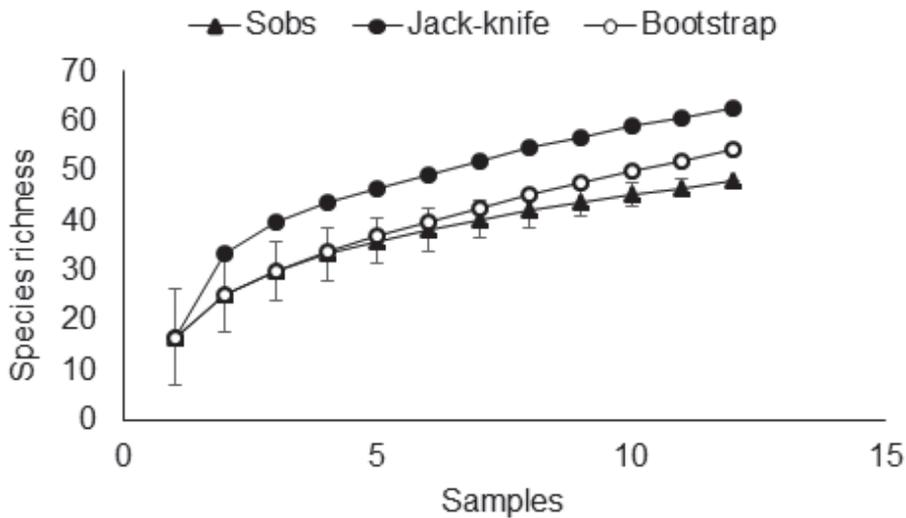


Figure 4. Rarefaction curve of anuran species based on the species records and sampling effort (sampling days) in the Cancão Municipal Natural Park, Amapá state, northern Brazil. Richness estimators used: Jack-knife 1 and Bootstrap. Sobs = total number of species observed in a samples.

Scinax garbei is known from Ecuador, adjacent Peru, Bolivia, Colombia, and Venezuela (Frost 2018). In Brazilian Amazonia, it has been recorded from Amazonas (França and Venâncio 2010) and Pará states (Ávila-Pires et al. 2010). In this study, we present the first record of the species in the state of Amapá, extending the species distribution in the Brazilian Amazonia by 525 km northward from the two localities in the state of Pará: Rio Xingu and Rio Curuá-Una (Silva e Silva and Costa-Campos 2014).

The frog species richness estimated for the area by Bootstrap and Jack-knife was 54 and 63 species, respectively, and the rarefaction curve cumulative species did not reach an asymptote. We believe that site has potential for species that have not yet been recorded (Figure 4). The Hylidae was the most species-rich family (17 species), followed by the Leptodactylidae (10) and Craugastoridae (4).

Spearman correlations obtained with the studied period were not significant for rainfall data ($R = 0.564$, $P = 0.056$), temperature ($R = 0.467$, $P = 0.167$) and relative humidity ($R = 0.267$, $P = 0.877$). According to multiple regressions, amphibian abundance does not seem to be related to any of the abiotic factors considered ($F_{3, 8} = 1.240$; $P = 0.179$; $r = 0.563$ for the entire analysis; $F_{3, 8} = 3.422$; $P = 0.091$ for rainfall; $F_{3, 8} = 1.097$; $P = 0.320$ for temperature; and $F_{3, 8} = 0.720$; $P = 0.579$ for relative humidity (Figure 5). With regard to total frog abundance, higher values were computed in rainy seasons compared to dry seasons.

Nine species (*Allophryne ruthveni*, *Rhinella martyi*, *Pristimantis zimmermanae*, *Boana calcarata*, *B. dentei*, *Osteocephalus oophagus*, *Scinax garbei*, *Leptodactylus lineatus* and *Pipa pipa*) were represented by only one individual and were considered rare in

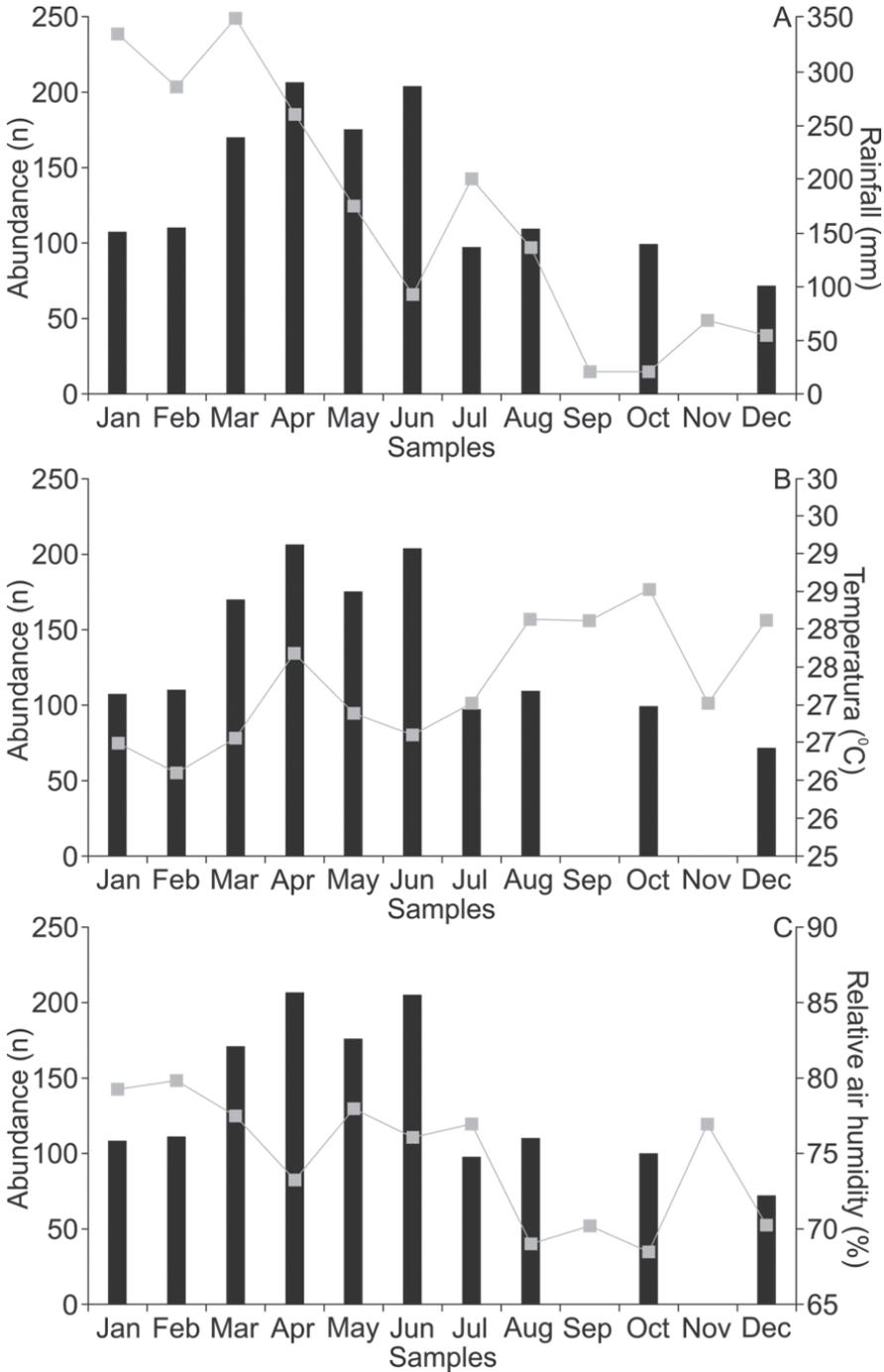


Figure 5. Correlation of recorded anuran abundance and abiotic factors in Cancão Municipal Natural Park, municipality of Serra do Navio Amapá state, from January through December 2013. **A** Anuran abundance (black bars) and rainfall (grey squares and line) **B** anuran abundance (black bars) and temperature (grey squares and line) **C** anuran abundance (black bars) and relative humidity (grey squares and line).

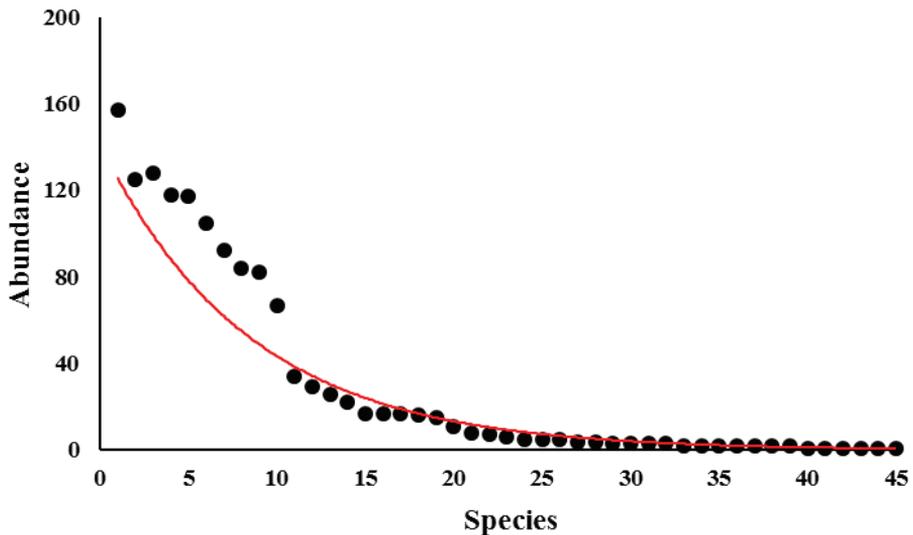


Figure 6. Whittaker diagram for the abundance distribution of amphibians recorded in the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá state.

the studied environments. Applying the number of singletons to the other end of the abundance distribution, eight species were defined as common, including *P. chiastonotus* (157 individuals), *B. multifasciata* (123 individuals), *R. margaritifera* complex (123 individuals), and *B. boans* (105 individuals). The 32 remaining species were classified as having intermediary abundance (Figure 6).

The dendrogram obtained from cluster analysis evidences three major groups: (A) sites located in the treefall gaps, (B) sites in the Terra firme forest, and (C) sites belonging to the Amapari River with temporary ponds. The group (A) is characterized by the higher *Boana multifasciata*, *B. cinerascens* and *Pithecopus hypochondrialis*, species occurring in open areas. For the group (B), the most frequent species were *Rhinella margaritifera* complex species and *Pristimantis chiastonotus*. The last group, (C), is characterized by the high frequency of occurrence of *Allobates femoralis* and *Adenomera andreae*. The coefficient of cophenetic correlation for the cluster analysis was 0.997 (Figure 7).

Discussion

Our data indicate that Cancão Municipal Natural Park contains a considerable portion of the anurans species richness of Amapá state. The anuran fauna corresponded to 65.7 % of the recorded species for the Tumucumaque Mountains National Park (Lima 2008), 86.9 % of the species found in the River Cajari Extractive Reserve (Queiroz et al. 2011), 60.9 % species recorded during the surveys conducted of the Rio Curiaú Environmental Protection Area (Lima et al. 2017), and 90.6 % species of anurans

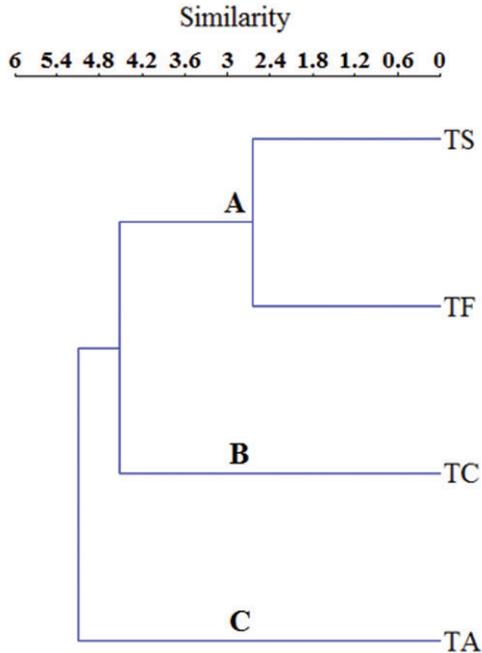


Figure 7. Dendrogram for cluster analysis (UPGMA) using the Jaccard's Similarity Index between the anurans of the Cancão Municipal Natural Park. Cophenetic correlation coefficient = 0.997. Sampled areas: Group **A** Treefall gap at stream Cancão (TS) and Treefall gap at Cancão forest (TF); Group **B** Terra firme trail at Cancão forest (TC); Group **C** Amapari trail (TA).

recorded in the Amapá National Forest (Bemício and Lima 2017). This high anurans richness for the Amazonian biome is highly underestimated considering taxonomic problems, recent descriptions of species and taxonomic revisions (Peloso et al. 2014; Vaz-Silva et al. 2015).

The results obtained from the rarefaction curve and the Jack-knife1 and Bootstrap estimators suggest that the species composition is still underestimated, and more long-term studies may reveal the presence of additional species in the area. Future studies should be complemented with combined and/or different approaches in fieldwork, such as the use of pitfall traps for leaf-litter species, increased visual search times (Freitas et al. 2017).

The finding of a large number of species of the families Hylidae and Leptodactylidae was similar to the results of other studies and follows the pattern found in neotropical environments (Segalla et al. 2016), including the Brazilian Amazon (Azevedo-Ramos and Galatti 2002, Neckel-Oliveira et al. 2013, Ramalho et al. 2016). In addition, three species of anurans (*Pristimantis* cf. *ockendeni*, *Hyalinobatrachium iaspidiense* and *Scinax garbei*) are new records in the Amapá state (Silva e Silva and Costa-Campos 2014, Silva e Silva et al. 2015, Silva e Silva and Costa-Campos 2016), evidence the incipience of knowledge in the regional context due to the lack of sampling.

The record of *Atelopus hoogmoedi* and *Ameerega pulchripecta* in the area studied is relatively important. *Atelopus hoogmoedi* is a terrestrial and diurnal species, and is most commonly found at small streams in primary forest (Ouboter and Jairam 2012). The species occurs in the Amazonian lowlands of Colombia, Ecuador, and eastern Peru, to Amazonas, Pará, Amapá (Brazil), and the Guianas (Frost 2018). *Ameerega pulchripecta* was hard to find, and it has been heard only during less than an hour around dawn and again around twilight (Costa-Campos et al. 2016). Additionally, its distribution appears restricted to Serra do Navio, in the state of Amapá, northeastern Brazilian Amazon. These species are classified as vulnerable and data deficient by the IUCN due to their areas of occurrence, status and little known ecological requirements (IUCN 2017).

The cluster analysis of the anuran assemblages generated three groups. The group A and B showed a more differentiated assemblage. Group C are located on the right bank of the Amapari River, and presented high values of abundance and richness. The results can be attributed to the similar characteristics between the sites. The main hypotheses proposed to explain barrier formation separating populations and causing the differentiation of species in Amazonia during the course of geological history are based on different factors (Haffer 2008). According the river hypothesis, rivers may play a major role in creating and maintaining high levels of spatial separation of populations (Vaz-Silva et al. 2015).

Conclusions

The results of the present study thus provide new data on geographic distribution of species showed three new records of the Brazilian Amazonian and important insights into the diversity of amphibians in the northern Brazil. The high amphibian richness recorded in this study for the eastern Amazon, combined with the presence of populations of Data Deficient or Vulnerable species, contributes to the knowledge on species, reinforcing the importance of the Cancão Municipal Natural Park for the conservation of anurans species.

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