# Two new species of the genus Milnesium Doyère, 1840 (Tardigrada, Apochela, Milnesiidae) from Madagascar 

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#### Abstract

The knowledge of the diversity and distribution of tardigrades on Madagascar is rather poor. To date, only 13 tardigrade taxa have been reported from this region (including one Milnesium species). We examined 46 specimens belonging to two new-to-science species of the genus Milnesium described herein using an integrative approach, including classical morphology and molecular marker (COI, ITS-2 and 28S rRNA) analysis. The species were found in two moss and lichen samples collected in the Ivohibory forest in Fianarantsoa Province. Milnesium matheusi sp. nov., with claw configuration [3-3]-[3-3] and rather wide buccal tube, morphologically is most similar to: Mil. beatae Roszkowska, Ostrowska \& Kaczmarek, 2015, Mil. bobleberi Bartels, Nelson, Kaczmarek \& Michalczyk, 2014, Mil. eurystomum Maucci, 1991, Mil. shilohae Meyer, 2015 and Mil. tumanovi Pilato, Sabella \& Lisi, 2016; however, it differs from these by morphometric characteristics. Milnesium wrightae sp. nov., by the presence of four points on secondary branches of claws IV, is most similar to Mil. quadrifidum Nederström, 1919. However, Mil. wrightae sp. nov. differs from Mil. quadrifidum by claw configuration ([4-4]-[4-4] in Mil. quadrifidum vs. [3-3]-[4-4] in Mil. wrightae sp. nov.), but also by the position of the fourth points on secondary branches of claws IV, which are located near the base of the claw in the new species and near the top of the claw in Mil. quadrifidum. Genotypic analysis showed that Mil. matheusi sp. nov. is most similar to Milnesium sp. (28S rRNA), Mil. variefidum (COI) and Mil. t. tardigradum (ITS-2) while Mil. wrightae sp. nov. is most similar to Milnesium sp. (28S rRNA), Mil. variefidum (COI) and Mil. matheusi (ITS-2). Five Milnesium taxa are recorded from the African region, including the two new species from Madagascar reported in this study.


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## Keywords

integrative taxonomy, Milnesiidae, Milnesium matheusi sp. nov., Milnesium wrightae sp. nov., tropical region

## Introduction

Madagascar stretches from $\sim 12^{\circ}$ to $-26^{\circ} \mathrm{S}$ latitude on the Indian Ocean, more than 400 km east of Africa. With an area of ca. $590,000 \mathrm{~km}^{2}$, Madagascar is the world's fourth largest island; however, it is sometimes considered a microcontinent due to its geological and biological history. First, it separated from Gondwana as part of East Gondwana, comprising the Antarctic, Madagascar, Indian, and Australian plates. After several subsequent breakups, it finally separated from the Seychelles and India ca. 66-90 My ago (de Witt 2003, Kusky et al. 2007). Madagascar is characterised by high biological endemism, estimated at $>90 \%$ for terrestrial vertebrates and $>80 \%$ for vascular plants (Goodman and Benstead 2003, Callmander et al. 2011). A great number of species occurring in Madagascar have restricted geographical ranges and are reported from only one or several localities (Wilmé et al. 2006). Several present taxa are assumed to be Gondwanan relicts. Most of the biota is believed to be derived from African and Asian colonizers (Yoder and Nowak 2006, Warren et al. 2010, Buerki et al. 2013; Hong-Wa and Besnard 2013). Madagascar has a tropical climate with two main climatic and biogeographic zones characterised by a substantially different vegetation cover, i.e., evergreen humid forests and deciduous forests divided by a mountain range that extends from north to south in eastern-central Madagascar (Du Puy and Moat 1996). Both zones are divided into several regions, each of which has distinctive climatic features and a set of unique habitats.

The area studied is located in south-central Madagascar (approximately 22.598830S, 46.720841E Ivohibe District, Fianarantsoa Province) on the eastern slopes of a hill located on the dry side of the main mountain range. The Ivohibory forest - which is a humid rainforest with some patches of grassy clearings - covers an area of approximately 1400 ha with an elevation gradient stretching from 900 to 1500 m asl, surrounded by human-created savannah, with a few lasting micro-patches of dry forest. It is situated on quartzite deposits, which is unique for this region (Wright and Houlihan 2017). This unusual geology strongly influences the species composition of the existing vegetation (Du Puy and Moat 1996).

The phylum Tardigrada currently consists of ca. 1200 species (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Degma et al. 2009-2018; Vicente and Bertolani 2013) that inhabit terrestrial and aquatic (freshwater and marine) environments throughout the world (Ramazzotti and Maucci 1983; Nelson et al. 2015). Our knowledge of the diversity and distribution of terrestrial tardigrades on Madagascar is very poor. To date, only 13 species (Bryodelphax parvulus Thulin, 1928, Calcarobiotus (Discrepunguis) polygonatus (Binda \& Guglielmino, 1991), Cornechiniscus madagascariensis Maucci, 1993, Doryphoribius flavus (Iharos, 1966), Echiniscus perarmatus Murray, 1907a, Ech. walteri Pilato \& Lisi, 2003, Macrobiotus hufelandi bufelandi C.A.S. Schultze, 1834, Mac. madegassus Maucci, 1993, Mesobiotus harmsworthi harmsworthi
(Murray, 1907b), Mil. tardigradum tardigradum Doyère, 1840, Minibiotus intermedius (Plate, 1888), Paramacrobiotus (Paramacrobiotus) richtersi (Murray, 1911) and Pseudechiniscus suillus (Ehrenberg, 1853)) have been reported from this region (Maucci 1993; Pilato and Lisi 2003).

Species of the genus Milnesium Doyère, 1840 are large and carnivorous, feeding mainly on rotifers, nematodes and other tardigrades, but single reports show that they can also feed on amoebas (Miller and Williams 2012; Roszkowska et al. 2015, 2016). Species in this genus reproduce parthogenetically and/or bisexually, and are characterised by sexual dimorphism (e.g., Suzuki 2003; Ciobanu et al. 2015). Thirty-eight species of the genus Milnesium have been reported mostly from mosses and lichens from many localities, ranging from the Antarctic through tropical and temperate to Arctic regions. Most have been described in recent years (Degma et al. 2009-2018; Kaczmarek et al. 2014, 2015, 2016; McInnes et al. 2017). According to its unique morphology (and based on molecular data) the genus Milnesium is classified in the class Apotardigrada (Schuster, Nelson, Grigarick \& Christenberry, 1980) (Guil et al. 2019). Until now, only three Milnesium species (Mil. dornensis Ciobanu, Roszkowska \& Kaczmarek, 2015, Mil. t. tardigradum and Mil. tetralamellatum Pilato \& Binda, 1991) have been reported in the so-called African region (McInnes et al. 2017), in which Madagascar is placed. This paper describes two new species from Madagascar using integrative taxonomy.

## Material and methods

## Sample processing

Two moss and lichen samples from tree and rocks were collected in the Ivohibory forest on June 4, 2017 (permits No 122/17/MEEF/SG/DGF/DSAP/SCB.Re and 150NEV06/MG17). The samples were packed in paper envelopes, dried at a temperature of ca. $30^{\circ} \mathrm{C}$ and delivered to the laboratory at the Faculty of Biology, Adam Mickiewicz University, Poznań, Poland. Tardigrades were extracted from the samples and studied following the protocol of Stec et al. (2015).

## Microscopy and imaging

Specimens for light microscopy were mounted on microscope slides in a small drop of Hoyer's medium, prepared according to Ramazzotti and Maucci (1983) as in the English translation by Beasley (1995), and secured with a cover slip. The slides were then placed in an incubator and dried for two days at ca. $60{ }^{\circ} \mathrm{C}$. Dried slides were sealed with a transparent nail polish and examined under an Olympus BX41 phase contrast light microscope (PCM) associated with an ARTCAM-300Mi digital camera (Olympus Corporation, Shinjuku-ku, Japan).

All figures were assembled in Corel Photo-Paint 2017. For deep structures that could not be fully focused in a single photograph, a series of $2-10$ images were taken every ca. $0.5 \mu \mathrm{~m}$ and then manually assembled into a single deep-focus image in Corel Photo-Paint 2017.

## Morphometrics and morphological nomenclature

All measurements are given in micrometres $[\mu \mathrm{m}]$. Structures were measured only if their orientation was suitable. Body length was measured from the anterior extremity to the end of the body, excluding the hind legs. All measurements (except buccal tube width) followed protocols in Tumanov (2006). Buccal tube width was measured at three points as suggested by Michalczyk et al. (2012). The $p t$ ratio is the ratio of the length of a given structure to the length of the buccal tube, expressed as a percentage (Pilato 1981). The pt values are always provided in [square brackets and in italics]. Configuration of the number of claw points on the secondary branches ("claw configuration") is given according to Michalczyk et al. (2012).

Morphometric data were handled using the "Apochela" ver. 1.1 template available from the Tardigrada Register (Michalczyk and Kaczmarek 2013). Tardigrade taxonomy follows Bertolani et al. (2014) and Guil et al. (2019). Genus abbreviations follow Perry et al. (2019).

## Comparative material

Species were identified using the key in Morek et al. (2016) and other original de-scriptions/re-descriptions (Nederström 1919; Maucci 1991; Bartels et al. 2014; Meyer 2015; Roszkowska et al. 2015; Pilato et al. 2016), or based on direct examination of fixed specimens (holotype and paratypes of Mil. bohleberi and specimens of Mil. eurystomum from Spitsbergen, Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland). Tardigrade taxonomy follows Marley et al. (2011).

## Genotyping

All specimens were preliminarily identified using light microscopy (LM) before DNA extraction. Later, each specimen was placed individually in a 1.5 ml Eppendorf microcentrifuge tube in $20 \mu$ l of sterile $\mathrm{MQ} \mathrm{H}_{2} \mathrm{O}$ and kept frozen at $-80^{\circ} \mathrm{C}$ until DNA isolation. DNA was extracted from individual animals following a modified Chelex100 resin (Bio-Rad) extraction method (Casquet et al. 2012), modified in order to obtain tardigrade exoskeletons, according to Zawierucha et al. (2016). DNA was extracted by incubating each specimen in $40 \mu \mathrm{l}$ of $10 \%$ Chelex 100 resin solution in sterile MQ $\mathrm{H}_{2} \mathrm{O}$ with the addition of 0.02 mg of Proteinase K (Genoplast) at $55^{\circ} \mathrm{C}$ for 5 h with shaking (500 RPM, Eppendorf Thermomixer 5436) and occasionally centrifuged. In

Table I. Primers used for amplification and sequencing of DNA fragments.

| DNA fragment | Direction | Code | Sequence (5'-3') | Reference |
| :--- | :---: | :---: | :---: | :---: |
| COI | Forvard | bcdF01 | CATTTTCHACTAAYCATAARGATATTGG | Dabert et al. 2010 |
|  | Reverse | bcdR04 | TATAAACYTCDGGATGNCCAAAAAA | Dabert et al. 2008 |
| ITS-2 | Forvard | ITS2_Eutar_Ff | CGTAACGTGAATTGCAGGAC | Stec et al. 2018 |
|  | Reverse | ITS2_Eutar_Rr | TGATATGCTTAAGTTCAGCGG |  |
| 28S rRNA | Forvard | 28SF0001 | ACCCVCYNAATTTAAGCATAT | Mironov et al. 2012 |
|  | Reverse | 28SR0990 | CCTTGGTCCGTGTTTCAAGAC |  |

Table 2. PCR cocktails used for the amplification of DNA fragments.

| Component | Concentration | Additional note |
| :--- | :---: | :---: |
| $\mathrm{H}_{2} \mathrm{O}$ | - | sterile MQ |
| buffer | $1 \times$ | 5X Phusion HF Buffer; Thermo Scientific |
| dNTPs | $200 \mu \mathrm{M}$ | dNTP Mix; Thermo Scientific |
| forward primer | $0.5 \mu \mathrm{M}$ | - |
| reverse primer | $0.5 \mu \mathrm{M}$ | - |
| polymerase | $0.02 \mathrm{U} / \mu \mathrm{l}$ | Phusion High-Fidelity DNA Polymerase; Thermo Scientific |
| DNA | - | - |

Table 3. PCR programmes used for the amplification of COI, ITS-2 and 28 S rRNA.

| Step | COI |  |  | ITS-2 and 28S rRNA |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Cycles | Time [min.:sec.] | Temp. $\left[{ }^{\circ} \mathbf{C}\right]$ | Cycles | Time [min:sec] | Temp. $\left[{ }^{\circ} \mathbf{C}\right]$ |
| initial denaturation | - | $05: 00$ | 98 | - | $05: 00$ | 98 |
| denaturation | 5 | $00: 30$ | 98 | - | - | - |
| annealing |  | $00: 30$ | 45 | - | - | - |
| extension | $01: 00$ | 72 | - | - | - |  |
| denaturation | 30 | $00: 30$ | 98 | 35 | $00: 30$ | 98 |
| annealing | $00: 30$ | $01: 00$ | 72 |  | $00: 30$ | 50 |
| extension |  | 72 | - | $01: 00$ | 72 |  |
| final extension | - |  |  | $-07: 00$ | 72 |  |

the next step, Proteinase K was inactivated by incubating at $70{ }^{\circ} \mathrm{C}$ for 15 min . Subsequently, $20 \mu \mathrm{l}$ of sterile $\mathrm{MQ} \mathrm{H}_{2} \mathrm{O}$ was added to the tube and centrifuged for 2 min at 8000 G . For further analysis, ca. $40 \mu \mathrm{l}$ of DNA extract (to the level of remaining Chelex beads at the bottom) was carefully transferred from each tube to a new 1.5 ml Eppendorf microcentrifuge tube. The tardigrade exoskeleton, present in a pellet after centrifugation, containing Chelex beads on the bottom of each tube, was extracted under stereomicroscope and then mounted in Hoyer's medium for further morphological analysis. Polymerase chain reaction (PCR) amplifications were carried out for three DNA fragments differing in mutation rates: mitochondrial cytochrome oxidase subunit I (COI), nuclear internal transcribed spacer 2 (ITS-2) and cytoplasmic ribosome large subunit component ( 28 S rRNA) in a total volume of $15-50 \mu \mathrm{l}$ (see Table 1 for primers, Table 2 for PCR cocktail recipes and Table 3 for PCR programmes). PCR products were verified by agarose gel electrophoresis (1-1.2\% agarose) with ethidium bromide. Prior to sequencing, PCR products were purified with thermosensitive Exonuclease I and FastAP Alkaline Phosphatase (Fermentas, Thermo Scientific) to improve their quality. Properly prepared PCR products were sequenced bidirectionally with BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems,

Foster City, CA, USA), according to the manufacturer's protocol. The sequences were edited and manually checked against non-conservative alignments using BioEdit, version 7.0.5 (Hall 1999), and submitted to GenBank (see Results section).

## Comparative molecular analysis

In the first step, the sequences of Mil. wrightae sp. nov. and Mil. matheusi sp. nov. were analysed by Standard Nucleotide BLAST to confirm their uniqueness. Then, a comparison was performed with COI, ITS-2 and 28 S rRNA sequences of the genus Milnesium, deposited in GenBank, using only the sequences of good quality and length. All sequences were aligned with the ClustalW Multiple Alignment tool (Thompson et al. 1994) implemented in BioEdit and trimmed to 510 (COI), 184 (ITS-2), 625 ( 28 S rRNA) bp, respectively. Based on the recommendation of Srivathsan and Meier (2012), pairwise distances were calculated using MEGA7 in preference to the genetic distances corrected by the Kimura 2 parametric model (K2P). All positions with less than $95 \%$ site coverage were eliminated.

## Results

## Taxonomic account

Phylum Tardigrada Doyère, 1840
Class Apotardigrada (Schuster, Nelson, Grigarick \& Christenberry, 1980)
Order Apochela Schuster, Nelson, Grigarick \& Christenberry, 1980
Family Milnesiidae Ramazzotti, 1962
Genus Milnesium Doyère, 1840

## Milnesium matheusi sp. nov.

http://zoobank.org/3EB072A7-1C84-4EF6-B6D2-D9486BBF6C4F
Figures 1-5, Tables 4, 5

Material examined. Holotype and 18 paratypes, all from sample No 139: Ivohibory forest, Madagascar, lichen sample from quartz rocks, coll. Marta Kepel and Andrzej Kepel.

Description. Adult females (Fig. 1, Table 4) with no modified claws I. Body light yellow before fixation and transparent afterwards, eyes present (in $89 \%$ of measured specimens). Dorsal cuticle sculptured with pseudopores, not arranged in bands, sparsely distributed and not forming a reticular design (Fig. 2). Six peribuccal papillae and six peribuccal lamellae present around the mouth opening. Two cephalic papillae positioned laterally. Peribuccal papillae slightly longer than lateral papillae.

The buccal apparatus of the Milnesium type (Figs 1, 3). The buccal tube wide and short (standard width, on average $46 \%$ of its length), and slightly funnel-shaped, wider


Figures I-3. Milnesium matheusi sp. nov. I Habitus (ventral view) (holotype) $\mathbf{2}$ dorsal cuticle with pseudopores (holotype) $\mathbf{3}$ buccal tube (holotype). All in PCM.


Figures 4, 5. Milnesium matheusi sp. nov. 4 Claws II (paratype), arrow indicates bar under claw $\mathbf{5}$ claws IV (holotype), arrowhead indicates small accessory point. All in PCM.
anteriorly (posterior diameter on average $89 \%$ of the anterior diameter) (Table 4). The pharyngeal bulb elongated, pear-shaped and without placoids or septulum.

Claws of the Milnesium type, slender (Figs 4, 5). Primary branches on all legs with small, but distinct accessory points detaching from the branch at its greatest curvature (Fig. 5, arrowhead). Secondary branches with rounded basal thickenings (Figs 4, 5).

Table 4. Measurements and $p t$ values of selected morphological structures of adult females of Milnesium matheusi sp. nov. mounted in Hoyer's medium ( N - number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD - standard deviation, $p t$ - ratio of the length of a given structure to the length of the buccal tube expressed as a percentage).

| Character | N | Range |  |  |  |  |  | Mean |  | SD |  | Holotype |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | $\mu \mathrm{m}$ |  |  | $p t$ |  |  | $\mu \mathrm{m}$ | $p t$ | $\mu \mathrm{m}$ | pt | $\mu \mathrm{m}$ | $p t$ |
| Body length | 6 | 630 | - | 766 | - | - | - | 691 | - | 45 | - | 766 | - |
| Peribuccal papillae length | 5 | 10.0 | - | 12.0 | 18.6 | - | 22.1 | 11.0 | 19.9 | 0.8 | 1.5 | 11.8 | 18.9 |
| Lateral papillae length | 7 | 9.4 | - | 10.7 | 16.5 | - | 19.7 | 10.0 | 18.1 | 0.4 | 1.2 | 10.3 | 16.5 |
| Buccal tube |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Length | 9 | 51.3 | - | 62.5 | - | - | - | 56.6 | - | 3.8 | - | 62.5 | - |
| Stylet support insertion point | 9 | 34.5 | - | 42.3 | 66.1 | - | 69.4 | 38.4 | 67.8 | 2.4 | 1.3 | 41.5 | 66.4 |
| Anterior width | 9 | 25.2 | - | 35.9 | 47.6 | - | 57.9 | 28.9 | 51.0 | 3.2 | 3.1 | 31.4 | 50.2 |
| Standard width | 9 | 23.1 | - | 31.1 | 42.4 | - | 50.8 | 26.3 | 46.5 | 2.7 | 3.0 | 29.4 | 47.0 |
| Posterior width | 9 | 23.0 | - | 30.2 | 41.1 | - | 50.3 | 25.7 | 45.3 | 2.6 | 3.1 | 28.9 | 46.2 |
| Standard width/length ratio | 9 | 42\% | - | 51\% | - | - | - | 46\% | - | 3\% | - | 47\% | - |
| Posterior/anterior width ratio | 9 | 84\% | - | 94\% | - | - | - | 89\% | - | 4\% | - | 92\% | - |
| Claw 1 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 9 | 17.2 | - | 21.8 | 30.2 | - | 35.2 | 18.9 | 33.3 | 1.5 | 1.6 | 21.8 | 34.9 |
| External base + secondary branch | 9 | 13.3 | - | 16.7 | 23.5 | - | 27.9 | 15.0 | 26.5 | 1.2 | 1.5 | 16.6 | 26.6 |
| External spur | 7 | 3.5 | - | 5.3 | 6.4 | - | 9.6 | 4.4 | 7.8 | 0.7 | 1.3 | ? | ? |
| External branches length ratio | 9 | 76\% | - | 82\% | - | - | - | 80\% | - | 2\% | - | 76\% | - |
| Internal primary branch | 9 | 16.0 | - | 21.1 | 30.2 | - | 34.5 | 18.3 | 32.3 | 1.6 | 1.6 | 21.1 | 33.8 |
| Internal base + secondary branch | 9 | 13.3 | - | 16.6 | 24.5 | - | 27.3 | 14.8 | 26.2 | 1.1 | 1.0 | 16.3 | 26.1 |
| Internal spur | 9 | 3.3 | - | 5.5 | 6.1 | - | 10.5 | 4.4 | 7.7 | 0.8 | 1.4 | 5.5 | 8.8 |
| Internal branches length ratio | 9 | 77\% | - | 88\% | - | - | - | 81\% | - | 4\% | - | 77\% | - |
| Claw 2 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 8 | 17.4 | - | 21.2 | 32.9 | - | 36.5 | 19.5 | 34.9 | 1.4 | 1.4 | 21.2 | 33.9 |
| External base + secondary branch | 7 | 13.7 | - | 17.0 | 24.5 | - | 27.5 | 15.0 | 26.7 | 1.1 | 1.2 | 17.0 | 27.2 |
| External spur | 3 | 3.9 | - | 4.9 | 7.2 | - | 7.8 | 4.4 | 7.6 | 0.5 | 0.4 | 4.9 | 7.8 |
| External branches length ratio | 7 | 72\% | - | 81\% | - | - | - | 77\% | - | 3\% | - | 80\% | - |
| Internal primary branch | 8 | 16.8 | - | 20.5 | 31.1 | - | 35.7 | 18.7 | 33.3 | 1.3 | 1.5 | 20.2 | 32.3 |
| Internal base + secondary branch | 9 | 13.0 | - | 16.3 | 25.0 | - | 27.9 | 14.7 | 26.0 | 1.1 | 0.9 | 16.3 | 26.1 |
| Internal spur | 9 | 3.4 | - | 5.8 | 6.1 | - | 10.3 | 4.4 | 7.8 | 0.8 | 1.5 | 4.7 | 7.5 |
| Internal branches length ratio | 8 | 74\% | - | 81\% | - | - | - | 78\% | - | 3\% | - | 81\% | - |
| Claw 3 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 5 | 19.7 | - | 21.0 | 32.3 | - | 38.3 | 20.5 | 35.7 | 0.6 | 2.5 | ? | ? |
| External base + secondary branch | 6 | 14.2 | - | 16.3 | 24.5 | - | 28.4 | 15.4 | 27.1 | 0.7 | 1.5 | ? | ? |
| External spur | 5 | 3.5 | - | 5.2 | 6.4 | - | 9.3 | 4.4 | 7.7 | 0.7 | 1.1 | ? | ? |
| External branches length ratio | 5 | 72\% | - | 82\% | - | - | - | 75\% | - | 4\% | - | ? | - |
| Internal primary branch | 5 | 18.9 | - | 20.4 | 31.3 | - | 36.5 | 19.7 | 34.3 | 0.6 | 2.0 | ? | ? |
| Internal base + secondary branch | 6 | 13.7 | - | 16.0 | 23.7 | - | 28.2 | 14.9 | 26.2 | 0.8 | 1.9 | ? | ? |
| Internal spur | 5 | 3.8 | - | 5.6 | 7.0 | - | 9.7 | 4.8 | 8.3 | 0.7 | 1.1 | ? | ? |
| Internal branches length ratio | 5 | 70\% | - | 79\% | - | - | - | 75\% | - | 4\% | - | ? | - |
| Claw 4 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Anterior primary branch | 7 | 19.6 | - | 23.0 | 35.1 | - | 39.8 | 20.9 | 37.2 | 1.3 | 1.5 | 23.0 | 36.8 |
| Anterior base + secondary branch | 7 | 14.6 | - | 17.2 | 26.3 | - | 29.4 | 15.8 | 28.2 | 0.9 | 1.1 | 17.2 | 27.5 |
| Anterior spur | 6 | 4.1 | - | 6.3 | 7.5 | - | 11.5 | 5.4 | 9.7 | 0.9 | 1.6 | 6.0 | 9.6 |
| Anterior branches length ratio | 7 | 71\% | - | 80\% | - | - | - | 76\% | - | 4\% | - | 75\% | - |
| Posterior primary branch | 7 | 20.5 | - | 24.0 | 38.1 | - | 41.3 | 21.8 | 38.9 | 1.1 | 1.1 | 24.0 | 38.4 |
| Posterior base + secondary branch | 7 | 15.2 | - | 17.7 | 26.9 | - | 29.6 | 16.1 | 28.6 | 0.8 | 1.0 | 17.7 | 28.3 |
| Posterior spur | 7 | 4.4 | - | 5.8 | 7.6 | - | 10.3 | 5.2 | 9.3 | 0.6 | 1.1 | 5.5 | 8.8 |
| Posterior branches length ratio | 7 | 70\% | - | 76\% | - | - | - | 74\% | - | 2\% | - | 74\% | - |

Table 5. Measurements and $p t$ values of selected morphological structures of adult males (with modified claws I) of Milnesium matheusi sp. nov. mounted in Hoyer's medium ( N - number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD standard deviation, $p t$ - ratio of the length of a given structure to the length of the buccal tube expressed as a percentage).

| Character | N | Range |  |  |  |  |  | Mean |  | SD |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | $\mu \mathrm{m}$ |  |  | $p t$ |  |  | $\mu \mathrm{m}$ | $p t$ | $\mu \mathrm{m}$ | $p t$ |
| Body length | 2 | 409 | - | 428 | - | - | - | 419 | - | 13 | - |
| Peribuccal papillae length | 3 | 3.0 | - | 3.9 | 8.9 | - | 11.3 | 3.5 | 10.2 | 0.5 | 1.2 |
| Lateral papillae length | 3 | 5.6 | - | 6.0 | 16.2 | - | 17.8 | 5.9 | 17.1 | 0.2 | 0.8 |
| Buccal tube |  |  |  |  |  |  |  |  |  |  |  |
| Length | 3 | 33.8 | - | 34.5 | - | - | - | 34.2 | - | 0.4 | - |
| Stylet support insertion point | 2 | 21.2 | - | 22.3 | 62.7 | - | 64.6 | 21.8 | 63.7 | 0.8 | 1.4 |
| Anterior width | 3 | 9.4 | - | 11.2 | 27.8 | - | 32.6 | 10.5 | 30.8 | 1.0 | 2.6 |
| Standard width | 3 | 9.1 | - | 9.8 | 26.9 | - | 28.5 | 9.5 | 27.8 | 0.4 | 0.8 |
| Posterior width | 3 | 9.4 | - | 10.2 | 27.8 | - | 29.6 | 9.8 | 28.7 | 0.4 | 0.9 |
| Standard width/length ratio | 3 | 27\% | - | 28\% | - | - | - | 28\% | - | 1\% | - |
| Posterior/anterior width ratio | 3 | 88\% | - | 100\% | - | - | - | 94\% | - | 6\% | - |
| Claw 1 lengths |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 2 | 15.8 | - | 16.3 | 45.9 | - | 48.2 | 16.1 | 47.1 | 0.4 | 1.6 |
| External base + secondary branch | 3 | 14.1 | - | 15.0 | 41.7 | - | 43.5 | 14.6 | 42.7 | 0.5 | 0.9 |
| External spur | 2 | 3.2 | - | 3.4 | 9.3 | - | 9.9 | 3.3 | 9.6 | 0.1 | 0.4 |
| External branches length ratio | 2 | 87\% | - | 94\% | - | - | - | 90\% | - | 5\% | - |
| Internal primary branch | 3 | 14.9 | - | 15.7 | 43.2 | - | 46.4 | 15.4 | 45.1 | 0.5 | 1.7 |
| Internal base + secondary branch | 3 | 14.0 | - | 14.5 | 40.6 | - | 42.9 | 14.2 | 41.4 | 0.3 | 1.3 |
| Internal spur | 3 | 3.0 | - | 3.7 | 8.9 | - | 10.7 | 3.4 | 9.9 | 0.4 | 0.9 |
| Internal branches length ratio | 3 | 89\% | - | 94\% | - | - | - | 92\% | - | 2\% | - |
| Claw 2 lengths |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 2 | 16.9 | - | 17.9 | 49.0 | - | 53.0 | 17.4 | 51.0 | 0.7 | 2.8 |
| External base + secondary branch | 1 | 13.2 | - | 13.2 | 39.1 | - | 39.1 | 13.2 | 39.1 | ? | ? |
| External spur | 1 | 3.5 | - | 3.5 | 10.4 | - | 10.4 | 3.5 | 10.4 | ? | ? |
| External branches length ratio | 1 | 74\% | - | 74\% | - | - | - | 74\% | - | ? | - |
| Internal primary branch | 3 | 16.4 | - | 16.9 | 47.5 | - | 50.0 | 16.7 | 48.8 | 0.3 | 1.2 |
| Internal base + secondary branch | 2 | 12.7 | - | 12.8 | 37.2 | - | 37.6 | 12.8 | 37.4 | 0.1 | 0.3 |
| Internal spur | 2 | 3.5 | - | 5.0 | 10.4 | - | 14.5 | 4.3 | 12.4 | 1.1 | 3.0 |
| Internal branches length ratio | 2 | 75\% | - | 76\% | - | - | - | 76\% | - | 1\% | - |
| Claw 3 lengths |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 3 | 16.2 | - | 17.4 | 47.1 | - | 51.5 | 16.8 | 49.0 | 0.6 | 2.3 |
| External base + secondary branch | 2 | 12.1 | - | 12.8 | 35.2 | - | 37.9 | 12.5 | 36.5 | 0.5 | 1.9 |
| External spur | 1 | 3.9 | - | 3.9 | 11.3 | - | 11.3 | 3.9 | 11.3 | ? | ? |
| External branches length ratio | 2 | 74\% | - | 75\% | - | - | - | 74\% | - | 1\% | - |
| Internal primary branch | 3 | 14.8 | - | 17.0 | 43.0 | - | 50.3 | 16.0 | 46.7 | 1.1 | 3.6 |
| Internal base + secondary branch | 2 | 12.7 | - | 13.0 | 37.6 | - | 37.8 | 12.9 | 37.7 | 0.2 | 0.2 |
| Internal spur | 2 | 2.9 | - | 4.0 | 8.4 | - | 11.8 | 3.5 | 10.1 | 0.8 | 2.4 |
| Internal branches length ratio | 2 | 75\% | - | 88\% | - | - | - | 81\% | - | 9\% | - |
| Claw 4 lengths |  |  |  |  |  |  |  |  |  |  |  |
| Anterior primary branch | 3 | 16.3 | - | 17.0 | 47.4 | - | 49.3 | 16.6 | 48.5 | 0.4 | 1.0 |
| Anterior base + secondary branch | 2 | 12.4 | - | 12.9 | 36.7 | - | 37.5 | 12.7 | 37.1 | 0.4 | 0.6 |
| Anterior spur | 1 | 3.8 | - | 3.8 | 11.0 | - | 11.0 | 3.8 | 11.0 | ? | ? |
| Anterior branches length ratio | 2 | 75\% | - | 79\% | - | - | - | 77\% | - | 3\% | - |
| Posterior primary branch | 3 | 17.7 | - | 18.8 | 51.3 | - | 54.7 | 18.3 | 53.4 | 0.6 | 1.8 |
| Posterior base + secondary branch | 3 | 12.7 | - | 13.7 | 37.1 | - | 39.8 | 13.1 | 38.2 | 0.6 | 1.5 |
| Posterior spur | 2 | 3.0 | - | 4.1 | 8.7 | - | 11.9 | 3.6 | 10.3 | 0.8 | 2.3 |
| Posterior branches length ratio | 3 | 69\% | - | 73\% | - | - | - | 72\% | - | 2\% | - |

All secondary branches on all legs with three points (claw configuration: [3-3]-[3-3]). Single, long transverse, cuticular bars present under claws I-III (Fig. 4, arrow).

Adult males (Table 5) with modified claws I. Similar to females but clearly smaller, with secondary branches of claws I modified into strong hooks and with a different proportion of peribuccal and lateral papillae length (peribuccal papillae clearly shorter than lateral), eyes present only in $33 \%$ of measured specimens.

Eggs oval, smooth and deposited in the exuvium as in all other known Milnesium species.

DNA sequences. We obtained good quality sequences for the applied molecular markers: 28 S rRNA sequence (GenBank: MN191503), 756 bp long; COI sequence (GenBank: MN187056), 628 bp long; ITS-2 sequence (GenBank: MN239906), 218 bp long.

Type locality. Madagascar, $22^{\circ} 37^{\prime} 07.7^{\prime \prime} \mathrm{S}, 46^{\circ} 43^{\prime} 14.5^{\prime \prime} \mathrm{E}, \mathrm{ca} .1187 \mathrm{~m}$ asl, Fianarantsoa Province, Ivohibory forest.

Etymology. The second author with great pleasure dedicates this species to her fiance - Mateusz Wojciechowski.

Type depositories. The holotype and 13 paratypes (slides: MAD139/14, MAD139/16, MAD139/18, MAD139/19, MAD139/34, MAD139/35, MAD139/42, MAD139/56, MAD139/72) are deposited at the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University in Poznań, Uniwersytetu Poznańskiego 6, Poznań, Poland; five paratypes (slides: MAD139/12, MAD139/13, MAD139/15) are deposited at the Natural History Museum, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark.

Morphological differential diagnosis. The new species with three points on the secondary branches of all claws (claw configuration [3-3]-[3-3]) and a rather wide buccal tube, in relation to its length, is most similar to: Mil. beatae Roszkowska, Ostrowska \& Kaczmarek, 2015, Mil. bohleberi Bartels, Nelson, Kaczmarek \& Michalczyk, 2014, Mil. eurystomum Maucci, 1991, Mil. shilohae Meyer, 2015 and Mil. tumanovi Pilato, Sabella \& Lisi, 2016, but it differs from:

1. Milnesium beatae, only reported from Argentina and USA (Roszkowska et al. 2015; Tibbs et al. 2016) by: narrower buccal tube (25.2-35.9 [47.6-57.9] and 23.1-31.1 [42.4-50.8] anterior and standard width, respectively, in the new species vs. 37.0-53.5 [70.3-78.9] and 32.0-42.5 [58.1-65.0] anterior and standard width respectively in Mil. beatae), smaller standard width/length ratio of the buccal tube ( $42 \%-51 \%$ in new species vs. $58 \%-66 \%$ in Mil. beatae) and larger posterior/anterior width ratio ( $84 \%-94 \%$ in new species vs. $69 \%-76 \%$ in Mil. beatae).
2. Milnesium bohleberi, only recorded from North Carolina and Tennessee (USA) (Bartels et al. 2014) by: presence of pseudopores on dorsal cuticle, shorter peribuccal papillae (10.0-12.0 [18.6-22.1] in new species vs. $15.5-20.3$ [27.2-32.3] in Mil. bohleberi), smaller $p t$ values of anterior, standard and posterior widths of the buccal tube (47.6-57.9, 42.4-50.8, 41.1-50.3, respectively, in new species vs. 63.4-74.7, 54.5-64.0, 52.4-62.0, respectively, in Mil. bohleberi), smaller standard
width/length ratio of the buccal tube ( $42 \%-51 \%$ in new species vs. $54 \%-64 \%$ in Mil. bohleberi) and slightly shorter claws (see Table 4 below and Bartels et al. (2014: Table 1) for the exact differences in claw dimensions).
3. Milnesium eurystomum reported from a few localities in Argentina, Chile, Greenland, Mongolia and USA (see review by Kaczmarek et al. 2016) by: shorter buccal tube (51.3-62.5 in new species vs. 70.8-77.5 in Mil. eurystomum), stylet supports inserted in a more posterior position ( $p t=66.1-69.4$ in new species vs. ca. $p t=$ 60.0-60.3 in Mil. eurystomum), narrower buccal tube (25.2-35.9 [47.6-57.9], 23.1-31.1 [42.4-50.8] and 23.0-30.2 [41.1-50.3] anterior, standard and posterior width, respectively, in new species vs. 53.7-55.9 [72.1-75.8], 45.9-47.9 [61.864.8] and 33.9-41.0 [43.7-57.9] anterior, standard and posterior width, respectively, in Mil. eurystomum), smaller standard width/length ratio of the buccal tube ( $42 \%-51 \%$ in new species vs. $62 \%-65 \%$ in Mil. eurystomum) and larger posterior/ anterior width ratio ( $84 \%-94 \%$ in new species vs. $61 \%-76 \%$ in Mil. eurystomum).
4. Milnesium shilohae, only reported from the type locality in Hawaii (USA) (Meyer 2015) by: presence of pseudopores on dorsal cuticle, presence of similar in length spurs on internal and external claws (internal and posterior spurs larger than external and anterior spurs in Mil. shilohae), slightly longer lateral papillae (9.4-10.7 in new species vs. 5.0-9.0 in Mil. shilohae), slightly longer buccal tube (51.3-62.5 in new species vs. 38.4-50.3 in Mil. shilohae), stylet supports inserted in a more anterior position ( $p t=66.1-69.4$ in new species vs. $p t=75.5-77.5$ in Mil. shilohae) and larger spurs on some external and anterior claws (see Table 4 below and Table 3 in Meyer (2015) for the exact differences in claw dimensions).
5. Milnesium tumanovi, only recorded from the type locality in the Crimea (Ukraine) (Pilato et al. 2016) by: presence of pseudopores on dorsal cuticle, funnel-shaped buccal tube (cylindrical in Mil. tumanovi) and stylet supports inserted in a more posterior position ( $p t=66.1-69.4$ in new species vs. ca. $p t=52-54$ in Mil. tumanovi).

Genotypic differential diagnosis. The ranges of uncorrected genetic p-distances between Mil. matheusi sp. nov. and species of the genus Milnesium, for which molecular marker sequences are available from GenBank (see Table 6 for details), are as follows:

1. 28 S rRNA: $4.5-6.7 \%$ ( $5.4 \%$ on average), with the most similar being Milnesium sp. from North America (JX888585.1, JX888586.1, JX888587.1) (unpublished) and the least similar being Mil. wrightae sp. nov. (MN191504.1) (present studies);
2. COI: $20.1-38.8 \%$ ( $23.3 \%$ on average), with the most similar being Mil. variefidum Morek, Gąsiorek, Stec, Blagden \& Michalczyk, 2016 from UK (KT951663.1) (Morek et al. 2016) and the least similar being Mil. t. tardigradum from Spain (FJ435810.1) (Guil and Giribet 2012);
3. ITS-2: 17.8-31.1\% ( $23.7 \%$ on average), with the most similar being Mil. t. tardigradum from Germany (JF951049.1) (Michalczyk et al. 2012) and the least similar being Mil. cf. granulatum from USA (MK681879.1) (Jackson and Meyer 2019).

Table 6. Sequences of 28 S rRNA, COI and ITS-2 of Milnesium taxa available in GenBank and used in differential diagnosis.

| DNA marker | Taxon | Accession number | Source |
| :---: | :---: | :---: | :---: |
| 28S rRNA | Milnesium sp. | JX888585.1 | Adams et. al. unpublished |
|  |  | JX888586.1 | Adams et. al. unpublished |
|  |  | JX888587.1 | Adams et. al. unpublished |
|  | Milnesium tardigradum | JX888541.1 | Adams et. al. unpublished |
|  |  | JX888540.1 | Adams et. al. unpublished |
|  |  | KC138808.1 | Zawierucha unpublished |
|  |  | KC138809.1 | Zawierucha unpublished |
|  | Milnesium sp. | AY210826.1 | Mallatt et. al. 2004 |
|  | Milnesium tardigradum | FJ435780.1 | Guil and Giribet 2012 |
|  |  | FJ435779.1 | Guil and Giribet 2012 |
|  | Milnesium berladnicorum | KT951661.1 | Morek et. al. 2016 |
|  | Milnesium variefidum | KT951665.1 | Morek et. al. 2016 |
| COI | Milnesium sp. | KX306950.1 | Fox et al. unpublished |
|  | Milnesium tardigradum | EU244603.1 | Schill unpublished |
|  |  | EU244604 | Schill unpublished |
|  |  | FJ435810.1 | Guil and Giribet 2012 |
|  | Milnesium t. tardigradum | JN664950.1 | Michalczyk et al. 2012 |
|  | Milnesium cf. tardigradum | JX683824.1 | Vicente et al. 2013 |
|  |  | JX683823.1 | Vicente et al. 2013 |
|  |  | JX683822.1 | Vicente et al. 2013 |
|  | Milnesium sp. | KJ857002.1 | Velasco-Castrillón et al. 2015 |
|  |  | KJ857001.1 | Velasco-Castrillón et al. 2015 |
|  | Milnesium cf. alpigenum | KU513422.1 | Kosztyła et al. 2016 |
|  | Milnesium variefidum | KT951663.1 | Morek et al. 2016 |
|  | Milnesium berladnicorum | KT951659.1 | Morek et al. 2016 |
|  | Milnesium sp. | EF632553.1 | Sands et. al unpublished |
|  | Milnesium cf. granulatum | MH751517.1 | Jackson and Meyer 2019 |
|  | Milnesium lagniappe | MH751518.1 | Jackson and Meyer 2019 |
|  | Milnesium tardigradum | MG923558.1 | Morek et al. 2019 |
|  |  | MG923559.1 | Morek et al. 2019 |
|  |  | MG923560.1 | Morek et al. 2019 |
|  |  | MG923561.1 | Morek et al. 2019 |
|  |  | MG923562.1 | Morek et al. 2019 |
|  |  | MG923563.1 | Morek et al. 2019 |
|  |  | MG923564.1 | Morek et al. 2019 |
|  |  | MG923565.1 | Morek et al. 2019 |
|  | Milnesium dornensis | MG923566.1 | Morek et al. 2019 |
| ITS-2 | Milnesium alpigenum | MH000382.1 | Morek et al. unpublished |
|  | Milnesium sp. | MH000386.1 | Morek et al. unpublished |
|  |  | MH000387.1 | Morek et al. unpublished |
|  | Milnesium tardigradum | HM150648.2 | Wetnicz et. al. 2010 |
|  |  | GQ403682.1 | Schill et al. 2010 |
|  |  | GQ403681.1 | Schill et al. 2010 |
|  | Milnesium t. tardigradum | JF951049 | Michalczyk et al. 2012 |
|  | Milnesium variefidum | KT951667.1 | Morek et. al. 2016 |
|  |  | KT951666.1 | Morek et. al. 2016 |
|  | Milnesium berladnicorum | KT951662.1 | Morek et. al. 2016 |
|  | Milnesium cf. granulatum | MK681875.1 | Jackson and Meyer 2019 |
|  |  | MK681876.1 | Jackson and Meyer 2019 |
|  |  | MK681877.1 | Jackson and Meyer 2019 |
|  |  | MK681878.1 | Jackson and Meyer 2019 |
|  |  | MK681879.1 | Jackson and Meyer 2019 |
|  |  | MK681880.1 | Jackson and Meyer 2019 |
|  |  | MK681881.1 | Jackson and Meyer 2019 |
|  |  | MK681882.1 | Jackson and Meyer 2019 |
|  |  | MK681883.1 | Jackson and Meyer 2019 |
|  |  | MK681884.1 | Jackson and Meyer 2019 |
|  |  | MK681885.1 | Jackson and Meyer 2019 |
|  |  | MK681886.1 | Jackson and Meyer 2019 |

## Milnesium wrightae sp. nov.

http://zoobank.org/A62CF1FF-8BDA-42D1-A090-6AE72608E529
Figures 6-11, Table 7

Material examined. Holotype and 28 paratypes, all from sample No 109: Ivohibory forest, Madagascar, moss sample from tree, coll. Marta Kepel and Andrzej Kepel.

Description. Adult females (Fig. 6, Table 7) with no modified claws I. Body light yellow before fixation and transparent afterwards, eyes present only in $39 \%$ of measured specimens. Dorsal cuticle sculptured with pseudopores not arranged in bands, sparsely distributed and not forming reticular design (Fig. 7). Six peribuccal papillae and six peribuccal lamellae present around the mouth opening. Two cephalic papillae positioned laterally. Peribuccal papillae slightly longer than lateral papillae.

The buccal apparatus of the Milnesium type (Figs 6, 8). The buccal tube with standard width on average $62 \%$ of its length, and funnel-shaped, wider anteriorly (posterior diameter on average $91 \%$ of the anterior diameter) (Table 7). The pharyngeal bulb elongated, pear-shaped and without placoids or septulum.

Claws of the Milnesium type, stout (Figs 8-11). Primary branches on all legs with small, but distinct accessory points detaching from the branch at its greatest curvature (Fig. 10, empty arrowhead). Secondary branches of claws similar in length to primary branches and sometimes even longer. Secondary branches with rounded basal thickenings (Figs 9-11). Secondary branches on legs I-III with three points, secondary claws of anterior and posterior claws IV with four points (claw configuration: [3-3]-[4-4]). The fourth point on secondary branches is always very small and located near the base of the claw (Figs 10-11, arrowheads). Single, long transverse, cuticular bars present under claws I-III (Fig. 9, arrow).

Males unknown.
Eggs oval, smooth and deposited in the exuvium as in all other known Milnesium species.

DNA sequences. We obtained good quality sequences for the applied molecular markers: 28 S rRNA sequence (GenBank: MN191504), 638 bp long; COI sequence (GenBank: MN187057), 638 bp long; ITS-2 sequence (GenBank: MN239907), 392 bp long.

Remarks. The fourth points on secondary branches of posterior claws can be barely visible or not visible at all in some positions of the specimens.

Type locality. Madagascar, $22^{\circ} 37^{\prime} 04.5^{\prime \prime} \mathrm{S}, 46^{\circ} 43^{\prime} 14.1^{\prime \prime} \mathrm{E}$, ca. 1198 m asl, Fianarantsoa Province, Ivohibory forest.

Etymology. This species is named after Patricia Chapple Wright, an American primatologist and conservationist, best known for her studies on lemurs. She contributed to the establishment of the Ranomafana National Park in Madagascar. She also organized and led the expedition to the Ivohibory forest, during which several new species of tardigrades were found, including this species.

Type depositories. The holotype and 23 paratypes (slides: MAD109/1, MAD109/3, MAD109/4, MAD109/5, MAD109/7) are deposited at the Department


Figures 6-8. Milnesium wrightae sp. nov. 6 Habitus (ventral view) (holotype) $\mathbf{7}$ dorsal cuticle with pseudopores (holotype) $\mathbf{8}$ buccal tube (paratype). All in PCM.


Figures 9-I I.Milnesium wrightae sp. nov. 9 Claws I (paratype), arrow indicates bar under claw $\mathbf{1 0}$ claws IV (holotype), empty arrowhead indicates small accessory point, filled arrowheads indicate the fourth points on secondary branches near the base of the claw II focus on the fourth points on secondary branches near the base of the claw IV (holotype, filled arrowheads). All in PCM.
of Animal Taxonomy and Ecology, Adam Mickiewicz University in Poznań, Uniwersytetu Poznańskiego 6, Poznań, Poland, five paratypes (slides: MAD109/2) are deposited at the Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9,30-387, Kraków, Poland.

Morphological differential diagnosis. The new species, by the presence of four points on secondary branches of claws IV, is most similar to Mil. quadrifidum Nederström, 1919, which is the only valid Milnesium species with four points on secondary branches of all claws. However, Mil. wrightae sp. nov. differs from Mil. quadrifidum

Table 7. Measurements and $p t$ values of selected morphological structures of females of Milnesium wrightae sp. nov. mounted in Hoyer's medium ( N - number of specimens/structures measured, RANGE refers to the smallest and the largest structure among all measured specimens; SD - standard deviation, pt - ratio of the length of a given structure to the length of the buccal tube expressed as a percentage).

| Character | N | Range |  |  |  |  |  | Mean |  | SD |  | Holotype |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | $\mu \mathrm{m}$ |  |  | $p t$ |  |  | $\mu \mathrm{m}$ | $p t$ | $\mu \mathrm{m}$ | $p t$ | $\mu \mathrm{m}$ | $p t$ |
| Body length | 17 | 329 | - | 553 | - | - | - | 448 | - | 60 | - | 515 | - |
| Peribuccal papillae length | 12 | 6.8 | - | 10.4 | 13.2 | - | 16.6 | 9.0 | 14.9 | 1.1 | 1.0 | 9.3 | 15.3 |
| Lateral papillae length | 8 | 5.1 | - | 8.4 | 10.0 | - | 13.0 | 6.7 | 11.3 | 1.0 | 0.9 | 6.1 | 10.0 |
| Buccal tube |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Length | 17 | 44.8 | - | 65.6 | - | - | - | 58.4 | - | 6.5 | - | 60.9 | - |
| Stylet support insertion point | 15 | 31.2 | - | 45.8 | 69.3 | - | 73.2 | 40.8 | 70.6 | 4.7 | 1.1 | 43.9 | 72.1 |
| Anterior width | 16 | 14.0 | - | 23.0 | 29.5 | - | 37.9 | 19.0 | 32.7 | 2.5 | 2.4 | 20.1 | 33.0 |
| Standard width | 14 | 13.0 | - | 20.7 | 27.5 | - | 36.4 | 17.7 | 31.0 | 2.3 | 2.6 | 19.8 | 32.5 |
| Posterior width | 14 | 12.7 | - | 20.1 | 26.6 | - | 33.5 | 16.9 | 29.6 | 2.2 | 2.3 | 18.9 | 31.0 |
| Standard width/length ratio | 14 | 28\% | - | 36\% | - | - | - | 31\% | - | 3\% | - | 33\% | - |
| Posterior/anterior width ratio | 14 | 88\% | - | 97\% | - | - | - | 91\% | - | 3\% | - | 94\% | - |
| Claw 1 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 16 | 11.0 | - | 15.2 | 19.5 | - | 24.6 | 13.0 | 21.9 | 1.2 | 1.6 | 13.8 | 22.7 |
| External base + secondary branch | 15 | 9.6 | - | 14.9 | 19.5 | - | 23.4 | 12.6 | 21.2 | 1.4 | 1.0 | 13.8 | 22.7 |
| External spur | 7 | 2.8 | - | 3.7 | 4.7 | - | 5.6 | 3.2 | 5.2 | 0.3 | 0.3 | ? | ? |
| External branches length ratio | 14 | 87\% | - | 103\% | - | - | - | 97\% | - | 5\% | - | 100\% | - |
| Internal primary branch | 16 | 10.9 | - | 14.0 | 19.4 | - | 24.6 | 12.4 | 20.9 | 0.9 | 1.4 | 12.7 | 20.9 |
| Internal base + secondary branch | 16 | 9.0 | - | 14.0 | 18.9 | - | 21.7 | 12.1 | 20.4 | 1.4 | 0.8 | 12.8 | 21.0 |
| Internal spur | 13 | 2.8 | - | 3.6 | 4.7 | - | 6.5 | 3.1 | 5.2 | 0.3 | 0.5 | 3.2 | 5.3 |
| Internal branches length ratio | 15 | 83\% | - | 103\% | - | - | - | 98\% | - | 7\% | - | 101\% | - |
| Claw 2 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 15 | 10.6 | - | 15.3 | 20.5 | - | 26.0 | 13.3 | 22.4 | 1.2 | 1.5 | 14.6 | 24.0 |
| External base + secondary branch | 14 | 9.3 | - | 13.7 | 18.8 | - | 21.5 | 12.2 | 20.5 | 1.4 | 0.7 | 12.5 | 20.5 |
| External spur | 8 | 3.1 | - | 4.1 | 4.9 | - | 6.7 | 3.4 | 5.5 | 0.3 | 0.6 | 4.1 | 6.7 |
| External branches length ratio | 13 | 78\% | - | 103\% | - | - | - | 92\% | - | 7\% | - | 86\% | - |
| Internal primary branch | 14 | 10.9 | - | 15.0 | 19.2 | - | 24.3 | 12.5 | 21.3 | 1.1 | 1.7 | 13.5 | 22.2 |
| Internal base + secondary branch | 15 | 9.0 | - | 14.2 | 18.0 | - | 22.3 | 12.1 | 20.2 | 1.5 | 1.0 | 12.9 | 21.2 |
| Internal spur | 12 | 2.6 | - | 4.6 | 4.3 | - | 6.9 | 3.4 | 5.7 | 0.6 | 0.7 | 3.7 | 6.1 |
| Internal branches length ratio | 13 | 82\% | - | 103\% | - | - | - | 95\% | - | 8\% | - | 96\% | - |
| Claw 3 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| External primary branch | 17 | 10.8 | - | 15.2 | 19.0 | - | 26.5 | 13.2 | 22.6 | 1.4 | 1.7 | ? | ? |
| External base + secondary branch | 16 | 9.5 | - | 15.7 | 19.1 | - | 24.7 | 12.0 | 20.7 | 1.6 | 1.4 | ? | ? |
| External spur | 7 | 3.0 | - | 4.0 | 4.9 | - | 6.8 | 3.3 | 5.5 | 0.4 | 0.7 | ? | ? |
| External branches length ratio | 16 | 79\% | - | 103\% | - | - | - | 92\% | - | 6\% | - | ? | - |
| Internal primary branch | 17 | 10.7 | - | 14.1 | 19.2 | - | 25.0 | 12.4 | 21.3 | 1.1 | 1.7 | ? | ? |
| Internal base + secondary branch | 16 | 9.0 | - | 14.1 | 17.8 | - | 21.8 | 11.5 | 19.7 | 1.5 | 1.1 | ? | ? |
| Internal spur | 10 | 2.4 | - | 4.0 | 4.1 | - | 6.8 | 3.3 | 5.7 | 0.5 | 0.9 | ? | ? |
| Internal branches length ratio | 16 | 80\% | - | 102\% | - | - | - | 93\% | - | 7\% | - | ? | - |
| Claw 4 lengths |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Anterior primary branch | 12 | 12.6 | - | 18.4 | 23.7 | - | 28.9 | 15.2 | 25.8 | 1.6 | 1.9 | 15.8 | 25.9 |
| Anterior base + secondary branch | 12 | 11.2 | - | 17.4 | 22.6 | - | 27.5 | 14.7 | 25.0 | 1.8 | 1.6 | 16.5 | 27.1 |
| Anterior spur | 7 | 2.7 | - | 5.2 | 5.6 | - | 8.2 | 3.7 | 6.3 | 0.8 | 0.9 | 4.2 | 6.9 |
| Anterior branches length ratio | 11 | 85\% | - | 104\% | - | - | - | 97\% | - | 6\% | - | 104\% | - |
| Posterior primary branch | 12 | 11.7 | - | 20.0 | 23.7 | - | 31.4 | 16.0 | 27.3 | 2.2 | 2.0 | 17.5 | 28.7 |
| Posterior base + secondary branch | 11 | 12.1 | - | 18.5 | 24.0 | - | 28.9 | 15.6 | 26.5 | 2.2 | 1.8 | 17.5 | 28.7 |
| Posterior spur | 7 | 2.9 | - | 5.2 | 5.3 | - | 8.2 | 3.8 | 6.7 | 0.9 | 1.0 | 4.4 | 7.2 |
| Posterior branches length ratio | 10 | 92\% | - | 103\% | - | - | - | 98\% | - | 4\% | - | 100\% | - |

not only by claw configuration ([4-4]-[4-4] in Mil. quadrifidum vs. [3-3]-[4-4] in Mil. wrightae sp. nov.), but also by the position of fourth points on secondary branches of claws IV (located near the base of the claw in the new species $v s$. near the top of the claw in Mil. quadrifidum). Additionally, all secondary branch points have similar length in Mil. quadrifidum, whereas the fourth points are very clearly smaller than the others in Mil. wrightae sp. nov.

Genotypic differential diagnosis. The ranges of uncorrected genetic p-distances between the Mil. wrightae sp. nov. and species of the genus Milnesium, for which molecular marker sequences are available from GenBank (see Table 6 for details), are as follows:

1. 28 S rRNA: $5.7-8.0 \%$ ( $6.7 \%$ on average), with the most similar being Milnesium sp. from North America (JX888585.1, JX888586.1, JX888587.1) (unpublished) and the least similar being Mil. t. tardigradum from Poland (KC138808.1 and KC138809.1) (unpublished);
2. COI: 17.7-38.4\% ( $22.0 \%$ on average), with the most similar being Mil. variefidum from UK (KT951663.1) (Morek et al. 2016) and the least similar being Mil. t. tardigradum from Spain (FJ435810.1) (Guil and Giribet 2012);
3. ITS-2: 25.6-36.3\% ( $31.5 \%$ on average), with the most similar being Mil. matheusi sp. nov. (present studies) and the least similar being Mil. cf. granulatum from USA (MK681879.1) (Jackson and Meyer 2019).

## Conclusions

Milnesium matheusi sp. nov. and Mil. wrightae sp. nov. are new for science taxa, based on morphological as well as molecular characteristics. Until now, five Milnesium taxa have been reported from the African region, including Madagascar (i.e. Mil. dornensis, Mil. matheusi sp. nov. Mil. tardigradum s.s., Mil. tetralamellatum and Mil. wrightae sp. nov.). The presence of Mil. tardigradum s.s. in Madagascar needs confirmation and currently this record should be considered dubious.

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## References

Bartels PJ, Nelson DR, Kaczmarek Ł, Michalczyk Ł (2014) The genus Milnesium (Tardigrada: Eutardigrada: Milnesiidae) in the Great Smoky Mountains National Park (North Carolina and Tennessee, USA), with the description of Milnesium bohleberi sp. nov. Zootaxa 3826(2): 356-368. https://doi.org/10.11646/zootaxa.3826.2.5
Beasley CW (1995) The phylum Tardigrada [Third Edition by G. Ramazzotti and W. Maucci, English Translation]. Published by the translator Clark Beasley, Abilene, 1014 pp.
Bertolani R, Guidetti R, Marchioro T, Altiero T, Rebecchi L, Cesari M (2014) Phylogeny of Eutardigrada: New molecular data and their morphological support lead to the identification of new evolutionary lineages. Molecular Phylogenetics and Evolution 76: 110-126. https://doi.org/10.1016/j.ympev.2014.03.006
Binda MG, Guglielmino A (1991) Tardigradi dell'Africa. VI: Macrobiotus polygonatus nuova specie di Eutardigrado del Kilimangiaro (Tanzania). Animalia 18: 223-227. http://hdl. handle.net/2067/2225
Buerki S, Devey DS, Callmander MW, Phillipson PB, Forest F (2013) Spatio-temporal history of the endemic genera of Madagascar. Botanical Journal of the Linnaean Society 171: 304-329. https://doi.org/10.1111/boj. 12008
Callmander MW, Phillipson PB, Andriambololonera S, Rabarimanarivo M, Rakotonirina N, Raharimampionona J, Chatelain C, Gautier L, Lowry II PP (2011) The endemic and non-endemic vascular flora of Madagascar updated. Plant Ecology and Evolution 144: 121-125. https://doi.org/10.5091/plecevo.2011.513
Casquet J, Thebaud C, Gillespie RG (2012) Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. Molecular Ecology Resources 12: 136-141. https://doi.org/10.1111/j.1755-0998.2011.03073.x
Ciobanu DA, Roszkowska M, Kaczmarek Ł (2015) Two new tardigrade species from Romania (Eutardigrada: Milnesiidae, Macrobiotidae), with some remarks on secondary sex characters in Milnesium dornensis sp. nov. Zootaxa 3941(4): 542-564. https://doi.org/10.11646/ zootaxa.3941.4.4
Ciobanu DA, Zawierucha K, Moglan I, Kaczmarek Ł (2014) Milnesium berladnicorum sp. n. (Eutardigrada, Apochela, Milnesiidae), a new species of water bear from Romania. ZooKeys 429: 1-11. https://doi.org/10.3897/zookeys.429.7755
Dabert J, Ehrnsberger R, Dabert M (2008) Glaucalges tytonis sp. nov. (Analgoidea: Xolalgidae) from the barn owl Tyto alba (Strigiformes: Tytonidae): compiling morphology with DNA barcode data for taxa descriptions in mites (Acari). Zootaxa 1719: 41-52. http://www. mapress.com/zootaxa/2008/f/zt01719p052.pdf

Dabert M, Witalinski W, Kazmierski A, Olszanowski Z, Dabert J (2010) Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long-branch attraction artifacts. Molecular Phylogenetic and Evolution 56(1): 222-241. https://doi.org/10.1016/j.ympev.2009.12.020
de Witt MJ (2003) Madagascar: Heads It's a Continent, Tails It's an Island, Annual Review of Earth and Planetary Science 31: 213-248. https://doi.org/10.1146/annurev. earth.31.100901.141337
Degma P, Guidetti R (2007) Notes to the current checklist of Tardigrada. Zootaxa 1579: 4153. https://doi.org/10.11646/zootaxa.1579.1.2

Degma P, Bertolani, R, Guidetti R (2009-2018) Actual checklist of Tardigrada species. [Version 34: Edition: 30-06-2018] http://www.tardigrada.modena.unimo.it/miscellanea/Actual \%20checklist\%20of\%20Tardigrada.pdf
Doyère M (1840) Memoire sur les tardigrades. Annales des Sciences Naturelles. Zoologie Series 2.14: 269-362.

Du Puy DJ, Moat J (1996) A refined classification of the primary vegetation of Madagascar based on the underlying geology: using GIS to map its distribution and to assess its conservation status. In: Lourenço WR (Ed.) Proceedings of the International Symposium on the 'Biogeography de Madagascar', Paris (France), September 1995. Editions de l'ORSTOM, Paris, 205-218.
Ehrenberg CG (1853) Diagnoses novarum formarum. Verhandlungen der Königlichen Preussische der Akademie Wissenschaften zu Berlin 8: 526-533.
Goodman SM, Benstead JP (2003) The Natural History of Madagascar. Journal of Mammalogy 84(4): 813-814. https://doi.org/10.1644/1545-1542(2004)085\<0813:BR\>2.0.CO;2
Guidetti R, Bertolani R (2005) Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. Zootaxa 845: 1-46. https://doi.org/10.11646/ zootaxa.845.1.1
Guil N, Giribet G (2012) A comprehensive molecular phylogeny of tardigrades adding genes and taxa to a poorly resolved phylum-level phylogeny. Cladistics 28(1): 21-49. https://doi. org/10.1111/j.1096-0031.2011.00364.x
Guil N, Jorgensen A, Kristensen R (2019) An upgraded comprehensive multilocus phylogeny of the Tardigrada tree of life. Zoologica Scripta 48: 120-137. https://doi.org/10.1111/ zsc. 12321
Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95-98. http://brownlab.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf
Hong-Wa C, Besnard G (2013) Intricate patterns of phylogenetic relationships in the olive family as inferred from multi-locus plastid and nuclear DNA sequence analyses: a close-up on Chionanthus and Noronhia (Oleaceae). Molecular Phylogenetic and Evolution 67(2): 367-378. https://doi.org/10.1016/j.ympev.2013.02.003
Iharos G (1966) Neue Tardigraden-arten aus Ungarn. Acta Zoologica Academiae Scientiarum Hungaricae 12: 111-122.
Jackson KJA, Meyer HA (2019) Morphological and genetic analysis of Milnesium cf. granulatum (Tardigrada: Milnesiidae) from Northeastern North America. Zootaxa 4604(3): 497-510. https://doi.org/10.11646/zootaxa.4604.3.6

Kaczmarek Ł, Michalczyk Ł, McInnes SJ (2014) Annotated zoogeography of non-marine Tardigrada. Part I: Central America. Zootaxa 3763(1): 1-62. https://doi.org/10.11646/ zootaxa.3763.1.1
Kaczmarek Ł, Michalczyk Ł, McInnes SJ (2015) Annotated zoogeography of non-marine Tardigrada. Part II: South America. Zootaxa 3923(1): 1-107. https://doi.org/10.11646/ zootaxa.3923.1.1
Kaczmarek Ł, Michalczyk Ł, Sandra JM (2016) Annotated zoogeography of non-marine Tardigrada. Part III: North America and Greenland. Zootaxa 4203(1): 1-249. https://doi. org/10.11646/zootaxa.4203.1.1
Kosztyła P, Stec D, Morek W, Gąsiorek P, Zawierucha K, Michno K, Ufir K, Małek D, Hlebowicz K, Laska A, Dudziak M, Frohme M, Prokop ZM, Kaczmarek Ł, Michalczyk Ł (2016) Experimental taxonomy confirms the environmental stability of morphometric traits in a taxonomically challenging group of microinvertebrates. Zoological Journal of the Linnaean Society 178(4): 765-775. https://doi.org/10.1111/zoj. 12409
Kusky TM, Toraman E, Raharimahefa T (2007) The Great Rift Valley of Madagascar: An extension of the Africa-Somali diffusive plate boundary? Gondwana Research 11(4): 577579. https://doi.org/10.1016/j.gr.2006.11.009

Mallatt JM, Garey JR, Shultz JW (2004) Ecdysozoan phylogeny and Bayesian inference: first use of nearly complete 28 S and 18 S rRNA gene sequences to classify the arthropods and their kin. Molecular Phylogenetic and Evolution 31(1): 178-191. https://doi. org/10.1016/j.ympev.2003.07.013
Marley NJ, McInnes SJ, Sands CJ (2011) Phylum Tardigrada: A re-evaluation of the Parachela. Zootaxa 2819: 51-64. https://doi.org/10.11646/zootaxa.2819.1.2
Maucci W (1991) Tre nuove specie di eutardigradi della Groenlandia meridionale. Bollettino del Museo Civico di Storia Naturale di Verona 15: 279-289.
Maucci W (1993) Prime notizie su Tardigradi "terrestri" del Madagascar con descrizione di tre specie nuove. Bollettino del Museo Civico di Storia Naturale di Verona, 17: 381-392.
McInnes SJ, Michalczyk Ł, Kaczmarek Ł (2017) Annotated zoogeography of non-marine Tardigrada. Part IV: Africa. Zootaxa 4284(1): 1-74. https://doi.org/10.11646/zootaxa.4284.1.1
Meyer HA (2015) Water bears (Phylum Tardigrada) of Oceania, with the description of a new species of Milnesium. New Zealand Journal of Zoology 42(3): 173-186. https://doi.org/1 0.1080/03014223.2015.1062402

Michalczyk $£$, Kaczmarek $£(2013)$ The Tardigrada Register: a comprehensive online data repository for tardigrade taxonomy. Journal of Limnology 72(S1): 175-181. https://doi. org/10.4081/jlimnol.2013.s1.e22
Michalczyk Ł, Wełnicz W, Frohme M, Kaczmarek Ł (2012) Redescriptions of three Milnesium Doyère, 1840 taxa (Tardigrada: Eutardigrada: Milnesiidae), including the nominal species for the genus. Zootaxa 3154: 1-20. https://doi.org/10.11646/zootaxa.3154.1.1
Miller WR, Williams S (2012) A note on a Greenland tardigrade: Milnesium eurystomum Maucci, 1991 eating an amoeba. Transactions of the Kansas Academy of Science 115: 26-27. https://doi.org/10.1660/062.115.0104
Mironov SV, Dabert J, Dabert M (2012) A new feather mite species of the genus Proctophyllodes Robin, 1877 (Astigmata: Proctophyllodidae) from the Long-tailed Tit Aegithalos caudatus
(Passeriformes: Aegithalidae): morphological description with DNA barcode data. Zootaxa 3253: 54-61. https://doi.org/10.11646/zootaxa.3253.1.2
Morek W, Gąsiorek P, Stec D, Blagden B, Michalczyk Ł (2016) Experimental taxonomy exposes ontogenetic variability and elucidates the taxonomic value of claw configuration in Milnesium Doyère, 1840 (Tardigrada: Eutardigrada: Apochela). Contributions to Zoology 85(2): 173-200. https://doi.org/10.1163/18759866-08502003
Morek W, Stec D, Gąsiorek P, Surmacz B, Michalczyk Ł (2019) Milnesium tardigradum Doyère, 1840: The first integrative study of interpopulation variability in a tardigrade species. Journal of Zoological Systematics and Evolutionary Research 57: 1-23. https://doi. org/10.1111/jzs. 12233
Murray J (1907a) Some South African Tardigrada. Journal of the Royal Microscopical Society 5: 515-524. https://doi.org/10.1111/j.1365-2818.1907.tb01665.x
Murray J (1907b) Arctic Tardigrada, collected by Wm. S. Bruce. Transactions of the Royal Society of Edinburgh 45: 669-681. https://doi.org/10.1017/S0080456800011789
Murray J (1911) Scottish Tardigrada, a review of our present knowledge. Annals of Scottish Natural History 78: 88-95.
Nederström P (1919) Die bis jetzt aus Finland bekannten Tardigraden. Acta Societatis pro Fauna et Flora Fennica 46(8): 1-25.
Nelson DR, Guidetti R, Rebecchi L (2015) Phylum Tardigrada. In: Throp JH, Rogers DC (Eds) Ecology and General Biology. Vol. 1. Thorp and Covich's Freshwater Invertebrates. Academic Press, Cambridge, 347-380. https://doi.org/10.1016/B978-0-12-385026-3.00017-6

Perry E, Miller WR, Kaczmarek $Ł(2019)$ Recommended abbreviations for the names of genera of the phylum Tardigrada. Zootaxa 4608(1): 145-154. https://doi.org/10.11646/ zootaxa.4608.1.8
Pilato G (1981) Analisi di nuovi caratteri nello studio degli Eutardigradi. Animalia 8: 51-57.
Pilato G, Binda MG (1991) Milnesium tetralamellatum, new species of Milnesiidae from Africa (Eutardigrada). Tropical Zoology 4: 103-106. https://doi.org/10.1080/03946975.1991.1 0539480
Pilato G, Lisi O (2003) Echiniscus walteri, new species of tardigrade from Madagascar. Bollettino Museo Civico di Storia Naturale di Verona 27: 65-70.
Pilato G, Sabella G, Lisi O (2016) Two new species of Milnesium (Tardigrada: Milnesiidae). Zootaxa 4132(4): 575-587. https://doi.org/10.11646/zootaxa.4132.4.9
Plate L (1888) Beiträge zur Naturgeschichte der Tardigraden. Zoologische Jahrbücher 3: 487550. https://doi.org/10.5962/bhl.part. 1265

Ramazzotti G (1962) Tardigradi del Cile, con descrizione di quattro nuove specie e di una nuova varieta. Atti della Società italiana di scienze naturali e del Museo civico di storia naturale di Milano 101: 275-287.

Ramazzotti G, Maucci W (1983) Il Phylum Tardigrada. III edizione riveduta e aggiornata. Memorie dell'Istituto Italiano di Idrobiologia 41: 1-1012.
Richters F (1926) Tardigrada. In: Kükenthal W, Krumbach T (Eds) "Handbuch der Zoologie Vol. 3. Walter de Gruyter \& Co. Berlin and Leipzig, 58-61.
Roszkowska M, Ostrowska M, Kaczmarek Ł (2015) The genus Milnesium Doyère, 1840 (Tardigrada) in South America with descriptions of two new species from Argentina and dis-
cussion of the feeding behaviour in the family Milnesiidae. Zoological Studies 54(1): 1-12. https://doi.org/10.1186/s40555-014-0082-7
Roszkowska M, Bartels PJ, Gołdyn B, Ciobanu D, Fontoura P, Michalczyk Ł, Nelson DR, Ostrowska M, Moreno-Talamantes A, Kaczmarek Ł (2016) Is the gut content of Milnesium (Eutardigrada) related to buccal tube size? Zoological Journal of the Linnean Society 178(4): 794-803. https://doi.org/10.1111/zoj. 12459
Schill RO, Forster F, Dandekar T, Wolf M (2010) Using compensatory base change analysis of internal transcribed spacer 2 secondary structures to identify three new species in Paramacrobiotus (Tardigrada). Organism Diversity and Evolution 10(4): 287-296. https://doi. org/10.1007/s13127-010-0025-z
Schultze CAS (1834) Macrobiotus Hufelandii animal e crustaceorum classe novum, reviviscendi post diuturnam asphixiam et aridiatem potens, etc. 8, 1 tab . C. Curths, Berlin.
Schuster RO, Nelson DR, Grigarick AA, Christenberry D (1980) Systematic criteria of the Eutardigrada. Transactions of the American Microscopical Society 99: 284-303. https:// doi.org/10.2307/3226004
Srivathsan A, Meier R (2012) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics 28: 190-194. https://doi.org/10.1111/ j.1096-0031.2011.00370.x

Stec D, Smolak R, Kaczmarek Ł, Michalczyk Ł (2015) An integrative description of Macrobiotus paulinae sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: hufelandi group) from Kenya. Zootaxa 4052(5): 501-526. https://doi.org/10.11646/zootaxa.4052.5.1
Stec D, Morek W, Gąsiorek P, Michalczyk Ł (2018) Unmasking hidden species diversity within the Ramazzottius oberhaeuseri complex, with an integrative redescription of the nominal species for the family Ramazzottiidae (Tardigrada: Eutardigrada: Parachela). Systematics and Biodiversity 16(4): 357-376. https://doi.org/10.1080/14772000.201 8.1424267

Suzuki AC (2003) Life history of Milnesium tardigradum Doyère (Tardigrada) under the rearing environment. Zoological Science 20(1): 49-57. https://doi.org/10.2108/zsj.20.49
Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positionspecific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673-4680. https:// doi.org/10.1093/nar/22.22.4673
Thulin G (1928) Über die Phylogenie und das System der Tardigraden. Hereditas, Lund 11: 207-266. https://doi.org/10.1111/j.1601-5223.1928.tb02488.x
Tibbs L, Emanuels A, Miller WR (2016) Tardigrades of the canopy: Argentine species Milnesium beatae Roszkowska, Ostrowska and Kaczmarek, 2015 (Eutardigrada, Milnesidae) discovered in the trees of Kansas, USA. Transactions of the Kansas Academy of Science 119(2): 173-178. https://doi.org/10.1660/062.119.0207
Tumanov D (2006) Five new species of the genus Milnesium (Tardigrada, Eutardigrada, Milnesiidae). Zootaxa 1122: 1-23. https://doi.org/10.11646/zootaxa.1122.1.1
Velasco-Castrillón A, McInnes SJ, Schultz MB, Arróniz-Crespo M, D’Haese CA, Gibson JAE, Adams BJ, Page TJ, Austin AD, Cooper SJB, Stevens MI (2015) Mitochondrial DNA analyses reveal widespread tardigrade diversity in Antarctica. Invertebrate Systematics 29: 578-590. https://doi.org/10.1071/IS14019

Vicente F, Bertolani R (2013) Considerations on the taxonomy of the Phylum Tardigrada. Zootaxa 3626(2): 245-248. https://doi.org/10.11646/zootaxa.3626.2.2
Vicente F, Cesari M, Serrano A, Bertolani R (2013) The impact of fire on terrestrial tardigrade biodiversity: a first case-study from Portugal. Journal of Limnology 72(S1): 152-159. https://doi.org/10.4081/jlimnol.2013.s1.e19
Warren BH, Strasberg D, Bruggemann J, Prys-Jones RP, Thébaud C (2010) Why does the biota of Madagascar region have such a strong Asiatic flavor? Cladistics 26: 526-538. https:// doi.org/10.1111/j.1096-0031.2009.00300.x
Wełnicz W, Grohme MA, Kaczmarek Ł, Schill RO, Frohme M (2010) ITS-2 and 18S rRNA data from Macrobiotus polonicus and Milnesium tardigradum (Eutardigrada, Tardigrada). Journal of Zoological Systematic and Evolutionary Research 49(Suppl. 1): 34-39. https:// doi.org/10.1111/j.1439-0469.2010.00595.x
Wilmé L, Goodman S, Ganzhorn J (2006) Biogeographic evolution of Madagascar's microendemic biota. Science 312: 1063-1065. https://doi.org/10.1126/science. 1122806
Wright PC, Houlihan PR (2017) Vohibory Rainforest: Biological Inventory Report - September $27^{\text {th }}$ to October 12 ${ }^{\text {th }} 2016.23 \mathrm{pp}$.
Yoder AD, Nowak MD (2006) Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. Annual Review of Ecology Evolution and Systematics 37: 405-431. https://doi.org/10.1146/annurev.ecolsys.37.091305.110239
Zawierucha K, Kolicka M, Kaczmarek Ł (2016) Re-description of the Arctic tardigrade Tenuibiotus voronkovi (Tumanov, 2007) (Eutardigrada; Macrobiotidea), with the first molecular data for the genus Zootaxa 4196(4): 498-510. https://doi.org/10.11646/zootaxa.4196.4.2

# Three new species and one subspecies of the Amynthas corticis-group from Guangxi Zhuang Autonomous Region, China (Oligochaeta, Megascolecidae) 

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#### Abstract

Three new species and one subspecies of the genus Amynthas are described from Guangxi Zhuang Autonomous Region, China: The new species are: Amynthas maximus Qiu \& Dong, sp. nov. and Amynthas tortuosus Qiu \& Dong, sp. nov., and Amynthas shengtangmontis Dong \& Jiang, sp. nov., the subspecies is Amynthas shengtangmontis minusculus subsp. nov. All have four pairs of spermathecal pores in 5/6-8/9, which indicates that they should belong to the corticis-group. Their morphological characteristics are compared to other similar species in the corticis-group from China and other Asian countries, such as Amynthas pulvinus Sun \& Jiang, 2013, Amynthas homosetus (Chen, 1938), Amynthas corticis (Kinberg, 1867), Amynthas dorsualis Sun \& Qiu, 2013, and Amynthas carnosus (Goto \& Hatai, 1899). In addition, the results presented are confirmed by the pairwise comparison of COI barcode sequences. The pairwise distances between each new species and the other eighteen corticis-group species are greater than $14.7 \%$ on average. Furthermore, the pairwise distance between $A$. shengtangmontis shengtangmontis and $A$. shengtangmontis minusculus is 10.7-11.4\%.


## Keywords

Earthworm, cytochrome c oxidase subunit I gene, new species

[^1]
## Introduction

The genus Amynthas Sims \& Easton, 1972 is the dominant genus of Megascolecidae in China (Jiang 2016, Zhao 2015) and the Amynthas corticis-group consists of a large number of species. Before 1972, only 99 species names had been recorded in the group (Sims and Easton 1972). Amynthas diffringens (Baird, 1869), Amynthas divergens divergens (Michaelsen, 1892), Amynthas yunnanensis (Stephenson, 1912), and Amynthas heterochaetus (Michaelsen, 1891) are synonyms of Amynthas corticis (Kinberg, 1867) (Blakemore 2004). Since then, 22 more species were reported: two species were described from mainland China (Chen et al. 1975, Chen and Xu 1977), seven species from Hainan Island in China (Sun et al. 2012, 2013), eight species from Taiwan Island (James et al. 2005, Tsai et al. 2001, 2007, 2010, Wang and Shih 2010), and five species from Korea (Hong and James 2001, Hong and Kim 2002).

Guangxi Zhuang Autonomous Region is located in the southeast edge of Yun-nan-Guizhou plateau, and west of Guangzhou-Guangxi hilly land. The landforms in Guangxi include mountains, hills and plains. Guangxi has a subtropical monsoon climate and the Pearl River, the Yangtze River, the Red River, and the coastal water systems flow through it. Guangxi has an average annual temperature between $16.5-23.1^{\circ} \mathrm{C}$, which is suited to the survival and dispersal of earthworms. In order to investigate the diversity of earthworms in China, we conducted a field survey in Guangxi Zhuang Autonomous Region in 2013 and have found both a number of described species and also species that are new to science. The previously described species are Amynthas dissimilis Qiu \& Jiang, 2018 (Jiang et al. 2018), Amynthas anteporus Jiang \& Dong, 2018 (Jiang et al. 2018), Amynthas marsupiformis Jiang \& Yuan, 2018 (Jiang et al. 2018), Amynthas crassitubus Qiu \& Dong, 2018 (Dong et al. 2018), and Amynthas stabilis Dong \& Jiang, 2018 (Dong et al. 2018). In this paper, we describe three new species and a subspecies of Amynthas which were collected from the Shiwan Mountain National Nature Reserve ( $22.6750^{\circ} \sim 22.07167^{\circ} \mathrm{N}, 107.49972^{\circ} \sim 108.21972^{\circ} \mathrm{E}$ ) and the Dayao Mountain National Nature Reserve ( $25.11667^{\circ} \sim 25.23334^{\circ} \mathrm{N}, 113.18333^{\circ} \sim 113.26667^{\circ} \mathrm{E}$ ) in Guangxi Zhuang Autonomous Region, China. Distributions of known species in Guangxi Zhuang Autonomous Region and sampling points for this paper are shown in Figure 1.

All of the newly described species and subspecies have four pairs of spermathecal pores in 5/6-8/9; hence, they belong to the Amynthas corticis-group.

## Materials and methods

The earthworms were collected in 2013, anaesthetized in $10 \%$ ethanol solution, and preserved in $99 \%$ ethanol solution. DNA was extracted from several specimens of $A$. maximus, $A$. tortuosus, $A$. shengtangmontis shengtangmontis, and $A$. shengtangmontis minusculus using the EZNA Mollusk DNA Kit (Omega Bio-tek, Norcross, GA, USA). The gene cytochrome oxidase subunit I (COI) was amplified. The PCR was carried out as follows:


Figure I. Distribution of known species in Guangxi Zhuang Autonomous Region and sampling points of this paper.

5 min at $94^{\circ} \mathrm{C}$ followed by 32 cycles $94^{\circ} \mathrm{C}$ for $30 \mathrm{~s}, 50^{\circ} \mathrm{C}$ for 30 s and $72^{\circ} \mathrm{C}$ for 1 min , with an extension of 10 min at $72^{\circ} \mathrm{C}$. Primers used in the research were: $5^{\prime}$-GGT-CAACAAATCATAAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACCAAAAAAT-CA-3' (Folmer et al. 1994), or 5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TATACTTCTGGGTGTCCGAAGAATCA-3' (Bely and Wray 2004). Sequencing was performed in the Beijing Genomics Institute (Shanghai, China). Sequencing was submitted to NCBI GenBank and accession numbers were shown in Table 1. All holotypes and paratypes are deposited in the Shanghai Natural History Museum.

Sequences were aligned with ClustalX (Thompson 1997), and then pairwise distances between these species were calculated using Kimura two-parameter model of DNA evolution with MEGA 5 (Tamura et al. 2011). Images were produced using the Affinity Photo and SketchBook software.

## Taxonomy

## Amynthas maximus Qiu \& Dong, sp. nov.

http://zoobank.org/E84CFBE5-4FF8-4F53-B49A-233EC5D04298
Figure 2, Table 2

Material. Holotype:1 clitellate (C-GX201304-01A): China, Guangxi Zhuang Autonomous Region, Shiwan Mountain Nature Reserve (21.50299N, $107.3035^{\circ} \mathrm{E}$ ), 449 m asl, black sandy soil under bryophytes in a subtropical evergreen forest, 12 May 2013, JP Qiu, Y Hong, JB Jiang, LL Zhang, Y Dong legit. Paratypes: 8 clitellates (C-GX201304-01B): same date as for holotype.

Table I. Species with molecular data used in this study. Abbreviations: HT holotype, PT paratype.

| Species | Species No. | Locality | Reference | GenBank <br> Acc. No |
| :---: | :---: | :---: | :---: | :---: |
| Amynthas maximus sp. n. (HT) | C-GX201304-01A | China: Guangxi | This paper | MG450707 |
| Amynthas tortuosus sp. n. (HT) | C-GX201306-06A | China: Guangxi | This paper | MG450708 |
| Amynthas tortuosus sp. n. (PT) | C-GX201301-09 | China: Guangxi | This paper | MK606425 |
| Amynthas tortuosus sp. n. (PT) | C-GX201305-07 | China: Guangxi | This paper | MK606426 |
| Amynthas shengtangmontis shengtangmontis sp. n. (HT) | C-GX201312-03A | China: Guangxi | This paper | MG450709 |
| Amynthas shengtangmontis minusculus subsp. n. (HT) | C-GX201316-02A | China: Guangxi | This paper | MG450710 |
| Amynthas shengtangmontis minusculus subsp. n. (PT) | C-GX201316-02B | China: Guangxi | This paper | MK606427 |
| Amynthas fuscatus (Goto \& Hatai, 1898) |  | Japan: Tokyo | Minamiya, submitted to GenBank in 2010 | AB542475 |
| Amynthas pulvinus Sun \& Jiang, 2013 | C-HN201115-08 | China: Hainan | Sun et al. 2014 | JQ905266 |
| Amynthas robustus (Chen, 1936) | C-SC201009-01 | China: Sichuan | Sun 2013, in Chinese | KF179573 |
| Amynthas corticis (Kingberg, 1867) | C-HN201035-02 | China: Hainan | Sun 2013, in Chinese | KF205966 |
| Amynthas carnosus (Goto \& Hatai, 1899) | C-HN201002-01 | China: Hainan | Sun 2013, in Chinese | KF205962 |
| Amynthas mirifius Sun \& Zhao, 2013 | C-HN201103-02 | China: Hainan | Sun et al. 2013 | JQ905265 |
| Amynthas micronarius (Goto \& Hatai, 1898) |  | Japan: Tokyo | Minamiya, submitted to GenBank in 2010 | AB542498 |
| Amynthas alexandri (Beddard, 1900) |  | Thailand | Jeratthitikul et al. 2017 | KU565178 |
| Amynthas andersoni (Michaelsen, 1907) |  | Thailand | Jeratthitikul et al. 2017 | KU565179 |
| Amynthas comptus (Gates, 1932) |  | Thailand | Jeratthitikul et al. 2017 | KU565187 |
| Amynthas exiguus (Gates, 1930) |  | Thailand | Jeratthitikul et al. 2017 | KU565189 |
| Amynthas formosae (Michaelsen, 1922) |  | India | Farooqui, submitted to GenBank in 2019 | LC458750 |
| Amynthas longicauliculatus (Gates, 1931) |  | Thailand | Jeratthitikul et al. 2017 | KU565195 |
| Amynthas szechuanensis vallatus (Chen, 1946) | C-SC201102-05 | China: Sichuan | Sun 2013, in Chinese | KF205477 |
| Amynthas mediocus (Chen et al., 1975) | C-GD201108-02 | China: <br> Guangdong | Sun 2013, in Chinese | KF205405 |
| Amynthas wulinensis Tsai et al., 2001 |  | Taiwan island | Chang et al. 2007 | DQ224182 |
| Amynthas yunlongensis (Chen, 1977) | C-GZ201101-06 | China: <br> Guizhou | Sun 2013, in Chinese | KF179581 |
| Amynthas stricosus Qiu \& Sun, 2012 | C-HN201104-04 | China: Sichuan | Sun 2013, in Chinese | JX315345 |

Diagnosis. Dimensions $145-170 \mathrm{~mm}$ by $5.8-6.2 \mathrm{~mm}$ at clitellum, clitellum taupe in $2 / 5$ XIV-XVI, 78-101 segments. First dorsal pore in 13/14. Setae numbering 3338 at III, 32-36 at V, 29-33 at VIII, 18-22 at XX, 50-65 at XXV; 9-13 between male pores; setae between spermathecal pores numbering 14-16 at VI, 10-14 at VII, and 18-22 at VIII. Four pairs of spermathecal pores ventrally in $5 / 6-8 / 9$. Seven or eight (three specimens have seven papillae, and four specimens have eight papillae) postsetal genital papillae arranged in two rows in VI-IX, 0.33 circumference ventrally apart from each other. One pair of male pores in XVIII, each on the top of a central, round porophore surrounded by three or four circular ridges, with one presetal indentedtopped genital papilla medial of each porophore. Ampulla elongate oval, stout duct as


Figure 2. A Ventral view showing spermathecal pores, female pores and male pores of Amynthas maximus sp. nov. B spermathecae of Amynthas maximus sp. nov. C illustration of the details of the male pore region.
long as $3 / 5$ ampulla. Diverticulum slightly shorter than main pouch, a little twist in the middle, terminal $2 / 5$ dilated into a swollen, club-shaped seminal chamber. Prostate glands well developed.

Description. External characters: Light purple-brown pigment on pre-clitellum dorsum, no pigment on ventrum. Pigment from light purple-brown to brown on postclitellum dorsum, light yellowish on ventrum. Clitellum taupe in $2 / 5$ XIV-XVI. Dimensions 160 mm by 6.0 mm at clitellum, 92 segments. Prostomium $1 / 2$ epilobous. First dorsal pore in 13/14. Setae numbering 36 at III, 34 at V, 31 at VIII, 20 at XX, 60 at XXV; 12 between male pores; Setae numbering 15 at VI, 12 at VII, 20 at VIII between spermathecal pores. Setae formula: $\mathrm{AA}=1.1-1.4 \mathrm{AB}, \mathrm{ZZ}=1.2-2.0 \mathrm{ZY}$. Clitellum annular, yellowish, in $2 / 5$ XIV-XVI, setae not visible externally. Four pairs of spermathecal pores in 5/6-8/9, ventral, eye-like, 0.4 circumference ventrally apart from each other. Seven or eight (three specimens have seven papillae, and four specimens have eight papillae) postsetal genital papillae arranged in two rows in VI-X, 0.33 circumference ventrally apart from each other. One pair of male pores in XVIII, 0.4 circumference apart ventrally, each on the top of a central, round porophore surrounded by three or four circular ridges, with one presetal indented-topped genital papilla medial of each porophore (Figure 1A). Single female pore in XIV, ovoid.

Internal characters. Septa 5/6-7/8, 10/11-13/14 thick and muscular, 8/9-9/10 absent. Gizzard bucket-shaped, in VIII-X. Intestine enlarged distinctly from XV onwards. Intestinal caeca paired in XXVII, extending anteriorly to XXII, transition state, ventral margin smooth, four pointed saccules in dorsal margin. Four pairs of esophageal hearts in X-XIII, developed. Ovaries in XIII. Four pairs of spermathecae in VIIX, short, approx. 1.6 mm long, ampulla elongate-oval; duct as long as $3 / 5$ ampulla. Diverticulum slightly shorter than main pouch (ampulla and duct), a little twist in middle, terminal $2 / 5$ dilated into a swollen, club-shaped seminal chamber (Figure 1B). One or two stalked accessory glands observed near ventral median line in VI-IX. Holandric: two pairs of testis sacs in X and XI, separated from each other, well developed. Two pairs of seminal vesicles in XI and XII, developed. Prostate glands undeveloped, inserting in XVIII and extending from XVII-XIX, coarsely lobate, prostatic duct Ishaped, of uniform thickness. No accessory glands observed in male pore region.

Etymology. The species is named after its large accessory glands observed in the spermathecal area.

Remarks. Amynthas maximus sp. nov. keys to the corticis-group in Sims and Easton (1972) with four pairs of spermathecal pores intersegmentally in 5/6-8/9. Amynthas maximus sp. nov. is similar to Amynthas carnosus (Goto \& Hatai, 1899) as re-described by Chang et al. (2016) with respect to body size, the distance between spermathecal pores and male pores, shorter diverticulum than main spermathecal axis, and no accessary glands near prostates. In contrast, the pigment on its ventrum is lighter than A. carnosus and other differences include the first dorsal pore, clitellum location, spermathecal pores, and male pores characters. The first dorsal pore in $A$. maximus sp. nov. is located in $13 / 14$, versus $12 / 13$ in $A$. carnosus; the clitellum occupies less than three segments; four pairs of spermathecal pores while sometimes three pairs in $A$. carnosus; the porophore is surrounded by three or four circular ridges, but no ridges are present in $A$. carnosus; several accessory glands observed in the spermathecal region in the new species but none in $A$. carnosus.

We also compare the new species with Amynthas corticis (Kinberg, 1867) which has been recognized as the typical species in the corticis-group. They share several common characters such as body size, pigment, clitellum extent, setal number, and both have stalked accessory glands. Other than that, the first dorsal pore in the new species is in $13 / 14$, but in $10 / 11$ or $12 / 13$, usually in $11 / 12$ in $A$. corticis. The diverticulum of $A$. maximus sp. nov. has a small twist in the middle compared with $A$. corticis, which has a long stalk. Moreover, $A$. maximus sp. nov. always exhibits genital markings in the male pore region, whereas in $A$. corticis, these markings are occasionally absent.

We further compare the new species with another species Amynthas dorsualis Sun \& Qiu, 2013 described from Hainan, China, and its clitellum also occupies fewer than three segments. In our results, we find the two species share several common characters, including the first dorsal pore location, setal formula, male pore characters, and in the diverticulum being shorter than the main pouch. However, the morphological dissimilarity of the two species is substantial. For instance, the locations of spermathecal pores are different between $A$. dorsualis and $A$. maximus sp. nov. In $A$. dorsualis, the spermathecal pores are located on the dorsum, while the pores are located on the ven-

Table 2. A comparison of characters of $A$. maximus sp. nov., $A$. dorsualis, 2013, A. carnosus, $A$. corticis and $A$. wulinensis.

| Characteristics | A. maximus sp. nov. | A. dorsualis | A. carnosus | A. corticis | A. wulinensis |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Body length (mm) | 145-170 | 121-? | 110-340 | 45-170 | 128-174 |
| Body width (mm) | 5.8-6.2 | 2.7-? | 4.0-9.0 | 3.0-6.0 | 5.6-6.1 |
| Pigment |  |  |  |  |  |
| dorsum | Light purple brown before clitellum, from light purple brown to brown after | Dark grey before clitellum, dark brown after | Dark brown or purple | Greenish brown | Whitish purple |
| ventrum | No pigment before clitellum, yellowish after | Light grey before VII, no pigment after | Dark brown or purple | No | Whitish gray |
| First dorsal pore | 13/14 | 13/14 | 12/13 | $10 / 11$ or $12 / 13$, usually at $11 / 12$ | 11/12 |
| Clitellum locality | 2/5XIV-XVI | 1/10XIV-7/10XVI | XIV-XVI | XIV-XVI, <br> occasionally shorter | XIV-XVI |
| Spermathecal pores | $\begin{gathered} 4 \text { pairs, in } 5 / 6-8 / 9, \\ 0.33 \mathrm{C} \end{gathered}$ | 4 pairs, in 5/6-8/9, dorsally, 0.6 C | 4 pairs in 5/6-8/9 or 3 pairs in 6/7-8/9, 0.33 C | $\begin{gathered} 4 \text { pairs, } 5 / 6-8 / 9 \\ 0.33 \mathrm{C} \end{gathered}$ | 4 pairs, 5/6-8/9, ventral, 0.29 C |
| Male pores | Middle, round, surrounded by 3-4 circular ridges, 0.4 C | Slightly raised, glandular, surrounded by 5-6 elliptic circular folds, 0.33C | Round or elliptic | Small, circular to transverse elliptical disc, $0.24-0.30 \mathrm{C}$ | Round or ovalshaped on setal line with depressed center, 2-3 circular folds, $0.24-0.28 \mathrm{C}$ |
| Papillae preclitellum | 7-8 post-setal indented-topped genital papillae arranged in two rows in VI-IX | Invisible | 8 papillae just overhead 8 spermathecal pores, 2 pairs preclitellar arranged on VIII and IX | Paired presetal and/or postsetal in some or all, near spermathecal pores | Absent |
| Papillae postclitellum | 2 presetal indentedtopped genital papillae medial of porophore | Invisible | 2 paired presetal genital on XVIII and IX, 1 pair postsetal genital on XVIII | Present or absent, occasionally one or more near male pore | Oval-shaped, medial to male pore in each of XVII and XIX, occasionally XX |
| Prostate glands | XVII-XIX | XVI-XX | Well developed | XVII-XX, rudimentary or absent | XV-XX, racemose, follicular |
| Spermathecae | About 1.6 mm long, ampulla long-oval; duct as long as $3 / 5$ ampulla | About 2.2 mm long, ampulla heart-shaped; duct as long as $2 / 5$ ampulla | Ampulla oval or pearshaped, duct equal to or slightly shorter than ampulla | Ampulla ovoid | Very short and stout stalk |
| Diverticulum | Shorter, lightly twist in middle, terminal $2 / 5$, swollen, club-shaped seminal chamber | Shorter, terminal $1 / 5$, ovoid plump seminal chamber | One-third to half of ampulla, slender stalk and a wider seminal chamber | Blunt ovioid, straight stalk | Oval, shining white seminal chamber, a slender and straight stalk |
| Accessory glands | 1 or 2 stalked accessory glands observed near ventral median line in VI, VII, VIII, IX | Invisible | - | Stalked, coelomic, bound down to parietes or retained within body wall | Paired in XVII and XIX, sessile, flowery |

trum in $A$. maximus sp. nov. The distance between male pore is shorter on the ventral side in $A$. dorsualis than Amynthas maximus sp. nov. In addition, $A$. dorsualis has no genital markings and no accessory glands, whereas $A$. maximus sp. nov. exhibits genital markings near the spermathecal pores and the male pores region, and stalked accessory glands are present in spermathecal pores region.

The body size of $A$. maximus sp. nov. is similar to $A$. wulinensis described from Taiwan Island. But the other characters of $A$. maximus sp. nov. differ from $A$. wulinensis. Amynthas maximus sp. nov. has no pigment before clitellum, yellowish after clitellum on dorsum, first dorsal pore in 13/14, 0.33 C between spermathecal pores, seven or eight indented-topped genital papillae in VI-IX and accessory glands in spermathecal pores region. In contrast, $A$. wulinensis has whitish gray on dorsum, first dorsal pore in $11 / 12,0.29 \mathrm{C}$ between spermathecal pores, no genital papillae observed in spermathecal pores region, and accessory glands observed in male pores region. Table 2 shows the comparison of characters of $A$. maximus sp. nov. with $A$. dorsualis, $A$. carnosus, $A$. corticis and $A$. wulinensis.

## Amynthas tortuosus Qiu \& Dong, sp. nov.

http://zoobank.org/898F2A2C-68E7-415D-B5BE-95876D8C672E
Figure 3, Table 3
Material. Holotype: 1 clitellate (C-GX201306-06A): China, Guangxi Zhuang Autonomous Region, Shiwan Mountain Nature Reserve ( $21.84739^{\circ} \mathrm{N}, 107.88989^{\circ} \mathrm{E}$ ), 553 m asl, black soil besides road, 13 May 2013, JP Qiu, Y Hong, JB Jiang, LL Zhang, Y Dong legit. Paratypes: 7 clitellates: 2 clitellates (C-GX201306-06B): same data as for holotype. 1 clitellate (C-GX201301-09): China, Guangxi Zhuang Autonomous Region, Shiwan Mountain Nature Reserve ( $21.48588^{\circ} \mathrm{N}, 107.57018^{\circ} \mathrm{E}$ ), 130 m asl, black sandy soil at riverside, 11 May 2013, JP Qiu, Y Hong, JB Jiang, LL Zhang, Y Dong legit. 4 clitellates (C-GX201305-07): China, Guangxi Zhuang Autonomous Region, Shiwan Mountain Nature Reserve ( $21.50396^{\circ} \mathrm{N}, 107.53350^{\circ} \mathrm{E}$ ), 494 m asl, black sandy soil besides road, 13 May 2013, JP Qiu, Y Hong, JB Jiang, LL Zhang, Y Dong legit.

Diagnosis. Dimensions $55-86 \mathrm{~mm}$ by $2.5-2.8 \mathrm{~mm}$ at clitellum, $55-83$ segments. First dorsal pore in 13/14. Setae numbering 24-26 at III, 34-36 at V, 34-36 at VIII, 32-36 at XX, 36-40 at XXV; 8-9 between male pores; setae between spermathecal pores numbering 9-12 at VI, 10-12 at VII, 12-13 at VIII. Four pairs of spermathecal pores in 5/6-8/9, eye-like. Four pairs of postsetal genital papillae in VI-IX, 0.20 circumference ventrally apart from each other. One pair of male pores in XVIII, each on the top of a central, round porophore surrounded by three or four rhombic ridges, with one presetal crescent indented-topped genital papilla medial of each male pore. Ampulla slender, heart-shaped; duct short. Diverticulum shorter than main pouch, terminal $4 / 5$ slightly dilated into a swollen, S-shaped twisted seminal chamber. Prostate glands well developed.

Description. External characters: Pre-clitellum, purple-brown pigment on dorsum, light purple-brown on ventrum. Post-clitellum, light purple-brown on dorsum, no pigment on ventrum. Clitellum taupe. Dimensions 76 mm by 27 mm at clitellum. 75 seg ments. Prostomium $1 / 2$ epilobous. First dorsal pore in 13/14. Setae numbering 24 at III, 34 at V, 36 at VIII, 36 at XX, 40 at XXV; 8 between male pores; Setae between spermathecal pores numbering 11 at VI, 10 at VII, 13 at VIII. Setae formula $A A=1.2-2.0 \mathrm{AB}$,


Figure 3. A Ventral view showing spermathecal pores, female pores and male pores of Amynthas tortuosus sp. nov. B spermathecae of Amynthas tortuosus sp. nov. C illustration of the details of the male pore region.

ZZ $=1.4-2.0 \mathrm{ZY}$. Clitellum annular, pale taupe, in XIV-XVI, setae not visible externally. Four pairs of spermathecal pores in $5 / 6-8 / 9$, eye-like, 0.25 circumference ventrally apart from each other. Four pairs of postsetal genital papillae in VI-IX, 0.20 circumference ventrally apart from each other. One pair of male pores in XVIII, 0.25 circumference apart ventrally, each on the top of a central, round porophore surrounded by three or four rhombic ridges, with one presetal crescent indented-topped genital papilla in the center of each male pore region (Figure 2A). Single female pore in XIV.

Internal characters. Septa 5/6-7/8 thick and muscular, 10/11-12/13 slightly thickened, 8/9-9/10 absent. Gizzard ball-shaped, in VIII-X. Intestine enlarged distinctly from XVI onwards. Intestinal caeca paired in XXVII, simple, smooth, extending anteriorly to XXIV. Four pairs of esophageal hearts in X-XIII, the first pair very thin, the last three pairs developed. Ovaries in XIII. Four pairs of spermathecae in VI-IX, small, 2.4 mm long. Ampulla slender, heart-shaped; duct short. Diverticulum 2.0 mm long, slightly shorter than main pouch, terminal $4 / 5$ slightly dilated into a swollen, $S$-shaped twisted seminal chamber (Figure 2B). One stalked accessory gland observed medial to each spermathecal duct. Holandric: two pairs of testis sacs in X-XI, separated from each other, developed. Two pairs of seminal vesicles in XI-XII, well developed. Prostate

Table 3. A comparison of characters of $A$. tortuosus sp. nov., A. carnosus, $A$. corticis, A. homosetus, $A$. exiguus aquilonius, and $A$. stricosus.

| Characteristics | A. tortuosus sp. nov. | A. carnosus | A. corticis | A. homosetus | A. exiguus aquilonius | A. stricosus |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Body length (mm) | 55-86 | 110-340 | 45-170 | 116 | 39-63 | 72-97 |
| Body width (mm) | $2.5-2.8$ | 4.0-9.0 | 3.0-6.0 | 5.2 | 1.9-2.6 | 2-2.8 |
| Pigment dorsum | Purple brown before clitellum, light purple brown after | Dark brown or purple | Greenish brown | Dark chocolate on anterior, grey on other parts | Dark reddish brown | No pigment |
| ventrum | Light purple brown before clitellum, no after | Dark brown or purple | No | Grey | Light gray on ventrum | No pogment |
| First dorsal pore | 13/14 | 12/13 | $10 / 11$ or $12 / 13$, usually at $11 / 12$ | 12/13 | 6/7 | 11/12 or 12/13 |
| Clitellum locality | XIV-XVI | XIV-XVI | XIV-XVI, occasionally shorter | XIV-XVI | XIV-XVI | XIV-XVI |
| Setal formula | $\begin{aligned} & \mathrm{AA}=1.2-2.0 \mathrm{AB}, \\ & \mathrm{ZZ}=1.4-2.0 \mathrm{ZY} \end{aligned}$ |  | - |  |  |  |
| Spermathecal pores | $\begin{gathered} 4 \text { pairs, in } 5 / 6-8 / 9, \\ 0.25 \mathrm{C} \end{gathered}$ | $\begin{gathered} 4 \text { pairs in } 5 / 6-8 / 9 \\ \text { or } 3 \text { pairs in } \\ 6 / 7-8 / 9,0.33 \mathrm{C} \end{gathered}$ | $\begin{gathered} 4 \text { pairs, } 5 / 6-8 / 9 \\ 0.33 \mathrm{C} \end{gathered}$ | $\begin{gathered} 4 \text { pairs, in } \\ 5 / 6-8 / 9,0.25 \mathrm{C} \end{gathered}$ | 4 pairs, 5/6-8/9, ventral, 0.45 C | $\begin{aligned} & 4 \text { pairs, } 5 / 6- \\ & 8 / 9,0.40 \mathrm{C} \end{aligned}$ |
| Male pores | Middle, round, surrounded by 3-4 rhombic ridges, 0.25 C | Round or elliptic | Small, circular to transverse elliptical di sc, $0.24-0.30 \mathrm{C}$ | Roundish glandular area, about 1.5 mm in diameter, 0.25 C | Round, smooth, slightly elevated with a male aperture inconspicuous on lateral concave area, 0.23-0.30C | on a coniform glandular disc surrounded by a round pad, 0.33 C |
| Papillae preclitellum | Four pairs of postsetal genital papillae in VI-IX | 8 papillae just overhead 8 spermathecal pores, 2 pairs preclitellar arranged on VIII and IX | Paired presetal and/or postsetal in some or all, near spermathecal pores | Invisible | presetal and postsetal, widely paired in 7,8 and 9 , number highly variable | Invisible |
| Papillae postclitellum | 2 presetal crescent indented-topped genital papilla medial of male pores | 2 paired presetal genital on XVIII and IX, 1 pair postsetal genital on XVIII | Present or absent, occasionally one or more near male pore | Invisible | presetal and postsetal, widely paired in XVII, XVIII and XIX, number highly variable | postsetal, single or paired in XVII, XIX and XX |
| Prostate glands | XVII-XXII | Well developed | XVII-XX, rudimentary or absent | XVI-XXI | XVI-XX, wrinkled | XVI-XX, coarsely lobate |
| Spermathecae | About 2.4 mm long, ampulla slender heart-shaped; duct short | Ampulla oval or pear-shaped, duct equal to or slightly shorter than ampulla | Ampulla ovoid | - | Ampulla peach-shaped, stalk straight, much shorter than ampulla | About 1.6 mm long; ampulla heart-shaped, gradually slender duct as long as ampulla |
| Diverticulum | About 2.0 mm long, terminal $4 / 5$, swollen, S-shaped twisted seminal chamber | One-third to half of ampulla, slender stalk and a wider seminal chamber | Blunt ovioid, straight stalk | Shorter, seminal chamber ovoid and whitish | Shorter, seminal chamber rudimentary or absent, straight or slightly bent | As long as main spermathecal axis, slender, terminal 0.4 dilated into a band shaped chamber |
| Accessory glands | 1 stalked accessory gland observed near the ental part of each spermatheca | - | Stalked, coelomic, bound down to parietes or retained within body wall | Invisible | round, stalked observed in spermathecal pores and male pores region | Invisible |

glands well developed, inserting in XVIII and extending from XVII-XXII, coarsely lobate, prostatic duct C-shaped, uniform thickness. No accessory glands observed.

Etymology. The species is named after the crooked shape of its diverticulum.
Remarks. Amynthas tortuosus sp. nov. is a comparatively small earthworm and shares some similarities to $A$. carnosus and $A$. corticis. All of them have genital markings both on the spermathecal and the male pore regions and share similar setal numbers. However, the body size of $A$. tortuosus sp. nov. is very distinct from others. The new species has roughly half the body size of $A$. carnosus and $A$. corticis, both in length and width. The first dorsal pore is in 13/14 and the distance between the spermathecal pores and male pores is less than those of $A$. carnosus and $A$. corticis. Moreover, $A$. tortuosus sp. nov. has long diverticulum with an S-shaped twisted seminal chamber and eight stalked accessory glands observed near the spermathecal duct, rather than a straight or slender stalk and an absence of accessory glands in $A$. carnosus.

We also compare the new species with Amynthas homosetus (Chen 1938) described from Hainan Island, which has a very similar distance between the male pores and spermathecal pores, and similar setal numbers. The differences between the two species are as follows: the body size of $A$. tortuosus is much smaller than $A$. homosetus; the size of prostate glands is larger in $A$. tortuosus; the shape of the seminal chamber is $S$-shaped twisted in $A$. tortuosus, while it is ovoid in $A$. homosetus; and several genital markings are present in the spermathecal pore region and male pore region in $A$. tortuosus sp. nov., while these markings are absent in $A$. homosetus.

The body size of $A$. tortuosus sp. nov. is similar to Amynthas exiguus aquilonius Tsai et al., 2001 described from Taiwan Island and Amynthas stricosus Qiu \& Sun, 2012 described from Hainan Island. The first dorsal pore of the new species is in 13/14, but in $6 / 7$ in $A$. exiguus aquilonius, and in $11 / 12$ or $12 / 13$ in $A$. stricosus. The new species has more closely spaced spermathecal pores than $A$. exiguus aquilonius and $A$. stricosus. Additionally, A. exiguus aquilonius has more genital papillae observed in spermathecal pores and male pores region than the new species. Accessory glands are observed in spermathecal pores and male pores region in A. exiguus aquilonius, but in the new species, accessory glands are only observed in spermathecal pores region. Furthermore, A. stricosus has a band shaped chamber, no genital papillae near spermathecal pores region, no accessory glands, and papillae observed in XVII, XIX and XX, but the new species has a S-shaped twisted seminal chamber, four pairs of genital papillae in spermathecal pores region, accessory glands near spermathecal duct, and no genital papillae observed in XVII, XIX and XX. Details of the comparison are showed in table 3.

## Amynthas shengtangmontis Dong \& Jiang, sp. nov. <br> http://zoobank.org/6831C1EE-6B6B-4B4C-8C9F-4A9B903EBCB6

Figure 4, Table 4

Material. Holotype: 1 clitellate (C-GX201312-03A): China, Guangxi Zhuang Autonomous Region, Dayao Mountain National Nature Reserve ( $23.97299^{\circ} \mathrm{N}$, $110.11106^{\circ} \mathrm{E}$ ), 1210 m asl, black sandy soil in bamboo forest, 15 May 2013, JP Qiu,

Y Hong, JB Jiang, LL Zhang, Y Dong legit. Paratypes: 4 clitellates (C-GX20131203A): same data as for holotype.

Diagnosis. Dimension $100-134 \mathrm{~mm}$ by $4.2-5.1 \mathrm{~mm}$ at clitellum, 116-138 segments. First dorsal pore in 12/13. Setae numbering 26-28 at III, 24-32 at V, 26-32 at VIII, 33-40 at XX, 35-42 at XXV; 8-11 between male pores. Setae between spermathecal pores numbering 11-15 at VI, 10-13 at VII, 12-16 at VIII. Four pairs of spermathecal pores in 5/6-8/9. Four pairs of postsetal genital papillae arranged in VI-IX, 0.25 circumference ventrally apart from each other. One pair of male pores in XVIII, each on the top of a large raised, round porophore, surrounded by two circular ridges, with one presetal indented-topped genital papilla medial of each porophore. First ampulla of the three pairs is heart-shaped, duct stalked, diverticulum as long as main chain, U-shaped twist in the middle, terminal $4 / 5$ dilated into a club-shaped seminal chamber. Ampulla of the fourth pair elongate-oval, duct as long as $1 / 6$ ampulla, diverticulum as long as main chain, U-shaped twist in the middle, terminal $1 / 3$ dilated into a chili-shaped seminal chamber. One round, semitransparent accessory gland presents near the medial area of each spermatheca. The prostate glands are developed.

Description. External characters: Pigment from brown to no pigment on dorsum, from light brown to no pigment on ventrum. Dimensions 102 mm by 4.5 mm at clitellum, 117 segments. Prostomium $1 / 2$ epilobous. First dorsal pore in segments 12/13. Setae numbering 26 at III, 26 at V, 29 at VIII, 36 at XX, 37 at XXV; 9 between male pores. Setae between spermathecal pores numbering 13 at VI, 12 at VII, 14 at VIII. Setal formula: $\mathrm{AA}=1.0-1.4 \mathrm{AB}, \mathrm{ZZ}=2.0-2.2 \mathrm{ZY}$. Clitellum annular, in XIVXVI, setae not visible externally. Four pairs of spermathecal pores in 5/6-8/9, 0.40 circumference apart ventrally. Four pairs of genital papillae on VI-IX, 0.25 circumference ventrally apart from each other. One pair of male pores in XVIII, 0.40 circumference ventrally apart from each other, each on the top of a larger raised, round porophore, surrounded by two circular ridges, with one presetal indented-topped genital papilla medial of each porophore (Figure 3A). Singled female pore in XIV, pale grey.

Internal characters. Septa 5/6-7/8 thick and muscular, 10/11-11/12 slightly thickened, 8/9-9/10 absent. Gizzard bucket-shaped, wider below than above, in VIIIX. Intestine enlarged distinctly from XV. Intestinal caeca paired in XXVII, simple, smooth, extending anteriorly to XXI. Esophageal hearts in X-XIII. Ovaries in XIII, four pairs of spermathecae in VI-IX, 2.2-3.0 mm long. Spermathecae of two shapes: ampulla of the first three pairs heart-shaped, duct stalked, diverticulum as long as main chain, U-shaped twist in the middle, terminal $4 / 5$ dilated into a club-shaped seminal chamber. The ampulla of the fourth pair elongate-oval, duct as long as $1 / 6$ ampulla (Figure 3C), and diverticulum as long as main chain, U-shaped twisted in the middle, terminal $1 / 3$ dilated into a chili-shaped seminal chamber (Figure 3B). One round semitransparent accessory gland presents near the medial area of each spermatheca. Holandric: two pairs of testis sacs in X-XI, separated from each other, well developed. Two pairs of seminal vesicles, in XI-XII, well developed. Prostate glands developed, thick, inserting in XVIII and extending from XV to XXII, coarsely lobate, prostatic


Figure 4. A Ventral view showing spermathecal pores, female pores and male pores of Amynthas shengtangmontis sp. nov. B, C spermathecae of Amynthas shengtangmontis sp. nov. D illustration of the details of the male pore region.
duct U-curved, slightly thicker at the ental part. Two stalked accessory glands near the medial area of the distal part of the prostatic duct.

Etymology. The species is named after the name of the collection site Shengtang Mountain, a famous peak of the Dayao Mountain National Nature Reserve.

Remarks. Amynthas shengtangmontis sp. nov. keys to the corticis-group in Sims and Easton (1972). In terms of morphology, it is closely related to $A$. carnosus, $A$. corticis, and Amynthas pulvinus Sun \& Jiang, 2013 (described from Hainan Island). Body size, body pigment, setal numbers, the first dorsal pore location, and simple intestinal caeca are similar among the four species.

In contrast, distance between the spermathecal pores and the male pores of the new species is 0.40 C body circumference compared with 0.33 C in $A$. carnosus, $A$. corticis, and $A$. pulvinus. In addition, there are eight postsetal genital markings on VI-IX in the new species, but the markings are present on V-VIII in $A$. carnosus, and there are more than two pairs of markings on VIII and IX in A. carnosus. The porophore of the new species is large, raised, round, and surrounded by two circular ridges, whereas the porophore is small in A. corticis and no genital markings apparent on the spermathecal pore region in A. pulvinus. Moreover, the new species has two different shapes of spermathecae, heart-shaped ampulla and diverticulum with club-shaped seminal chamber; and long-oval ampulla and diverticulum with chili-shaped seminal chamber, which are very different from those in the other species (Table 4).

Table 4. A comparison of characters of $A$. shengtangmontis shengtangmontis, A. shengtangmontis minusculus, A. carnosus, A. corticis. and A. pulvinus.

| Characteristics | A. shengtangmontis <br> shengtangmontis | A. shengtangmontis <br> minusculus |  <br> Hatai, 1899) | A. corticis <br> (Kinberg, 1867) | A. pulvinus <br> Sun \& Qiu, |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| 2013 |  |  |  |  |  |

Amynthas shengtangmontis minusculus Dong \& Law, subsp. nov. http://zoobank.org/AE048B96-5A37-4C48-A734-22EA11119010
Figure 5, Table 4
Material. Holotype: 1 clitellate (C-GX201316-02A): China, Guangxi Zhuang Autonomous Region, Dayao Mountain Nature Reserve ( $24.16658^{\circ} \mathrm{N}, 110.24313^{\circ} \mathrm{E}$ ), 1285 m asl, black sandy soil under bryophytes beside road, 16 May 2013, JP Qiu, Y


Figure 5. A Ventral view showing spermathecal pores, female pores and male pores of Amynthas shengtangmontis minusculus subsp. nov. B spermathecae of Amynthas shengtangmontis minusculus subsp. nov. C illustration of the details of the male pore region.

Hong, JB Jiang, LL Zhang, and Y Dong legit. Paratypes: 6 clitellate (C-GX20131602B): same date as for holotype.

Diagnosis. Dimensions $75-83 \mathrm{~mm}$ by $3.0-3.2 \mathrm{~mm}$ at clitellum, $75-87$ segments. First dorsal pore in 11/12. Setae numbering 21-26 at III, 18-21 at V, 27-32 at VIII, 29-34 at XX, 36-40 at XXV; 5-7 between male pores; Setae between spermathecal pores numbering 9-11 at VI, 10-12 at VII, 10-12 at VIII. Four pairs of spermathecal pores in 5/6-8/9. Three pairs of postsetal genital papillae arranged in VI-VIII, 0.13 circumference apart ventrally. One pair of male pores in XVIII, each on the top of a raised, elliptic porophore surrounded by six circular ridges, with one small indentedtopped genital papilla medial of each male pore. Ampulla heart-shaped; stout duct as long as $1 / 2$ ampulla. Diverticulum as long as main pouch, terminal $1 / 2$ dilated into a long club-shaped seminal chamber. Prostate glands developed.

Description. External characters: Purple brown pigment on dorsum, no pigment on ventrum. Dimensions 83 mm by 3.2 mm at clitellum, 87 segments. Prostomium $1 / 2$ epilobous. First dorsal pore in 11/12. Setae numbering 26 at III, 21 at V, 32 at VIII, 34 at XX, 38 at XXV; 6 between male pores; setae between spermathecal pores numbering

10 at VI, 11 at VII, 12 at VIII. Setal formula $\mathrm{AA}=1.2-1.4 \mathrm{AB}, \mathrm{ZZ}=1.4-1.8 \mathrm{ZY}$. Clitellum annular in XIV-XVI, setae not visible externally. Four pairs of spermathecal pores in 5/6-8/9, 0.40 circumference ventrally apart from each other. Three pairs of postsetal genital papillae arranged in VI-VIII. One pair of male pores in XVIII, 0.40 circumference apart ventrally, each on the top of a raised, elliptic porophore surrounded by six circular ridges, with one small indented-topped genital papilla in the center of each male pore (Figure 4A). Single female pore in XIIV.

Internal characters. Septa 5/6-7/8, thick and muscular, 10/11-11/12 slightly thickened, 8/9-9/10 absent. Gizzard bucket-shaped, wider below than above, in IXX. Intestine enlarged distinctly from XV. Intestinal caeca paired in XXVII, simple, smooth, extending anteriorly to $1 / 2$ XXIV. Esophageal hearts in X-XIII. Ovaries in XIII, four pairs of spermathecae in VI-IX, heart-shaped, $2.2-2.7 \mathrm{~mm}$ long, duct as long as $1 / 2$ ampulla. Diverticulum as long as main pouch, terminal $1 / 2$ dilated into a long club-shaped seminal chamber. Six semitransparent elliptical accessory glands observed near the ental part of the last three pairs spermathecae (Figure 4B). Holandric: two pairs of testis sacs, separated from each other, in X-XI, oval, the first pair extremely developed. Two pairs of seminal vesicles, in XI-XII, developed. Prostate glands, thick, inserting in XVIII and extending from XVI to XXI, developed, coarsely lobate; prostatic duct U-curved, long, slightly thicker at the ental part.

Remarks. We compare $A$. shengtangmontis minusculus subsp. nov. with $A$. shengtangmontis shengtangmontis and find that they share similar characters of spermathecal pores, male pores, intestinal caeca, and prostate glands. However, there is a level of difference between them. For instance, $A$. shengtangmontis minusculus subsp. nov. has a smaller body size, fewer and more closely spaced genital papillae, longer spermathecal duct, accessory glands only observed in the spermathecal area. On the other hand, the first dorsal pore of $A$. shengtangmontis minusculus subsp. nov. is located in 11/12 compared to in 12/13 in $A$. shengtangmontis shengtangmontis. The pairwise distance of COI between $A$. shengtangmontis shengtangmontis and $A$. shengtangmontis minusculus subsp. nov. is $10.7 \%-11.4 \%$, which is acceptable to differentiate subspecies.

Etymology. The subspecies is named after its small body size, compared to the nominate species.

## Molecular results

In addition to the morphological comparison between the species, we also compared the COI gene sequences of the three proposed new species and one subspecies and the results of the pairwise distances of COI, ranging from $10.7 \%-25.2 \% \%$ are shown in Table 5. Studies show that pairwise distances of COI of interspecies in the same genus are 17-23\% (Sun 2013), 16-23\% (Huang et al. 2007), 15-16\% (Admassu et al. 2006), 16-22\% (Novo et al. 2009), and 15-28\% (Chang et al. 2008). In general, pairwise distances between three new species and the other eighteen corticis-group species are greater than $14.7 \%$. Together with the different morpho-
Table 5. Percentage of pairwise distances obtained for the sequences of COI genes in Amynthas species.

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| S1 HT |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| S2 HT | 19.2\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| S2 PT | 19.2\% | 0.0\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| S2 PT | 19.2\% | 0.0\% | 0.0\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| S3 HT | 16.4\% | 15.7\% | 15.7\% | 15.7\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| S4 HT | 16.7\% | 14.7\% | 14.7\% | 14.7\% | 10.7\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| S4 PT | 16.9\% | 15.4\% | 15.4\% | 15.4\% | 11.4\% | 0.2\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A. fuscatus | 15.9\% | 17.4\% | 17.4\% | 17.4\% | 20.4\% | 17.4\% | 17.0\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A. pulvinus | 20.0\% | 18.2\% | 18.2\% | 18.2\% | 19.7\% | 18.4\% | 18.8\% | 18.6\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A. robustus | 18.9\% | 16.9\% | 16.9\% | 16.9\% | 17.8\% | 18.0\% | 18.0\% | 17.1\% | 22.7\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A. corticis | 15.6\% | 18.0\% | 18.0\% | 18.0\% | 18.2\% | 18.7\% | 18.8\% | 18.0\% | 16.3\% | 18.0\% |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A. carnosus | 19.8\% | 17.4\% | 17.4\% | 17.4\% | 20.2\% | 20.0\% | 19.9\% | 18.3\% | 18.7\% | 16.7\% | 16.7\% |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A. micronarius | 20.7\% | 19.8\% | 19.8\% | 19.8\% | 19.9\% | 19.5\% | 20.1\% | 21.0\% | 19.1\% | 20.9\% | 17.5\% | 21.2\% |  |  |  |  |  |  |  |  |  |  |  |  |
| A.alexandri | 24.3\% | 21.3\% | 21.3\% | 21.3\% | 23.7\% | 22.4\% | 22.0\% | 21.3\% | 21.5\% | 20.7\% | 21.5\% | 20.8\% | 22.6\% |  |  |  |  |  |  |  |  |  |  |  |
| A.andersoni | 23.2\% | 20.1\% | 20.1\% | 20.1\% | 23.0\% | 21.0\% | 20.9\% | 18.9\% | 21.2\% | 19.6\% | 17.3\% | 18.4\% | 20.9\% | 23.6\% |  |  |  |  |  |  |  |  |  |  |
| A.comptus | 21.9\% | 19.2\% | 19.2\% | 19.2\% | 20.7\% | 19.4\% | 20.2\% | 19.1\% | 21.2\% | 19.6\% | 18.5\% | 20.0\% | 20.5\% | 23.5\% | 19.2\% |  |  |  |  |  |  |  |  |  |
| A.exiguus | 18.2\% | 18.0\% | 18.0\% | 18.0\% | 18.1\% | 18.5\% | 18.7\% | 18.2\% | 18.6\% | 18.7\% | 18.5\% | 19.1\% | 18.3\% | 24.2\% | 21.3\% | 22.1\% |  |  |  |  |  |  |  |  |
| A.formosae | 21.4\% | 21.0\% | 21.0\% | 21.0\% | 19.3\% | 21.9\% | 22.0\% | 23.2\% | 19.8\% | 22.7\% | 20.3\% | 22.7\% | 20.8\% | 20.5\% | 24.4\% | 24.1\% | 23.5\% |  |  |  |  |  |  |  |
| A. longicauliculatus | 20.2\% | 19.5\% | 19.5\% | 19.5\% | 21.6\% | 21.8\% | 22.1\% | 19.6\% | 21.5\% | 21.3\% | 19.8\% | 22.6\% | 21.7\% | 22.9\% | 18.3\% | 18.1\% | 20.2\% | 23.9\% |  |  |  |  |  |  |
| A.mirifus | 17.6\% | 16.6\% | 16.6\% | 16.6\% | 18.7\% | 17.1\% | 17.1\% | 16.1\% | 16.7\% | 17.9\% | 15.3\% | 17.4\% | 20.5\% | 19.2\% | 20.7\% | 19.8\% | 18.9\% | 21.3\% | 19.7\% |  |  |  |  |  |
| A.szechuanensis | 22.5\% | 20.4\% | 20.4\% | 20.4\% | 22.6\% | 20.4\% | 20.5\% | 19.3\% | 22.4\% | 21.8\% | 18.2\% | 19.8\% | 21.5\% | 24.7\% | 19.3\% | 18.9\% | 20.0\% | 24.0\% | 18.3\% | 21.4\% |  |  |  |  |
| A.mediocus | 19.4\% | 19.4\% | 19.4\% | 19.4\% | 19.4\% | 23.8\% | 23.6\% | 18.0\% | 19.8\% | 19.1\% | 18.5\% | 20.5\% | 21.4\% | 25.2\% | 22.5\% | 18.7\% | 18.2\% | 24.2\% | 20.9\% | 18.8\% | 22.5\% |  |  |  |
| A.wulinensis | 20.2\% | 18.3\% | 18.3\% | 18.3\% | 21.8\% | 18.4\% | 18.4\% | 19.4\% | 20.9\% | 20.2\% | 17.8\% | 19.7\% | 18.2\% | 25.1\% | 20.7\% | 21.5\% | 20.4\% | 23.0\% | 23.2\% | 19.9\% | 19.2\% | 19.7\% |  |  |
| A.yunlongensis | 19.7\% | 18.6\% | 18.6\% | 18.6\% | 18.4\% | 16.8\% | 17.3\% | 19.2\% | 18.0\% | 17.4\% | 17.8\% | 17.9\% | 20.3\% | 21.3\% | 22.2\% | 19.8\% | 17.1\% | 21.0\% | 20.0\% | 17.0\% | 16.2\% | 20.3\% | 20.5\% |  |
| A.stricosus | 16.3\% | 15.0\% | 15.0\% | 15.0\% | 18.2\% | 16.6\% | 16.4\% | 13.1\% | 18.4\% | 17.1\% | 16.1\% | 17.2\% | 20.8\% | 18.4\% | 17.3\% | 17.6\% | 21.7\% | 18.8\% | 18.4\% | 19.5\% | 20.8\% | 18.1\% | 16.5\% | 21.1\% |

Notes: S1 represent $A$. maximus, S 2 represent $A$. tortuosus, S 3 represent $A$. shengtangmontis shengtangmontis, S 4 represent $A$. shengtangmontis minusculus
logical characters of each, we could conclude that $A$. maximus, $A$. tortuosus, $A$. shengtangmontis shengtangmontis, and $A$. shengtangmontis minusculus are different from the previously described species and each other. Since the pairwise distance between $A$. shengtangmontis shengtangmontis and $A$. shengtangmontis minusculus is $10.7 \%-$ $11.4 \%$, which is more than $1 \%$ and less than $15 \%$, by definition we conclude that both subspecies are valid.

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## References:

Admassu B, Juen A, Traugott M (2006) Earthworm primers for DNA-based gut content analysis and their cross-reactivity in a multi-species system. Soil Biology and Biochemistry 38(6): 1308-1315. https://doi.org/10.1016/j.soilbio.2005.08.019
Blakemore RJ (2004) Checklist of Pheretimoid earthworms after Sims \& Easton (1972). In: Moreno AG, Borges S (Eds) Advances in earthworm taxonomy (Annelida: Oligochaeta). Madrid: Editorial Complutense, SA, 127-154.
Bely AE, Wray GA (2004) Molecular phylogeny of naidid worms (Annelida: Clitellata) based on cytochrome oxidase I. Molecular Phylogenetics and Evolution 30(1): 50-63.
Chang CH, Snyder BA, Szlavecz K (2016) Asian pheretimoid earthworms in North America north of Mexico: an illustrated key to the genera Amynthas, Metaphire, Pithemera, and Polypheretima (Clitellata: Megascolecidae). Zootaxa 4179(3): 495-529. https://doi. org/10.11646/zootaxa.4179.3.7
Chang CH, Lin SM, Chen JH (2008) Molecular systematics and phylogeography of the gigantic earthworms of the Metaphire formosae species group (Clitellata, Megascolecidae). Molecular Phylogenetics and Evolution 49(3): 958-968. https://doi.org/10.1016/j. ympev.2008.08.025
Chang CH, Lin YH, Chen IH, Chuang SC, Chen JH (2007) Taxonomic re-evaluation of the Taiwanese montane earthworm Amynthas wulinensis Tsai, Shen \& Tsai, 2001 (Oligochaeta: Megascolecidae): Polytypic species or species complex? Organisms Diversity \& Evolution 7(3): 231-240. https://doi.org/10.1016/j.ode.2006.06.001
Chen Y (1938) Oligochaeta from Hainan, Kwangtung. Contributions from the Biological Laboratory of the Science Society of China Zoological series 12: 375-427.
Chen Y, Hsü CF, Yang T, Feng HY (1975) On some New Earthworms from China. Acta Zoologica Sinica 21: 89-99.

Chen Y, Xu ZF (1977) On some New Earthworms from China II. Acta Zoologica Sinica 23: 175-181.
Dong Y, Yuan Z, Jiang JB, Zhao Q, Qiu JP (2018) Two new species of earthworms belonging to the genus Amynthas (Oligochaeta: Megascolecidae) from Guangxi Province, China. Zootaxa 4496(1): 259-268. https://doi.org/10.11646/zootaxa.4496.1.21
Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology Biotechnology 3: 294-299. https://doi.org/10.4028/www.scientific.net/DDF.7.460
Hong Y, James SW (2001) Five new earthworms of the genus Amynthas Kinberg (Megascolecidae) with four pairs of spermathecae. Zoological Studies 40: 269-275. http://www. sinica:edu.tw/zool/zoolstud/40.4/269.pdf
Hong Y, Kim TH (2002) Four new earthworms of the genus Amynthas (Oligochaeta: Megascolecidae) from Korea. Korean Journal of Biological Sciences 6: 195-199. https://doi.org/ 10.1080/12265071.2002.9647651

Huang J, Xu Q, Sun ZJ (2007) Identifying earthworms through DNA barcode. Pedobiologia 51(4): 301-309. https://doi.org/10.1016/j.pedobi.2007.05.003
James SW, Shih HT, Chang HW (2005) Seven new species of Amynthas (Clitellata: Megascolecidae) and new earthworm records from Taiwan. Journal of Natural History 39: 10071028. https://doi.org/10.1080/00222930400001434

Jiang JB (2016) Taxonomy and Molecular Phylogeny of the Family Megascolecidae Earthworms from China. PhD Thesis, Shanghai Jiao Tong University, Shanghai.
Jiang JB, Dong Y, Yuan Z, Qiu JP (2018) Three new earthworm species of the tokioensis-group in the genus Amynthas (Oligochaeta: Megascolecidae) from Guangxi Province, China. Zootaxa 4496(1): 269-278. https://doi.org/10.11646/zootaxa.4496.1.22
Jeratthitikul E, Bantaowong U, Panha S (2017) DNA barcoding of the Thai species of terrestrial earthworms in the genera Amynthas and Metaphire (Haplotaxida: Megascolecidae). European Journal of Soil Biology 81:39-47. https://doi.org/10.1016/j.ejsobi.2017.06.004
Kinberg JGH (1867) Annulata nova. Öfversigt af Kongliga Vetenskaps-akademiens förhandlingar 23: 97-103.
Novo M, Almodovar A, Diaz-Cosin DJ (2009) High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications. Zoologica Scripta 38(5): 537-552. https://doi.org/10.1111/j.14636409.2009.00389.x

Sims R, Easton E (1972) A numerical revision of the earthworm genus Pheretima auct. (Megascolecidae: Oligochaeta) with the recognition of new genera and an appendix on the earthworms collected by the Royal Society North Borneo Expedition. Biological Journal of the Linnean Society 4: 169-268. https://doi.org/10.1111/j.1095-8312.1972.tb00694.x
Sun J (2013) Taxonomy and Molecular Phylogeny of Amynthas Earthworms from China. PhD Thesis, Shanghai Jiao Tong University, Shanghai.
Sun J, Jiang JB, Qiu JP (2012) Four new species of the Amynthas corticis-group (Oligochaeta: Megascolecidae) from Hainan Island, China. Zootaxa 3458: 149-158. https://doi. org/10.11646/zootaxa.3458.1.8

Sun J, Zhao Q, Jiang JB, Qiu JP (2013) New Amynthas species (Oligochaeta: Megascolecidae) from south and central Hainan Island, China and estimates of evolutionary divergence among some corticis-group species. Journal of natural history 47(17-18): 1143-1160. https://doi.org/10.1080/00222933.2012.743613
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular biology and evolution 28(10): 2731-2739. https:// doi.org/10.1093/molbev/msr121
Tsai CF, Shen HP, Tsai SC (2001) Some new earthworms of the genus Amynthas (Oligochaeta: Megascolecidae) from Mt. Hohuan of Taiwan. Zoological Studies 40: 276-288. http:// www.sinica.edu.tw/zool/zoolstud/40.4/276.pdf
Tsai CF, Shen HP, Tsai SC, Lee HH (2007) Four new species of terrestrial earthworms belonging to the genus Amynthas (Megascolecidae: Oligochaeta) from Taiwan with discussion on speculative synonyms and species delimitation in oligochaete taxonomy. Journal of Natural History 41: 357-379. https://doi.org/10.1080/00222930701201279
Tsai CF, Shen HP, Tsai SC (2010) Four new species of Amynthas earthworms (Oligochaeta: Megascolecidae) from the Central Mountain Range of southern Taiwan. Journal of Natural History 44: 1251-1267. https://doi.org/10.1080/00222931003624788
Wang YH, Shih HT (2010) Earthworm fauna of Eastern Taiwan, with descriptions of two new species (Oligochaeta: Megascolecidae). Zootaxa 2341: 52-68. https://doi. org/10.1186/1742-9994-7-5
Zhao Q (2015) Taxonomy, Phylogeny and Paleogeography of Pheretimoid Earthworm Species in Hainan Island (China). PhD Thesis, Shanghai Jiao Tong University, Shanghai.

# Tryphactothripini of India (Thysanoptera, Thripidae, Panchaetothripinae), with identification keys and a new record of Opimothrips 

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#### Abstract

An identification key is provided to the four genera of Panchaetothripinae from India that are members of the Tribe Tryphactothripini, together with a key to identify all the known six Indian species of Astrothrips. Furthermore, the genus Opimothrips is newly reported from India.


## Keywords

Astrothrips, Opimothrips tubulatus

## Introduction

The Thripidae subfamily Panchaetothripinae currently comprises approximately 145 species in 42 genera (ThripsWiki 2019). These species breed on the leaves of a wide range of plants in tropical countries, and include the widespread pest, the Greenhouse Thrips, Heliothrips haemorrhoidalis (Bouché) (Xie et al. 2019). Within this subfamily is a tribe, Tryphactothripini, that comprises species in which the second abdominal tergite is sharply constricted anteriorly and bears complex sculpture anterolaterally. This sculpture is in the form of closely spaced, small ridges that often give the optical impression of
being stout, sharply recurved, microtrichia. Twenty species in nine genera are currently placed in this tribe, and four of these genera, with nine species, are recorded from India.

Members of this tribe are restricted to the tropics, with a few Astrothrips species also found in subtropical areas. Despite being leaf feeders, a few adults have also been taken from grass. Recently, an additional genus of Tryphactothripini was found in India, Opimothrips. This monotypic genus was described from Thailand and also recently reported from China (Nonaka and Okajima 1992; Xie et al. 2019). The objective here is to give an account of the tribe Tryphactothripini in India and to report a new record of genus Opimothrips with a note on $O$. tubulatus.

## Taxonomy

## Key to genera of Tryphactothripini from India

1 Fore wing distal half with costal setae longer than fringe cilia........Noathrips

- Fore wing distal half with costal setae shorter than fringe cilia..................... 2

2 Genae without protruding fringe of pale sculpture; fore wing uniformly shaded with apex sharply paler Opimothrips

- Genae with protruding fringe of pale sculpture, fore wing with dark and light bands 3
3 Abdominal tergites III-VII with paired clusters of round sculptured areolae, striated across anterior third, bearing sigmoidal setae .........Tryphactothrips*
- Abdominal tergites III-VII without paired clusters of round sculptured areolae; reticulated across anterior third, sigmoidal setae present or absent Astrothrips


## Astrothrips Karny, 1921

Astrothrips Karny, 1921: 239. Type species Heliothrips globiceps Karny, 1913.

Notes. This genus was based originally on a single species that had been described from a single female collected in New Guinea, but four generic synonyms are listed in ThripsWiki (2019).

It is an Old World genus that is restricted to tropical countries, with two species from Africa and 10 distributed between Pakistan and New Guinea (ThripsWiki 2019). These are leaf-feeding thrips, with the occasional adult found in flowers, and although adults have been taken from a wide variety of plants, suggesting the possibility of polyphagy, larvae remain unknown for most of the described species. The many published host-plant associations (Table 1) involve more than 30 plant families, with little indication of any specificity. These records are based on the collection of one or more adults

[^2]Table I. Astrothrips distributions and host-associations of adults. Data from: Akram (2000); Kudô (1979, 1995); Mirab-balou et al. 2011; Reyes (1994); Saeed and Yousuf (1994); Tillekaratne et al. (2011).

| Species | No. of antennal segments | Distribution | Host associations | Larvae found |
| :---: | :---: | :---: | :---: | :---: |
| A. asiaticus | 8 | India | Colocasia; Lantana; Ricinus; Wedelia; "weeds" | No |
| A. aucubae | 7 | China; Japan; Philippines | Aucuba; Thalictrum | No |
| A. aureolus | 5 | Malaysia; Australia | Hymenocallis | No |
| A. bhattii | 5 | Nigeria | Citrus; Colocasia; Cucurbita; "palm" | On Citrus grandis |
| A. chisinliaoensis | 6 | Malaysia; China; Taiwan | Canthium; Carallia; Morus; Rbus | No |
| A. globiceps | 6 | India; Myanmar; Malaya; Thailand; Philippines; New Britain; New Guinea; Japan | Angiopteris; Calopogonium; Centrosema; Colocasia; Crinum; Dysoxylum; Hyptis; Lantana; Microdesmis; Pleocnemia; Plumeria; Portulaca; "bamboo" | No |
| A. lantana | 7 | India; Nepal | Lantana; Quercus; "evergreen tree" | No |
| A. parvilimbus | 6 | India | Antirrbinum; Boerhavia; Crinum; Erythrina; Ipomoea; Musa; Ricinus; Sida; "fern" | No |
| A. roboris | 5 | Nigeria; Ghana; Sudan | Colocasia; Cucurbita; Musa; Nicotiana; Phaseolus; Piper, Thunbergia; "climbing legume" "palm" | On Colocasia esculenta |
| A. stannardi | 7 | India; Pakistan | Celosia; Ipomoea; Lantana; Mirabilis; Verbascum | No |
| A. strasseni | 8 | Myanmar; China | "bamboo" | No |
| A. tumiceps | 7 | India, Pakistan; Sri Lanka; Malaysia; Myanmar; Indonesia; Philippines; Thailand; Japan; Australia | Artocarpus; Canna; Centrosema; Dysoxylum; Dolichos; Eleusine; Erythrina; Glycine; Gossypium; Lantana; Mallotus; Phaseolus; Ricinus; "fern" | No |

from any given plant, and thus may involve a flying adult simply resting on a plant surface without feeding. Possibly these records are more of a measure of the dispersive behavior of adults, rather than an indication of the plants on which they might breed. Moreover, most of the species are known from few specimens. This combination of small sample size and lack of biological information leads to a lack of confidence in the relatively trivial structural differences that have been used to distinguish some of the species. The species of Astrothrips from India were reviewed by Bhatti (1967), with many clear illustrations in the form of line drawings, and Ananthakrishnan and Sen (1980) provided a further key to the species from India but without illustrations.

Diagnosis. Small, dark brown, strongly reticulate Panchaetothripinae usually with banded fore wings bearing stout veinal setae. Antennae with 5-8 segments, sense cones on III and IV forked or simple. Head with conspicuous raised reticulate sculpture, setae all small, ocellar area sometimes elevated; maxillary palps bi-segmented. Pronotum transverse, strongly sculptured with some raised reticulation. Mesoscutum usually deeply notched;
metascutum with prominent triangle. Tarsi 1-segmented. Fore wing slender, both longitudinal veins with prominent setae; costal setae shorter than costal cilia; posteromarginal cilia wavy. Tergite II of abdomen with anterior margin strongly constricted, and anterolaterally with a group of prominent strongly recurved microtrichia; tergites III-VII with transverse reticulation on anterior half; VIII with no posteromarginal comb; X divided longitudinally. Male smaller, sternites with or without slender, deeply curved, pore plates.

Antennal segmentation. The 8 -segmented condition of antennae is considered plesiotypic for the family Thripidae (Zhang et al. 2019). However, among some Panchaetothripinae genera, including Astrothrips, there are species with the distal segments fused in different combinations, such as segments VI+VII or VII+VIII, or sometimes VI-VIII or even V-VIII forming a terminal group. As a result of this fusion the number of apparent segments is reduced to seven, six, or even five. It is important to recognise that reduction in the number of segments is not necessarily a shared apomorphy, because the 7-segmented condition could arise by fusion of either VII+VIII or by VI+VII (Zhang et al. 2019).

Species recognition. Species level taxonomy in this genus is based on some relatively trivial character states, each of which may have been observed on very few specimens. Stannard and Mitri (1962) described aureolus from only two females and distinguished this new species from globiceps and parvilimbus. The three diagnostic characters selected by the authors (shape of antennal segment III, colour of costal setae, body colour) are now considered to be variable among more recently collected specimens. Some other character states used by authors to distinguish species in this genus have been found to be more variable with the discovery of more specimens. Bhatti (1967) described stannardi as having the major sense cone on antennal segment VI surpassing the apex of the antenna, but this has been found to be untrue on various specimens of the species collected from South India and Thailand. Wilson (1975) in his key to species treated stannardi in the group with five or six antennal segments, but in the main text under that species, he states that there are seven segments; this confusion is repeated by Ananthakrishnan and Sen (1980). Subsequent identifications that are based solely on such original descriptions may not be reliable. The male of aureolus has been unknown, but a male identified as this species from Timor Leste (in ANIC) has U-shaped pore plates on sternites IV-VII as in stannardi. Similar problems are involved in host-plant associations. For example, Bhatti (1967) described lantana from two females, but Wilson (1975) mentions weekly collections from Lantana camara near the type locality without finding this thrips. In contrast, Kudô (1995) identified three females from Nepal as lantana that were taken from a species of Quercus and an unidentified tree.

## Key to Indian species of Astrothrips

1 Antennae 8 segmented; segments separated by clear sutures........A. asiaticus

- Antennae with 5 to 7 segments .................................................................... 2

2 Pronotum posterior margin without a sub-marginal transverse apodeme [male with no sternal pore plates]
A. tumiceps

- Pronotum posterior margin with distinct transverse sub-marginal apodeme .... 3

3 Pronotal sub-marginal transverse apodeme weak, present only on median third (Fig. 7); posterolateral angles of pronotum with raised sculpture $\qquad$ A. globiceps

- Pronotal sub-marginal transverse apodeme strong, extending fully across pronotum (Fig. 8); no raised sculpture on pronotal posterolateral angles. 4
4 Antennae 6-segmented; mesoscutal anterior notch shorter than median intact part [male with pore plates on sternites V-VII]. A. parvilimbus
- Antennae 7-segmented................................................................................ 5

5 Antennal segments V-VII separated by clear sutures; male not known
A. lantana*

- Antennal segments V-VII not separated by clear sutures; male with pore plates on sternites IV-VII
A. stannardi


## Noathrips Bhatti, 1967

Noathrips Bhatti, 1967: 9. Type species Noathrips prakashi Bhatti, 1967 by monotypy.

Notes. The genus was erected with the type species prakashi, collected on herbage and Lantana leaves from Jabalpur, Madhya Pradesh (Bhatti 1967). Later, this species was collected in Sri Lanka and China (Kudô 1979; Xie et al. 2019).

Diagnosis. Antennae 8 -segmented, III and IV with forked sense cones. Head without conspicuous raised structure, postocular seta 4 strongly developed, ocellar hump small; maxillary palps bi-segmented. Pronotum with weakly raised sculpture. Mesoscutum not notched anteriorly; metascutum with prominent reticulate triangle. Tarsi 1-segmented. Fore wing with slender pointed setae; costal setae longer than fringe cilia; posteromarginal setae wavy. Abdominal tergite I with a postmarginal flange; II anterior margin constricted, with narrow plate like cuticular processes laterally; III-VII with transverse reticulations on anterior half, posterior half smooth; X asymmetrical, divided longitudinally. Males smaller; sternites IV-VII each with transversely elongated anteriorly concave pore plates.

## Opimothrips Nonaka \& Okajima, 1992

Opimothrips Nonaka and Okajima 1992: 106. Type species: Opimothrips tubulatus Nonaka \& Okajima, 1992 by monotypy.

Notes. The reports of $O$. tubulatus from Thailand and China are from grass, and hence Xie et al. (2019) reported the species as being associated with grasses. However, the present specimens have been collected from an unidentified weed.

[^3]Diagnosis. Antennae 8 -segmented, III and IV with thin, Y-shaped sense cones with the arms curving around the segment. Head polygonally reticulate, cheeks constricted at base; maxillary palps bi-segmented. Pronotum uniformly reticulate, two pairs of campaniform sensilla, one pair of long setae. Mesoscutum entire; metascutum with reticulate triangle. Tarsi 1 -segmented. Fore wing with prominent veins, costal setae shorter than fringe cilia; posteromarginal setae wavy. Abdominal tergite I reticulate, median pair of setae minute; II strongly constricted, wart-like tubercles laterally; III-VII with thick antecostal line; X asymmetrical, divided longitudinally.

## Opimothrips tubulatus Nonaka \& Okajima, 1992

Figures 1-6
Material studied. Three females, Chitradurga, Karnataka, India, on unidentified weed, 04 December 2017, Rachana R.R. leg. Two females deposited in the Insect Museum, National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru, India. One female deposited in ANIC - Australian National Insect Collection, CSIRO, Canberra.

Female macroptera. Body yellowish brown (Fig. 1), fore legs yellow, tarsi yellow, tibiae brown, yellow in apical half and basally, femora brown in basal half, rest yellow; antennal segments I-III golden yellow, IV-V yellow with shaded brown distally, VI-VIII dark brown; fore wing uniformly shaded with apex pale, clavus brown; first vein with 11 setae, not uniformly arranged; second vein with 6 setae. Antennae 8 -segmented; sutures complete and distinct between all segments; III-IV with thin, Yshaped sense cones, the arms unusually thin and curving around the segment, narrow apex on IV shorter, wider, more abruptly constricted than III; outer sense cone on VI extending to midpoint of VIII (Fig. 3). Head wider than long; ocellar hump weakly developed, ocelli visible; major setae rudimentary; eyes not bulged, covering lateral side almost completely; genae much reduced, without protruding transparent fringe (Fig. 2). Pronotum reticulate, raised sculpture on lateral margins; median area with transverse reticulations (Fig. 2). Mesonotum anterior margin shallowly notched, not reaching beyond anterior one third; 2 pairs of small setae, the inner pair anterior to the outer pair (Fig. 4). Metanotal median triangle weakly indicated; polygonally reticulate, extending beyond posterior margin, median setae anterior to campaniform sensilla (Fig. 4). Fore wing base humped, costal setae shorter than fringe; first vein with 7 basal setae, 2 at middle and 2 distally, thin and pointed; second vein with 6 setae, curved except last three; clavus with 4 veinal setae but no discal seta; posteromarginal cilia wavy (Fig. 6). Fore tibia with a spine at apex; hind tibia with a row of 11 conspicuous spines on inner side and two stout ones at apex; hind tarsi with a spine at median on inner side and two short, stout ones at apex. Abdominal tergite I reticulations extending beyond margin; median area of II with weak reticulations, laterally with wartlike tubercles; thick sublateral antecostal line on III-VII, laterally forming a posterior directed notch; VIII with complete posteromarginal comb of minute teeth; IX with campaniform sensilla; X asymmetric, median split complete, terminal setae almost half


Figures I-6. Opimothrips tubulatus I female $\mathbf{2}$ head and prothorax $\mathbf{3}$ antenna $\mathbf{4}$ pterothorax and female abdominal tergite I $\mathbf{5}$ female abdominal tergites IX-X $\mathbf{6}$ fore wing.
as long as the segment (Fig. 5). Sternites II-VII with 2 pairs of marginal setae on broad craspedum; antecostal lines on III-VII with median concave invagination. Ovipositor long, well developed, exceeding abdominal apex.


Figures 7, 8. Astrothrips species, head and pronotum 7 globiceps; female from Java, compared to holotype and identified by J.S. Bhatti $\mathbf{8}$ parvilimbus; female from Madras on Erythrina, identified by T.N. Ananthakrishnan. [images by Manfred Ulitzka].

## Tryphactothrips Bagnall

Tryphactothrips Bagnall, 1919: 256. Type species Dinurothrips rutherfordi Bagnall, 1915, by original designation.

Notes. Various workers on Thysanoptera [Bagnall (1919); Ramakrishna and Margabandhu (1931, 1940); Shumsher (1947); Patel and Patel (1953); Ananthakrishnan (1954); Wilson (1975)] reported T. rutherfordi from India. However, Bhatti (1967 and 1990) clarified the report of Ramakrishna and Margabandhu (1931) as Astrothrips tumiceps and of Patel and Patel (1953) from Pune as a species of Astrothrips. The records by Shumsher (1947) and Ananthakrishnan (1954) have never been validated, but Wilson (1975) collected three females from a forest tree in Tamil Nadu and compared these with the female holotype of T. rutherfordi in London. This is the only authenticated report of rutherfordi from India, and is only the second reliable report since the original description of the species from Ceylon.

Diagnosis. Antennae with six segments, terminal segments fused into an elongate unit. Head with raised sculpture covering cheeks and vertex; maxillary palps bi-segmented. Pronotum with raised sculpture anteriorly and on anterolateral angles. Mesoscutum
slightly notched. Tarsi 1-segmented. Fore wing with anterior vein fused to costa at fork of veins; costal setae shorter than costal cilia; posteromarginal cilia wavy. Abdominal tergite II sharply constricted, laterally with double based rod like processes; III-VII with paired clusters of round sculptured areolae, striated across anterior third, bearing pair of sigmoidal setae laterally; VIII with no posteromarginal comb; X asymmetric, divided longitudinally.

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## References

Akram W (2000) Systematics of Thysanoptera of Pakistan. PhD Thesis, University of Agriculture, Faisalabad.

Ananthakrishnan TN (1954) New and little known Indian Thysanoptera. Journal of Zoological Society of India 6(2): 159-166.
Ananthakrishnan TN, Sen S (1980) Taxonomy of Indian Thysanoptera. Zoological Survey of India, Handbook Series No.1, 234 pp.
Bagnall RS (1915) Brief descriptions of new Thysanoptera V. Annals and Magazine of Natural History 15(8): 315-324. https://doi.org/10.1080/00222931508693644
Bagnall RS (1919) Brief descriptions of new Thysanoptera X. Annals and Magazine of Natural History 4(9): 253-277. https://doi.org/10.1080/00222931908673889
Bhatti JS (1967) The Tryphactothrips complex in India, with a key to the world genera. Oriental Insects 1: 139-191. https://doi.org/10.1080/00305316.1967.10433858
Bhatti JS (1990) Catalogue of insects of the order Terebrantia from the Indian sub-region. Zoology 2(4): 205-352.
Karny H (1913) Beitrag zur Thysanopteren-Fauna von Neu-Guinea und Neu-Britannien. Archiv für Naturgeschichte 79: 123-136.
Karny H (1921) Zur Systematik der Orthopteroiden Insekten, Thysanoptera. Treubia 1: 211-269.
Kudô I (1979) Some panchaetothripine Thysanoptera from Southeast Asia. Oriental Insects 13: 345-355. https://doi.org/10.1080/00305316.1979.10433628
Kudô I (1995) Some Panchaetothripinae from Nepal, Malaysia and the Philippines (Thysanoptera: Terebrantia: Thripidae). Insecta Matsumurana New Series 52: 81-103.
Mirab-balou M, Tong XL, Feng JN, Chen XX (2011) Thrips (Insecta: Thysanoptera) of China. Check List: Journal of Species Lists and Distribution 7: 720-744. https://doi. org/10.15560/11009

Nonaka T, Okajima S (1992) Two new genera and species of the subfamily Panchaetothripinae (Thysanoptera: Thripidae) from Southeast Asia. Bulletin of the Biogeographical Society of Japan 47(11): 103-107.
Patel NG, Patel GA (1953) Host plants, distribution and abundance of thrips (Thysanoptera) of Bombay state. Journal of Bombay Natural History Society 51: 597-607.
Ramakrishna TV, Margabandhu V (1931) Notes on Indian Thysanoptera with brief descriptions of new species. Journal of the Bombay Natural History Society 34: 1025-1040.
Ramakrishna TV, Margabandhu V (1940) Catalogue of Indian insects - Thysanoptera. New Delhi, 64 pp .
Reyes CP (1994) Thysanoptera (Hexapoda) of the Philippine Islands. Raffles Bulletin of Zoology 42: 107-507.
Saeed M, Yousuf M (1994) Checklist of Thysanoptera of the Punjab (Pakistan). Pakistan entomologist 16: 59-61.
Shumsher Singh (1947) Studies on Indian Thysanoptera II. Records of Indian Museum 40: 201.
Stannard LJ, Mitri TJ (1962) Preliminary studies on the Tryphactothrips complex in which Anisopilothrips, Mesostenothrips and Elixothrips are erected as new genera (Thripidae: Heliothripinae). Transactions of the American Entomological Society 88: 183-224.
ThripsWiki (2019) ThripsWiki - providing information on the world thrips. http://thrips.info/ wiki/Main_Page [10.viii.2019]
Tillekaratne K, Edirisinghe JP, Gunatilleke CVS, Karunaratne WAIP (2011) Survey of thrips in Sri Lanka: a checklist of thrips species, their distribution and host plants. Ceylon Journal of Science 40(2): 89-108. https://doi.org/10.4038/cjsbs.v40i2.3926
Wilson TH (1975) A monograph of the subfamily Panchaetothripinae (Thysanoptera: Thripidae). Memoirs of the American Entomological Institute 23: 1-354.
Xie Y, Li Y, Li Z, Zhang H (2019) Two genera of Panchaetothripinae newly recorded from China, with first description of males of Opimothrips tubulatus (Thysanoptera, Thripidae). Zootaxa 4567(3): 583-586. https://doi.org/10.11646/zootaxa.4567.3.10
Zhang S, Mound LA, Feng J (2019) Systematic significance of antennal segmentation and sense cones in Thripidae (Thysanoptera: Terebrantia). Zootaxa 4554(1): 239-254. https:// doi.org/10.11646/zootaxa.4554.1.8

# Dealing with a hairy beast-larval morphology and chaetotaxy of the Australian endemic diving beetle genus Spencerhydrus (Coleoptera, Dytiscidae, Cybistrini) 

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#### Abstract

In this contribution, the larval morphology of Spencerhydrus Sharp, 1882 was studied, an Australian endemic genus in the diving beetle tribe Cybistrini. All instars of the only two species included in the genus (S. latecinctus Sharp, 1882 and S. pulchellus Sharp, 1882) are described and illustrated with the exception of the third instar of $S$. latecinctus. Detailed morphometric and primary chaetotaxic analyses were performed to discover useful characters for generic diagnosis and species distinction. Spencerhydrus can be distinguished from other Cybistrini genera by the medial projection of frontoclypeus slightly indented apically, with lamellae clypeales directed forward in a characteristic V-shaped pattern, the median process of prementum strongly developed, the presence of a single ventral sclerite on prothorax, the presence of basoventral spinulae on claws, and the reduced sclerotization of the abdominal segment VII which covers only the anterior half. Larvae of the two species of Spencerhydrus can readily be distinguished by the shape of the median process of prementum, which is visibly broader in $S$. pulchellus than in S. latecinctus.


## Keywords

Water beetle, sensilla, larva, morphometry

## Introduction

Spencerhydrus Sharp, 1882 is a small, Australian endemic genus of large diving beetles (adult length $17-18 \mathrm{~mm}$ ) included in the tribe Cybistrini. It is made up of two species restricted to southern Australia and with clearly separated distributions, S. latecinctus Sharp, 1882 in the south-east, and S. pulchellus Sharp, 1882 in the south-west (Watts 1978). Biological information for the genus is scarce; adults are rarely collected, mainly in lentic habitats with considerable emergent vegetation (Miller and Bergsten 2016). Recent studies based mainly on adult and molecular characters placed Spencerhydrus in a clade of Australian cybistrines together with Austrodytes Watts, 1978, Onychohydrus Schaum \& White, 1847, and Sternhydrus Brinck, 1945 (Miller et al. 2007; Miller and Bergsten 2014). Studies of larval characters (Michat et al. 2015, 2017) supported this placement, although Austrodytes was not included because its larva is unknown. Even though larval characters of Spencerhydrus were included in these phylogenetic analyses, larvae were not described or illustrated, and therefore morphology of members of this genus remains little known.

The only treatment of larvae of Spencerhydrus found in the literature is Watts (2002), in which the genus was included in a key to larvae of Australian Dytiscidae and illustrated with a photograph of the head and a drawing of the last two abdominal segments. A thorough morphometric and chaetotaxic treatment of dytiscid larvae, as that developed in the past three decades to complement the more traditional morphological study (see Alarie and Michat 2014 for a review), allows the discovery of useful characters at various taxonomic levels both for the diagnosis of taxa and for phylogenetic studies. In this context, we provide detailed descriptions and illustrations of the two species of Spencerhydrus with an emphasis on morphometry and chaetotaxy. With this treatment, we aim to recognize suitable characters to distinguish larvae of this genus from those of other cybistrine genera, and also to differentiate the larvae of its two known species, i.e., S. latecinctus and S. pulchellus.

## Materials and methods

Larvae were first cleared by submerging them for several days in lactic acid, then dissected in the standard way and mounted on slides with polyvinyl-lacto-glycerol as the medium. Examination (at magnifications up to $1,000 \times$ ) and drawings were made using an Olympus CX31 (Olympus Corporation, Japan) compound microscope equipped with a camera lucida. Drawings were scanned and digitally inked using a Genius PenSketch tablet (KYE Corporation, Taiwan). After study, the material will be held in the collection of the South Australian Museum.

The methods and terms used herein follow those employed in Michat $(2006,2010)$, Alarie et al. (2011), and Michat et al. (2015). The reader is referred to those papers for a complete list and additional explanations of the terms used here. The criterion of similarity of position (Wiley 1981) was primarily used to propose homology hypotheses. It is worth mentioning, however, that larvae of Cybistrini (and of Spencerhydrus in particular) bear numerous additional sensilla (i.e., those evolved secondarily in the
first instar) that obscure the establishment of positional homologies with the ancestral systems of other Dytiscidae. Alarie et al. (2011) showed that first instars of Cybistrini are characterized by the presence of multifid setae (i.e., setae that are split into two or more branches beyond the base), which are distributed similarly to the ancestral pattern of primary setae depicted for the subfamily Dytiscinae. The presence of a multifid seta was therefore considered as an additional argument for homology when more than one seta of Spencerhydrus larvae was potentially homologous with a given seta of other dytiscine genera. The ancestral chaetotaxy pattern thus established for Spencerhydrus is in good agreement with that observed in other genera (Alarie et al. 2011).

## Results

## Description of the larvae of Spencerhydrus Sharp, 1882

## Spencerhydrus Sharp, 1882

Diagnosis. Larvae of Spencerhydrus can be distinguished from those of other Cybistrini genera by the following combination of characters: medial projection of frontoclypeus slightly indented apically, with lamellae clypeales directed forward in a characteristic V-shaped pattern (Figs 1, 2, 31, 32); lateral projections of frontoclypeus entire, with lamellae clypeales directed forward (Figs 1, 2, 31, 32); notches separating medial and lateral projections of frontoclypeus wide (Figs 1, 2, 31, 32); antennomere 1 with two or three additional pores proximally (Figs 5-7); mandible lacking crown of multifid setae on distal fourth (Figs 9, 10); maxillary palpomere 1 subdivided into two articles (Figs 11-14); median process of prementum strongly developed (Figs 15-18); prothorax with a single ventral sclerite; seta TI7 short, spine-like (Figs 20, 22); ventral row of setae on tibia and tarsus formed by setae of different lengths (Figs 19, 21); claws with basoventral spinulae (Figs 19-22); urogomphi very small but still longer than broad, included together with anus in the membranous ventrodistal area of abdominal segment VIII (Figs 25, 26); urogomphus bearing a single additional pore (Figs 27, 29).

Instar I (Figs 1-30). Color. Cephalic capsule pale yellow with small, irregular, light brown maculae on central portion of FR, on posterior half of PA dorsally, and few on central portion of PA ventrally (maculae weakly marked in some specimens); a light brown ring-like band present on neck region contiguous to occipital suture; AN, MX, and LA either completely pale yellow or with A4 and apices of A3 and MP3 light brown; MN with distal region light brown; thoracic tergites pale yellow, protergite with some small light brown maculae, meso- and metatergite each with four small light brown maculae marginally; abdominal tergites I-VI pale yellow, each with 4-6 small light brown maculae marginally; tergite VII pale yellow; sclerite of segment VIII uniformly pale yellow to light brown; membranous parts creamy white; legs pale yellow, distal portion and claws sometimes light brown; urogomphus light brown.

Body. Elongate, subcylindrical. Measurements and ratios that characterize the body shape are shown in Table 1.

Table I. Measurements and ratios for the larvae of Spencerhydrus.

| Measure | S. latecinctus |  | S. pulchellus |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | Instar I $(\boldsymbol{N = 4} \mathbf{4})$ | Instar II $(\boldsymbol{N}=\mathbf{1})$ | Instar I $(\boldsymbol{N}=\mathbf{3})$ | Instar II $(\boldsymbol{N}=\mathbf{4})$ | Instar III $(\boldsymbol{N}=\mathbf{4})$ |
| HL (mm) | $2.55-2.63$ | 3.73 | $2.14-2.28$ | $2.93-3.14$ | $3.94-4.40$ |
| HW (mm) | $1.86-1.98$ | 2.93 | $1.75-1.89$ | $2.38-2.63$ | $3.20-3.51$ |
| FRL (mm) | $1.08-1.10$ | 1.44 | $0.94-0.98$ | $1.11-1.19$ | $1.51-1.58$ |
| OCW (mm) | $0.71-0.83$ | 1.19 | $0.60-0.64$ | $0.86-0.99$ | $1.33-1.55$ |
| HL/HW | $1.32-1.38$ | 1.27 | $1.21-1.22$ | $1.19-1.26$ | $1.20-1.27$ |
| HW/OCW | $2.33-2.70$ | 2.46 | $2.92-2.96$ | $2.47-2.84$ | $2.27-2.42$ |
| COL/HL | $0.57-0.59$ | 0.61 | $0.56-0.57$ | $0.62-0.63$ | $0.62-0.65$ |
| FRL/HL | $0.41-0.43$ | 0.39 | $0.43-0.44$ | $0.37-0.38$ | $0.35-0.38$ |
| A/HW | $1.07-1.13$ | 0.97 | $1.08-1.16$ | $0.93-1.06$ | $0.84-0.92$ |
| A2/A1 | $0.63-0.69$ | 0.58 | $0.58-0.60$ | $0.56-0.60$ | $0.52-0.59$ |
| A3/A1 | $0.34-0.37$ | 0.28 | $0.32-0.34$ | $0.31-0.32$ | $0.28-0.31$ |
| A4/A3 | $0.18-0.22$ | 0.15 | $0.23-0.24$ | $0.15-0.17$ | $0.13-0.14$ |
| A3'/A4 | $0.75-1.00$ | 0.77 | $0.76-0.88$ | $0.67-0.86$ | $0.69-0.85$ |
| MNL/MNW | $3.40-3.59$ | 3.73 | $3.28-3.41$ | $3.23-3.50$ | $3.31-3.95$ |
| MNL/HL | $0.48-0.50$ | 0.51 | $0.54-0.55$ | $0.51-0.54$ | $0.50-0.52$ |
| A/MP | $1.44-1.47$ | 1.56 | $1.40-1.48$ | $1.52-1.56$ | $1.51-1.61$ |
| PPF/MP1 | $0.49-0.54$ | 0.58 | $0.56-0.60$ | $0.53-0.60$ | $0.57-0.63$ |
| MP1/MP2 | $2.09-2.17$ | 2.22 | $2.17-2.19$ | $2.08-2.16$ | $2.13-2.30$ |
| MP3/MP2 | $1.50-1.60$ | 1.27 | $1.48-1.53$ | $1.27-1.32$ | $1.14-1.18$ |
| MP/LP | $2.62-2.92$ | 2.63 | $2.71-2.81$ | $2.72-2.89$ | $2.51-2.71$ |
| LP2/LP1 | $1.00-1.04$ | 0.75 | $1.00-1.04$ | $0.81-0.97$ | $0.74-0.80$ |
| L3 (mm) | $5.50-6.06$ | 7.84 | $5.01-5.28$ | $6.46-6.72$ | $8.14-8.46$ |
| L3/L1 | $1.21-1.24$ | 1.21 | $1.20-1.23$ | $1.20-1.23$ | $1.20-1.23$ |
| L3/L2 | $1.10-1.13$ | 1.12 | $1.09-1.12$ | $1.10-1.12$ | $1.10-1.12$ |
| L3/HW | $2.95-3.07$ | 2.68 | $2.80-2.87$ | $2.56-2.73$ | $2.40-2.54$ |
| L3 (CO/FE) | $1.04-1.15$ | 1.11 | $1.00-1.05$ | $1.04-1.07$ | $1.04-1.10$ |
| L3 (TI/FE) | $0.64-0.68$ | 0.65 | $0.65-0.67$ | $0.62-0.63$ | $0.60-0.62$ |
| L3 (TA/FE) | $0.75-0.78$ | 0.65 | $0.77-0.80$ | $0.67-0.72$ | $0.60-0.64$ |
| L3 (CL/TA) | $0.44-0.53$ | 0.45 | $0.44-0.47$ | $0.40-0.48$ | $0.37-0.45$ |
| LAS (mm) | $3.45-3.58$ | 5.09 | $3.00-3.23$ | $3.99-4.04$ | $5.38-5.70$ |
| LAS/HW | $1.79-1.88$ | 1.74 | $1.69-1.73$ | $1.54-1.68$ | $1.54-1.68$ |
| U (mm) | $0.09-0.10$ | 0.12 | $0.11-0.12$ | $0.08-0.10$ | $0.10-0.11$ |
|  |  |  |  |  |  |

Head. Cephalic capsule (Figs 1-4). Flattened, subtriangular, longer than broad; maximum width at level of stemmata, constricted at level of occipital region; occipital suture present; ecdysial suture well marked, coronal line long; occipital foramen deeply emarginate ventrally; posterior tentorial pits close to well visible ventral midline; FR subtriangular, anterior margin divided into three well developed projections: medial projection broad, well projected forward, slightly indented apically; lateral projections broad, rounded, less projected forward; anterolateral lobes rounded, not projected beyond anterior margin; egg bursters large, rounded to somewhat pointed, located laterally close to ecdysial line; four stemmata on upper side of head and two on underside, arranged in two vertical series. Antenna (Figs 5-8). Elongate, slender, somewhat longer than HW, composed of four antennomeres; A1 longest, subdivided into two articles, distal one somewhat less than twice longer than basal one; A2 shorter than A1, subdivided into three articles, basal one shortest, medial one longest; A3 shorter than A2, subdivided into three articles, basal one shortest, distal one longest, bearing a


Figures I-4. Cephalic capsule of Spencerhydrus species, instar I I S. Latecinctus, dorsal aspect $\mathbf{2}$ S. pulchellus, dorsal aspect $\mathbf{3}$ S. latecinctus, ventral aspect $\mathbf{4}$ S. pulchellus, ventral aspect. Numbers and lowercase letters indicate primary setae and pores, respectively. Additional setae not labelled. Color patterns not represented. Abbreviations: EB: egg burster; FR: frontoclypeus; PA: parietal; LC: lamellae clypeales; TP: tentorial pit. Scale bar: 0.70 mm .
ventroapical spinula; apical lateroventral process of A3 slender, elongate; A4 shortest, with a small lateroventral process on distal third. Mandible (Figs 9, 10). Strong, falciform, broad at base, narrowing to acute apex, more abruptly narrowed on distal fourth; mandibular channel present. Maxilla (Figs 11-14). Premaxillary lobes well developed; cardo sub-ovate to subtriangular; stipes elongate, slender, subcylindrical; galea absent; PPF elongate, palpomere-like; MP elongate, slender, shorter than antenna, composed of three palpomeres, MP1 longest, MP2 shortest; MP1 and MP2 subdivided into two articles, distal one longer than basal one; MP3 subdivided into three articles, basal one


Figures 5-14. Head appendages of Spencerhydrus species, instar I 5 S. latecinctus, right antenna, dorsal aspect 6 S. latecinctus, left antenna, ventral aspect 7 S. pulchellus, right antenna, dorsal aspect $\mathbf{8}$ S. pulchellus, left antenna, ventral aspect 9 S. latecinctus, right mandible, dorsal aspect 10 S. pulchellus, right mandible, dorsal aspect IIS. latecinctus, right maxilla, dorsal aspect I 2 S. latecinctus, left maxilla, ventral aspect I3 S. pulchellus, right maxilla, dorsal aspect I4 S. pulchellus, left maxilla, ventral aspect. Numbers and lowercase letters indicate primary setae and pores, respectively. Additional setae and pores not labelled. Abbreviations: A1-4: antennomeres $1-4$; AN: antenna; MN: mandible; MP1-3: maxillary palpomeres 1-3; MX: maxilla; PPF: palpifer; SP: spinula. Scale bar: 0.30 mm .


Figures 15-18. Labium of Spencerhydrus species, instar I 15 S. latecinctus, dorsal aspect 16 S. latecinctus, ventral aspect $\mathbf{1 7}$ S. pulchellus, dorsal aspect $\mathbf{1 8}$ S. pulchellus, ventral aspect. Numbers and lowercase letters indicate primary setae and pores, respectively. Additional setae and pores not labelled. Abbreviations: LA: labium; LP1-2: labial palpomeres 1-2. Scale bar: 0.15 mm .
shortest, distal one longest. Labium (Figs 15-18). Prementum broader than long, anterodorsal margin projected forward into strongly developed, apically rounded median process; LP much shorter than MP, composed of two palpomeres subequal in length; LP1 more or less weakly subdivided into two articles, distal one somewhat longer than basal one; LP2 subdivided into two articles, distal one much longer than basal one.

Thorax. Terga convex, pronotum shorter than subequal meso- and metanotum combined; protergite subrectangular, margins somewhat truncate, much more developed than meso- and metatergite; meso- and metatergite small, subrectangular to subtrapezoidal with posterior margin indented; all three tergites with sagittal line, lacking anterotransverse carina; sterna membranous except for a single sclerite on anterior portion of prothorax; spiracles absent. Legs (Figs 19-22). Long, composed of six articles,


Figures 19-22. Left metathoracic leg of Spencerhydrus species, instar I 19 S. latecinctus, anterior aspect 20 S. latecinctus, posterior aspect 21 S. pulchellus, anterior aspect 22 S. pulchellus, posterior aspect. Numbers and lowercase letters indicate primary setae and pores, respectively. Additional setae and pores not labelled. Abbreviations: CO: coxa; FE: femur; PT: pretarsus; TA: tarsus; TI: tibia; TR: trochanter. Scale bar: 0.20 mm .


Figures 23-26. Abdominal segment VIII of Spencerhydrus species, instar I 23 S. latecinctus, dorsal aspect 24 S. pulchellus, dorsal aspect 25 S. latecinctus, ventral aspect 26 S. pulchellus, ventral aspect. Numbers and lowercase letters indicate primary setae and pores, respectively. Additional setae and pores not labelled. Abbreviation: AB: abdominal segment VIII. Scale bar: 0.50 mm .


Figures 27-30. Urogomphus of Spencerhydrus species, instar I 27 S. latecinctus, right urogomphus, dorsal aspect $\mathbf{2 8}$ S. latecinctus, left urogomphus, ventral aspect 29 S. pulchellus, right urogomphus, dorsal aspect $\mathbf{3 0}$ S. pulchellus, left urogomphus, ventral aspect. Numbers and lowercase letters indicate primary setae and pores, respectively. UR: urogomphus. Scale bar: 0.05 mm .

L1 shortest, L3 longest; CO elongate, subcylindrical, TR divided into two parts by an annulus, FE, TI and TA slender, subcylindrical, PT with two long, slender, slightly curved claws, anterior claw slightly longer than posterior claw on L1 and L2, claws subequal in length on L3; proTA with a row of well-developed, bifid, ventral spinulae, those on basal third shorter, multifid, forming a dense patch (cleaning device); claws with elongate ventral spinulae on basal $1 / 2$ to basal $2 / 3$.

Abdomen. Eight-segmented; segments I-VI subequal, membranous except for a small anterodorsal sclerite and a minute sclerite on each lateral; tergites I-VI subrectangular, lacking anterotransverse carina, sagittal line visible, posterior half covered with short spinulae; segment VII narrower, main sclerite covering about anterior half of dorsal surface, lacking anterotransverse carina, covered with short spinulae, sagittal line not found; segments I-VII with a minute lateral sclerite, lacking spiracles; segment VIII (= LAS, Figs 23-26) longest and narrowest, completely sclerotized except ventrodistally around anus and urogomphi, lacking anterotransverse carina and sagittal line, basal half covered with short spinulae; siphon short. Urogomphus (Figs 27-30). Strongly reduced, approximately two or three times longer than broad, composed of one urogomphomere.


Figures 3I-32. Head of Spencerhydrus species 3I S. latecinctus, instar II, dorsal aspect 32 S. pulchellus, instar III, dorsal aspect. Scale bar: 1.00 mm .

Chaetotaxy (Figs 1-30). Similar to that of generalized Cybistrini larva (Alarie et al. 2011) with the following remarks: FR with numerous lamellae clypeales distributed on the apices of the anterior projections, those on medial projection longer than the others, arranged in a characteristic V-shaped pattern; A1 with some additional multifid setae distally and two or three additional pores proximally; crown of elongate additional (usually multifid) setae on distal fourth of MN absent; seta LA8 present; ventral row of setae on TI and TA formed by setae of different lengths; setae CO7, TR2, TR3, FE8, FE9, FE10, and pore ABa most likely present but obscured by the presence of additional sensilla; seta AB4 bifid or multifid; $U$ bearing all ancestral setae and one pore; several short additional setae present on membranous area of abdominal segment VIII, near urogomphal base.

Instar II (Fig. 31). As instar I except for the following features:
Color. Light brown maculae on cephalic capsule more numerous; thoracic tergites with some light brown maculae on disc; maculae on abdominal tergites I-VI more extended; sclerite of segment VII with some small light brown maculae.

Body. Measurements and ratios that characterize the body shape are shown in Table 1.
Head (Fig. 31). Egg bursters absent; A subequal in length to HW; LP1 somewhat longer than LP2.

Thorax. Anterotransverse carina present on metatergite, present or absent on mesotergite; spinulae of claws restricted to middle region.

Abdomen. Tergites I-VI with anterotransverse carina, sometimes weakly marked; sclerites I-VIII lacking spinulae.

Chaetotaxy. Secondary setae most likely present on different body parts, although difficult to evaluate due to large number of additional setae.

Instar III (Fig. 32). As instar II except for the following features:
Color. Somewhat darker in general; abdominal tergites I-VII predominantly light brown with longitudinal creamy white area centrally.

Body. Measurements and ratios that characterize the body shape are shown in Table 1.
Head (Fig. 32). Somewhat more robust and parallel sided. Antenna shorter than HW.

Thorax. Meso- and metatergite covering most of dorsal surface; well-developed spiracles present on mesothorax.

Abdomen. Well-developed spiracles present on segments I-VII.

## Spencerhydrus latecinctus Sharp, 1882

Source of material. The descriptions of this species are based on nine specimens of instar I and one of instar II (no instar III was available). Larvae were collected in Australia at the following localities: 1) SW Victoria, Kinhil, 14.x. 1983 and 9.xi.1983; 2) SA, Watervalley SE 8 km N Mt. Rough, 15.x.2000; 3) SA, 6 km W Penola, 30.x.2001. The association is firm as $S$. latecinctus is the only species of Spencerhydrus in south-east Australia, and the larvae of the other Cybistrini genera potentially present in the area (Cybister Curtis, 1827, Onychohydrus and Sternhydrus) clearly differ morphologically from the studied material (Alarie et al. 2011; Michat et al. 2015).

Differs from S. pulchellus as follows. Larger size (Table 1); median process of prementum relatively slender (Figs 15, 16); anterior margin of prementum with one additional seta (Fig. 16); setae LA2 and LA11 unified (Fig. 16); additional setae on ventral margin of meso- and metatibia more robust, dissimilar in length but not much so (Fig. 19); ratio LAS/HW (Table 1).

## Spencerhydrus pulchellus Sharp, 1882

Source of material. The descriptions of this species are based on nine specimens of instar I, four of instar II, and four of instar III. Larvae were collected in Australia at the following localities: 1) WA, Ellenbrook Nat. Res., 14.ix.2000; 2) WA, 6 km S Pinjarra, 23.x.1996, 23.ix.2000, and 3.x.2003. The association is firm as $S$. pulchellus is the only species of Spencerhydrus in south-west Australia, and the larvae of the other Cybistrini genera potentially present in the area (Cybister and Onychohydrus) clearly differ morphologically from the studied material (Alarie et al. 2011).

Differs from S. latecinctus as follows. Smaller size (Table 1); median process of prementum relatively broader (Figs 17, 18); anterior margin of prementum lacking additional setae (Fig. 18); setae LA2 and LA11 bifid (Fig. 18); additional setae on ventral margin of meso- and metatibia less robust and highly dissimilar in length (Fig. 21); ratio LAS/HW (Table 1).

## Discussion

Similarly to all other members of the tribe Cybistrini known with sufficient chaetotaxic detail (Michat 2006, 2010; Alarie et al. 2011; Michat et al. 2015), larvae of Spencerhydrus are characterized by bearing a large number of additional setae on almost all body regions. This particularly high number of additional setae (with many of them multifid or variously modified) distinguishes members of this tribe from all other known diving beetles, and summed to the very large size of cybistrine larvae somewhat justifies the title's opening statement. An exception to this pattern, however, is seen in the urogomphi. The miniaturization of this structure within Cybistrini, although showing a different degree of reduction among genera (see Alarie et al. 2011), may have prevented the development of additional sensilla, to the point that they bear the same number of (or even less) setae than in other dytiscids, and a highly reduced number of pores.

As mentioned above (see Introduction) the genus Spencerhydrus was included in recent phylogenetic analyses of the Cybistrini (Michat et al. 2015) and Dytiscidae (Michat et al. 2017) based on larval morphology. Most of the characters supporting monophyly and relationships of this genus are corroborated in our study, and therefore we find worth mentioning them. Spencerhydrus was resolved as part of a clade of Australian Cybistrini together with Onychohydrus and Sternhydrus, and within this clade, it is sister to the clade formed by the other two genera. Synapomorphies in support of a clade Spencerhydrus + Onychohydrus + Sternhydrus are the presence of additional pores on antennomere 1 , the subdivision of the maxillary palpomere 1 into two articles, the short and spine-like aspect of the tibial seta TI7, the presence of very small urogomphi, although still longer than broad, included together with anus in the non-sclerotized ventrodistal area of abdominal segment VIII, and the absence of a crown of multifid setae on the distal fourth of mandible (this last character shared with members of the subgenus Trifurcitus Brinck, 1945 of Megadytes Sharp, 1882). On the other hand, the following autapomorphies characterize the genus Spencerhydrus within Cybistrini: medial projection of frontoclypeus slightly indented apically, with lamellae clypeales directed forward in a characteristic V-shaped pattern (Figs 1, 2); median process of prementum strongly developed (Figs 15-18); presence of a single ventral sclerite on prothorax; and presence of basoventral spinulae on claws (Figs 19-22). Watts (2002), in his key to larvae of the Australian genera of Dytiscidae, gives the reduced sclerotization (covering only anterior half) of the abdominal segment VII as diagnostic for Spencerhydrus. This is another good character to separate the genus from the other Cybistrini because, given the large size of the larvae, it is easily visible under low magnifications.

Although very similar morphologically, we were able to find some characters to confidently separate larvae of the two species of Spencerhydrus (see earlier). The most conspicuous of these characters is the shape of the median process of prementum, which is visibly broader in S. pulchellus than in S. latecinctus (compare Figs 15 and 16 with Figs 17 and 18). This difference seems to be constant between larval instars, as the comparison of the second instar of both species offers the same picture. Unfortunately, the third instar of $S$. latecinctus was not available for comparison, but we estimate that this difference most likely remains the same in this stage.

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## References

Alarie Y, Michat MC (2014) Bridging Ecology and Systematics: 25 Years of Study of Larval Morphology of World Dytiscidae (Coleoptera). In: Yee DA (Ed.) Ecology, Systematics, and the Natural History of Predaceous Diving Beetles (Coleoptera: Dytiscidae). Springer, New York, 17-47. https://doi.org/10.1007/978-94-017-9109-0_2
Alarie Y, Michat MC, Miller KB (2011) Notation of primary setae and pores on larvae of Dytiscinae (Coleoptera: Dytiscidae), with phylogenetic considerations. Zootaxa 3087: 1-55. https://doi.org/10.11646/zootaxa.3087.1.1
Michat MC (2006) Descriptions of larvae of Megadytes (Coleoptera: Dytiscidae: Dytiscinae): the hypothesis of monophyletic origin revisited. European Journal of Entomology 103: 831-842. https://doi.org/10.14411/eje.2006.114
Michat MC (2010) Descriptions of larvae of Megadytes (Coleoptera: Dytiscidae: Dytiscinae): the subgenera Trifurcitus and Megadytes s. str., ground plan of chaetotaxy of the genus and phylogenetic analysis. European Journal of Entomology 107: 377-392. https://doi. org/10.14411/eje.2010.047
Michat MC, Alarie Y, Watts CHS (2015) Phylogenetic placement of the genus Sternhydrus (Coleoptera: Dytiscidae: Cybistrini) based on larval morphology, with description of S. atratus. Annals of the Entomological Society of America 108: 881-892. https://doi. org/10.1093/aesa/sav067
Michat MC, Alarie Y, Miller KB (2017) Higher-level phylogeny of diving beetles (Coleoptera: Dytiscidae) based on larval characters. Systematic Entomology 42: 734-767. https://doi. org/10.1111/syen. 12243
Miller KB, Bergsten J (2014) The Phylogeny and Classification of Predaceous Diving Beetles (Coleoptera: Dytiscidae). In: Yee DA (Ed.) Ecology, Systematics, and the Natural History of Predaceous Diving Beetles (Coleoptera: Dytiscidae). Springer, New York, 49-172. https://doi.org/10.1007/978-94-017-9109-0_3
Miller KB, Bergsten J (2016) Diving Beetles of the World. Johns Hopkins University Press, Baltimore, 320 pp. https://doi.org/10.1093/ae/tmx033
Miller KB, Bergsten J, Whiting M (2007) Phylogeny and classification of diving beetles in the tribe Cybistrini (Coleoptera, Dytiscidae, Dytiscinae). Zoologica Scripta 36: 41-59. https://doi.org/10.1111/j.1463-6409.2006.00254.x
Watts CHS (1978) A revision of the Australian Dytiscidae (Coleoptera). Australian Journal of Zoology (Suppl. series) 57: 1-166. https://doi.org/10.1071/AJZS057

Watts CHS (2002) Checklists and Guides to the Identification, to Genus, of Adult and Larval Australian Water Beetles of the Families Dytiscidae, Noteridae, Hygrobiidae, Haliplidae, Gyrinidae, Hydraenidae and the Superfamily Hydrophiloidea (Insecta: Coleoptera). Identification guide No. 43, The Cooperative Research Centre for Freshwater Ecology. Albury, New South Wales, 110 pp.
Wiley EO (1981) Phylogenetics. The Theory and Practice of Phylogenetic Systematics. John Wiley and Sons, New York, 439 pp. https://doi.org/10.1002/9781118017883

# A new Peruvian species of Scybalocanthon Martínez, 1948 (Coleoptera, Scarabaeidae, Scarabaeinae, Deltochilini) and some remarkable intrapopulational variation in the endophallus of S. pinopterus (Kirsch, I873) 

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#### Abstract

Scybalocanthon ashei sp. nov. from Madre de Dios, Peru, is described based on differences in external and male genital morphology. Its diagnostic characters and an updated identification key to the species of the genus are provided as well as new distributional data for the following species: S. acrianus Silva \& Valois, 2019, S. aereus (Schmidt, 1922), S. kaestneri (Balthasar, 1939) and S. pinopterus (Kirsch, 1873).


## Keywords

Dung beetles, neotropical fauna, taxonomy

## Introduction

The genus Scybalocanthon Martínez, 1948 comprises species distributed from Costa Rica to northern Argentina (Pereira and Martínez 1956; Silva and Valois 2019). The genus was recently revised by Silva and Valois (2019), who recognized 23 valid spe-
cies: S. acrianus Silva \& Valois, 2019; S. adisi Silva \& Valois, 2019; S. aereus (Schmidt, 1922); S. arnaudi Silva \& Valois, 2019; S. batesi Vaz-de-Mello \& Silva, 2017; S. chamorroi Silva \& Valois, 2019; S. cyanocephalus (Harold, 1868); S. darlingtoni (Paulian, 1939); S. federicoescobari Silva \& Valois, 2019; S. haroldi Silva \& Valois, 2019; S. kaestneri (Balthasar, 1939); S. kelleri Pereira \& Martínez, 1956; S. korasakiae Silva, 2011; S. maculatus (Schmidt, 1920); S. martinezi Silva \& Valois, 2019; S. moniliatus (Bates, 1887); S. nigriceps (Harold, 1868); S. papaxibe Silva \& Valois, 2019; S. pinopterus (Kirsch, 1873); S. pygidialis (Schmidt, 1922); S. sexspilotus (Guérin-Méneville, 1855); S. trimaculatus (Schmidt, 1922); and S. uniplagiatus (Schmidt, 1922) (see Silva and Valois 2019 for taxonomic background).

According to the current definition of the genus, Scybalocanthon is characterized by the first meso- and metatarsomeres with external margin with one-half the length of the second tarsomeres, and with apex obliquely truncated; lateral borders of tarsomeres parallel, forming a continuous border for all tarsomeres; overall shape of tarsomeres 2-4 square to rectangular; and dorsal surface of mesotibiae with dense, randomly distributed setae (Silva and Valois 2019).

Examination of the collection at the Canadian Museum of Nature (CMNC), Ottawa, has revealed a new species. In this paper, we describe this newly discovered species and provide an updated key to the species of Scybalocanthon. Besides, we also present new distributional data for S. acrianus, S. aereus, S. kaestneri and S. pinopterus. The results also show remarkable intrapopulational variations in the endophallus of S. pinopterus.

## Materials and methods

The material studied was deposited in the following collections: CEMT/UFMT (Seção de Entomologia da Coleção Zoológica, Cuiabá, curator Fernando Vaz-de-Mello); CMNC (Canadian Museum of Nature, Ottawa, Canada, curator François Génier); MZUFPA (Coleção de Scarabaeinae do Museu de Zoologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Brazil, curator Fernando A. B. Silva).

Examination of the aedeagus and endophallic sclerites allowed clarifying the differences between species. In describing these structures, we followed Tarasov and Solodovnikov (2011) and Tarasov and Génier (2015). The endophallus was removed from the aedeagus through the basal foramen of the phallobase, and its sclerites were illustrated in ventral view, except for the superior right peripheral sclerite (SRP), which was illustrated from the right side of the aedeagus. The following sclerites were found to be taxonomically useful: Superior Right Peripheral Sclerite (SRP) and Fronto-Lateral Peripheral Sclerite (FLP).

Images of specimens and key characters were taken with Leica stereomicroscope M205A, using image stacking software (Leica Application Suite, version 3.7.0), and they were edited using Adobe Photoshop CS4.

## Results

## Taxonomy

## Scybalocanthon ashei sp. nov.

http://zoobank.org/9E58A29A-B2F0-4893-8AF4-5EAA15821193
Figs 1A-G, 5C

Material studied. Holotype. PERU: MADRE DE DIOS, Reserva Cuzco Amazonica, 15 km NE Puerto Maldonado, $6^{\circ} 03^{\prime} \mathrm{W}, 12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}, 4 . \mathrm{VII} .1989$, Ashe and Leschen legs ( $1 \sigma^{\Uparrow} \mathrm{CMNC}$ ). Paratypes [ $5 \sigma^{\widehat{ }}$ and 7 ? ]. PERU: MADRE DE DIOS, Reserva Cuzco Amazonica, 15 km NE Puerto Maldonado, $69^{\circ} 03^{\prime} \mathrm{W}, 12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}$, 7.VII.1989, Ashe and Leschen legs ( $1 q$ CMNC); Reserva Cuzco Amazonica, 15 km NE Puerto Maldonado, $69^{\circ} 03^{\prime} \mathrm{W}, 12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}, 13 . \mathrm{VII} .1989$, Ashe and Leschen legs ( 1 Q CMNC); Reserva Cuzco Amazonica, 15 km NE Puerto Maldonado, $69^{\circ} 03^{\prime} \mathrm{W}$, $12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}, 17 . V I .1989$, Ashe and Leschen legs ( $1 \uparrow$ CMNC); Reserva Cuzco Amazonica, 15 km NE Puerto Maldonado, $69^{\circ} 03^{\prime} \mathrm{W}, 12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}, 24 . \mathrm{VI} .1989$, Ashe and Leschen legs ( 1 Q CMNC); Reserva Cuzco Amazonica, 15 km NE Puerto Maldonado, $69^{\circ} 03^{\prime} \mathrm{W}, 12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}, 26 . \mathrm{VI} .1989$, Ashe and Leschen legs ( $1 \delta^{\top} \mathrm{CMNC}$,
 donado, $69^{\circ} 03^{\prime} \mathrm{W}, 12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}, 20 . \mathrm{VI} .1989$, Ashe and Leschen legs ( $1 \widehat{\mathrm{O}}^{\lambda} \mathrm{CMNC}$ ); Reserva Cuzco Amazonica, 15 km NE Puerto Maldonado, $69^{\circ} 03^{\prime} \mathrm{W}, 12^{\circ} 33^{\prime} \mathrm{S}, 200 \mathrm{~m}$, 30.VI.1989, Ashe and Leschen legs ( 1 q CMNC); Parque Nacional del Manú, 15-30. VIII.1986, A. Forsyth leg. ( $1 \delta^{\lambda}$ and $1 q$ CMNC, $1 q$ MZUFPA).

Diagnosis. Specimens of Scybalocanthon ashei sp. nov. (Fig. 1A) are similar to those of S. arnaudi (Fig. 2D), S. federicoescobari (Fig. 2F), S. martinezi (Fig. 2I), S. papaxibe (Fig. 2J), S. pinopterus (Fig. 2K-M), and S. uniplagiatus (Fig. 2O) in having the pronotum uniformly colored, or with one elliptical spot on the central portion; femora almost completely yellow or brown, with black spots only on the tips (Fig. 1B); eighth elytral stria with thin carina anteriorly (see Silva and Valois 2019, fig. 3D); endophallus with bristles right beside the FLP sclerite (Fig. 1E); and additional sclerite (AS) absent. They can be distinguished from those of $S$. arnaudi and $S$. pinopterus, however, by the strongly asymmetrical parameres; left paramere with acute projection on dorsal margin and bilobate excavation on ventral margin (Fig. 1C, D) (other species with slightly asymmetrical parameres, lacking acute projection and bilobate excavation on dorsal and ventral margins (Fig. 3D, K-N); from those of S. federicoescobari, S. martinezi, S. papaxibe, and S. uniplagiatus by the bilobate excavation of the ventral margin of the left paramere wider, extending along two-third of the paramere margin (Fig. 1D) (in S. federicoescobari (Fig. 3F) and S. papaxibe (Fig. 3J) the excavation extending along one-fourth of the paramere margin; in S. uniplagiatus (Fig. 3P) the excavation extending along one-third of the paramere margin; in S. martinezi (Fig. 3I) the excavation is deeper, extending about one-half of the paramere margin).


Figure I. Scybalocanthon ashei sp. nov. A Holotype (CMNC), dorsal view B holotype (CMNC), ventral view $\mathbf{C}$ aedeagus (right side) $\mathbf{D}$ aedeagus (left side) $\mathbf{E}$ set of bristles $\mathbf{F}$ fronto-lateral peripheral (FLP) sclerite (left), and Complex of axial and subaxial ( $\mathrm{A}+\mathrm{SA}$ ) sclerites (right) G Superior right peripheral (SRP) sclerite.

Description. Body: Oval-elongated (Fig. 1A). General surface opaque, completely microgranulated. Pronotum with silky sheen. Color: Most of pronotum, hypomera (except internal and posterior margins), metaventrite, metepisterna, abdominal ven-


Figure 2. Species of Scybalocanthon, dorsal views. A S. acrianus B S. adisi C S. aereus D S. arnaudi E S. chamorroi $\mathbf{F}$ S. federicoescobari $\mathbf{G}$ S. kaestneri (specimen from Pastaza, Ecuador) H S. kaestneri (specimen from Napo, Ecuador) IS. martinezi JS. papaxibe K S. pinopterus (specimen from Madre de Dios, Peru) LS. pinopterus (specimen from Tingo Maria, Huanuco, Peru) M S. pinopterus (specimen from Orellana, Ecuador) $\mathbf{N}$ S. pygidialis $\mathbf{O}$ S. uniplagiatus, Scale bars: 2 mm .
trites, pygidium, and middle of femora yellow or light brown. Head, internal and posterior margins of hypomera, prosternum, mesoventrite, mesepisterna, elytra, trochanters, proximal and distal portions of femora, tibiae and tarsomeres dark brown. Length: $8.8-11.5 \mathrm{~mm}$. Head: Dorsal surface with fine microgranulation. Clypeal margin with two small, triangular central teeth. Eye comma-shaped in dorsal view. Thorax: Pronotum twice as wide as long, anterior angles acute, directed forward. Pronotum usually with one brown rounded spot at the anterocentral portion; in some specimens, spot absent. Anterior angles of pronotum approximately $80^{\circ}$. Lateral margin strongly curved outward. Elytra: Striae thin and shiny, punctures conspicuous. Eighth stria with a thin carina anteriorly. Legs: Protibiae with three lateral teeth. Anterior and posterior edge of meso- and metafemora not margined. Mesotibiae smoothly arched toward body. Metatibiae almost straight. First meso- and metatarsomeres short, external margin half the length of second tarsomere, and obliquely truncated apically. Lateral margins of tarsomeres parallel, forming even margin along length of tarsus. Overall shape of meso- and metatarsomeres $2-4$ varying from quadrate to rectangular. Dorsal (internal) surface of mesotibiae with dense setae, randomly distributed. Secondary


Figure 3. Aedeagus, detail of parameres in left and right view. A S. acrianus $\mathbf{B}$ S. adisi $\mathbf{C}$ S. aereus $\mathbf{D}$ S. arnaudi E S. chamorroi F S. federicoescobari G S. kaestneri (specimen from Pastaza, Ecuador) H S. kaestneri (specimen from Napo, Ecuador) I S. martinezi J S. papaxibe K S. pinopterus (specimen from Madre de Dios, Peru) L S. pinopterus (specimen from Tingo Maria, Huanuco, Peru) M S. pinopterus (specimen from Tingo Maria, Huanuco, Peru) N S. pinopterus (specimen from Orellana, Ecuador) O S. pygidialis P S. uniplagiatus, Scale bars: 0.5 mm .
sexual characters: Females can be distinguished from males by the sixth abdominal ventrite longer than in males, and the anterocentral portion of sixth abdominal ventrite more swollen than the posterocentral portion in lateral view (males have, in general, the posterocentral portion more swollen). Genital capsule: Parameres strongly asymmetrical (Fig. 1C, D). Dorsal margin of right paramere curved inward, apex obliquely truncated. Ventral margin of right paramere with a rounded excavation at the basal portion (Fig. 1C). Dorsal margin of left paramere curved inward from basal to medial portions, medial portion with a short and pointed projection, apex obliquely truncated (Fig. 1D). Ventral margin of left paramere with a bilobate excavation extending along two-third of the paramere margin; apical third obliquely truncated (Fig. 1D). Endophallus: SRP circular, with rounded handle-shaped extension (Fig. 1G). FLP short (Fig. 1F, left), comma-shaped, with three sets of bristles (Fig. 1E) right beside it. $\mathrm{A}+\mathrm{SA}$ with two superposed and elongate sclerites (Fig. 1F, right).


Figure 4. Set of bristles and microbristles of endophallus. A S. pygidialis (specimen from Amazonas, Brazil) B S. pygidialis (specimen from Cayenne, French Guiana) C S. pinopterus (specimen from Madre de Dios, Peru) D S. pinopterus (specimen from Tingo Maria, Huanuco, Peru) E S. pinopterus (specimen from Tingo Maria, Huanuco, Peru) F S. pinopterus (specimen from Orellana, Ecuador) G S. kaestneri (specimen from Pastaza, Ecuador) H S. kaestneri (specimen from Napo, Ecuador).

Etymology. Named in honor of the late James S. Ashe, collector of most of the known specimens.

Habitat. Amazon rainforest. Known from Peru (Fig. 5C). Endemism areas: Brazilian sub-region: South Brazilian dominion: Rondônia province (see Morrone 2014; fig. 12).

Remarks. According to aedeagus characters, S. ashei sp. nov. (Fig. 1C, D) is closely related to S. federicoescobari (Fig. 3F), S. martinezi (Fig. 3I), S. papaxibe (Fig. 3J), S. pygidialis (Fig. 3O), and S. uniplagiatus (Fig. 3P) by having the parameres strongly asymmetric, with different shape and length (dorsal margin of left paramere with projection; ventral margin of left paramere with bilobate excavation; ventral margin of right paramere with rounded excavation), and FLP sclerite short (Fig. 1F, left), with three sets of bristles right beside it (Fig. 1E).

## Updated key to males of species of Scybalocanthon Martínez, 1948 (based on Silva and Valois 2019)

1 Femora completely black. (Scybalocanthon aereus (Schmidt, 1922) (in part), Scybalocanthon maculatus (Schmidt, 1920), and Scybalocanthon kelleri Pereira \& Martínez, 1956) .......See Silva and Valois (2019): 307 for these species.

- Femora bicolored, central portion pale yellow, with dark spots at least on the tips (Fig. 1B)
2 (1) Pronotum with a longitudinal dark band on midline
See Silva and Valois (2019) for these species.
- Pronotum uniformly colored or, if bicolored, lacking longitudinal dark band on midline
3
3 (2) Black spots on the tips of femora covering approximately $1 / 15$ length of femora 4
- Black spots on the tips of femora covering approximately $1 / 5$ length of femora, central portion with elliptical yellow spot. (Scybalocanthon korasakiae Silva, 2011, Scybalocanthon aereus (Schmidt, 1922) (in part), Scybalocanthon batesi Vaz-de-Mello \& Silva, 2017, Scybalocanthon haroldi Silva \& Valois, 2019, Scybalocanthon nigriceps (Harold, 1868), Scybalocanthon darlingtoni (Paulian, 1939), Scybalocanthon sexspilotus (Guérin-Méneville, 1855), Scybalocanthon maculatus (Schmidt, 1920), Scybalocanthon trimaculatus (Schmidt, 1922), and Scybalocanthon moniliatus (Bates, 1887))
See Silva and Valois (2019): 308, step 16, for these species.

4 (3) Pronotum with four black spots, two central spots with triangular shape, and one rounded spot on each side (Fig. 2G). In some individuals, one central spot can be absent (Fig. 2H). Ecuador (Fig. 5B)

Scybalocanthon kaestneri (Balthasar, 1939)

- Pronotum uniformly colored, lacking spots, or with one elliptical spot on the central portion5

5 (4) Eighth elytral stria lacking carina at the anterior portion.
See Silva and Valois (2019) for these species.

- Eighth elytral stria with very fine and sharp carina at the anterior portion... 6

6 (5) Left paramere lacking acute projection on dorsal margin and lacking bilobate excavation on ventral margin (Fig. 3D, K)

See Silva and Valois (2019) for these species.

- Left paramere with acute projection on dorsal margin and bilobate excavation on ventral margin (Fig. 3F, I, J, O, P)7

7 (6) Bilobate excavation of ventral margin of left paramere wide and deep, extending at least one-half of the paramere margin in the lateral view (Figs 1D, 3I)........ 8

- Bilobate excavation of ventral margin of left paramere short, not reaching one-half of paramere margin in the lateral view (Fig. 3F, J, P).

See Silva and Valois (2019) for these species.
8 (7) Bilobate excavation of ventral margin of left paramere extending about onehalf of the paramere margin in the lateral view (Fig. 3I). Ecuador and Colombia (Fig. 5C) .................... Scybalocanthon martinezi Silva \& Valois, 2019

- Bilobate excavation of ventral margin of left paramere extending along twothird of the paramere margin (Fig. 1D). Peru (Fig. 5C)


## New distributional data for species of Scybalocanthon

## S. acrianus Silva \& Valois, 2019

New material examined. In addition to those mentioned by Silva and Valois 2019.
Non-type material. PERU: MADRE DE DÍOS, 15 km N.E. Puerto Maldonado, Reserva Cuzco Amazonica, $12^{\circ} 33^{\prime} \mathrm{S}, 69^{\circ} 03^{\prime} \mathrm{W}, 20 . V I .1989,200 \mathrm{~m}$, Ashe and Leschen leg. (1ठ CMNC); Manu National Park, 15-30.VIII.1986, A. Forsyth leg. (1才 CMNC).

Distribution. Known from Brazil (Acre), Bolivia and Peru (Fig. 5B). Endemism areas: Brazilian subregion: South Brazilian dominion: Rondônia province (see Morrone 2014; fig. 12).

## S. aereus (Schmidt, 1922)

New material examined. In addition to those mentioned by Silva and Valois 2019.
Non-type material. BRAZIL: AMAZONAS, Vila Nova ( 1 Q CMNC)
Distribution. Known from Brazil (Acre, Amazonas, Mato Grosso), Bolivia, and Peru (Fig. 5A). Endemism areas: Brazilian sub-region: South Brazilian dominion: Madeira, Ucayale, Yungas, and Rondônia provinces (see Morrone 2014; fig. 12).

## S. kaestneri (Balthasar, 1939)

New material examined. In addition to those mentioned by Silva and Valois 2019.
Non-type material. ECUADOR: NAPO, P. Misahualli, 18-22.II.1983, 350m, M. Sharkey leg. (1ठ CMNC); 20km S Tena, 9-11.VII.1976, 600m, S. Peck leg. (2 $q$ CMNC).

Distribution. Known from Ecuador (Fig. 5B). Endemism areas: Brazilian subregion: Boreal Brazilian dominion: Napo province (see Morrone 2014; fig. 12).


Figure 5. Known distributions of species of Scybalocanthon. A S. adisi and S. aereus B S. acrianus, S. arnaudi, S. chamorroi, S. kaestneri and S. pinopterus C S. ashei sp. nov., S. federicoescobari, S. martinezi, S. papaxibe, S. pygidialis, and S. uniplagiatus.

## S. pinopterus (Kirsch, 1873)

New material examined. In addition to those mentioned by Silva and Valois 2019.
Non-type material. ECUADOR: ORELLANA, Limoncocha, 10-15.III.1975, J.M. Campbell leg. ( $1 \circlearrowleft^{\lambda}$ and $1 q$ CMNC); Limoncocha, 13III.1976, J.M. Campbell leg.
 Lago Agrio, VI.1976, 250m, Martínez leg. ( 2 § CMNC); PERU: HUANUCO, Tingo Maria, Universidad, XII.1974, Martínez leg. ( 7 § and $16 \nrightarrow$ CMNC); Tingo Maria, Universidad, VII.1974, Martínez leg. (2q CMNC); Tingo Maria, VII.1974, 700m, Bordón leg. ( $2 \delta^{\top}$ and $4 \not \subset$ CMNC); Cucharas, Valley Huallaga, VI.1954, Felix Woytkowski leg. (1才 CMNC); Huallaga, n.r. Tocache, 17.X.1976, 500m, J. Schunke leg. (1§ CMNC).

Distribution. Known from Ecuador and Peru (Fig. 5B). Endemism areas: Brazilian sub-region: Boreal Brazilian dominion: Napo province; South Brazilian dominion: Rondônia and Yungas provinces (see Morrone 2014; fig. 12).

## Discussion

Scybalocanthon now includes 24 valid species. Based on the aedeagus morphology, including endophallic sclerites, two major patterns within the genus are found: slight paramere asymmetry, namely, parameres with similar shape, but slightly different lengths; and parameres strongly asymmetric, with shapes and lengths conspicuously different. The species which have the second pattern also present the dorsal margin of left paramere with a projection (Figs 1D, 3F, I, J, O, P); ventral margin of left paramere with bilobate excavation (Figs 1D, 3F, I, J, O, P); ventral margin of right paramere with rounded excavation (Figs 1C, 3F, I, J, O, P); and FLP sclerite short, with three sets of bristles right beside it (Fig. 1E, F). Heretofore, only S. federicoescobari, S. martinezi, S. papaxibe, S. pygidialis, and S. uniplagiatus were known having this shape of aedeagus. We described here a new species, $S$. ashei sp. nov., which have the same characteristics mentioned above (Fig. 1C-F). However, it can be distinguished from those species by the wider bilobate excavation of the ventral margin of the left paramere, extending along two-third of the paramere margin (Fig. 1D).

Some of those species mentioned above have similar external morphology, including similarities with other species with slightly asymmetrical parameres. Characters of external morphology can also vary within the same species, such as body coloration and patterns of spots. Besides, some of these species overlap in their geographical distribution, which makes them difficult to tell apart without examining the male genitalia. According to the general external morphology and close geographical distribution, specimens of S. ashei sp. nov., S. acrianus, S. adisi, S. aereus, S. arnaudi, S. chamorroi, S. federicoescobari, S. martinezi, S. pinopterus, and S. uniplagiatus can be mistaken at first glance. All these species have distributional records in the western/central Amazon (Fig. $5 \mathrm{~A}-\mathrm{C}$ ) and, in general, they have the pronotum and elytra uniformly colored, or
with a small median spot anteriorly on pronotum (Figs 1A, 2A-F, I, K-M, O). However, $S$. ashei sp. nov. can be easily distinguished from S. aereus, S. adisi, S. acrianus, S. chamorroi, S. arnaudi, and S. pinopterus by the strongly asymmetrical parameres (Fig. 1C, D). From S. federicoescobari, S. martinezi, and S. uniplagiatus the genital differences have already been stated above.

Silva and Valois (2019) described variations in the patterns of bristles and microbristles in endophallus of S. pygidialis, as follows: specimens from eastern Amazon (French Guiana and Amapá) had two sets of bristles and one set of microbristles right beside the FLP sclerite (Fig. 4B), while specimens from western Amazon (Amazonas and Roraima) had instead three sets of bristles right beside the FLP sclerite (Fig. 4A). An intraspecific variation in number of bristles and microbristles also occur at least in other species of the genus. According to Silva and Valois (2019), based on the examination of 17 males from Huánuco and Madre de Dios, Peru, S. pinopterus would have endophallus with three sets of bristles and one set of microbristles right beside the FLP sclerite (Fig. 4C). Four newly examined males from Tingo Maria, Huánuco, Peru, however, have four sets of bristles and a set of microbristles right beside the FLP sclerite (Fig. 4D). The parameres in these specimens (Fig. 3L) are slightly thinner and longer than those of S. pinopterus examined by Silva and Valois (2019) (Fig. 3K), but they have the same shape. The other five males from Tingo Maria, Huánuco, Peru, have two sets of bristles and a set of microbristles (Fig. 4E). However, there are no differences in the shape and length of parameres between these specimens (Fig. 3M) and those examined by Silva and Valois (2019) (Fig. 3K). Finally, thirteen males from Orellana, Ecuador, have only a short set of bristles and a set of microbristles (Fig. 4F), but no differences have been verified in the shape of aedeagus between these specimens (Fig. 3N) and those examined by Silva and Valois (2019) (Fig. 3K).

These observations are in agreement with the hypothesis raised by Silva and Valois (2019: 333) that the apparent allopatric distribution between populations may be artificial due to a lack of collections. Therefore, the apparent discrete differences in the sets of bristles may turn out to be an artefact and will be continuous if more specimens are examined along the geographical distribution of the species, that is, one form may intergrade into the other across this putative chain of populations, or if the forms themselves end up being indeed discrete, the frequency between them may vary among these populations.

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## References

Morrone JJ (2014) Biogeographical regionalisation of the Neotropical region. Zootaxa 3782: 1-110. https://doi.org/10.11646/zootaxa.3782.1.1
Pereira FS, Martínez A (1956) Os gêneros de Canthonini americanos (Col. Scarabaeidae). Revista Brasileira de Entomologia 6: 91-192.
Silva FAB, Valois M (2019) A taxonomic revision of the genus Scybalocanthon Martínez, 1948 (Coleoptera: Scarabaeidae: Scarabaeinae: Deltochilini). Zootaxa 4629: 301-341. https:// doi.org/10.11646/zootaxa.4629.3.1
Tarasov SI, Génier F (2015) Innovative bayesian and parsimony phylogeny of dung beetle (Coleoptera, Scarabaeidae, Scarabaeinae) enhanced by ontology-based partitioning of morphological characters. Plos ONE 10: 1-86. https://doi.org/10.1371/journal.pone. 0116671
Tarasov SI, Solodovnikov AY (2011) Phylogenetic analyses reveal reliable morphological markers to classify mega-diversity in Onthophagini dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). Cladistics 27: 490-528. https://doi.org/10.1111/j.1096-0031.2011.00351.x

# Taxonomic review of the Themus (Telephorops) nepalensis species-group (Coleoptera, Cantharidae) 

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#### Abstract

The diagnosis of the Themus (Telephorops) nepalensis species-group is summarized. A catalogue, a key and a distribution map of all world species are provided. Two synonymies are proposed: Themus (Telephorops) subcaeruleiformis Wittmer, 1983, syn. nov. = T. (Telephorops) crassimargo Champion, 1926; T. (Telephorops) separandus Wittmer, 1975, syn. nov. = T. (Telephorops) laboissierei (Pic, 1929). The female internal genitalia are photographed and described in this species-group for the first time, the aedeagi of T. (Telephorops) crassipes Pic, 1929 and T. (Telephorops) impressipennis (Fairmaire, 1886) are illustrated and described for the first time, and some additional distribution information is provided for the species. Themus (Telephorops) cavipennis (Fairmaire, 1897) is a new record for the Chinese fauna.


## Keywords

female internal genitalia, new faunistic record, new synonym, soldier beetles, taxonomy

## Introduction

Themus Motschulsky, 1858 is one of the largest cantharid genera and comprises about 250 species in total (Yang et al. 2014; Kopetz 2016). It consists of four subgenera (Wittmer 1973, 1997), which were redefined by Švihla (2008) on the basis of the shapes and color of the pronotum and elytra.

The subgenus Telephorops Fairmaire, 1886 for T. impressipennis (by original and monotypic designation) was subdivided into two species groups, which however were
not given names (Švihla 2008). According to the Principle of Priority (ICZN 1999, Articles 23.1 and 23.3.3), the valid name of a taxon is the oldest available name applied to it, so the earliest-named member of an aggregate of vicarious species will be the species-group name. They have been named the davidis species-group (Yang et al. 2019) and the nepalensis species-group (including the type species for the genus) respectively. The latter was characterized by the reduced and shortened laterophyses of the aedeagus and the enlarged elytra with depressions (Švihla 2008).

Most species of the nepalensis species-group were described by early taxonomists, such as Fairmaire (1886, 1897), Hope (1831), Pic (1911, 1912, 1926, 1929a,b), Gorham (1889) and Champion (1926). Recently, those species were revised and more species were added by Wittmer (1954, 1975, 1983a, b, 1997). A few more species and additional morphological or distributional information were added by Okushima (1999, 2003), Kopetz $(2004,2010,2016)$ and Yang et al. (2013).

Up to now, 15 species were included in the nepalensis species-group. This group has not previously been reviewed globally, and some sibling species remain difficult to diagnose from others due to there being few characters known for the females when males are unavailable. Furthermore, attribution of species to the groups is difficult because species diagnoses are often imprecise. For example, T. minor Wittmer, 1997, T. subcaeruleus (Pic, 1911) and T. crassimargo Champion, 1926, whose elytra are enlarged and laterophyses well developed, give contradictory information about their placement in the species-group defined by Švihla (2008). Thus, in the present study, all known species are reviewed to evaluate morphological evidence supporting species groups, and both are redefined where necessary.

## Material and methods

The material is deposited in the following collections:
BMNH British Museum of Natural History, London, UK;
CAUB Chinese Agriculture University, Beijing, China;
IZAS Institute of Zoology, Chinese Academy of Sciences, Beijing, China;
MHBU Museum of Hebei University, Baoding, China;
MNHN Muséum national d'Histoire naturelle, Paris, France;
NHMB Naturhistorisches Museum Basel, Switzerland.

Genitalia of both sexes and abdominal sternites VIII of females were dissected and cleared in $10 \% \mathrm{KOH}$ solution, and female genitalia were stained with hematoxylin. The female internal genitalia is attached to the ventral side of abdominal tergite IX and the vulva opens between the coxites. The dorsal or ventral side of vagina is established according to the tergite IX. The situation of median oviduct opening is on the opposite side of tergite IX and established as the ventral side of vagina. The diverticulum and spermatheca arise from apex of vagina.

Habitus photos were taken using a Leica M205 A stereomicroscope, multiple image layers were stacked using Combine ZM (Helicon Focus 5.3). Line drawings were made using a camera lucida attached to a Nikon SMZ1500 stereomicroscope, then edited in CorelDRAW 12 and Adobe Photoshop 8.0.1. Body length was measured from the anterior edge of the clypeus to the elytral apex and body width across the humeri of elytra. Morphological terminology of female genitalia followed Brancucci (1980). The key to the species was prepared mainly based on the characters of the aedeagus. If the aedeagi of different species were too similar to be described, the female abdominal sternite VIII and internal genitalia were compared; body size and coloration was also referred to when necessary.

In the checklist, valid scientific names and original sources, synonyms and publications for the taxonomical changes, type localities and depositories, additional material information and all distributions were included, as well as additional description or remarks were added if necessary. Complete label data were cited for type specimens, using square brackets "[ ]" for our remarks and comments, [p] indicating that the following data were machine printed and [h] that they were handwritten, quotation marks to separate data from different labels. A distribution map was prepared using the geographic information system software ArcGIS (ver. 10.2), based literature records and the author's databases of specimens examined for this study.

The specimens were identified based on examination of types if available and original literature. In practice, species were determined mainly by the aedeagus of male, and the females were associated with males based on evidence that they were collected at the same locality and date. Also, the female could be identified by the structure of abdominal sternite VIII, which was useful in species' recognition and illustrated in the literature by cantharid specialists. For each species, compared with males, the females have smaller eyes, shorter and narrower antennae, simple middle antennomeres, without smooth narrow impressions along the outer edges, wider pronotum and elytra, and only seven abdominal ventrites.

## Taxonomy

## Themus (Telephorops) nepalensis species-group

Diagnosis. Elytra enlarged posteriorly and widest near apical third. Aedeagus: conjoint dorsal plate of parameres narrowed apically in dorsal view, emarginate at middle of apical edge; laterophyses flattened dorsoventrally, reduced and not reaching apices of conjoint dorsal plate except in a few species. Female internal genitalia: diverticulum situated at end of vagina, presenting with a sclerotized ring around at base, confluent in middle and extending to median oviduct; spermatheca arising from middle of the sclerotized ring.

Distribution. Most species are restricted in their distribution (Figs 1, 2), except T. impressipennis (Fairmaire, 1886) and T. coelestis (Gorham, 1889), which are widely distributed in China.


Figure I. Distribution map of Themus (Telephorops) nepalensis species-group (part I).

Remarks. The diagnosis is developed from the definition of the species-group by Švihla (2008). Characters of the elytra and aedeagus, the female internal genitalia are supplemented in the present study. This differs from the davidis species-group in the female genitalia having a sclerotized ring around the base of the diverticulum, delimit-


Figure 2. Distribution map of Themus (Telephorops) nepalensis species-group (part II).
ing it from the vagina; and spermatheca opening on the opposite side to the median oviduct. While in the davidis species-group, there are only a pair of short conjoint sclerotized ridges below the diverticulum, hardly delimitated from the vagina; and spermatheca opening on the same side as the median oviduct (Yang et al. 2019).

## Key to species (adults) of Themus (Telephorops) nepalensis species-group

(characters based on illustrations in the present study or those from Wittmer (1983a, b, 1997)).

1 Aedeagus: laterophyses reaching apices of conjoint dorsal plate of parameres... 2

- Aedeagus: laterophyses reduced, not reaching apices of conjoint dorsal plate of parameres.4

2 Antennae, mid and hind legs uniformly black; aedeagus (Wittmer 1997: fig. 104): laterophyses without processes on both sides
T. minor Wittmer, 1997

Antennae and legs mixed yellow and black; aedeagus: laterophyses with a narrow process each side3

3 Aedeagus (Wittmer 1983b: fig. 2): conjoint dorsal plate of parameres triangularly emarginate in middle of apical edge in dorsal view; female abdominal sternite VIII (Wittmer 1983b: fig. 62) with lateral protuberances of posterior edge nearly as wide as distance between them....T. subcaeruleus (Pic, 1911) Aedeagus (Wittmer 1983b: fig. 3a): conjoint dorsal plate of parameres rectangularly emarginate in middle of apical edge in dorsal view; female abdominal sternite VIII (Fig. 9D) with lateral protuberances of posterior edge about half as wide as distance between them
T. crassimargo Champion, 1926

4 Aedeagus: ventral process of each paramere hooked at apex in lateral view..... 5

- Aedeagus: ventral process of each paramere not hooked at apex ................. 9

5 Aedeagus: ventral process of each paramere expanded and obtusely hooked dorsally at apex (e.g. Fig. 5C) 6

- Aedeagus: ventral process of each paramere narrowed and acutely hooked ventrally at apex8

Elytra dark green or blue, strongly metallic ....... T. nepalensis (Hope, 1831)

- Elytra purple-black, weakly metallic 7
7 Body longer than 16.0 mm ; female internal genitalia (Fig. 6D) with diverticulum narrowed apically T. crassipes Pic, 1929
- $\quad$ Body 13.0-15.0 mm in length; female internal genitalia (Fig. 7C) with diverticulum expanded apically. T. masatakai Okushima, 2003 Aedeagus (Fig. 6B): ventral process of each paramere triangularly protuberant apicolaterally in dorsal view; female abdominal sternite VIII (Fig. 9B) with each protuberance narrower than the distance between it and apicolateral angle. T. cavipennis (Fairmaire, 1897)
- Aedeagus (Wittmer 1983b: fig. 5): ventral process of each paramere normal, not protuberant in dorsal view; female abdominal sternite VIII (Fig. 9J) with each protuberance wider than the distance between it and apicolateral angle
$9 \quad$ Elytra no more than 1.5 times as long as maximal width; aedeagus (Wittmer 1983b: fig. 4): ventral process of each paramere with apex slightly bent inwards in ventral view, nearly as long as conjoint dorsal plate in lateral view...
T. laboissierei Pic, 1929
- Elytra about twice as long as maximal width; aedeagus: ventral process of each paramere with apex unlike above, not bent inwards in ventral view, longer than conjoint dorsal plate in lateral view 10

10 Aedeagus (Wittmer 1983a: fig. 47): ventral process of each paramere abruptly narrowed at apex in ventral view; female abdominal sternite VIII (Fig. 9I) with acute apicolateral angles T. sauteri (Pic, 1912)

- Aedeagus (Fig. 6C): ventral process of each paramere expanded at apex in ventral view; female abdominal sternite VIII with rounded apicolateral angles 11
11 Tibiae mixed yellow and black; aedeagus (Wittmer 1983b: fig. 1): ventral process of each paramere narrowed apically in ventral view, conjoint dorsal plate widely emarginate medially at apical edge in dorsal view
T. coelestis (Gorham, 1889)
- Tibiae uniformly black or yellow; aedeagus: ventral process of each paramere almost even in width in ventral view, conjoint dorsal plate narrowly emarginate medially at apical edge in dorsal view 12
12 Femora mixed yellow and black, tibiae black; female abdominal sternite VIII (Fig. 9F) with protuberances of posterior edge not reaching apices of apicolateral angles in ventral view T. impressipennis (Fairmaire, 1886)
- Femora and tibiae uniformly yellow; female abdominal sternite VIII (Fig. 9A) with protuberances of posterior edge exceeding apices of apicolateral angles in ventral view
T. bicoloricornis Wittmer, 1983


## Themus (Telephorops) bicoloricornis Wittmer, 1983

Figs 6A, 9A
Themus (Telephorops) bicoloricornisWittmer, 1983a: 153, figs 48 (aedeagus illustration), 51 (female abdominal sternite VIII illustration).

Type material examined. $1 \widehat{\text { § }}$ (paratype, NHMB), [h] "Idabon \Musha $\backslash$ 23.7.1928", [p] "PARATYPUS", [h] "Themus (Tryblius) \bicoloricornis \Wittm. \det. W. Wittmer", [p] "Naturhist. \Museum Basel \ coll. W. Wittmer", [p] "CANTHARIDAE \} CANTH00002241".

Other material examined. $1 \delta^{\lambda}, 1 q$ (IZAS), Taiwan, Nantou, Tzuei-feng, 1997. VII.9, leg. K. Mizota.

Supplementary description. Female. Like male, but antennomeres V-X without impressions along outer edges (while present with smooth narrow longitudinal
or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9A) with posterior edge narrowly and triangularly emarginate medially and paired rounded middle protuberances, which are wider than the distance between protuberance and apicolateral angle and exceeding apex of the latter. Internal genitalia (Fig. 6A): diverticulum hardly narrowed apically and rounded at apex, about 2.5 times as long as its maximal width; spermatheca expanded apically.

Distribution. Taiwan.

Themus (Telephorops) cavipennis (Fairmaire, 1897)
Figs 3A, 6B, 9B
Tryblius cavipennis Fairmaire, 1897: 228.
Themus ancoralis Champion, 1926: 128. Synonymized by Wittmer 1975: 251.
Themus (Tryblius) cavipennis: Pic 1929a: 195; Wittmer 1975: 251, fig. 1 (aedeagus illustration).
Themus (Telephorops) cavipennis: Wittmer 1983b: 197; Okushima 1999: 59, figs 10 (habitus photo), 34 (female abdominal sternite VIII illustration).


Figure 3. Male habitus, dorsal view A Themus cavipennis Champion, 1926 (the specimen of Xizang) B T. crassimargo Champion, 1926 (the specimen of Sichuan). Scale bars: 5.0 mm .

Type material examined. $1 \circlearrowleft^{\lambda}$ (holotype, MNHN), [p]"Himalaya $\backslash$ Sikkim", [h]"Tryblius \cavipennis \Fairm., Sikkim", [p]"HOLOTYPUS", [h]"Themus \ (Tryblius) \cavipennis \Fairm. \det. W. Wittmer".

Other material examined. CHINA: Xizang: $1 \delta, 1 q$ (IZAS), Bomi, Tangmai, 2300m, 2005.VIII.31, leg. X.J. Wang; 1 Q (IZAS), Nyingchi, Pêlong, 2100 m, 2005. IX.2, leg. X.L. Chen; $1 q$ (IZAS), same data, 2005.IX.1; $1 q$ (IZAS), same locality and date, 2115 m, leg. X.J. Wang; 1 q (IZAS), Nyingchi, Zayü, Shang Zayü, 1960m, 2005. VIII.23, leg. X.L. Chen; 1 (IZAS), Zayü, Zhowagoin, Xungjug, 1938 m, 28.6067N, 97.2816E, 2014.VIII.29, leg. H. Liu; $1 q$ (MHBU), Shang Zayü, 2005.VII.14, leg. A.M. Shi; 1 (MHBU), Nyingchi, Pêlong, 2007.IX.23.-28, leg. F.M. Shi.

Supplementary description. Male (Fig. 3A). Female. Like male, but antennomeres IV-X without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9B) with posterior edge narrowly and triangularly emarginate medially between paired rounded middle protuberances, each protuberance narrower than the distance between it and apicolateral angle and exceeding apex of apicolateral angle. Internal genitalia (Fig. 6B): diverticulum narrowed apically and nearly pointed at apex, about twice as long as its maximal width; spermatheca abruptly expanded apically.

Distribution. China (new record: Xizang), Bhutan, Nepal, northern India.

## Themus (Telephorops) coelestis (Gorham, 1889)

Figs 6C, 9C
Telephorus coelestis Gorham, 1889: 104, t.10, fig. 7.
Themus (Telephorops) coelestis: Wittmer 1983b: 197, figs 1 (aedeagus illustration), 59 (female abdominal sternite VIII illustration).
Themus rugosus Pic, 1929b: 8. Synonymized by Wittmer 1983b: 197.
Themus violetipennis Wang \& Yang, 1992: 265, fig. 2 (habitus illustration). Synonymized by Yang et al. 2013: 3.

Type material examined. $1 \overbrace{}^{\Uparrow}$ (NHMB, lectotype of Telephorus coelestis): without locality information, [h]"coelestis $\widehat{\sigma}^{\lambda "},[\mathrm{~h}]$ " ${ }^{\lambda \prime \prime}$, [h]"Themus \(Telephorops) \coelestis \} (Gorh.) \det. W. Wittmer", [h] "Type", [p] "LECTOTYPUS", [p]"Naturhist. \ Museum Basel \coll. W. Wittmer", [p]"CANTHARIDAE \CANTH00001277". The lectotype was designated by Wittmer (1983b).

1 (MNHN, holotype of Themus rugosus), [h]"Fokien" (China, Fujian), [h]"Themus $\backslash$ rugosus $\backslash \mathrm{n}$. sp.", [h]"Themus $\backslash$ (Telephorops) \coelestis $\backslash$ (Gorh.) \det. W. Wittmer", [h]"type", [p]"TYPE". The holotype is damaged, lacking antennae and right meta-leg.
$1 q$ (IZAS, neotype of Themus violetipennis), [p] "Hunan, Yongshun, Shanmuhe forestry station $\backslash 600 \mathrm{~m}$ ", [p] "4.VIII. $1988 \backslash$ leg. Shu-Yong Wang". The neotype was designated by Yang et al. (2013).

Other material examined. CHINA, Shaanxi: 2 (MHBU), Chushui, Niubeiliang, 1056 m, 2011.VIII.22-29, leg. X.C. Zhu \& Y. Zhao. Hubei: 19 (MHBU), Badong, Lvcongpo, 1700 m, 2006.VII.14, leg. M. Li; 1 q (MHBU), same data, leg. J. H. Wan; $1 q$ (MHBU), Yuan'an, Hehua, 2009.VII.12, leg. X.M. Sun; $1 q$ (MHBU), same data, leg. Y. Dong; $1 q$ (MHBU), Yichang, Xianrenxi, 2009.VI.25, leg. G.L. Xie; $1 q$ (MHBU), Yichang, Dalaoling Forestry, 2009.VI.26, leg. Y. Tian; 1 Q (MHBU), Yidu, Niejiahe, 2008.VI.16, leg. G. L. Xie; 1 q (MHBU), Yichang, Hejiaping, Qinggangping, 2013.VII-XI, leg. T.H. Du; 1 Q (MHBU), Changyang, Langping, Changfeng, 900 m, 2013.VII.11, leg. Y.Q. Wu; $1 ठ^{\lambda}$ (MHBU), Jingshan, Huzhuashan Forestry, 2007.VII.15, leg. G.L. Xie; $1 \widehat{ }^{\Uparrow}$ (MHBU), Wufeng, Houhe, 2002.VII.16, leg. F.Y. Wang; $1 \delta^{\AA}(\mathrm{MHBU})$, same locality, 2002.VII.21, C.H. Shi. Guangxi: 19 (MHBU), Luocheng, Pingying, 2004.V.29, leg. J.M. Zhang. Guangdong: 1 q (MHBU), Nanling, 2010.VIII.8-18, leg. H.Y. Liu; 1q (MHBU), same locality and collector, 2010. VIII.17; 1 Q (MHBU), same locality and collector, 2010.VIII.8-11. Hebei: $1 \delta^{\AA}$ (MHBU), Changli, Huangjin seaside, 1999.VIII.18, leg. H.Z. Liang; $1 \sigma^{\top}$ (MHBU), same data, leg. Z.J. Ma; $1 \delta^{\top}(\mathrm{MHBU})$, Zushan, 1998.VII.14, leg. X.J. Li. Zhejiang: $1 q$ (MHBU), Longquan, Fengyangshan, 2007.VII.25, leg. L.K. Tan; $1 q$ (MHBU), same locality and collector, 2007.VII.30; 1 q (MHBU), same locality, 2007.VII.26, leg. G.L. Xie; 1 q (MHBU), same locality and collector, 2007.VII.27; 1 q (MHBU), same locality and collector, 2007.VII.31; 3才, 1 (MHBU), same locality, 2007. VII.25-VIII.1, leg. H.Y. Liu \& Z.H. Gao; $1 \widehat{o}^{\top}$ (MHBU), same locality, 2012.VII.18, leg. G.L. Xie \& J. Jiao; 2 ( P (MBU), Lin’an, Tianmushan, 2013.VI.26-VII.2, leg. J.Y. Su; 2 ( MHBU ), Hangzhou, Lin'an, Dajingwu, 2012.VI.10, leg. H. Xu; $1 \varnothing^{\Uparrow}$ (MHBU), Qingyuan, Baishanzu, 2012.VII.24, G.L. Xie \& X. Wang. Yunnan: $2{ }^{\text {§ }}$ (MHBU), Dali, 2008.VIII.18, leg. G. L. Xie.

Supplementary description. Female. Like male, but antennomeres IV-X without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9C) with posterior edge triangularly emarginate medially and largely and triangularly emarginate on both sides, lateral emargination about twice as deep as middle one, the protuberances between middle and lateral emarginations acute, exceeding the rounded apices of apicolateral angles. Internal genitalia (Fig. 6C): diverticulum hardly narrowed apically and rounded at apex, about twice as long as its maximal width; spermatheca expanded apically.

Distribution. China (Shaanxi, Gansu, Henan, Anhui, Zhejiang, Hubei, Jiangxi, Hunan, Fujian, Hainan, Guangxi, Sichuan, Guizhou).

## Themus (Telephorops) crassimargo Champion, 1926

Figs 3B, 4A, B, 9D
Themus crassimargo Champion, 1926: 127.
Themus (Tryblius) crassimargo: Wittmer 1975: 251, fig. 2 (aedeagus illustration).


Figure 4. Habitus, dorsal view A Themus crassimargo Champion, 1926 (lectotype) B Themus (Telephorops) subcaeruleiformis Wittmer, 1983 (holotype) C Triblius Laboissierei Pic, 1929 (lectotype) D Themus (Tryblius) separandus Wittmer, 1975 (holotype).

Themus (Telephorops) subcaeruleiformis Wittmer, 1983b: 199, fig. 3, 3a (aedeagus illustration), syn. nov.
Themus (Telephorops) crassimargo: Okushima 1999: 59, figs 11 (habitus photo), 35 (female abdominal sternite VIII illustration).

Type material examined. $1 \delta^{\lambda}$ (BMNH, lectotype of Themus crassimargo), [p]"Gopaldhara, Sikkim, vii.1924, H. Stevens", [p] "LECTOTYPUS", [p] "Themus \} crassimargo \Champ.". The lectotype was designated by Wittmer (1975).
$1{ }^{\top}$ (NHMB, holotype of Themus (Telephorops) subcaeruleiformis), [h] "Kuanshien (Guanxian, now is Dujiangyan) \Umg. VIII.1934", "600-1300m \Szechwan (Sichuan), China", [p] "HOLOTYPUS", [h] "T. (Telephorops) subcaerulei- I formis Wittm. \det. W. Wittmer", [p] "Naturhist. \Museum Basel \coll. W. Wittmer", [p] "CANTHARIDAE \CANTH00001233".

Other material examined. CHINA, Xizang: $1 \AA$ (CAUB), Zayü, Shajiong, 1700 m, 1978.VI.26, leg. F.S. Li. Sichuan: 1 ¢ (IZAS), 70 km West Chengdu, Qingcheng, Hou Shan mts., 1360 m, $30^{\circ} 44^{\prime} \mathrm{N}, 103^{\circ} 08^{\prime} \mathrm{E}, 2004 . V I I I .28, ~ S . ~ M u r z i n$.

Distribution. China (Xizang, Sichuan), N. India, Bhutan, Nepal.
Supplementary description. Female (Fig. 3B). Like male, but antennomeres VXI without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9D) with posterior edge triangularly protuberant on each side, space between lateral protuberances about twice as wide as each width.

Remarks. Themus (T.) subcaeruleiformis Wittmer, 1983 was originally described based on a single male type, from China, Szechwan, Kuanshien Umg. (now in Sichuan, Dujiangyan). Here a female (Fig. 3B) collected from Qingcheng, which is near the type locality, is discovered for the first time. The structure of its abdominal sternite VIII (Fig. 9D) is like that of T. crassimargo Champion, 1926, illustrated by Okushima (1999: fig. 35). Furthermore, T. subcaeruleiformis was only compared with T. subcaeruleus located in Yunnan, China in the original publication (Wittmer 1983b), but not with species from the Himalayas (Wittmer 1975).Moreover, the types of T. subcaeruleiformis and T. crassimargo were compared, but no differences between them were found in external morphology (Fig. 4A, B) and aedeagi illustrated by Wittmer (1975: fig. 2; 1983b: fig. 3, 3a).Therefore, T. subcaeruleiformis is proposed here to be junior synonym of T. crassimargo, according to the Principle of Priority (ICZN 1999, Article 23.1).

## Themus (Telephorops)crassipes Pic, 1929

Figs 5A-C, 6D, 9E
Themus crassipes Pic, 1929b: 8.
Themus (Telephorops) crassipes: Wittmer 1983b: 191, 200, fig. 64 (female abdominal sternite VIII illustration).


Figure 5. Aedeagus (A, D ventral view B, E dorsal view C, F lateral view) A-C Themus crassipes Pic, 1929 D-F T. impressipennis (Fairmaire, 1886). Scale bar: 1.0 mm . (vp: ventral process of each paramere; dp : conjoint dorsal plate of parameres; lp: laterophyse; bp: basal piece).

Type material examined. $1 \widehat{\delta}^{\widehat{ }}$ (MNHN, holotype), [p]"CHAPG. prov. De \Laokay. Ht.-Tonkin", [h]"Themus crassipes \ n. sp.", [h]"type", [p]"HOLOTYPUS", [h]"Themus <br>(Telephorops)\crassipes $\backslash$ Pic det. W. Wittmer".

Other material examined. CHINA, Guangxi: $1 \delta^{\lambda}, 1 \nsubseteq$ (IZAS), Leye, Yachang Forestry, Nanchao, 1130 m, 2004.VII.26, leg. X. Yu; 1 if (MHBU), Tianlin, Cenwanglaoshan, 2014.VIII.16, leg. J.H. Huang; 1o (IZAS), Jinxiu, Rd. Jinzhong, 1100 m, 1999.V.12, leg. X.K. Yang; 2 (IZAS), same locality, 1000 m, leg. X. Z. Zhang.


Female. Like male, but antennomeres $\mathrm{V}-\mathrm{X}$ without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9E) with posterior edge narrowly and triangularly emarginate medially between paired rounded protuberances, each protuberance nearly as wide as space between it and apicolateral angle and hardly exceeding apex of apicolateral angle. Internal genitalia (Fig. 6D): diverticulum hardly narrowed apically and rounded at apex, about 2.5 times as long as its maximal width; spermatheca expanded apically.

Distribution. China (Guangxi, Yunnan); Vietnam.

## Themus (Telephorops) impressipennis (Fairmaire, 1886)

Figs 5D-F, 7A, 9F
Telephorops impressipennis Fairmaire, 1886: 339.
Telephorops violaceipennis Gorham, 1889: 105. Synonymized by Wittmer 1983b: 199. Themus (Telephorops) impressipennis: Wittmer 1983b: 199, fig. 60 (female abdominal sternite VIII illustration); 1983a: 153, fig. 51a (female abdominal sternite VIII illustration).

Type material examined. $1 \circlearrowleft$ (MNHN, holotype of Telephorops impressipennis), [h]"KouyTcheou (China, Guizhou)", [h]"Telephorops \ impressipennis \ Fairm.", [p]"HOLOTYPUS", [h] "Themus $\backslash$ (Telephorops) \impressipennis $\backslash$ (Fairm.) \det. W. Wittmer".

1 ( MNHN , holotype of Telephorops violaceipennis), [p]"Kiukiang (China, Jiangxi) \June $1887 \backslash$ A.E. Pratt", [h]"Type", [h]"violaceipennis", [h]"Themus $\backslash$ (Telephorops) \impressipennis <br>(Fairm.) \det. W. Wittmer".

Other material examined. CHINA, Guizhou: $2 q$ (MHBU), Daozhen, Xiannvhe, 2004.VIII.24-26, leg. X.J. Yang \& H.R. Hua; 1 q (MHBU), Suiyang, Baishaogou, 2010.VIII.14, leg. L.Y. Guo; $1 \circlearrowleft^{\lambda}$ (IZAS), Fanjingshan, Huguosi, 1350 m, 2001. VIII.3, leg. Q.Z. Song; 1q (IZAS), Fanjingshan, Heihaihe, 500 m, 2001.VII.27, leg. Q.Z. Song; $1 \delta^{\top}, 2 q$ (NHMB), Dakua, 35 km NE Leishan, 1994.VI.20.-24, lgt. Bolm; 1 ${ }^{\top}$, 1 q (NHMB), Leigongshan, Xijiang, 1200-1900 m,1997.V.29-VI.2, lgt. Bolm. Sichuan: $1 \sigma^{\Uparrow}$ (IZAS), Nanyang, 1200 m, 1987.VII. 17, leg. L.L. Yang; 1 q (IZAS), Emei Shan, Xixiangchi, 550-750 m, 1957.VI.8, leg. F.X. Zhu; 1 q (NHMB), Kwanhsien, 1928.VII.18, collector unknown; $10^{\uparrow}, 1 q$ (NHMB), Chuanxian, 600 m ,
 15, coll. D. C. Graham; 1 Q (NHMB), Guanxian, Dujiangyan Park, 1996.VIII.2, A. Zamotajiov \& A. Mirostrikov; 1 q (NHMB), Guanxian, 1992.VII.8, lgt. R. Dunda; 1 ( FHMB ), Gonggashan, Moxi, $1300 \mathrm{~m}, 29^{\circ} 13^{\prime} \mathrm{N}, 102^{\circ} 10^{\prime} \mathrm{E}, 1996 . \mathrm{VII} .10 .-11$, J. Farkač, P. Kabátek\&Smetana; 1o (NHMB), Emei Shan, 2500-1800 m, 1992. VII. Yunnan: $1 q$ (NHMB),Vallis flumin, Soling-ho., coll. R. Hicker. Hubei: $1 \sigma^{\top}, 3 q$ (MHBU), Dabieshan, Taohuachong, 2014.VI.23-27, leg. X.R. Li; $1 \delta^{\lambda}, 2$ (MHBU), Dabieshan, Wujiashan, 2014.VI.28-30, leg. X.R. Li; $2 q$ (MHBU), Wufeng, Houhe,


Figure 7. Female internal genitalia, lateral view A Themus impressipennis (Fairmaire, 1886) B T. laboissierei (Pic, 1921) C T. masatakai Okushima, 2003. Scale bars: 1.0 mm (sp: spermatheca; sr: sclerotized ring; va: vagina; di: diverticulum; ov: median oviduct; ag: accessory gland).
2002.VII.15, leg. S.X. Zhou; 1 ¢ (MHBU), same data, leg. Z.L. Xiang; 1 (MHBU), same locality, 2002.VII.16, leg. H.M. Zhang; 1 ¢ (MHBU), same locality, 2002.VII.16, leg. F.Y. Wang; 1 ¢ (MHBU), same locality, 2002.VII.16, leg. J. Guo; 1 ( (MHBU), same locality, 2002.VII.17, leg. L. Wang; 2 (MHBU), same locality, 2002.VII.18,
leg. Y. Liu; 19 (MHBU), same data, leg. F. P. Fu; 1 ( $q$ (MHBU), same locality and collector, 2002.VII.19; 1 q (MHBU), same data, leg. M. Wang; 2 q (MHBU), same data, J.B. Yan; $1 q$ (MHBU), same locality, 2002.VII.10, S.H. Yu; $1 q$ (MHBU), same locality, 2002.VII.20, leg. P.B. Luo; 1 q (MHBU), same data, leg. H.M. Zhang; 1 q (MHBU), same data, leg. J.R. Zheng; $10^{\text {đ }}$ (MHBU), same data, leg. C.H. Shi; $1 \delta^{\text {® }}$ (MHBU), same data, leg. H.F. Li; 1 q (MHBU), same locality, 2002.VII.26, leg. P. Shen; $1 \delta^{\lambda}, 1 q$ (MHBU), same locality, 2002.VII.14, leg. S.X. Zhou; $10^{\top}$ (MHBU), same data, leg. H.F. Li; $1 \widehat{N}^{\lambda}$ (MHBU), same data, leg. C.H. Shi; 10 (MHBU), same locality, 2002.VII.15, leg. X.Q. Yu; $1 \jmath^{\lambda}$ (MHBU), same locality, 2002.VII.19, leg. F.P. Fu; 1 Q (MHBU), Wufeng, Changleping, 2008.VII.17, leg. H.P. Zhang; 1 ( MHBU ), Xingshan, Gaolan, $1000 \mathrm{~m}, 2004 . V I I I .11$, leg. P. Jia; 1 q (MHBU), same data, leg. J. Xu; 1 q (MHBU), Xingshan, Huangliang, 1000 m, 2004.VII.12, leg. X.G. Zhou; 1 q (MHBU), same locality, 2004.VII.13, leg. H. Pan; 1 q (MHBU), same locality, 2004. VII.16, leg. D.W. Chen; $1 \delta^{\uparrow}$ (MHBU), same locality, 2004.VII.13, leg. Y.P. Zou; 1 q (MHBU), Xingshan, Nanyang, 1000 m, 2004.VII.13, P.P. Wang; 1 Q (MHBU), same locality, 2004.VII.14, leg. D.X. Tan; $2 q$ (MHBU), Shennongjia, Jiuhuping, 1900 m, 2006.VII.29, leg. L.K. Tan; $1 \uparrow$ (MHBU), Shennongjia, Muyu, 900 m, 2004.VIII.12, leg. Z.X. Liu; 1 ¢ (MHBU), same data, leg. R.L. Han; $1 \delta^{\top}$ (MHBU), same locality, 1200 m, 2004.VIII.12, leg. D.Y. Pan; 1q (MHBU), Changyang, Tianzhushan, 2005. VII.13, leg. X. Ming; 1 q (MHBU), same locality, 2005.VII.12, leg. Q. W. Wang; 1 q (MHBU), Changyang, Langping, Changfeng, $900 \mathrm{~m}, 2012 . \mathrm{VII} .4$, leg. H. Zheng; 1 q (MHBU), Badong, Lvcongpo, 1700 m, 2006.VII.14, leg. H.Y. Bao; 1 q (MHBU), Badong, Tiansanping, $1500 \mathrm{~m}, 2006 . V I I .14$, leg. Y.L. Chen; $1{ }^{\lambda}$ (MHBU), same data, leg. F. Xia; 1 q (MHBU), same data, leg. F. Yang; 1 q (MHBU), Yichang, Xiabaoping, 1000 m, 2004.VIII.11, leg. Q. Xie; 1 q (MHBU), same data, 2004.VIII.11, leg. W. M. Li; 1 iq (MHBU), same locality, 2004.VIII.13, leg. S.J. Huang; $1 \delta^{\AA}$ (MHBU), same data, leg. J. Li; $1 \sigma^{\lambda}$ (MHBU), same data, leg. H.Y. Lei; 1 ( T (MBU), same locality, 2004. VIII.14, leg. B.J. Yu; 1 Q (MHBU), Yichang, Dalaoling Forestry, 2010.VI.24, leg. W. Li; 1 q (MHBU), Yichang, Xianrenxi, 2009.IX.12, leg. G.L. Xie; $10^{\lambda}$ (MHBU), same locality and collector, 2009.VI.25; $1 才$ (IZAS), Xingshan, Longmenhe, 1350 m, 1993. VII.18, leg. B.W. Sun; 1 (I (IZAS), same data, 1993.VII.14; $1 \sigma^{\text {§ }}$ (NHMB), Lichuan, Shaoho, 1948.VIII.13, coll. Gressitt \& Djou; $1 \lesssim$ (NHMB), same data, 1948.VIII.12;
 Shaanxi: 1 (MHBU), Meixian, Songping, 2012.VII.12,leg. G.D. Ren; $1 \delta^{\lambda}(\mathrm{MHBU})$, Nanzheng, Beiba, 2005.VI.19-22, leg. Y.B. Ba; 1 Q (MHBU), Chushui, Niubeiliang, 2011.VIII.22-29, leg. X.C. Zhu \& Y.C. Zhao; $1 \delta^{\lambda}, 1 q$ (IZAS), Ningshan, Huoditang, 1580-1650 m, 1999.VI.27, leg. D. C. Yuan. Gansu: $10^{\lambda}$ (IZAS), Kangxian, Qinghelinchang, 1400 m, 1998.VII.8, leg. J. Yao; 1q (IZAS), Kangxian, Douba, 1050m, 1999. VII.6, leg. H. J. Wang. Henan: $1{ }^{\Uparrow}$ (IZAS), Songxian, Baiyunshan, 1600 m, 2002. VII.19, leg. W.Z. Li; 1 Q (IZAS), Lushixian, Jihelinchang, 1200 m, 2001.VII.20, leg. K.Z. Dong. Hunan: 1 q (MHBU), Changsha Agriculture University, 2012.VII.23, leg. H. Xu; $1 \widehat{c}^{\top}$ (IZAS), Yongshun, Shanmuhe Forestry, 600-820 m, 1988.VIII.7, leg. S.Y. Wang; 1 q (IZAS), Sangzhi, Tianpingshan, 1370-1570 m, 1988.VIII.13, leg. S.Y. Wang; 1 ( NH MB),Wulingshan, Tianzishan Nat. Res., 800 m, 1997.VI.16.-18, lgt.

Bolm; $1 q$ (NHMB), Kiang Jia Jie, 1200-1600 m, 1992.VII. Zhejiang: $1 q$ (MHBU), Hangzhou, Lin'an, Dajingwu, 2012.V.10, leg. H. Xu; 4才, 1 (MHBU), Lin’an, Qingliangfeng, Shunxi, 2012.VI.25, leg. H. Xu; 1 ${ }^{\top}, 1 q$ (MHBU), Longquan, Fengyangshan, 2007.VII.29, leg. L.K. Tan; 1 (MHBU), same locality, 2007.VII.27, leg. G.L. Xie; 1 q (MHBU), same locality and collector, 2007.VII.26; 1ठ (IZAS), Tienmushan, 1935. VII.15, collector unknown; $1 \uparrow$ (IZAS), same data, 1935.VIII.4. Jiangxi: $2{ }^{\top}$ (NHMB), Kuling, 1934.IX.4, coll. O. Piel. Taiwan: $1 q(\mathrm{NHMB})$, Formosa, T. Kano. Fujian: $1 q$ (MHBU), Wuyishan, Tongmu, Tongmuguan-Sangang, 740-1160 m, 2004.VIII.20, leg. D.K. Zhou; 19 (IZAS),Dehua, Lishan, $900-1200 \mathrm{~m}, 1960 . V I .12$, leg. F.J. Pu; $1 q$ (IZAS), Jiangle, Longqishan, 1991.V.25, leg. Y. S. Shi; $2 \widehat{1}$ (NHMB), Kuatun, 1946. IX.18. Anhui: $6{ }^{\top}, 4 \not \subset(\mathrm{MHBU})$, Shexian, Qingliangfeng, 2013.VI.5-9, leg. J.S. Xu \& C.X. Yuan; $2 \oint^{\top}$ (NHMB), Kiuhua Shan, 1932.IX, G. Liu Fukien; 1§, $2 \nrightarrow$ (NHMB), Yuexi, Miaodaoshan mts., $600-1300 \mathrm{~m}, 30^{\circ} 48^{\prime} \mathrm{N}, 116^{\circ} 05^{\prime} \mathrm{E}, 1995 . V I I .18 .-20$, lgt. L. R. Businský. Guangdong: $1 \uparrow$ (MHBU), Nanling, 2010.VIII.10, leg. H.Y. Liu. Guangxi: 1 q (MHBU), Jiuwandashan, Jiuren Reserve Station, 2003.VIII.3, leg. L.L. Zhang; 1才, $1 \not \subset$ (IZAS), Longsheng, Tianpingshan, $740 \mathrm{~m}, 1963 . V I .17$, leg. S.Y. Wang.

Supplementary description. Male. Aedeagus (Figs 5D-F): ventral process of each paramere about 3 times as long as wide in ventral view, expanded and nearly globose at apex in lateral view; conjoint dorsal plate of parameres hardly shorter than ventral processes, depth of middle emargination about one-third of entire length.

Female. Like male, but antennomeres V-X without impressions along outer edges(while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9F) with posterior edge narrowly and triangularly emarginate medially and paired rounded protuberances, each nearly as wide as the distance between it and apicolateral angle and not reaching apex of the latter. Internal genitalia (Fig. 7A): diverticulum little thinned apically and rounded at apex, about 2.5 times as long as its maximal width; spermatheca expanded apically.

Distribution. China (Gansu, Shaanxi, Henan, Jiangsu, Anhui, Zhejiang, Hubei, Jiangxi, Hunan, Fujian, Taiwan, Guangxi, Sichuan, Guizhou, Yunnan).

## Themus (Telephorops) laboissierei (Pic, 1929)

Figs 4C-D, 7B, 9G
Triblius laboissierei Pic, 1929a: 195, 196.
Themus (Tryblius) separandus Wittmer, 1975: 252, fig. 3 (aedeagus illustration). syn. n.
Themus (Telephorops) laboissierei: Wittmer 1983b: 200, figs 4 (aedeagus illustration), 65 (female abdominal sternite VIII illustration).
Themus (Telephorops) separandus: Kazantsev and Brancucci 2007: 271 (distributional data).

Type material examined. $1 \delta^{\lambda}$ (MNHN, lectotype of Triblius laboissierei), [ph]"TONKIN \Chapa \3.VII. 1917 \JEANVOINE", [h]"Tryblius \laboissierei \n.
sp.", [h] "type", [p]"LECTOTYPUS", [h]"Themus \(Telephorops) \ laboissierei \Pic \} det. W. Wittmer". The lectotype was designated by Wittmer (1983b).

1才 (BMNH, holotype of Themus (Telephorops) separandus), [p] "Gopaldhara, Darjeeling, 25.VII.1914, 3440-4720', leg. H. Stevens", [p] "HOLOTYPUS", [h] "Themus <br>(Tryblius) \ separandus \ Wittm. \det. W. Wittmer".

Other material examined. CHINA, Yunnan: $1 \delta^{\hbar}$ (IZAS), Jinping, Hetouzhai, 1700m, 1956.V.9, leg. K.R. Huang; same data, 1500-1700 m, 1956.V.11; 1ô, 1 ㅇ (IZAS), Xishuangbanna, Menghai, $1200-1600 \mathrm{~m}, 1958 . \mathrm{VII} .18$, leg. S.Y. Wang; 1 § (IZAS), same locality, 1958.VII.21, leg. F.J. Pu;1才 (MHBU), Qushi, Jiangmu, 2011. VII.16,leg. H.Y. Liu. Guangxi: $1{ }^{\text {hen }}$, 3 (MHBU), Tianlin, Cengwanglaoshan, 2014. VIII.16, leg. J.H. Huang; 1 it (MHBU), same locality, 1300-1400 m, 2009.V.16-19, collector unknown.

Supplementary description. Female. Like male, but antennomeres V-X without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (narrower and triangular in male) (Fig. 9G) with posterior edge narrowly and triangularly emarginate medially between paired obtuse protuberances, each protuberance nearly as wide as the distance between it and apicolateral angle and not reaching apex of the latter. Internal genitalia (Fig. 7B): diverticulum hardly narrowed apically and rounded at apex, about three times as long as its maximal width; spermatheca expanded apically.

Distribution. China (Yunnan, Guangxi); northern Laos, northern Vietnam, northern India.

Remarks. Themus (Tryblius) separandus was described based on a single male type and its aedeagus was illustrated by Wittmer (1975). Except the original publication, no additional information was available. The type locality is "Gopaldhara, Darjeeling" (N. India), not Bhutan as that listed by Kazantsev and Brancucci (2007).

Wittmer (1975) noted that the single specimen designated as holotype of T. separandus was separated from the collection of T. crassimargo in BMNH. Wittmer differentiated T. separandus from T. crassimargo by the structure of aedeagus, also from T. cavipennis and T. nepalensis in the body coloration and aedeagus. He made no comparison with other species.

In the present study, the habitus (Fig. 4C-D) and aedeagi of T. separandus and T. laboissierei were compared (Wittmer 1975: fig. 2; Wittmer 1983b: 4), but no differences found. Thus we recommend T. separandus Wittmer, 1975 to be junior synonym of T. laboissierei, according to the Principle of Priority (ICZN 1999, Article 23.1).

## Themus (Telephorops) masatakai Okushima, 2003

Figs 7C, 9H
Themus (Telephorops) masatakai Okushima, 2003: 280, figs 1-4 (habitus photo, aedeagus illustrations); Kopetz 2010: 185 (distributional data), fig. 4 (aedeagus illustration); Kopetz 2016: 255, figs 15 (habitus photo), 44 (female abdominal sternite VIII photo).

Material examined. LAOS: $1 \AA^{\lambda}, 1 q$ (NHMB), Oudomxai, 17 km NEE, 1100 m , $20^{\circ} 45^{\prime} \mathrm{N}, 102^{\circ} 09^{\prime} \mathrm{E}, 2002 . \mathrm{V} .1 .-9$, leg. Vit Kubáň; $10^{\top}$ (NHMB), Phongsaly, Ban Sano Mai, $1150 \mathrm{~m}, 21^{\circ} 21^{\prime} \mathrm{N}, 102^{\circ} 03^{\prime} \mathrm{E}, 2004 . \mathrm{V} .19 .-26$, M. Brancucci; $1 \delta^{\top}$ (NHMB), Phongsaly, $1500 \mathrm{~m}, 21^{\circ} 41^{\prime} \mathrm{N}, 102^{\circ} 06^{\prime} \mathrm{E}, 2004 . \mathrm{V} .6 .-17$, M. Brancucci; $1 \delta^{\top}$ (NHMB), 20 km NW Louang Namtha, $900-1000 \mathrm{~m}, 21^{\circ} 09.2^{\prime} \mathrm{N}, 101^{\circ} 18.7^{\prime} \mathrm{E}, 1997 . \mathrm{V} .5 .-30$, C. Holzschuh.

CHINA, Yunnan: $1 \delta^{\lambda}$ (IZAS), Xishuangbanna, Meng'a, 1050-1080 m, 1958.V.13, leg. F.J. Pu; $1 \delta^{\top}$ (IZAS), same data, leg. S.Y. Wang; 1 Q (IZAS), same locality and collector, 1958.VIII.10; $1 \oint^{\Uparrow}$ (IZAS), Simao, Rd. Kunluo 591 km, 1350 m, 1957.V.11, leg. F.J. Pu; 1 q (IZAS), Simao, 1957.V.23, leg. A. Мэнцяский; 1 q (IZAS), Simao, $1200 \mathrm{~m}, 1957 . \mathrm{V} .11$, leg. S.Y. Wang.

Supplementary description. Female. Like male, but antennomeres VII-XI without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9H) with posterior edge narrowly and triangularly emarginate medially between paired rounded protuberances, each protuberance nearly as wide as the distance between it and apicolateral angle and exceeding apex of the latter. Internal genitalia (Fig. 7C): diverticulum expanded apically and rounded at apex, about twice as long as its maximal width; spermatheca expanded apically.

Distribution. China (Yunnan, Guangxi); Laos, northernVietnam.

## Themus (Telephorops) minor Wittmer, 1997

Themus (Telephorops) minor Wittmer, 1997: 272, fig. 104 (aedeagus illustration); Kopetz 2010: 185, fig. 44 (female abdominal sternite VIII illustration).

Type specimens examined. $1 \delta$ (holotype, NHMB), [p] "YUNNAN, 23.-24.JUN I YULONG Mts., 1993 \27.00N 100.12E \ Bolm lgt. 3200m", [p] "HOLOTYPUS", [h] "Th. (Tryblius) $\backslash$ minor Wittm. $\$ det. W. Wittmer", [p] "CANTHARIDAE \ CANTH00001283".

Distribution. China (Yunnan).

## Themus (Telephorops) nepalensis (Hope, 1831)

Telephorus nepalensis Hope, 1831: 26.
Themus (Tryblius) nepalensis: Wittmer 1975: 252.
Themus (Telephorops) nepalensis: Okushima 1999: 58 (distributional data), figs 9 (habitus photo), 31-33 (aedeagus and female abdominal sternite VIII illustrations).

Distribution. Northern India, Nepal.

## Themus (Telephorops) sauteri (Pic, 1912)

Figs 8A, 9I
Cantharis sauteri Pic, 1912: 46.
Themus sauteri: Wittmer 1954: 276.
Themus (Telephorops) sauteri: Wittmer 1983a: 197, figs 47 (aedeagus illustration), 50 (female abdominal sternite VIII illustration).

Material examined. Taiwan: $1 \circlearrowleft$ (NHMB), Nanshanchi, 1978.VI.18, H. Akiyama; $1 \delta^{\lambda}, 1 q(\mathrm{NHMB})$, Formosa, T. Kano; $1 q$ (NHMB), Wushe, 1975.VI.9, K. Akiyama; $1 \sigma^{\top}$ (NHMB), Taichung Hsien, Kukuan, 1996.VII.12, leg. C. Lou; $1 \sigma^{\Uparrow}$ (NHMB), same data, 1994.VI.20; 1 Q (NHMB), Nantou Hsien, Sungkang, 1995.VII.17, leg. C. Lou; 1 ¢ (NHMB), Nantou Hsien, Shintzetou, 1994.VII.14, leg. C. Lou; 1才, $2 q($ IZAS), Taichung Hsien, Kukuan,1996.VII.12, leg. C. Lou; $1 q$ (IZAS), Mt. Nantou Hsien, Hohwangshan, 1997.VIII. 27, leg. C. Lou.

Supplementary description. Female. Like male, but antennomeres VI-X without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9I) with posterior edge triangularly emarginate medially and largely and triangularly emarginate on both sides, lateral emargination about 3 times as deep as middle one, the protuberances between middle and lateral emarginations acute, exceeding the acute apices of apicolateral angles. Internal genitalia (Fig. 8A): diverticulum hardly narrowed apically and rounded at apex, about twice as long as its maximal width; spermatheca expanded apically.

Distribution. Taiwan.

## Themus (Telephorops) subcaeruleus (Pic, 1911)

Tryblius cavipennis var. subcaeruleus Pic, 1911: 132.
Themus (Tryblius) subcaeruleus: Pic 1929a: 195.
Themus (Telephorops) subcaeruleus: Wittmer 1983b: 199, figs 2 (aedeagus illustration), 62 (female abdominal sternite VIII illustration).

Type material examined. $1 \bigcirc$ (MNHN, lectotype), [p]"Yünan \China", [h]"type", [h]"Tryblius \ subcaeruleus \ Pic", [p]"LECTOTYPUS", [h]"Themus \ (Telephorops) I subcaeruleus \ Picl det. W. Wittmer". The lectotype was designated by Wittmer (1983b).

Other material examined. CHINA: $1 q$ (NHMB), Yunnan; $1 \AA^{\lambda}$ (NHMB), Pe Yen Tsing; 1 ( NHMB ), Tche-Ping-Tcheou.

Distribution. China (Yunnan), northern Vietnam.


Figure 8. Female internal genitalia, lateral view A Themus sauteri (Pic, 1912) B T. uncinatus Wittmer, 1983. Scale bars: 1.0 mm .

Themus (Telephorops) uncinatus Wittmer, 1983
Figs 8B, 9J
Themus (Telephorops) uncinatus Wittmer, 1983b: 200, figs 5 (aedeagus illustration), 66 (female abdominal sternite VIII illustration).

Type material examined. $1 \circlearrowleft^{\lambda}$ (holotype, MNHN), [p] "MUSEUM PARIS \SETSCHOUEN (China, SE. Sichuan) \ ENV DE TA_TSIEN-LOU (Dajianlu, now is Kangding) \MO-SY-MIEN \Père AUBERT 1902", [p] "HOLOTYPUS", [h] "Themus (Telephorops) \uncinatus \Wittm. \det. W. Wittmer".

Other material examined. CHINA, Sichuan: $1 \delta^{\top}$ (NHMB), Jinfo Shan, 1700$1950 \mathrm{~m}, 29^{\circ} 01^{\prime} \mathrm{N}, 107^{\circ} 14^{\prime} \mathrm{E}, 1998 . \mathrm{VI} .24 .-29$, D. Král; 1 中 (NHMB), Chadiping, 1200-1500m, 1996.VIII.5.-7, A. Miroshnikov \& A. Zamatajiov; $10^{\top}, 1$ (IZAS), Luding, Moxi, 1500 m, 1983.VI.17, leg. S.Y. Wang; $1{ }^{\top}$ (IZAS), Emei Shan, Jiulaodong, 1800-1900 m, 1957.VII.28, leg. K.R. Huang. Yunnan: $4 \widehat{c}^{\lambda}, 1 q$ (MHBU),


Figure 9. Female abdominal sternite VIII, ventral view A Themus bicoloricornis Wittmer, 1983 B T. cavipennis Champion, 1926 C T. coelestis (Gorham, 1889) D T. crassimargo Champion, 1926 E T. crassipes Pic, 1929 F T. impressipennis (Fairmaire, 1886) G T. laboissierei (Pic, 1921) H T. masatakai Okushima, 2003 I T. sauteri (Pic, 1912) J T. uncinatus Wittmer, 1983. Scale bars: 1.0 mm .

Lushui, Laowo, 1500 m, 2008.VII.26.-28, leg. J.S. Xu \& Z.H. Gao; 1 §, 2 q (MHBU), Lushui, Pianma, 2005.VII.22.-23, leg. B.Y. Mao \& J.S. Xu; 1 (MHBU), Longling, Longxin, Heishan, 2008.XII.22.-23, leg. J.S. Xu \& Z.H. Gao.

Supplementary description. Female. Like male, but antennomeres V-X without impressions along outer edges (while present with smooth narrow longitudinal or oblong impressions in male), terminal abdominal ventrite wide (while narrow and triangular in male) (Fig. 9J) with posterior edge narrowly and triangularly emarginate medially between paired protuberances, each protuberance wider than the distance between it and apicolateral angle and hardly exceeding apex of the latter. Internal genitalia (Fig. 8B): diverticulum narrowed apically and pointed at apex, about twice as long as its maximal width; spermatheca moderately expanded apically.

Distribution. China (Sichuan, Yunnan), northern Vietnam.

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## References

Brancucci M (1980) Morphologie comparée, évolution et systématique des Cantharidae (Insecta: Coleoptera). Entomologica Basiliensia 5: 215-388.
Champion GC (1926) Some Indian (and Tibetan) Coleoptera (19). The Entomologist's Monthly Magazine 62: 118-137.
Fairmaire L (1886) Descriptions de coléoptères de l'intérieur de la Chine (2. partie). Annales de la Société Entomologique de France 6(6): 303-356.
Fairmaire L (1897) Coléoptères de l'Inde et de la Malaisie. Notes from the Leyden Museum 18[1896/97]: 225-240. https://doi.org/10.5962/bhl.part. 29501
Gorham HS (1889) Descriptions of new species and a new genus of Coleoptera of the family Telephoridae. Proceedings of the Zoological Society of London1889: 96-111. https://doi. org/10.1111/j.1469-7998.1889.tb06759.x
Hope FW (1831) Synopsis of the new species of Nepal Insects in the collection of Major General Hardwicke. In: Gray JE (Ed.) Zoologica Miscellanea 1: 21-32.
ICZN (1999) International Code of Zoological Nomenclature (Fourth Edition).

Kazantsev SV, Brancucci M (2007) Cantharidae. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera, Vol. 4. Apollo Books, Stenstrup, 234-298.
Kopetz A (2004) Zur Kentnis der Gattung Themus Motschulsky, 1857 im Himalaya (Coleoptera, Cantharidae). Entomologica Basiliensia 26: 113-153.
Kopetz A (2010) Ein weiterer Beitrag zur Kenntnis der Gattung Themus Motschulsky, 1858 (Coleoptera: Cantharidae). Veröffentlichungen des Naturkundemuseums Erfurt 29: 165-188.
Kopetz A (2016) Zur Kenntnis der Gattungen Themus Motschulsky, 1858 und Cyrebion Fairmaire, 1891 in Mittel- und Ostasien (Coleoptera, Cantharidae). Entomologische Blätter und Coleoptera 112(1): 245-267.
Okushima Y (1999) Cantharidae collected by the Hokkaido University Expeditions to Nepal Himalaya (Coleoptera). Insecta Matsumurana 56: 51-68. http://hdl.handle.net/ 2115/9897
Okushima Y (2003) A new beautiful species of the genus Themus (Coleoptera, Cantharidae) from Laos. Special publication of the Japan Coleopterological Society 6: 279-282.
Pic M (1911) Coléoptères exotiques nouveaux ou peu connus. L'Échange, Revue Linnéenne 27: 132-134. https://doi.org/10.5962/bhl.part. 2978
Pic M (1912) Coléoptères exotiques nouveaux ou peu connus. L'Échange, Revue Linnéenne 28: 46-47.
Pic M (1926) Hetéromères et Malacodermes nouveaux. Bulletin du Muséun d'Histoire Naturelle (Paris) 32: 211-212.
Pic M (1929a) Sur le genre Triblius Fairm. (Col. Malacodermata). Bulletin de la Société Zoologique deFrance 54: 195-196.
Pic M (1929b) Coléoptères exotiques en partie nouveaux (Suite). L'Échange, Revue Linnéenne 45: 7-8.
Švihla V (2008) Redescription of the subgenera of the genus Themus Motschulsky, 1858, with description of five new species (Coleoptera: Cantharidae. Veröffentlichungen des Naturkundemuseums Erfurt 27: 183-190.
Wang S-J, Yang J-K (1992) Coleoptera: Cantharidae. In: Huang FS (Ed.) Insects of Wuling mountains area, SW China. Science Press, Beijing, 264-267.
Wittmer W (1954) Zur Kenntnis der Cantharidae und Malachiidae der Insel Formosa. Revue Suisse de Zoologie 61(7): 271-282. https://doi.org/10.5962/bhl.part. 75389
Wittmer W (1975) Ergebnisse der Bhutan-Expedition 1972 des Naturhistorischen Museums in Basel. Coleoptera: lam. Cantharidae (2.Teil). Entomologica Basiliensia 1: 249-278.
Wittmer W (1983a) Die Familie Cantharidae (Col.) auf Taiwan (2. Teil). Entomological Review of Japan 38(2): 147-172.
Wittmer W (1983b) Beitrag zur einer Revision der Gattung ThemusMotsch. Coleoptera: Cantharidae. Entomologischen Arbeiten aus dem Museum G. Frey 31/32: 189-239.
Wittmer W (1997) Neue Cantharidae (Col.) aus dem indo-malaiischen und palaearktischen Faunengebiet mit Mutationen. 2. Beitrag. Entomologica Basiliensia 20: 223-366.
Yang Y-X, Kopetz A, Yang X-K (2013) Taxonomic and nomenclatural notes on the genera Themus Motschulsky and Lycocerus Gorham (Coleoptera, Cantharidae). Zookeys 340: 1-19. https://doi.org/10.3897/zookeys.340.5470

Yang Y-X, Su J-Y, Kopetz A (2014) Descriptions of three new cantharid species related to Themus (Themus) senensis (Pic, 1922) (Coleoptera: Cantharidae). Annales Zoologici 64(4): 655-666. https://doi.org/10.3161/000345414X685938
Yang Y-X, Zong L, Yang X-K, Liu H-Y (2019) A taxonomic study on the Themus (Telephorops) davidis species-group, with description of a new species from China. Zootaxa 4612(3): 401-411. https://doi.org/10.11646/zootaxa.4612.3.6

# Revision of the New World genus Enderleiniella Becker, I912 (Diptera, Chloropidae) 

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#### Abstract

The genus Enderleiniella Becker, 1912 is revised. The genus is distinguished on the basis of a somewhat flattened head with the inner vertical setae located anteromedially to the outer vertical setae, three lightly incised lines on the scutum, trapezoidal or rectangular scutellum with marginal setae borne on tubercles, reduced alula and anal angle of the wing, and the structure of the male genitalia. The genus contains eleven species in the northern Neotropical and southern Nearctic Regions: E. caerulea sp. nov. (type locality: Blue Creek, Belize); E. cryptica sp. nov. (type locality: 24 km W Piedras Blancas, Costa Rica); E. flavida sp. nov. (type locality: Emerald Pool, Dominica); E. longiventris (Enderlein, 1911) (type species; type locality: Costa Rica); E. maculata sp. nov. (type locality: Xilitla, San Luis Potosi, Mexico); E. marshalli sp. nov. (type locality: Guanacaste, Costa Rica); E. maya sp. nov. (type locality: Las Escobas, Guatemala); E. punctata sp. nov. (type locality: Potrerillo, Bolivia); E. tripunctata (Becker, 1916) (type locality: San Mateo, Costa Rica); $E$. tumescens sp. nov. (type locality: San Esteban, Venezuela); and $E$. wheeleri $\mathbf{s p}$. nov. (type locality: Turrialba, Costa Rica).


## Keywords

Grass fly, frit fly, Neotropic, Nearctic, Central America, taxonomy

## Introduction

The genus Enderleiniella was proposed by Becker (1912) for the single species Tricimba longiventris Enderlein, 1911. Subsequently, Becker (1916) proposed a second genus, Anoscinella Becker, 1916, for the new species Anoscinella tripunctata Becker, 1916. Duda (1930) considered the two species congeneric and synonymised Anoscinella under Enderleiniella. That synonymy was accepted by subsequent authors (e.g., Sabrosky

[^4]and Paganelli 1984) and there has been no published taxonomic research on the genus since. As part of an inventory of Costa Rican Chloropidae and the chloropid chapter in the Central American Manual of Diptera (Wheeler 2010), several undescribed species of Enderleiniella were identified in the northern Neotropical and extreme southern Nearctic regions. The validity of the genus has recently been questioned because certain characters of Enderleiniella seem to fit into the definition of Tricimba Lioy, 1864 ( M von Tschinrhaus and JW Ismay pers. comm.).

The purpose of this paper is to revise the genus Enderleiniella, provide descriptions of those new species, present a morphological key to species, and discuss the validity of Enderleiniella as a genus.

## Materials and methods

Specimens studied are housed in the Canadian National Collection of Insects, Ottawa, Ontario, Canada (CNC); University of Guelph Insect Collection, Guelph, Ontario, Canada (DEBU); Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica (INBio); Lyman Entomological Museum, McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada (LEM); National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

For examination of genitalia, the abdomen was removed (older air-dried specimens were relaxed in a humidity chamber prior to dissection) and cleared in $85 \%$ lactic acid heated in a microwave oven for two periods of 10 seconds, separated by a one-minute cooling period. The cleared abdomen was then placed in glycerine for further dissection and examination. The dissected abdomen was stored in glycerine in a plastic microvial pinned beneath the source specimen. Morphological observations were done on a Leica M165C microscope, genitalic observations were done on a Leica DM6C microscope. The specimens were photographed with a Leica DFC 450 camera mounted on the microscope. Morphological terminology follows Cumming and Wood (2009). I sent specimens for sequencing of the insect barcode fragment of the Cytochrome c Oxidase one (CO1) to the Centre of Biodiversity Genomics, Biodiversity Institute of Ontario. Because most specimens are old, only eight individuals were successfully sequenced with the complete 658 bp fragment representing four species (GenBank: MK919190, MK919191, MK919192, MK919194, MK919195, and MK919196). To determine whether Enderleiniella should be synonymised with Tricimba, I chose seven species as outgroups in the subfamily Oscinellinae. These seven species were chosen based on the availability from the public records on BOLD and that they represent different groups within the subfamily Oscinellinae. To determine whether Enderleiniella should be synonymised with Tricimba, I chose the type species of Tricimba and two others from other biogeographical realms: Tricimba linealla (BOLD:AAH4184), Tricimba trisulcata (GenBank: JF867146, BOLD:AAN5667), and a specimen of Tricimba sp. (from the Neotropics; GenBank: MK919193). Sequences of Oscinella frit (GenBank: OPPFO330, BOLD:AAN5659), Aphanotrigonum scabrum (GenBank: JF874104, BOLD:AAQ0868), Eribolus nana (GenBank: JF873115, BOLD:AAH4175), and Ela-
chiptera nigriceps (BOLD:AAP5169) were also analysed as representatives of other tribes in the subfamily Oscinellinae.

The 658 bp cytochrome oxidase one barcode sequences were aligned in MUSCLE (Edgar 2004). A Maximum Likelihood (ML) tree using GTR+G+I evolution model (the best fit for the sequences; $\mathrm{AICc}=3788.63$; $\mathrm{BIC}=4053.86$ ) to determine whether there is molecular support to validity of Enderleiniella as a genus. Bootstrap value branch support was determined by replicating the analyses 1000 times (Felsenstein 1985). Evolutionary analyses and ML tree were conducted in MEGA 7 (Kumar et al. 2016).

## Taxonomy

## Enderleiniella Becker, 1912

Enderleiniella Becker, 1912: 192. Type species: Tricimba longiventris Enderlein (original description).
Anoscinella Becker, 1916: 448. Type species: Anoscinella tripunctata Becker (monotypy); Duda 1930: 70 (synonymy).

Diagnosis. Small to medium Oscinellinae with head as wide as or slightly wider than scutum in dorsal view, occiput posteriorly convex in dorsal view, eye usually hairy, gena thin always parallel with ventral portion of eye, scutum elongate, with three parallel lines of shallow incised punctures, wing long and slender, broadest in distal half, with reduced alula and anal angle, legs long and slender, abdomen long and narrow, male genitalia with sternite 6 present, in some species epandrium enlarged and cercus elongate.

Description. (Figs 1-4): Chloropidae, Oscinellinae. Head. Frontal triangle glossy or microtomentose, occiput convex; frons with many interfrontal setulae; cephalic setae short, $6-12$ reclinate fronto-orbitals with most dorsal seta proclinate; ocellars and postocellars erect, convergent, vertical setae stronger than other cephalic setae, inner vertical setae as long as outer vertical setae, in line with posterior ocelli; ocelli in most species large; eye large, oval, usually densely haired; postgena clearly visible; gena linear, densely pruinose, very pale except for brown ventral margin; vibrissal angle not projecting, vibrissa present but small; face flat, narrow, pruinose, very pale, facial carina short and small; antenna with scape and pedicel short, first flagellomere large, subquadrate; arista sparsely pubescent, aristal setulae usually longer than width of arista at base; proboscis variable from small to geniculate, but never long, palpus short. Scutum. Pronotum elongate and visible in dorsal view, sulcus between postpronotum and scutum deep and well defined; scutum usually glossy, rarely pruinose, with three parallel lines of finely incised punctures (treated as grooves by Ismay (1993)), less distinct in some species, with one anterior and either one (Enderleiniella flavida) or two posterior notopleural setae, one longer outer postalar bristle and one weak inner postalar setae, one postsutural dorsocentral bristle, all dark; postpronotal seta and other scutal setae weak; scutal setulae short, weak, evenly arranged in three distinct rows; in certain species scattered setulae present between and outside the three distinct rows; scutellum flattened, trapezoidal or rectangular dorsally, with two small marginal projections,


Figures I-4. I Enderleiniella longiventris, head lateral view. 2 Enderleiniella longiventris, scutum and scutellum 3 Enderleiniella flavida, scutum and scutellum 4 Enderleiniella maculata, wing. Scale bars: 0.5 mm .
bearing one strong bristle, lateral marginal scutellar setae present; Thoracic pleurites bare. Wing. Hyaline (a darkened spot in Enderleiniella maculata), long and slender, broadest in distal half, alula and anal angle reduced, cell c normal, second costal sector longer than third; cell $\mathrm{r}_{1}$ normal and long, $\mathrm{R}_{2+3}$ and $\mathrm{R}_{4+5}$ divergent at base, cell br narrow, crossvein $\mathrm{r}-\mathrm{m}$ near middle of cell dm, crossvein dm-cu fused with M4 in a right angle. Legs. Slender without outstanding setae or spurs; femoral organ present as a row or small patch of three or four sensillae, tibial organ long oval, velvety, with single longitudinal row of setulae along midline. Abdomen. Narrow, cylindrical; syntergites $1+2$ elongate; tergites broad; sternites small and narrow; spiracles 3-5 in abdominal membrane ventral to lateral margins of tergites;

Male specific characters. Sternite 6 present; dorsal pregenital sclerite symmetrical, left and right spiracles 6 and 7 within sclerite close to lateral margin; epandrium variable from small and simple to enlarged and inflated. Surstylus simple, straight, elongate; hypandrium open posteriorly; pregonite and postgonite simple, round and elongate; cercus variable from simple and elongate to multiple lobed; subepandrial sclerite usually small.

Female specific characters. Terminalia unmodified, typical of Oscinellinae.
Comments. The type species, Enderleiniella longiventris, is atypical in having a densely pruinose scutum and a long, rectangular scutellum.

## Key to described species of Enderleiniella

1 Scutellum pale yellow (Fig.16), contrasting strongly with dark scutum; occiput with single strong bristle behind eye; one anterior and one posterior notopleural bristle
E. flavida sp. nov.

- $\quad$ Scutellum brown or grey, not contrasting strongly with scutum; occiput without outstanding bristle behind eye; one anterior and two posterior notopleural setae 2
2 Scutum and frontal triangle densely pollinose (dull) (Figs 26, 33)............... 3
- $\quad$ Scutum and frontal triangle glossy or sparsely pollinose (Figs 21, 53, 59) ... 4 Wing hyaline; male epandrium expanded, broader and tall than pre-epandrium; large species, body length $2.7-3.6 \mathrm{~mm} . . . . .$. . E. longiventris (Enderlein)
- Wing with a dark apical spot (Fig. 33); male epandrium smaller, not excessively higher than pre-epandrium; smaller species, body length $2.4-2.9 \mathrm{~mm}$.. E. maculata sp. nov.

Occiput with dorsolateral pubescent swelling just behind outer vertical bristle (in males because females currently unknown); mouthparts not geniculate (the only species from Venezuela).
E. tumescens sp. nov.

- Occiput not modified, swelling absent; mouthparts geniculate or not (species not known from Venezuela)5
$5 \quad$ Scutum 1.2 times as long as wide; Abdominal tergite 3 with setae arising from enlarged punctate sockets (in males because females currently unknown) .....
E. punctata sp. nov.

Scutum at least 1.5 times as long as wide. Abdominal tergite 3 unmodified..... 6
6 Scutellum rectangular; apical scutellar tubercles long at least $1 / 5$ the length of the scutellum
E. maya sp. nov.

- $\quad$ Scutellum trapezoidal; apical scutellar tubercles short or long at most 1/8 the length of scutellum 7
7 Scutum sparsely pollinose ............................................E. marshalli sp. nov.
- Scutum polished......................................................................................... 8

8 Mouthparts geniculate .............................................................................. 9

- Mouthparts not geniculate or elongate...................................................... 10
$9 \quad$ Scutellum 0.8 times as wide as long, with distinct tubercles, Male epandrium large, surstylus parallel sided with round tip E. cryptica sp. nov.
- $\quad$ Scutellum 0.6 times as long as wide, with very small tubercles; Male genitalia small, surstylus triangular with pointed tip E. wheeleri sp. nov.

10 Hairs on scutum in well-defined rows between punctate dorsocentral rows (grooves). In male, epandrium very wide compared to high, cerci in lateral view directed posteroventrally . E. caerulea sp. nov.

- Hairs on scutum scattered between punctate rows. In male, epandrium square, cerci in lateral view directed ventrally.......... E. tripunctata (Becker)


## Enderleiniella caerulea sp. nov.

http://zoobank.org/0998D876-29F8-4195-AA88-3A04701C6EA5
Figs 5-9
Diagnosis. Medium Oscinellinae with a shiny frontal triangle and thorax. Hairs on scutum placed in well-defined rows between punctate dorsocentral rows (grooves). Scutellum small and trapezoidal with small tubercles. Male postabdomen large and bulbous.

Description. Total length 2.3-2.7 mm. Overall colour black. Head. Frontal triangle black, shiny, microtomentose, 0.6-0.7 times length of frons; ocellar tubercle black, shiny, microtomentose; frons brown to black, paler medially; cephalic setae dark, $10-$ 12 fronto-orbital setae well-developed, interfrontal setulae inside margin of frontal triangle and fronto-orbital setulae sparse, eight or nine post-ocellar setulae small; gena yellow, microtomentose, $0.06-0.08$ times eye height; face yellow; scape and pedicel yellow, first flagellomere yellow basally and ventrally, black dorsally and distally, first flagellomere round, arista brown, thin at base, pubescence sparse and short; palpus yellow in male, brown in female, proboscis and clypeus brown. Scutum. Black, shiny, acrostichal and dorsocentral setae in three punctuate rows, scutum longer than wide; scutellum black, slightly paler than thorax, trapezoidal, $1.4-1.5$ times wider than long, microtomentose; apical scutellar setae strong, on small tubercles on dorsal margin of scutellum, lateral scutellar setae much weaker than apical setae but longer and darker


Figures 5-9. Enderleiniella caerulea. $\mathbf{5}$ Lateral habitus $\mathbf{6}$ dorsal habitus $\mathbf{7}$ male genitalia (lateral) $\mathbf{8}$ male genitalia (posterior) 9 geographic distribution. Abbreviations: cer - cercus; epd - epandrium; hyp - hypandrium; phal - phallus; phap - phallapodeme; pog - postgonite.
than surrounding setae. Legs. Yellow, hind femur basally and tibia brown; femoral organ present as a very small patch of three sensillae, tibial organ oval, dark, occupying middle half of hind tibia. Wing hyaline, brown tint dorsally from M1; veins brown; ratio of costal sectors C1: C2: C3: C4-1 1.7: 1.25: 0.5; haltere yellow. Abdomen. Paler than thorax, sparsely microtomentose; syntergites $1+2$ membranous under scutellum, marginally longer than other tergites. Male postabdomen (Figs 7, 8). Epandrium large, bulbous, higher than long in lateral view, much wider than high in posterior view, flattened dorsally, with several setae; surstylus 0.6 times as high as epandrium, triangular with a slight anterior curve along length, with broadly rounded apex, surstylus with three or four anterior setae near base and short setae elsewhere; cercus elongate, straight with a narrow ventral projection, extending postero-ventrally, with sparse setae, three setae at tip of cercus longer than others; distiphallus weakly sclerotised.

Type material. Holotype $\delta^{\star}$ : BELIZE: Toledo District, Blue Creek $\left(16^{\circ} 12^{\prime} \mathrm{N}\right.$, $\left.89^{\circ} 3^{\prime} \mathrm{W}\right)$, 23.i.1982, A.T. Finamore, sweeping (LEM). Paratypes: same as holotype except 17.i. 1982 (1 , LEM); ECUADOR: Guare Los Rios, vii.1955, Levi-Castillo (1才, USNM; USNMENT01476001); Manabi Camarones 9.viii.1955, Levi-Castillo (1ठ, USNM; USNMENT01476000).

Etymology. The species name is from the Latin caeruleus (sky-blue), referring to the type locality.

## Enderleiniella cryptica sp. nov.

http://zoobank.org/59B9BAAD-E4BE-42F3-9D19-6D80741455DE
Figs 10-14
Diagnosis. Medium Oscinellinae with a shiny frontal triangle and thorax. Mouthparts geniculate. Scutellum with distinct tubercles. Male postabdomen large with parallel sided surstylus.

Description. Total length $2.2-2.5 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, shiny, microtomentose, 0.6-0.65 times length of frons; ocellar tubercle black, shiny, microtomentose; frons brown to black, paler antero-medially; cephalic setae dark, 11-17 fronto-orbital setae well-developed, interfrontal setulae on margin of frontal triangle and fronto-orbital setulae conspicuous, six or seven post-ocellar setulae small; gena yellow, microtomentose, $0.08-0.1$ times eye height; face yellow; scape and pedicel yellow, first flagellomere yellow basally and ventrally, darker dorsally and distally, first flagellomere round, arista brown, thin at base, pubescence sparse and short; palpus and clypeus yellow in males; proboscis brown, geniculate. Scutum. Black, shiny, acrostichal and dorsocentral setae in three punctuate rows, scutum longer than wide; scutellum black, trapezoidal, 1.3-1.5 times wider than long, microtomentose; apical scutellar setae strong, on small tubercles on upper margin of scutellum, lateral scutellar setae much weaker than apical setae but longer and darker than surrounding setae. Legs. Yellow, hind femur and tibia brown; femoral organ present as row of three or four sensillae, tibial organ oval, dark, occupying middle third of hind tibia. Wing. Hyaline;


Figures I0-14. Enderleiniella cryptica. I0 Lateral habitus II dorsal habitus $\mathbf{I} \mathbf{2}$ male genitalia (lateral) 13 male genitalia (posterior) 14 geographic distribution.
veins brown; ratio of costal sectors $\mathrm{C} 1: \mathrm{C} 2: \mathrm{C} 3: \mathrm{C} 4-1: 1.7$ : 1.1: 0.6 ; haltere yellow. Abdomen. Paler than thorax, sparsely microtomentose; syntergites $1+2$ membranous under scutellum, marginally longer than other tergites. Male postabdomen (Figs 12, 13). Epandrium large, bulbous, higher than long in lateral view, wider than high in posterior view, with several setae, rounded dorsally; surstylus 0.6 times as high as epandrium, straight, spoon shaped, surstylus with five anterior setae near base and short setae elsewhere; cercus straight with a narrow ventral projection, extending ventrally, cercus with sparse setae, five setae longer than others; distiphallus weakly sclerotised, straight, projecting posteriorly.

Molecular data. Accession numbers MK919190, MK919192, and MK919196.
Type material. Holotype $\delta^{\lambda}$ : COSTA RICA: Prov Cartago, Cartago, P.N. Barbilla, Camino a Valle Escondido, Rio Dantas, 400-500m, 17.ix.2001, E. Rojas, F. Umaña, Libre, L_N_218000_594300 \#64657 (INBio). Paratypes: same as holotype (1ठ, 1Q, INBio); Higuito, San Mateo, Pablo Schild Coll (1 ${ }^{\lambda}$, USNM; USNMENT01476002); Prov Alajuela, C.B. Guanacaste-Rincón de la Vieja, Estac. San Gerardo, Send al Perdido, 600m, 16-18.x.2002. D. Briceño, Red. L.N. 317994384374 \#68995 (1q, INBio); Prov Cartago, P.N. Barbilla, Send. Principal antes de Río Dantas, 200-300m 16.ix. 2000 E Rojas, Red de Barrido, L.N. 217400596700 \#58440 (4才, 2q, INBio); Prov Cartago, P.N. Barbilla, Send. Principal Río Dantas, 370m, 8.xii.2002, E. Rojas, Red de Golpe, L.N. 217250596250 \#70369 (2§̂, INBio); Prov Cartago, R.F. Río Pacuara, Turrialba, P.N. Barbilla, Send. Quebrada, 400m 11.x.2001, E. Rojas, Red de

Golpe, L.N. 217500596893 \#63565 (1 1 , INBio); Prov. Cartago, R.F. Río Pacuare, P.N. Barbilla, Send. Principal a Río Dantas, 370m 22.ix.2001, E. Rojas. W. Arana, R. Madigal, Golpe, L.N. 217500596893 \#64659 (1q, INBio); Prov Guanacaste, Nandayure, Cerro Azul 1018m, 5.ii.2003, Y. Cardenas. Red de Golpe, L.N. 214769397000 \#7288 (1 $q$ INBio); Prov. Límon, P.N. Barbilla, Camino a Valle Escondido, Orilla Río Dantas, 400m, 11.x.2001, E. Rojas, Red de Golpe, L.N. 218800594300 \#64954 (1 ${ }^{\text {§ }}$, 2 , INBio); Prov Límon, P.N. Cahuita, Sector Puerto Vargas, Orilla de la playa, Om, 16-17.i.2003, E Rojas, Red de Golpe, L.N. 190500666200 \#72723 (1 ${ }^{\lambda}$, INBio); Prov Límon, P.N. Cahuita, Sector Puerto Vargas, Orilla de la playa, Om, 15.i. 2003 E Rojas, Red de Golpe, L.N. 190500666200 \#72722 (1q, INBio); Prov Límon, PN. Barbilla, Sector Casa Negra, 1.5 km NO dela Estación, 300m, 13.xii.2002, E. Rojas, Libre, L.N. 219900598400 \#70494 (1 , INBio); Prov. Punta, Albergue Cerro de Oro, 200m, 4-14.v.1995, E. Alfaro, L.S. 280450517500 \#5919 (11 §, 8q, INBio); Prov. Punta, Albergue Cerro de Oro, 200m, 5-12.v.1995, M.A. Zumbado, L.S. 279660518450 \#6028 (1 q, INBio); Prov. Punta, Albergue Cerro de Oro, 200m, 5-9.v.1995, B. Gamboa, L.N. 279650518450 \#4745 (1 ふ, INBio); Prov. Puntarenas, Golfito. P.N. Corcovado, Salida de la Estac. a Río Rincón, 75m 16.x.2002. K. Caballero. Libre, L.S. 281050 516800 \#71799 (1q, INBio); Prov Puntarenas, Est. Agujas, Río Agujas,300m, 19-24. iii.1997. A. Azofeifa, L.S. 276750526550 \#46258 (1q, INBio); Prov. Puntarenas, Est, Río Bonito, 2.3 km al O. del Cerro al Gamba, $110 \mathrm{~m}, 17-21 . \mathrm{iii} .1997$. E. Fletes, L.S. 293900547075 \#45597 (1 §, INBio) Prov. Puntarenas R.F. Golfo Dulce, 24km W Piedras Biancas, 200m, xii.1990. P. Hanson (1 §, LEM); Prov Puntarenas, R. Priv. Karen Mogensen. Send. Quebrada Pérez, 315m, 24.ix.2003. W. Porras, Red de Golpe, L.N. 205300419750 \#75433 (9 ふ, 3q, INBio); Prov Puntarenas, Lepanto, R. Priv. Karen Mogensen. Send. Quebrada Pérez, 315m, 22-23.xi.2003. D. Briceño, Libre, L.N. 205300_419750\#74568 (3 ${ }^{\lambda}, 2$, INBio); Prov Puntarenas, Sendero Tres Ríos, 300m, 9.xii.2003. M.A. Zimbado. W. Porras Vega Libre, L.N. 205164419993 \#74577 (7 ${ }^{\top}, 3$, INBio); Prov Puntarenas, R. Priv. Karen Mogensen. Send. El Viejo Nisper, 300-500m, 23.xi.2003. Y. Cardenas, Red con Aguamiel, 205600420300 \#74531 (1 1 , INBio); ECUADOR: Rio Mulaute, 15 km N.E. Sto. Domingo de Colorados, 2.iii.1973, M. \& N. Deyrup (1ठ̃, USNM; USNMENT01476003).

Etymology. The species name is from the Latin crypticus (hidden), referring to the external similarity of this species to $E$. tripunctata.

## Enderleiniella flavida sp. nov.

http://zoobank.org/41D4A122-BC45-4255-9EA7-F42537546EAD
Figs 3, 15-19
Diagnosis. Medium Oscinellinae with a shiny frontal triangle and thorax. occiput with single strong bristle behind eye; one anterior and one posterior notopleural bristle Scutellum pale yellow, contrasting in colour with the dark scutum.

Description. Total length $2.2-2.5 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, shiny, $0.6-0.75$ times length of frons; ocellar tubercle black, shiny; frons


Figures 15-19. Enderleiniella flavida. 15 Lateral habitus 16 dorsal habitus 17 male genitalia (lateral) 18 male genitalia (posterior) 19 geographic distribution.
brown to black, paler antero-medially; cephalic setae pale, $7-10$ fronto-orbital setae well-developed, interfrontal setulae on margin of frontal triangle and fronto-orbital setulae sparse and small, 4-6 post-ocellar setulae small; gena yellow, microtomentose, 0.08-0.1 times eye height; eye bare; occiput with a strong and stout seta projecting from a short tubercle just dorsal to posterior midpoint of eye; face yellow; scape, pedicel and first flagellomere yellow, first flagellomere round, arista brown, thin at base, pubescence sparse and short; palpus, clypeus and proboscis yellow; proboscis geniculate. Scutum. Black, shiny, acrostichal and dorsocentral setae in three punctuate rows, notopleural bristle one anterior and one posterior relatively thick and long; outer postalar setae very short, gold, cryptic and fine; dorsocentral setae weak, scutum as long as wide; scutellum yellow, trapezoidal, 1.4-1.6 times wider than long, microtomentose; apical scutellar setae strong, on small tubercles on upper margin of scutellum, lateral scutellar setae as strong as apical setae (Fig. 3). Legs. Yellow; femoral organ small row of two or three tubercles, tibial organ oval, pale, occupying middle third of hind tibia. Wing. Hyaline; veins brown; ratio of costal sectors C1: C2: C3: C4-1: 2.1: 1.4: 0.6; haltere yellow. Abdomen. paler than thorax, sparsely microtomentose; syntergites $1+2$ membranous under scutellum, marginally longer than other tergites. Male postabdomen (Figs 17, 18). Epandrium small, higher than long in lateral view, wider than high in posterior view, with several setae; surstylus 0.7 times as high as epandrium, with a slight curve at the base, parallel-sided, apex rounded, surstylus with short setae; cercus broad with three narrow ventral projection, extending ventrally laterally, cercus separated by very
narrow anal membrane posteriorly, cercus with sparse setae, one setae longer than others; distiphallus weakly sclerotised.

Type material. Holotype $\delta^{\top}$ : DOMINICA: S. Chiltern Est, 20.ii.1965, W.W. Wirth (USNM; USNMENT01476004). Paratypes: same data as holotype ( $2 \widehat{3}, 4$, USNM; USNMENT1476005-USNMENT01476009); DOMINICA: W.I. 2mi E. Ponte Casse, 5.x.1966, R.J. Gagne. Bredin-Archibol-SmithsonianBio.Surv.Dominica (1q, USNM; USNMENT01476010); DOMINICA: W.I. d'LeauGommier, 16.iii.1965, W.W. Wirth (1 ${ }^{\lambda}$, USNM; USNMENT01476011) DOMINICA: St. David: Emerald Pool, rainforest, 20.xi.1994, L. Masner (2q, LEM).

Etymology. The species name is from the Latin flavida (yellow), referring to the colour of the scutellum.

Remarks. It is the first time that a strong and stout seta projecting from a short tubercle just dorsal to posterior midpoint of eye on occiput has been described in the Chloropidae. It could be that this seta has the same evolutionary origin as those found on many other Chloropidae species that possess a long (but not stout) seta on the postgena.

## Enderleiniella longiventris (Enderlein, 1912)

Figs 1, 2, 20-27
Tricimba longiventris Enderlein, 1911: 207 (type locality: Costa Rica).
Enderleiniella longiventris: Becker 1912: 192.
Description. Total length $2.5-3.0 \mathrm{~mm}$. Overall colour black. Head Frontal triangle black, shiny, microtomentose, $0.55-0.6$ times length of frons; ocellar tubercle black, shiny, microtomentose; frons brown to black, paler antero-medially; cephalic setae dark, 12-15 fronto-orbital setae well-developed, interfrontal setulae on margin of frontal triangle and, seven or eight post-ocellar setulae small, posterior setulae proclinate; gena yellow, microtomentose, $0.08-0.09$ times eye height; face yellow; scape and pedicel yellow, first flagellomere yellow apico-dorsally darker, first flagellomere round, arista brown, thin at base, pubescence sparse and short; palpus yellow clypeus and proboscis brown, proboscis geniculate and thin (Fig. 1). Scutum. Black, pruinose, acrostichal, and dorsocentral setae in three punctuate rows, scutum longer than wide; outer postalar bristle very robust and long, scutellum black, trapezoidal, 1.2 times wider than long, microtomentose, rugose; apical scutellar setae strong, on tubercles on upper margin of scutellum, lateral scutellar setae much weaker than apical setae, on tubercles, longer than surrounding setae (Fig. 2). Legs. Yellow, hind femur and hind tibia brown; femoral organ arranged in one line of three strong sensillae, tibial organ oval, pale, occupying middle third of hind tibia. Wing. Hyaline; veins brown; ratio of costal sectors C1: C2: C3: C4-1: 1.3: 1: 0.4; haltere yellow. Abdomen. As dark as thorax, sparsely microtomentose; syntergites $1+2$ membranous under scutellum, slightly longer than other tergites. Male postabdomen (Figs 23-27). Remnant of sternite 6 present; Epandrium large, bulbous, higher than long in lateral view, wider than high in posterior view, with several setae; surstylus half the height of epandrium, straight, parallel-sided, blunt-


Figures 20-22. Enderleiniella longiventris. $\mathbf{2 0}$ Lateral habitus $\mathbf{2 I}$ dorsal habitus $\mathbf{2 2}$ geographic distribution.
ended, surstylus with one or two anterior setae near base and short setae elsewhere; cercus elongate, straight with a narrow ventral projection, extending ventrally, cercus with sparse setae, one or two setae longer than others; distiphallus weakly sclerotised, straight, blunt ended.

Molecular data. Accession number MK919191.
Type material. Holotype ${ }^{3}$ : COSTA RICA, H. Schmidt (Warsaw).
Other material examined. COSTA RICA: Cartago: Rio Grande de Orosi nr Tapanti Natl. Pk. 1100-1150m, floodplain and forest, 9.x.1999, S. Marshall \& M. Buck. ( $1 \delta^{\top}$, DEBU; DEBU00103967). MEXICO: Chiapas: 7 km N Cacahoatan, 22.iv. 1983, W.N. Mathis ( 2 §', USNM; USNMENT01476013-USNMENT01476014, USNMENT01476026); Chiapas: Finca Prusia, 33 km S Jaltenango, 1000m, 10-12.v.1985, W.N. Mathis ( 3 'h, 1 우, USNM; USNMENT01476015-USNMENT01476018); Chiapas: 9 km S Union Juaréz, 23.iv,1983, W.N. Mathis (1 $\widehat{ } 1$, USNM; USNMENT01476019); Chiapas, 20-25 mi. N Huixtla 3000', 4.vi.1969, B.V. Peterson ( $6{ }^{2}, 1$ 우, CNC); Colima: 7 mi NE Colima, 3.xii. 1948 (1오, USNM; USNMENT01476020); Tamaulipas: Liera, 10-6-1956, reared ex Colocasia sp. probably antiquorom ( 1 §̂, 2 ㅇ, USNM; USNMENT01476021-USNMENT01476023); San Luis Potosi: Naranjo, xii.1960, A. Fabergé, reared ex aroid flower (19, USNM; US-


Figures 23-27. Enderleiniella longiventris. $\mathbf{2 3}$ Male abdomen (lateral) $\mathbf{2 4}$ male genitalia (lateral) $\mathbf{2 5}$ male genitalia (posterior) $\mathbf{2 6}$ male genitalia (ventral) $\mathbf{2 7}$ male genitalia (posteroventral). Abbreviations: cer cercus; hyp - hypandrium; phal - phallus; phap - phallapodeme; pog - postgonite; $\mathrm{S}_{6}$ - remnant sternite 6; $\mathrm{T}_{7+8}$ - tergite $7+8$.

NMENT01476024); Veracruz: Fortin de las Flores, 952m, 02.v.1985, W.N. Mathis (1ठ, USNM; USNMENT01476025).

Remarks. Although the holotype was collected in Costa Rica, most examined specimens of E. longiventris for this study are from Mexico. This is one of two species of Enderleiniella (the other is E. maculata) whose known range extends into the Nearctic Region, with specimens recorded in the Mexican states of San Luis Potosi and Tamaulipas.

## Enderleiniella maculata sp. nov.

http://zoobank.org/CE01F49F-CDF5-43A7-91D2-83D89AE7BC08
Figs 4, 28-34
Diagnosis. Medium Oscinellinae with a pruinose frontal triangle and thorax. Wing with a dark apical spot. Male postabdomen small, not excessively higher than pre-epandrium.

Description. Total length $2.4-2.9 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, pruinose, 0.5 times length of frons; ocellar tubercle black, microtomentose; frons yellow, heavily microtomentose; cephalic setae pale, eleven fronto-orbital setae weak-developed, interfrontal setulae on margin of frontal triangle, eight post-


Figures 28-30. Enderleiniella maculata. $\mathbf{2 8}$ Lateral habitus $\mathbf{2 9}$ dorsal habitus $\mathbf{3 0}$ geographic distribution.
ocellar setulae small, posterior ocellar setae proclinate; gena white anteriorly, black posteriorly, microtomentose, 0.08 times eye height; eye hairy; face yellow; scape, pedicel brown, first flagellomere brown, yellow basoventrally, arista black, thin at base, pubescence sparse and short; palpus yellow in male, brown in female, proboscis and clypeus brown.

Scutum. Black, pollinose, acrostichal, and dorsocentral lines pruinosity with setae in 3 faint punctuate rows, notopleural bristle one anterior and two posterior relatively thick; outer postalar setae strong, black; dorsocentral setae strong, scutum 1.2 times longer than wide; scutellum brown, trapezoidal, $1.35-1.50$ times wider than long, microtomentose, rugose; apical scutellar setae strong, on small tubercles on upper margin of scutellum, lateral scutellar setae small, barely longer than other dorsal setae. Legs. Yellow, mid femur, hind femur and hind tibia black; femoral organ small patch on baso-anterior part of mid femur, tibial organ linear, pale, occupying middle quarter of hind tibia. Wing. Hyaline with distinct apical brown spot (Fig. 4); veins brown; ratio of costal sectors C1: C2: C3: C4-1: 1.7: 1.1: 0.6 ; haltere white. Abdomen. Paler than thorax, sparsely microtomentose; Abdominal syntergites $1+2$ slightly longer than other tergites. Male postabdomen (Figs 31-34). Epandrium small, higher than long in lateral view, wider than high in posterior view, with many setae; surstylus 0.7 height of epandrium, straight, parallel-sided; cercus narrow with long ventral projection, cercus with sparse setae; Distiphallus weakly sclerotised.


Figures $\mathbf{3 I - 3 4}$. Enderleiniella maculata. 3 I Male genitalia (lateral) $\mathbf{3 2}$ male genitalia (posterior) $\mathbf{3 3}$ male genitalia (ventral) $\mathbf{3 4}$ male genitalia (posteroventral).

Type material. Holotype ठ': MEXICO: San Luis Potosi: Xilitla, 1800', 24.vii. 1954, J.G. Chillcott (CNC); Paratypes: same data as holotype ( $5{ }^{\text {® }}$, 7 오, CNC); Guerrero, Taxco, 8mi NE, 5154', 8.viii.1954, J.G. Chillcott (1ㅇ, CNC).

Etymology. The species name is from the Latin maculatus (spotted), referring to the wing pattern.

## Enderleiniella marshalli sp. nov.

http://zoobank.org/6DBF3CF0-A1A4-49C1-B11B-0C9D38853E0F
Figs 35-41
Diagnosis. Small Oscinellinae with a shiny frontal triangle and sparsely pruinose thorax. Scutellum trapezoidal. Male postabdomen small with parallel sided surstylus.

Description. Total length $2.0-2.5 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, shiny, microtomentose, $0.7-0.8$ times length of frons; ocellar tubercle black, shiny, microtomentose; frons black, paler antero-medially; cephalic setae dark, 6-8 fronto-orbital setae weak-developed, interfrontal setulae on margin of frontal triangle and fronto-orbital setulae sparse and small, five or six post-ocellar setulae small; gena white, margin of gena black, microtomentose, 0.08-0.1 times eye height; eye hairy; face yellow; scape yellow, pedicel yellow to brown, first flagellomere black, reniform, arista yellow distally darkening brown, thin at base, pubescence sparse and short; palpus, clypeus and proboscis brown to black in females, palpus yellow, clypeus and proboscis brown to black in male; proboscis regular. Scutum. Black, pruinose, acrostichal and dorsocentral setae in three punctuate


Figures 35-37. Enderleiniella marshalli. $\mathbf{3 5}$ Lateral habitus $\mathbf{3 6}$ dorsal habitus $\mathbf{3 7}$ geographic distribution.
rows, notopleural bristle one anterior and one posterior relatively thick and long; outer postalar setae strong, black; dorsocentral setae strong, scutum as long as wide or marginally longer than wide; scutellum black, trapezoidal, 1.4-1.5 times wider than long, microtomentose, rugose; apical scutellar setae strong, on small tubercles on upper margin of scutellum, lateral scutellar setae as small, marginally larger than other dorsal setae. Legs. Yellow, fore-tarsi, mid and hind femur and tibia dark distally; femoral organ present as a row of three sensillae, tibial organ linear, brown, paler than leg, occupying middle quarter of hind tibia. Wing. Hyaline; veins brown; ratio of costal sectors C1: C2: C3: C4-1: 1.6: 1.2: 0.6 ; haltere, white. Abdomen. paler than thorax, sparsely microtomentose; syntergites $1+2$ membranous under scutellum, marginally longer than other tergites. Male postabdomen (Figs 38-40). Epandrium small, higher than long in lateral view, wider than high in posterior view, with many setae; surstylus 0.7 height of epandrium, with slight curve basally, parallel-sided, with short setae elsewhere; cercus small, extending ventrally, with sparse setae, one seta longer than others; hypandrium open; distiphallus weakly sclerotised.


Figures 38-4I. Enderleiniella marshalli. $\mathbf{3 8}$ Male genitalia (lateral) $\mathbf{3 9}$ male genitalia (posterior) $\mathbf{4 0}$ male genitalia (ventral) 41 male genitalia (posteroventral).

Type material. Holotype ${ }^{\text {}}$ : COSTA RICA: Guanacaste: Cacao Field Stn., carrion traps, $700 \mathrm{~m}, 13-15 . \mathrm{ii} .1996$, S. A. Marshall (DEBU). Paratypes: same data as holotype ( 4 ¢ , DEBU); Guanacaste: Estacion Santa Rosa, 300m, 16.ii.1996, S. Marshall, Borde del Rio, L_N_313000_359800 \#6920, INBIO CRI002239665 (1 , INBio). ${ }^{\text {T}}$ : COSTA RICA: Prov Guanacaste, Cañas, Palmira, Sector Rio Corobici. 224m, 10-15.x.2002, J.D. Gutiérrez, Libre, L_N_281200_416500 \#71957 (INBio). Paratypes: Prov Guanacaste, Bagaces, Fortuna, Z.P. Miravalles, Send. Cabro Muco, $980 \mathrm{~m}, 1-15$. viii.2002. J.D. Gutiérrez, Red de Golpe L_N_299151_410000 \#64536 (1 ${ }^{\text {N, }}$, INBio); same data as except 12.iv.2002, L_N_299151_410000 \#67730 (19, INBio); Prov Cartago, Cartago, P.N. Barbilla, Camino a Valle Escondido, Rio Dantes, 400-500m, 17.ix.2001, F. Rojas, f. Umaña, Libre, L_N_281200_594300 \#64657 (1 ㅇ, INBio).

Etymology. This species is named in honour of Steve Marshall, collector of the type series, in recognition of his contributions to our knowledge of Central American acalyptrate Diptera.

## Enderleiniella maya sp. nov.

http://zoobank.org/74FBCE7B-165B-4BF5-BFA8-AA98D3018169
Figs 42-46
Diagnosis. Small Oscinellinae with a shiny frontal triangle and thorax. Scutellum rectangular with distinct apical scutellar bristles. Male postabdomen large with parallel sided surstylus with broadly rounded apex.

Description. Total length $2.4-2.6 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, shiny, microtomentose, 0.7 times length of frons; ocellar tubercle black, shiny, microtomentose; frons brown to black; cephalic setae dark, eight or nine fron-to-orbital setae well-developed, interfrontal setulae on margin of frontal triangle and fronto-orbital setulae sparse, five orbital setulae small, posterior orbital setae proclinate, proclinate bristle on vertex between postocellar and inner vertical; gena yellow, microtomentose, 0.09 - 0.1 times eye height; face yellow; scape and pedicel yellow, first flagellomere yellow, black dorsally and distally, first flagellomere round, arista brown, thin at base, pubescence sparse and short; palpus yellow, proboscis and clypeus brown, proboscis geniculate. Scutum. Black, pruinose, acrostichal and dorsocentral setae in three punctuate rows, scutum longer than wide; scutellum black, rectangular, 1-1.1 times wider than long, microtomentose, rugose; apical scutellar setae strong, on tu-


Figures 42-46. Enderleiniella maya. 42 Lateral habitus 43 dorsal habitus 44 male genitalia (lateral) 45 male genitalia (posterior) 46 geographic distribution.
bercles 0.2 times as long as length of scutellum, on upper margin of scutellum, lateral scutellar setae much weaker than apical setae but longer and darker than surrounding setae, on small tubercles. Legs. Yellow, mid and hind femurs basally and tibia brown; femoral organ present as a small patch of three sensillae, tibial organ linear, dark, occupying middle third of hind tibia. Wing. Hyaline; veins brown; ratio of costal sectors C1: C2: C3: C4-1: 1.5: 1: 0.5; haltere yellow. Abdomen. Same colour as abdomen, sparsely microtomentose; syntergites $1+2$ membranous under scutellum, marginally as long as tergites 3 and 4 together. Male postabdomen (Figs 44, 45). Epandrium large, bulbous, higher than long in lateral view, wider than high in posterior view, with several setae; surstylus 0.6 times height of epandrium, parallel-sided straight with broadly rounded apex, surstylus with short setae; cercus elongate, straight with a narrow ventral projection, extending postero-ventrally cercus with sparse setae, one seta longer than others; distiphallus weakly sclerotised.

Type material. Holotype $\delta^{\top}$ : GUATEMALA: Departamento Izabal: Las Escobas, 15.vii.1986, L. Lesage (CNC). Paratypes: Same as holotype ( $1 \delta^{\pi}$ ), MEXICO: Chiapas, Rio Izapa, 21.iv.1983, W.N. Mathis (1q, USNM; USNMENT01476012).

Etymology. The species name, to be treated as a noun in apposition, refers to the Maya people whose culture has been dominant in this region for more than 1000 years.

## Enderleiniella punctata sp. nov.

http://zoobank.org/57607AE9-E81F-4342-9825-2F346703C2BA
Figs 47-52

Diagnosis. Small Oscinellinae with a shiny frontal triangle and thorax. Scutellum trapezoidal with very small apical tubercles bristles. Tergite 3 with medial setulae arising from large distinct punctate sockets. Male postabdomen small with parallel sided surstylus.

Description. Total length 2.1 mm . Overall colour black. Head. Frontal triangle black, pollinose, shiny posteriorly, 0.6 times length of frons; ocellar tubercle black, microtomentose; occiput black, shiny; frons brown, yellow antero-medially, heavily microtomentose; cephalic setae dark, eight fronto-orbital setae weak-developed, interfrontal setulae on margin of frontal triangle, six post-ocellar setulae small; gena white, margin of gena black, microtomentose, 0.1 times eye height; eye hairy; face yellow; scape pedicel yellow, first flagellomere yellow, black anterodorsally, large, quadrate, arista yellow basally darkening distally, thin at base, pubescence sparse and short; palpus and clypeus yellow, proboscis brown, geniculate. Scutum. Black, shiny, acrostichal and dorsocentral setae in 3 faint punctuate rows, notopleural bristle one anterior and two posterior relatively thick; outer postalar setae strong, black; dorsocentral setae strong, scutum 1.1 times longer than wide; scutellum black, trapezoidal, 1.7 times wider than long, microtomentose, smooth; apical scutellar setae strong, on small tubercles on upper margin of scutellum, lateral scutellar setae as large, twice as long as other dorsal setae. Legs. Yellow, all tarsi, mid and hind femur and tibia dark distally; femoral organ present as a small patch of three sensillae, tibial organ


Figures 47-52. Enderleiniella punctata. 47 Lateral habitus 48 dorsal habitus 49 male abdomen (dorsal) $\mathbf{5 0}$ male genitalia (lateral) 51 male genitalia (posterior) $\mathbf{5 2}$ geographic distribution. Abbreviations: Epd epandrium; $\mathrm{T}_{1+2}$ - tergites $1+2$.
linear, brown, paler than leg, occupying middle quarter of hind tibia. Wing. Hyaline; veins brown; ratio of costal sectors C1: C2: C3: C4-1:1.2: 1.4: 0.6; haltere white. Abdomen. Paler than thorax, sparsely microtomentose; Abdominal syntergites $1+2$ elongate, tergite 3 with medial setulae arising from large distinct punctate sockets (Fig. 49). Male postabdomen (Figs 49-51). Epandrium small, higher than long in lateral view, wider than high in posterior view, with many setae; surstylus 0.7 height of epandrium, straight, parallel-sided, surstylus with one anterior setae near base and short setae elsewhere; cercus elongate with narrow, long ventral projection, cercus with sparse setae, one seta longer than others; hypandrium open; distiphallus weakly sclerotised, straight, blunt ended.

Type material. Holotype $\delta$ : BOLIVIA: Santa Cruz: Andres lbanez, Potrerillo ( $17^{\circ} 40^{\prime} \mathrm{S}, 63^{\circ} 27^{\prime} \mathrm{W}$ ), 438m, yellow pan trap B-17, 13-16.v.1997, L. Masner (CNC).

Etymology. The species name is from the Latin punctatus (punctured), referring to the structure of the third abdominal tergite.

## Enderleiniella tripunctata (Becker, 1916)

Figs 53-57
Anoscinella tripunctata Becker, 1916: 448 (type locality: Costa Rica: Higuito, San Mateo). Enderleiniella tripunctata: Duda 1930: 77.

Description. Total length $1.9-2.6 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, shiny, microtomentose, 0.6-0.7 times length of frons; ocellar tubercle black, shiny, microtomentose; frons brown to black, paler medially; cephalic setae dark, eight or nine fronto-orbital setae well-developed, interfrontal setulae on margin of frontal triangle and fronto-orbital setulae sparse, 5-7 orbital setulae small, posterior setae proclinate; gena yellow, microtomentose, $0.09-0.1$ times eye height; face yellow; scape and pedicel yellow, first flagellomere yellow basally and ventrally, black dorsally and distally, first flagellomere quadrate, arista brown, thin at base, pubescence sparse and short; palpus yellow in male, brown in female, proboscis and clypeus brown. Scutum. Black, shiny, acrostichal and dorsocentral setae in three punctuate rows, scutum longer than wide; scutellum black, trapezoidal, 1.2-1.3 times wider than long, microtomentose; apical scutellar setae strong, on small tubercles on upper margin of scutellum, lateral scutellar setae much weaker than apical setae but longer and darker than surrounding setae. Legs. Yellow, mid and hind femora basally and tibia brown; femoral organ present as a small patch of four sensillae, tibial organ linear, dark, occupying middle quarter of hind tibia. Wing. Hyaline, brown tint dorsally from M1+2; veins brown; ratio of costal sectors C1: C2: C3: C4-1:1.9: 1.4: 0.5; haltere yellow. Abdomen. Slightly paler than abdomen, sparsely microtomentose; syntergites $1+2$ membranous under scutellum, marginally longer than other tergites. Male postabdomen (Figs 55, 56). Epandrium large, bulbous, higher than long in lateral view, wider than high in posterior view, with several setae; surstylus half the height of epandrium, clavate with a slight anterior curve along length, with broadly rounded apex, surstylus with three or four anterior setae near base and short setae elsewhere; cercus elongate, straight with a narrow ventral projection, extending postero-ventrally cercus with sparse setae, three or four setae longer than others; distiphallus weakly sclerotised, straight, blunt ended.

Molecular data. Accession number MK919194
Type material. Holotype $\delta^{\lambda}$ : COSTA RICA: [Provincia de San José, about $9^{\circ} 56^{\prime} \mathrm{N}$, $84^{\circ} 32^{\prime} \mathrm{W}, 200 \mathrm{~m}$ asl], Higuito: San Mateo, 1914, P. Schild (Budapest).

Other material examined. BELIZE: BARC, near San Pedro Colombia ( $16^{\circ} 17^{\prime} \mathrm{N}$, $88^{\circ} 58^{\prime} \mathrm{W}$ ), Malaise trap and yellow pans, 10-12.iii.2002, J. Skevington (1才, LEM); Stan Creek District: Silk Grass Creek ( $16^{\circ} 54^{\prime} \mathrm{N}, 8^{\circ}{ }^{\circ} 6^{\prime} \mathrm{W}$ ), 3.iv.1993, W.N. Mathis ( $1 \delta^{\top}$, USNM; USNMENT01476027); Toledo District: Blue Creek ( $\left.16^{\circ} 12^{\prime} \mathrm{N}, 89^{\circ} 3^{\prime} \mathrm{W}\right)$, sweeping, 17.i.1982, A.T. Finnamore (1q, LEM); COSTA RICA: Puntarenas: 24 km W Piedras Blancas ( $8^{\circ} 47^{\prime} \mathrm{N}, 83^{\circ} 15^{\prime} \mathrm{W}$ ), 200m, Malaise trap, xi.1990, P. Hanson (1中, USNM; USNMENT01476028); Puntarenas: 3 km SW Rincon $\left(9^{\circ} 55^{\prime} \mathrm{N}, 84^{\circ} 13^{\prime} \mathrm{W}\right)$, 10 m , Malaise trap, x-xii.1990, P. Hanson (1ठ, USNM; USNMENT01476029); Higuito: San Mateo, P. Schild (1 ${ }^{\text {® }}, 2$ q, USNM; USNMENT01476030-USNMENT01476032); Heredia: 3


Figures 53-57. Enderleiniella tripunctata. 53 Lateral habitus $\mathbf{5 4}$ dorsal habitus 55 male genitalia (lateral) $\mathbf{5 6}$ male genitalia (posterior) $\mathbf{5 7}$ geographic distribution.
km S Puerto Viejo OTS-La Selva, 100m, Malaise traps, xi.1992, P. Hanson (1q, LEM); ECUADOR: Manabi, La Palma, viii.1955, Levi-Castillo (1 $q$, USNM); Manabi, Camarones, 9.ix.1955, Levi-Castillo (1才, USNM; USNMENT01476033); Rio Mulaute, 15 km NE Santo Domingo de los Colorados, 2.iii.1973, M. \& N. Deyrup (1 ${ }^{\lambda}, 3 q$, USNM; USNMENT01476034-USNMENT01476036, USNMENT01476041); Domingo de los Colorados, 5.iii.1973, M. \& N. Deyrup (1 ${ }^{\text {T, USNM, USNMENT01476066); }}$ Napo: Napo-Pastaza, Levi-Castillo (1q, USNM; USNMENT01476037); Balao Guayas, xii.1955, Levi-Castillo (3q, USNM; USNMENT01476038-USNMENT01476040); Guayas: Naranjal, xii.1955, Levi-Castillo (1q, USNM; USNMENT01476042); Guare: Los Rios,viii.1955. Levi-Castillo (1q, USNM; USNMENT01476043); Guayas: Taura, xii.1955, Levi-Castillo (3q, USNM; USNMENT01476044-USNMENT01476046); Pichincha Manabi, viii.1955, Levi-Castillo (3q, USNM; USNMENT01476047-USNMENT01476049); EL SALVADOR: San Salvador, x.1965,N.L.H. Krauss (1q, USNM; USNMENT01476050); GUATEMALA: Departamento Izabal: Las Escobas, 15.vii.1986, L. LeSage (1 $\widehat{ }$, CNC); MEXICO: Chiapas: 7 km N Cacahoatan, 22.iv.1983, W.N. Mathis (1ठ, 1q, USNM; USNMENT01476051-USNMENT01476052); Chiapas: Rio Izapa, 21.iv.1983, W.N. Mathis (1q, USNM; USNMENT01476055); Chiapas: Puenta Macalapa, light trap, 22.v.1964, F.S. Blanton (1q, USNM; USNMENT01476056); Vera Cruz: Cordoba, vii.1965, N.L.H. Krauss (1q, USNM; USNMENT01476057); PANAMA: Gamboa: Pipeline Road, Malaise traps, vii.1967, W.W. Wirth (1ठ, 1q, USNM; USNMENT01476058-USNMENT01476059); Gamboa: Rio Agua Salud, vii.1967,
W.W. Wirth (1q, USNM; USNMENT01476060); Summit, ix.1946, N.H.L. Krauss (1q, USNM; USNMENT01476061); Tabogal, 26.ii.1912, A. Busck (1q, USNM; USNMENT01476062); PERU: Canet, 17.v.1941, P.A. Berry (2q, USNM; USN-MENT01476063-USNMENT01476064); VENEZUELA: Zulia: El Tucuco, 45 km SW Machiques, 5-6.vi.1976, A.S. Menke \& D. Vincent (1q, USNM; USNMENT01476065).

## Enderleiniella tumescens sp. nov.

http://zoobank.org/E8291025-CEAB-4EA9-A6D9-586CE6869F89
Figs 58-63

Diagnosis. Small Oscinellinae with a shiny frontal triangle and sparsely pruinose thorax. Occiput with dorsolateral pubescent swelling just behind outer vertical bristle. Scutellum trapezoidal with very small apical tubercles bristles. Male postabdomen small with parallel sided surstylus.

Description. Total length $2.3-2.5 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, pruinose, 0.7 times length of frons; ocellar tubercle black, microtomentose; occiput with distinct pubescent swelling (Fig. 33); frons brown, yellow antero-medially, heavily microtomentose; cephalic setae dark, eleven fronto-orbital setae weak-developed, interfrontal setulae on margin of frontal triangle, six post-ocellar setulae small; gena white, microtomentose, 0.09 times eye height; eye hairy; face yellow; scape, pedicel yellow, first flagellomere and arista missing in both type specimens; palpus yellow, proboscis and clypeus brown. Scutum. Black, shiny, acrostichal and dorsocentral lines pruinose with setae in three faint punctuate rows, notopleural bristle one anterior and two posterior relatively thick; intra-alar setae strong, black; dorsocentral setae strong, scutum 1.2 times longer than wide; scutellum brown, trapezoidal, 1.7 times wider than long, microtomentose, smooth; apical scutellar setae strong, on small tubercles on upper margin of scutellum, lateral scutellar setae as large, twice as long as other dorsal setae. Legs. Yellow, hind tibia darker; femoral organ present as a small patch of three sensillae, tibial organ oval, pale, occupying middle third of hind tibia. Wing. Hyaline; veins brown; ratio of costal sectors C1: C2: C3: C4-1: 1.9: 1.2: 0.5; haltere white. Abdomen. Paler than thorax, sparsely microtomentose; abdominal syntergites $1+2$ elongate. Male postabdomen (Figs 61, 62). Epandrium small, higher than long in lateral view, wider than high in posterior view, with many setae; surstylus 0.8 height of epandrium, straight, parallel-sided; cercus elongate, narrow with long ventral projection, cercus with sparse setae, one seta near apex long; hypandrium open; distiphallus weakly sclerotised, blunt-ended.

Female unknown.
Molecular data. Accession number MK919195
Type material. Holotype ${ }^{\top}$ : VENEZUELA: San Esteban, xii.1939, P. J. Anduze (USNM; USNMENT01476067). Paratype: same data as holotype (1 ${ }^{\top}$, USNM; USNMENT01476068).

Etymology. The species name is from the Latin tumescens (swollen), referring to the distinctive structure of the occiput.


Figures 58-63. Enderleiniella tumescens. $\mathbf{5 8}$ Lateral habitus $\mathbf{5 9}$ dorsal habitus $\mathbf{6 0}$ head (dorsal) $\mathbf{6 1}$ male genitalia (lateral) 62 male genitalia (posterior) 63 geographic distribution.

## Enderleiniella wheeleri sp. nov.

http://zoobank.org/059F617B-9B40-43C1-B7B7-33E61C86E6F3
Figs 64-68

Diagnosis. Small Oscinellinae with a shiny frontal triangle and thorax. Mouthparts geniculate. Scutellum trapezoidal with very small apical tubercles bristles. Male postabdomen small with a triangular surstylus.

Description. Total length $1.9-2.0 \mathrm{~mm}$. Overall colour black. Head. Frontal triangle black, pruinose, 0.7 times length of frons; ocellar tubercle black, microtomentose; frons brown, yellow antero-medially, microtomentose; cephalic setae dark, six or seven fronto-orbital setae weak-developed, interfrontal setulae on margin of frontal triangle, six or seven ocellar setulae small, posterior setae proclinate; gena white, microtomentose, $0.07-0.08$ times eye height; eye hairy; face yellow; scape, pedicel, first flagellomere brown to black, first flagellomere subquadrate, arista black, thin at base, pubescence sparse; palpus yellow, clypeus and proboscis black in males. Scutum. Black, shiny, acrostichal and dorsocentral setae in three faint punctuate rows, notopleural bristle one anterior and two posterior relatively thick; outer postalar setae strong, black; dorsocentral setae strong, scu-


Figures 64-68. Enderleiniella wheeleri. 64 Lateral habitus $\mathbf{6 5}$ dorsal habitus 66 male genitalia (lateral) 67 male genitalia (posterior) 68 geographic distribution.
tum 1.2 times longer than wide; scutellum black, trapezoidal, 1.6 times wider than long, microtomentose, smooth; apical scutellar setae strong, on very small tubercles on dorsal margin of scutellum, lateral scutellar setae as large, twice as long as other dorsal setae. Legs. Yellow, mid and hind femur and tibia dark distally; femoral organ a line of three sensillae, tibial organ linear, brown, paler than leg, occupying middle third of hind tibia. Wing. Hyaline; veins brown; ratio of costal sectors C1: C2: C3: C4-1: 1.7: 1.2: 0.6; haltere white. Abdomen. Paler than thorax, sparsely microtomentose; abdominal syntergites $1+2$ slightly longer than other tergites. Male postabdomen (Figs 66, 67). Epandrium higher than long in lateral view, wider than high in posterior view, with many setae; surstylus 0.5 height of epandrium, triangular, with a slight posterior curve, surstylus with one anterior seta near base and short setae elsewhere; cercus narrow with long ventral projection, cercus with sparse setae, one seta longer than others; distiphallus weakly sclerotised, straight, blunt-ended.

Female unknown.
Type material. Holotype ${ }^{\lambda}$ : COSTA RICA: Prov. Cartago, Turrialba, P.N. Barbilla, 2km S. de Est. por la Quebrada, 200-300m, 25.ix.2000, E. Rojas, Red Barrido, L.N. 217500596893 \#58442 (INBio; INBIO0003466073). Paratype: same as holotype ( $1{ }^{\text {® }}$, INBio; INBIO0003466202).

Etymology. This species is named in honour of Terry Wheeler, in recognition of his contributions to our knowledge of New World Chloropidae.

## Discussion

Enderleiniella was distinguished by previous authors (Becker 1912, Duda 1930) on the basis of the incised lines on the scutum, the absence of the alula, and the reduced anal angle of the wing. The scutal character is consistent, although variable in known species, where the incised lines are much clearer in certain species such as E. longiventris but much more subtle in in species like E. tumescens. The alula is present, but small, in most species of Enderleiniella and the anal angle varies but it is not as pronounced as in many oscinelline genera (Fig. 4). As with many oscinelline genera, the phylogenetic relationships of Enderleiniella within the subfamily are unclear. The incised lines on the scutum are shared with the species-rich and cosmopolitan genus Tricimba Lioy, but the well-developed pronotal carina because of the rounded shape of the back of the head and the distinct postpronotal sulcus of Enderleiniella are absent in species of Neotropical and Nearctic Tricimba that have been examined. There are also some male genitalic differences between the two genera such as the overall size of the epandrium compared with the abdomen and the presence of the remnant of sternite 6 in several species. These pronotal and postpronotal characters are shared with other Neotropical oscinelline genera and may be indicative of close relationship but it would be premature to speculate without a broader and more comprehensive phylogenetic analysis. Some characters (linear gena, anteromedial-posterolateral placement of vertical setae, stout, tuberculate scutellar setae, large male epandrium) are shared with the Neotropical genus Agrophaspidium Wheeler \& Mlynarek, and the two may be related (Wheeler and Mlynarek 2008). Enderleiniella flavida has $1+1$ notopleural setae, as in Agrophaspidium, and the structure of the scutellum in that species is intermediate between the two genera. It would be difficult to construct a cladogram of species-level relationships within Enderleiniella based on morphological characters because most of the known species are defined on autapomorphies, with few synapomorphic character states uniting species within the genus.

The molecular barcode data also supports Enderleiniella as a valid genus distinct from Tricimba. All the sequences from Enderleiniella cluster together in $91 \%$ of the bootstrap replicates whereas fewer than $50 \%$ of the bootstrap replicates supported Tricimba as sister to Enderleiniella. If the clades with $<50 \%$ support are collapsed (Fig. 69), there remains only support to maintain Enderleiniella with completely unresolved relationships with all the other outgroup species. I must emphasise that this should not be considered a true phylogenetic analysis of Enderleiniella. Enderleiniella is a valid genus based on morphological and molecular (COI barcode fragment) support. This revision also demonstrates the need for revision and redefinition of the limits of Tricimba and Chloropidae using an integrated taxonomic approach.

Four of the eleven described species of Enderleiniella are known from only one or two specimens and mostly from single localities in an area extending from Mexico to Bolivia. This suggests that additional sampling effort in the Neotropical Region will result in discovery of more undescribed species.


Figure 69. Maximum likelihood tree based on Cytochrome oxidase I barcode fragment using GTR+G+I evolutionary model. Bootstrap values above the branches are based on 1000 replicates of the analysis. Branches with less than $50 \%$ support are collapsed. Accession numbers in parentheses.

The biology of Enderleiniella was unknown prior to this study. Even now, there is limited ecological information on the species assigned to the genus. Four specimens of E. longiventris were reared from plants of Colocasia (Araceae) and flowers of an unidentified aroid plant in Mexico, although there is no indication as to whether the larvae were phytophagous in live plants or secondary invaders of dead or damaged plant tissues. Other specimens examined in this study were collected in a broad range of localities and habitats, from primary rainforest to disturbed dry areas.

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## References

Becker T (1912) Chloropidae. Eine monographische Studie. V. Teil. Neotropische Region. Annales Historico-Naturales Musei Nationalis Hungarici 10: 121-256.
Becker T (1916) Neue Chloropiden aus dem ungarischen National Museum. Annales Histori-co-Naturales Musei Nationalis Hungarici 14: 423-453.
Cumming JM, Wood DM (2009) Adult morphology and terminology. In: Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado MA (Eds) Manual of Central American Diptera. Vol 1. NRC Research Press, Ottawa, Ontario, 2-50.

Duda O (1930) Die neotropischen Chloropiden (Dipt.). Folia Zoologica et Hydrobiologica 2: 46-128, v-xix.
Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy amd high throughput. Nucleic Acids Research 32(5): 1792-1797. https://doi.org/10.1093/nar/ gkh340
Enderlein G (1911) Klassifikation der Oscinosominen. Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin 1911(4): 185-244.
Enderlein G (1913) Dipterologische Studien. IV. Neues und altes über Chloropiden. Zoologischer Anzeiger 42: 352-374.
Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
Ismay JW (1993) Revision of Tricimba Lioy and Aprometopis Becker (Diptera: Chloropidae) from Australia and the Papuan region. Invertebrate Taxonomy 7: 297-499. https://doi. org/10.1071/IT9930297
Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870-1874. https://doi.org/10.1093/molbev/msw054
Sabrosky CW, Paganelli CH (1984) Family Chloropidae. In: Vanzolini EP, Papavero NA (Eds) A catalogue of the Diptera of the Americas south of the United States. Museu de Zoologia, Universidade de São Paulo 81: 1-63.
Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. https://www.simplemappr.net [accessed 01 August 2019]
Wheeler TA, Mlynarek JJ (2008) Systematics of Agrophaspidium, a new genus of Neotropical Chloropidae (Diptera). Zootaxa 1926: 41-52. https://doi.org/10.11646/zootaxa.1926.1.4
Wheeler TA (2010) Family Chloropidae (grass flies, frit flies, eye gnats). In: Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado M (Eds) Manual of Central American Diptera. Vol 2, 1137-1153.

# A new species of Caecilia (Gymnophiona, Caeciliidae) from the Magdalena valley region of Colombia 

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#### Abstract

A new species of the genus Caecilia (Caeciliidae) from the western foothills of the Serranía de los Yariguíes in Colombia is described. Caecilia pulchraserrana sp. nov. is similar to C. degenerata and $C$. corpulenta but differs from these species in having fewer primary annular grooves and a shorter body length. With this new species, the currently recognized species in the genus are increased to 35 . Mitochondrial DNA sequences, including newly sequenced terminals representing two additional, previously unanalyzed species, corroborate the phylogenetic position of the new species within Caecilia and the monophyly of the genus. This analysis also included newly sequenced terminals of Epicrionops aff. parkeri (Rhinatrematidae) and transAndean Microcaecilia nicefori (Siphonopidae). Evidence was found for the non-monophyly of the family Siphonopidae and the siphonopid genera Microcaecilia and Siphonops. The implications of these results for caecilian systematics are discussed and the status of the transAndean populations of Caecilia degenerata is commented upon.


## Keywords

Amphibia, Caecilia degenerata, Epicrionops, Microcaecilia, paraphyly, phylogeny, Siphonopidae, South America, taxonomy, tropical humid forest

## Introduction

The Neotropical caecilian amphibian genus Caecilia Linnaeus, 1758 (Gymnophiona: Caeciliidae) currently comprises 34 nominal species (Wilkinson et al. 2011; Frost 2018; Maciel and Hoogmoed 2018), 18 of which occur in Colombia, with eight being endemic to this country. Seven species occur in the Magdalena valley region of Colombia (Dunn 1942; Lynch 1999) and external morphology segregates them into two groups. A first group comprises four species that lack secondary annular grooves: C. caribea Dunn, 1942, endemic to Colombia, from the eastern slope of the Cordillera Central, Caldas Department, between 10-1700 m above sea level (a.s.l); C. corpulenta Taylor, 1968, from the type locality in Peru, with a Colombian record from the Andean forests on the 1750 m a.s.l., Santander Department; C. subdermalis Taylor, 1968, from northern Ecuador and eastern slopes of the Cordillera Central, Huila and Caldas Departments in Colombia, between 850-2320 m a.s.l.; and C. degenerata Dunn, 1942, endemic to Colombia, from both flanks of the Cordillera Oriental, between $800-2100 \mathrm{~m}$ a.s.l., Boyacá, Cundinamarca, and Santander Departments (Dunn 1942; Taylor 1968; Ruiz-Carranza et al. 1996; Lynch 1999; Acosta-Galvis 2000; Rivera-Correa 2006; Castro-Herrera et al. 2007; Frost 2018; Appendix 1).

A second group includes three species that have secondary annular grooves: $C$. guntheri Dunn, 1942, with a wide distribution from northern Ecuador to Colombia, where the records are discontinuous and include the sub-Andean forests of the Cordillera Occidental and the region of Muzo at Quípama Municipality, Boyacá Department, western slope of the Cordillera Oriental, 1000 m a.s.l.; C. subnigricans Dunn, 1942, from northern Venezuela and lowlands of the Caribbean and Magdalena Valley regions of Colombia, with a record from Mariquita Municipality, Tolima Department; and C. thompsoni Boulenger, 1902b, endemic to the middle Magdalena valley in Colombia, 240-1571 m a.s.l. (Dunn 1942; Taylor 1968; Ruiz-Carranza et al. 1996; Lynch 1999; Acosta-Galvis 2000; Bernal et al. 2005 Acosta-Galvis et al. 2006; Lynch and Romero 2012; Mueses-Cisneros and Moreno-Quintero 2012; PaterninaH et al. 2013; Acevedo-Rincón et al. 2014; Angarita-M et al. 2015; Restrepo et al. 2017; Frost 2018; Appendix 1).

During a recent herpetological survey in wet tropical forests of the Serranía de los Yariguíes, in the Department of Santander, Colombia (Fig. 1), we collected several specimens of a small Caecilia that lack secondary annular grooves and dermal scale pockets, suggesting that they correspond to either C. degenerata or C. corpulenta. However, a low number of primary annular grooves and a combination of morphometric characters indicate instead that these specimens belong to a new species, which we describe herein. To test the generic placement of the new species and to explore the relationships of other Neotropical caecilians, we perform a phylogenetic analysis of DNA sequences. We discuss the implications of our results for caecilian systematics and comment on the status of the trans-Andean populations of C. degenerata.

## Materials and methods

## Fieldwork and reference collections

The new species was collected during fieldwork carried out in the Serranía de los Yariguíes, vereda La Belleza, municipality of El Carmen de Chucurí, Santander Department, Colombia ( $06^{\circ} 34^{\prime} \mathrm{N}, 73^{\circ} 34^{\prime} \mathrm{W}, 731-789 \mathrm{~m}$ a.s.l.; Fig. 1), from 17 February to 1 May 2018, during the dry season. Specimens were found in two separate humid spots near the Río Cascajales, which drains Tropical moist broadleaf forests, within the ecoregion of the Magdalena valley montane forests, in the foothills of the Cordillera Oriental, Colombia (Dinerstein et al. 1995; Olson and Dinerstein 2002).

Previous fieldwork conducted between 1998-1999 by John Lynch in collaboration with the first author, successfully allowed the detection of microhabitats and several specimens of Microcaecilia nicefori (Lynch 1999); subsequently, between 2000 to date, fieldwork with caecilians such as Oscaecilia polyzona (Lynch and Acosta 2004), Caecilia sp., C. thompsoni, and C. isthmica (unpublished data) allowed successful detection of microhabitats and multiple specimens.

The collecting technique, which was used to obtain specimens of the new species, consists of first asking local people about the locations where they have spotted caecilians using the common names of "blind snakes", or "captain worms" ("lombrices capitanas"), or "motolas" (this common name is specific for the Department of Santander). Subsequently, the reported sites are visited and inspected to select sites under the shade of vegetation, and where the soil is not compact and very humid (usually associated with water springs that form a mosaic of marshy and dry areas). Collecting efforts are focused in the selected damp microhabitats, digging with a hoe to a depth of approximately 20 cm (approximate sampling effort of 2-person-hour to collect five specimens). Coordinates and elevations were obtained with a Garmin GPSMAP 64SC (map datum WGS 84). Collected specimens were euthanized using $20 \%$ benzocaine (Chen and Combs 1999), fixed in $10 \%$ formalin, and preserved in $70 \%$ ethanol. Tissue samples from two individuals were obtained immediately after euthanasia and preserved in $96 \%$ ethanol. Specimens were deposited at the Biological Collections of the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia (IAvH-Am and IAvH-CT) and the Amphibian Collection of the Universidad Industrial de Santander, Bucaramanga, Santander, Colombia (UIS-MHN-A).

## Phylogenetic analysis

To test the generic assignment of the new species and to explore the relationships of other endemic caecilians from Colombia, available mitochondrial DNA sequences of the genes 16 S and CO1 from members of all Neotropical caecilian families (Caeciliidae, Typhlonectidae, Siphonopidae, Dermophiidae, and Rhinatrematidae) were analyzed


Figure I. A Map of Colombia showing the known localities of the species of Caecilia that occur in the Magdalena valley region. Key: C. caribea (blue triangle), C. corpulenta (black dot), C. degenerata (black cross), C. guntheri (violet asterisk), C. subnigricans (yellow triangle), C. subdermalis (green star), C. thompsoni (black star), Caecilia pulchraserrana sp. nov. (red triangle) B Type locality of Caecilia pulchraserrana sp. nov. (red triangle) at Serranía de los Yariguíes, Santander Department, Colombia.
(Table 1). The analysis included a fragment of COI (ca. 651 bp ) and a fragment of 16 S (ca. 510 bp ). Sequences for most terminals were obtained from GenBank (Table 1). We added new sequences for eight Colombian terminals representing the new species, Caecilia thompsoni, C. isthmica, Typhlonectes natans, Epicrionops aff. parkeri, and Microcaecilia nicefori (Appendix 1). The cryptobranchid Cryptobranchus alleganiensis was used to root the tree. Laboratory protocols and primers are those of Palumbi (1996), Ivanova et al. (2006), and Carr et al.(2011). Bidirectional PCR products were used for Sanger sequencing at the Instituto de Genética of the Universidad Nacional de Colombia. Resulting sequences were visualized, assembled, checked for stop codons (COI), and edited in Geneious Pro v 10.2.3 (Kearse et al. 2012). All sequences were deposited in the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2007) and GenBank (Table 1). Sequences of each gene were aligned independently using the MAFFT plugin $v 7.388$ within Geneious, considering the secondary structure of RNA in 16 S and implementing the G-INS-I algorithm. Subsequently, sequences of both genes were concatenated in a single dataset using Geneious, which was used to construct a Maximum Likelihood phylogeny using IQ-TREE (Nguyen et al. 2015), performing a partitioned analysis based on four partitions (16S, COI first codon position, COI second codon position, COI third codon position) using the partition finder algorithm (-m option TESTMERGE; Lanfear et al. 2012) in IQ-TREE and

Table I. List of species examined and GenBank or Barcode of life Data Systems (BOLD) accession numbers for each gene analyzed in this study. See Appendix 1 for locality details.

| Species | Family | Tissue code | $\begin{array}{c}\text { 16S GenBank; } \\ \text { BOLD number }\end{array}$ | $\begin{array}{c}\text { CO1 GenBank; } \\ \text { BOLD number }\end{array}$ | Source |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Caecilia gracilis | Caeciliidae |  | KX757086 | NC_023508 | $\begin{array}{c}\text { Maciel et al. 2017, San Mauro } \\ \text { et al. 2014 }\end{array}$ |
| Caecilia isthmica | Caeciliidae | IAvH-CT-22982 | $\begin{array}{c}\text { MN555719; } \\ \text { SABIO393-19 }\end{array}$ | $\begin{array}{c}\text { MN555727; } \\ \text { SABIO393-19 }\end{array}$ | This study |
| Caecilia pulchraserrana | Caeciliidae | IAvH-CT-227334 | $\begin{array}{c}\text { MN555715; } \\ \text { sp. nov }\end{array}$ | $\begin{array}{c}\text { MN555723; } \\ \text { SABIO005-18 }\end{array}$ | SABIO005-18 |$]$| This study |
| :---: |

best fitting models for each partition selected by the same program (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). Each partition was allowed to have its own set of branch lengths (-sp option). Branch support analysis was performed with 1000 ultrafast bootstrap replicates (Hoang and Chernomor 2017).

## Morphology

Criteria and terminology for morphological descriptions, diagnostic characters, and data for other species of Caecilia follow Lynch (1999), Gower and Wilkinson (2002), Maciel et al. (2009), Maciel and Hoogmoed (2011), Kamei et al. (2009), Wilkin-
son and Kok (2010), Donnelly and Wake (2013), and Wilkinson et al. (2009, 2013, 2015). For comparative purposes, specimens of C. guntheri, C. isthmica, C. thompsoni, and C. subdermalis were examined (Appendix 1). Morphological observations were made using a stereoscope Nikon optical device SMZ-1B, with High Intensity Illuminator NI-150 Nikon and App Scope 3xSRA41. Measurements were taken using a Mitutoyo precision digital caliper to $\pm 0.1 \mathrm{~mm}$; and using the following abbreviations for anatomical features and ratios of measurements:

| ADD | anal disc diameter; | PA | primary annulus; |
| :--- | :--- | :--- | :--- |
| AM | anteromedial limit of the | PAG | primary annular groove; |
|  | mouth on the upper jaw; | PM | premaxillary-maxillary tooth; |
| BH | body height at midbody; | ST | snout tip; |
| C1 | first collar length; | STD | distance between snout |
| C2 | second collar length; |  | tip and anterior margin of |
| CM | corner of the mouth; |  | mouth; |
| CMB | circumference at midbody; | STND | distance between ST and naris; |
| D | diameter at midbody; | STLPD | distance between ST and lip; |
| ED | eye diameter; | STOD | distance between ST and eye; |
| END | distance between eye and naris; | TA | tentacular aperture; |
| HH | head height at level with CM; | INTA | distance between TAs; |
| HL | head length; | TAOD | distance between TA and eye; |
| HW | head width at CM; | TALPD | distance between TA and lip; |
| HWNG1 | head width at NG1; | TANRD | distance between TA and naris; |
| IND | distance between nares; | TASTD | distance between TA and ST; |
| IOD | interorbital distance; | VP | vomeropalatine tooth; |
| TL | total length; | WC2 | width at second collar; |
| TL/D | TL divided by diameter at | WCH | width of choanae; |
|  | midbody (ratio of length/di- | WBV | width of body at vent level; |
|  | ameter); | WMB | width at midbody; |
| LPOD | distance between eye and lip; | TL/HL | TL divided by HL; |
| ND | naris diameter; | TL/WMB | TL divided by WMB; |
| NG1 | first nuchal groove; | TL/HW | TL divided by HW; |
| NG2 | second nuchal groove; | HL/HW | HL divided by HW. |
| NG3 | third nuchal groove; |  |  |

Dermal scale pockets and subdermal scales were searched using the criteria proposed by Wilkinson et al. (2013) and sex and maturity were determined by examination of gonads. Live specimens were photographed with a digital camera model Canon EOS 70D and preserved specimens with a digital camera Canon EOS 5D Mark II.

## Results

## Phylogenetic analysis

The final concatenated molecular dataset consisted of a matrix of $1273 \mathrm{bp}, 567$ sites were parsimony-informative, 111 were singletons, and 595 were constant sites. The best fitting substitution model for both CO 1 and 16 S was TIM2+F+I+G4 after testing the large selection of models in IQ-TREE. The ML tree is shown in Fig. 2 (LnL: -15725.921 ). Our phylogenetic analysis recovered the new species nested within a moderately well-supported (84\%) monophyletic Caecilia, in a maximally supported monophyletic Caeciliidae. The new species appears most closely related, of the sampled species, to C. volcani but support for this relationship is not strong (58\%). Rhinatrema nigrum and $R$. bivittatum were recovered as monophyletic with the sister group Epicrionops. Siphonops was inferred to be paraphyletic with respect to Luetkenotyphlus (Siphonopidae), and Microcaecilia nicefori was recovered as the sister group of Dermophidae + Siphonophidae, the latter including the remaining Microcaecilia (with Brasilotyphlus guarantanus nested within it) and the paraphyletic Siphonops.

## Description of new species

Generic assignment. The new species is assignable to the genus Caecilia because its eyes are not covered by bone and it has tentacles below the nostrils (Type D sensu Lynch, 1999, Fig. 3 D-E). In addition, the new species is nested within the Caecilia clade (Fig. 2) in our Maximum Likelihood phylogenetic analysis.

## Caecilia pulchraserrana sp. nov.

http://zoobank.org/03F213A5-2148-4255-91BB-37719EF0E7B7
Figs 3-5; Tables 2-4

Holotype. IAvH-Am-15487 (field number ARA 7872; Figs 3, 4C), an adult female collected 25 February 2018 by A. R. Acosta-Galvis, Miguel Torres, and Daniela García.

Type Locality. (Fig. 1) Colombia, Santander Department, El Carmen de Chucurí Municipality, vereda La Belleza, Cascajales River, $06^{\circ} 34^{\prime} 8.9^{\prime \prime} \mathrm{N}, 73^{\circ} 34^{\prime} 20.2^{\prime \prime W} \mathrm{~W}, 789 \mathrm{~m}$ a.s.l.

Paratypes. Four specimens (Fig. 4), IAvH-Am-15488 (field number ARA 7871) and UIS-MHN-A-6575 (field number ARA 7689), adult females, collected with holotype, and IAvH-Am-15489-90 (field numbers ARA 7690-1, respectively), adult males (exhibiting phallus, Fig. 5 A-C), $06^{\circ} 34^{\prime} 41.1^{\prime \prime N}$ N, $73^{\circ} 34^{\prime} 28.9^{\prime \prime} \mathrm{W}, 731 \mathrm{~m}$ a.s.l., collected 19 February 2018 by A. R. Acosta-Galvis and Miguel Torres.

Referred specimens. UIS-MHN-A-6576-7 (field numbers ARA 7692-3, respectively), juveniles, $06^{\circ} 34^{\prime} 41.1^{\prime \prime} \mathrm{N}, 73^{\circ} 34^{\prime} 28.9^{\prime \prime} \mathrm{W}, 731 \mathrm{~m}$ a.s.l., collected 19 February


Figure 2. Maximum Likelihood tree inferred from the analysis of a concatenated dataset comprising partial sequences of two mitochondrial genes. Numbers above branches indicate bootstrap support values (percent) ( ${ }^{*}=100 \%$ bootstrap). Scale bar indicates nucleotide substitutions per site. The phylogenetic position of Caecilia pulchraserrana sp. nov. is shown in bold.

Table 2. Morphological data of the Colombian species of Caecilia that lack secondary annular grooves and possess incomplete primary annular grooves. Abbreviations are given in Material and methods.

| Species | PAG | TL (mm) | TL/D | Dermal scale Sample size <br> pockets | Source |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| C. caribea | $142-152$ | $390-585$ | $53-55$ | Absent | 4 | Dunn 1942, Lynch 1999 |
| C. corpulenta | $129-132$ | $152-441$ | $19-35$ | Absent | 6 | Taylor 1968, Lynch 1999 |
| C. degenerata | $123-137$ | $390-1050$ | $38-58$ | Absent | 9 | Lynch 1999 |
| C. orientalis | $114-124$ | $231-673$ | $29-55$ | Present | 8 | Lynch 1999 |
| C. subdermalis | $116-138$ | $131-680$ | $28-54$ | Present | 32 | Lynch 1999 |
| C. pulchraserrana sp. nov. | $100-104$ | $195-232$ | $9-12$ | Absent | 7 | This study |

Table 3. Morphometric (in mm ) and meristic data of the type series of Caecilia pulchraserrana sp. nov. Abbreviations are given in Materials and methods.

|  | IAvH-Am-15487 Holotype | IAvH-Am-15490 Paratype | IAvH-Am-15489 Paratype | IAvH-Am-15488 Paratype | UIS-MHN-A-6575 <br> Paratype |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Sex | F | M | M | F | F |
| PAG | 104 | 100 | 101 | 103 | 100 |
| TL | 206 | 214 | 200 | 232 | 195 |
| HW | 5.4 | 5.3 | 5.0 | 4.8 | 4.3 |
| HWNG1 | 5.2 | 4.2 | 4.4 | 4.9 | 4.3 |
| WC2 | 5.8 | 4.6 | 4.0 | 5.2 | 4.8 |
| WMB | 8.5 | 6.2 | 5.5 | 8.1 | 6.2 |
| CMB | 22 | 18 | 17 | 23 | 18 |
| WBV | 5.2 | 3.7 | 4.0 | 4.4 | 3.5 |
| HL | 7.4 | 5.8 | 6.4 | 6.0 | 5.1 |
| HH | 5.1 | 4.8 | 4.0 | 4.4 | 3.8 |
| IND | 1.7 | 1.6 | 1.5 | 2.0 | 1.2 |
| IOD | 2.9 | 2.6 | 2.3 | 2.8 | 2.5 |
| ED | 0.3 | 0.4 | 0.4 | 0.2 | 0.4 |
| ND | 0.18 | 0.18 | 0.16 | 0.16 | 0.15 |
| END | 2.3 | 2.3 | 1.6 | 2.5 | 2.1 |
| STD | 6.9 | 5.6 | 5.7 | 6.0 | 5.2 |
| STND | 0.8 | 0.6 | 0.4 | 0.7 | 0.7 |
| STLPD | 2.2 | 2.1 | 2.1 | 2.0 | 1.8 |
| STOD | 3.3 | 2.7 | 2.5 | 3.4 | 2.9 |
| TA | 0.27 | 0.19 | 0.30 | 0.26 | 0.33 |
| INTA | 2.3 | 2.2 | 1.8 | 2.3 | 1.9 |
| TAOD | 2.5 | 2.1 | 1.9 | 2.6 | 2.1 |
| TALPD | 1.0 | 1.3 | 0.6 | 1.4 | 0.99 |
| TANRD | 0.99 | 0.67 | 0.69 | 0.75 | 0.7 |
| TASTD | 0.6 | 0.7 | 0.7 | 0.2 | 0.7 |
| LPOD | 1.0 | 1.2 | 0.9 | 1.0 | 0.7 |
| WCH | 0.16 | 0.11 | 0.09 | 0.11 | 0.14 |
| C1 | 1.6 | 1.2 | 1.6 | 1.1 | 0.9 |
| C2 | 1.7 | 1.5 | 2.4 | 1.5 | 1.1 |
| BH | 7.0 | 4.4 | 4.1 | 6.5 | 5.1 |
| ADD | 2.9 | 2.6 | 2.9 | 2.7 | 2.6 |
| VP | 11 | 9 | 10 | 9 | 11 |
| Premaxillarymaxillary teeth | 13 | 11 | 14 | 14 | 12 |
| Dentary teeth | 12 | 13 | 10 | 11 | 12 |

2018 by A. R. Acosta-Galvis and Miguel Torres. Tissues for molecular analysis (IAvH-CT-22733-4) were extracted from these specimens.

Diagnosis. Caecilia pulchraserrana sp. nov. differs from its congeners by the combination of having 100-104 dorsally incomplete primary annular grooves, a small size
(195-232 mm), lips and ventral margin of upper jaw with a pink-orange (salmon) color (Fig. 4), and lacking secondary annular grooves and dermal scale pockets.

Species comparisons. Regarding the species of the genus Caecilia, the absence of secondary annular grooves distinguishes C. pulchraserrana sp. nov. from C. abitaguae Dunn, 1942, C. albiventris Daudin, 1803, C. armata Dunn, 1942, C. antioquiaensis Taylor, 1968, C. bokermanni Taylor, 1968, C. dunni Hershkovitz, 1938, C. flavopunctata Roze \& Solano, 1963, C. gracilis Shaw, 1802, C. guntheri Dunn, 1942, C. isthmica Cope, 1878, C. leucocephala Taylor, 1968, C. marcusi Wake, 1985, C. mertensi Taylor, 1973, C. museugoeldi Maciel \& Hoogmoed, 2018, C. nigricans Boulenger, 1902, C. occidentalis Taylor, 1968, C. pressula Taylor, 1968, C. perdita Taylor, 1968, C. subnigricans Dunn, 1942, C. subterminalis Taylor, 1968, C. tentaculata Linnaeus, 1758, C. tenuissima (Taylor, 1973), C. thompsoni Boulenger, 1902, and C. volcani Taylor, 1969.

Caecilia pulchraserrana sp. nov. shares with C. attenuata Taylor, 1968, C. caribea Dunn, 1942, C. corpulenta Taylor, 1968, C. crassisquama Taylor, 1968, C. degenerata Dunn, 1942, C. inca Taylor, 1973, C. orientalis Taylor, 1968, C. pachynema Günther, 1859, and C. subdermalis Taylor, 1968 the absence of secondary annular grooves and the presence of incomplete primary annular grooves. However, the new species can be distinguished from these nine species by having a lower number of primary annular grooves (100-104 vs. 114-199). Caecilia pulchraserrana sp. nov. most closely resembles C. degenerata, which also lacks subdermal scales, but differs from it in having fewer primary annuli.

Description of holotype. An adult female (Fig. 3). Head dorsoventrally flattened and slightly narrower than body; head width at CM $63 \%$ of width at midbody, head width at CM $72 \%$ of head length; head length $3.5 \%$ of total length; interorbital distance $40 \%$ of head width. Snout projects 1.6 mm beyond mouth; tip of snout rounded in dorsal and lateral view (Fig. 3); area between the eye and naris flattened. Eyes visible but small, eye diameter $4 \%$ of head length and $13.5 \%$ of eye-nostril distance; nares small, margins slightly protuberant, directed posterodorsally, visible from above. Tentacular openings circular and small, slightly raised above skin, laterally positioned near margin of mouth (Type D sensu Lynch 1999, Fig. 3D, E), slightly closer to corner of mouth than to nostrils. Tongue anteriorly attached, surface smooth with some longitudinally oriented grooves. Teeth pointed, recurved, with size decreasing posteriorly; premaxilla-ry-maxillary and dentary teeth monocuspid and visible externally. Premaxillary-maxillary teeth 13 , posterior maxillary teeth smaller. Premaxillary-maxillary series extending behind level of choanae. Vomeropalatine teeth 10, monocuspid, relatively uniform, moderately recurved, not visible externally, similar in size. Dentary teeth 12 , moderately recurved, faintly larger than premaxillary-maxillary teeth. Choanae subovoid; narial plugs visible (Fig. 3F). Nuchal grooves indistinct dorsally and ventrally, incompletely encircling body with transverse grooves on the collars, in ventral surfaces. First collar shorter than second. Body subcylindrical, slightly deeper than wide (Fig. 3A, B); body width at midbody $4 \%$ of total length. Width along body varies slightly, narrower at terminal region. Primary annuli 104 incomplete dorsally and ventrally. Primary annular


Figure 3. Holotype of Caecilia pulchraserrana sp. nov. Adult female, IAvH-Am-1548. A, B Lateral views of body $\mathbf{C}$ dorsal $\mathbf{D}$ ventral $\mathbf{E}$ lateral views of head $\mathbf{F}$ Frontal view of cephalic region, the arrow indicates the narial plug $\mathbf{G}$ dorsal and $\mathbf{H}$ lateral views of caudal region $\mathbf{I}$ ventral view of vent.


Figure 4. Caecilia pulchraserrana sp. nov. in life. A Adult female, paratype, IAvH-Am-15488, TL= 232 mm B adult female, paratype, IAvH-Am-15488, TL= 232 mm C adult female, holotype, IAvH-Am-15487, TL= 206 mm D-E adult female. paratype, UIS-MHN-A-6575, TL= 195 mm .
grooves completely encircling the body. Secondary grooves absent (Fig. 3G-I). Dermal scale pockets absent. Vent circular; disc around vent conspicuous enlarged (Fig. 3I) with seven denticulations anterior, seven nearly equal posterior denticulatios (Fig. 3I); anal papillae absent, and unsegmented terminal shield of 4.9 mm length.

Color in life (Fig. 4): Jaw margins, area between the eye and naris, and tentacular regions pink-orange (salmon); eyeballs completely violet blue (Fig 4b); periorbital region salmon; body dark brownish with thin salmon-colored chromatophores; ventral surface of body slightly paler than dorsum; annular grooves on sides of body slightly darker than general body color.

Table 4. Ratios and percentages of measurements of the type series of Caecilia pulchraserrana sp. nov. Abbreviations are given in Materials and methods.

|  | IAvH-Am-15490 <br> Paratype | IAvH-Am-15489 <br> Paratype | IAvH-Am-15488 <br> Paratype | IAvH-Am-15487 <br> Holotype | UIS-MHN-A-6575 <br> Paratype |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Sex | M | M | F | F | F |
| C1/C2 | 75.9 | 66.1 | 70.5 | 96.4 | 82.7 |
| TL/D | 11.8 | 11.7 | 10.0 | 9.3 | 10.8 |
| TL/HL | 39.9 | 40.0 | 48.2 | 38.1 | 44.5 |
| TL/ WMB | 34.1 | 35.9 | 28.3 | 24.1 | 31.5 |
| L/HW | 36.7 | 30.8 | 38.2 | 27.7 | 37.8 |
| HL/HW | 92.0 | 77.0 | 79.2 | 72.9 | 85.0 |

Color in preservative (ethanol 70\%; Fig. 3): Body dark slate gray dorsally with diffuse khaki chromatophores; jaw margins, rostral and periocular regions yellowish; ventral and lateral surfaces slightly paler than dorsum; vent disk jaw margins and area between the eye and naris yellowish.

Variation of type series (Tables 3, 4). There is little variation among type specimens. Head flattened and slightly narrower than body, head width at CM 58-97\% of width at midbody; head width at CM $72-92 \%$ of head length; head length $2-4 \%$ of total length; interorbital distance 36-50\% of head width. Eye diameter 4-8\% of the head length and $10-19 \%$ of eye-nostril distance. Nares small, slightly protuberant, directed posterodorsally, and visible from above. Premaxillary-maxillary teeth 11-13. Vomeropalatine teeth $9-12$. Dentary teeth $10-13$. First collar 66-96\% of second collar. Body width at midbody $2-4 \%$ of total length. Primary annuli incomplete dorsally and ventrally. Secondary grooves and dermal scales absent. Vent circular; disc around with 12-15 anal denticulations. Denticulations usually seven-eight anteriorly, and seven posteriorly, nearly equal in size (Fig. 3I).

Distribution and natural history. Caecilia pulchraserrana sp. nov. is currently known from two adjacent, relictual tropical wet forest localities on the western slope of the Cordillera Oriental of Colombia (Serranía de los Yariquíes; Fig. 1) at elevations between 731789 m a.s.l. The Serrania of the Yariguies corresponds to an isolated mountain range that is part of the western slope of the Cordillera Oriental of Colombia (Fig. 1). Caecilia pulchraserrana sp. nov. is a fossorial species associated with marshy areas surrounded by secondary vegetation at the forest edge (Fig. 6). The specimens were collected during the dry season in very wet soils lacking rocks (i.e., bogs; Fig. 6), in a slightly inclined area (nearly $5^{\circ}$ of slope) covered with vegetation of the family Heliconiaceae (Heliconia spp., Fig. 6).

Caecilia pulchraserrana sp. nov. was obtained during the initial 10 minutes of removal with a hoe.We extracted the first specimen in intermediate substrates between marshy and dry areas; after 40 minutes of excavation in these selected areas, we obtained four additional specimens. Using these same criteria, when moving two kilometers above the original point, an area with similar characteristics was located and within 20 minutes we collected two additional specimens. Caecilia pulchraserrana sp. nov. was collected on black sandy soils with high organic matter content. These caecilians move


Figure 5. Phallus (everted cloaca) of adult males Caecilia pulchraserrana sp. nov. A Ventro-lateral view (IAvH-Am-15489) B Ventro-lateral view of vent and C dorsal surface of the phallus (IAvH-Am-15490).


Figure 6. Habitat of Caecilia pulchraserrana sp. nov. in the Serranía de los Yariguíes in Santander Department, El Carmen de Chucurí Municipality, vereda La Belleza, Cascajales River, 06³4'8.9"N, $73^{\circ} 34^{\prime} 20.2^{\prime \prime} \mathrm{W}, 789 \mathrm{~m}$ a.s.l.. A View showing standing water in marshy area B Transitional change of wetter (right) to drier (left) microhabitat.
quickly under the substrate, so once the first specimen is detected it is important to quickly create channels to surround and block them from escaping.

Etymology. The specific epithet is formed from the Latin pulchra (nominative feminine singular of pulcher), meaning beauty, and the Spanish adjective serrana (feminine singular of serrano), from the sierra or serranía. This specific name refers to the type locality of the species: vereda La Belleza (beauty in English) in the western foothills of the Serranía de Los Yariguíes. The specific name was chosen using a citizen science approach. First, scientists and inhabitants of the El Carmen de Chucurí municipality gathered a list of possible names for the new species. Then, the list of potential names and their meanings was shared with the local people, who voted to choose their preferred name.

## Discussion

## Phylogenetic relationships

Our description of Caecilia pulchraserrana sp. nov. brings the number of known species of Caecilia to 35 (Frost 2018). Molecular data are currently available for only six of these species (including the three newly sequenced species analyzed here), which precludes a thorough analysis of the relationships within the genus. Consequently, our phylogenetic analysis (Fig. 2) was designed mainly to test the generic placement of $C$. pulchraserrana sp. nov. in addition to exploring the relationships of C. isthmica and C. thompsoni (two other species that are endemic to Colombia). Our results recovered $C$. pulchraserrana sp. nov., C. isthmica, and C. thompsoni within Caecilia (Fig. 2), corroborating the generic placement of the new species and the monophyly of the genus, as previously hypothesized by Wilkinson et al. (2011). Our analysis recovered Oscaecilia as the sister group of Caecilia, which agrees with the results of San Mauro et al. (2014) but disagrees with those of Pyron and Wiens (2011), who instead recovered Caecilia as paraphyletic with respect to Oscaecilia.

Our phylogenetic analysis only included two mitochondrial loci and a small number of species and should not be considered as a robust resolution of caecilian relationships. Nevertheless, our results highlight several potential cases of non-monophyletic taxa and suggest that a taxonomic revision, including a major generic rearrangement, is warranted. Our study includes, for the first time, the Colombian endemics Epicrionops aff. parkeri (Rhinatrematidae) and Microcaecilia nicefori in molecular phylogenetic analyses. On one hand, recent contributions (Maciel et al. 2018) have allowed taxonomic rearrangements within Rhinatrematidae, with Rhinatrema nigrum and $R$. bivittatum being recovered as monophyletic, supporting previous claims (Wilkinson and Gower 2010; Wilkinson et al. 2011; Pyron and Wiens 2011; San Mauro et al. 2014) that Epicrionops could be transferred to Rhinatrema. Our analysis recovers Epicrionops aff. parkeri nested within a monophyletic Epicrionops (E. marmoratus+ E. aff. parkeri with 89\%), which was sister to Rhinatrema (Fig. 2), corroborating the results obtained by Maciel et al. (2018).

On the other hand, Microcaecilia nicefori was recovered as the sister taxon to a clade formed by the dermophiids Gymnophis multiplicata + Dermophis mexicanus and the remaining siphonopids, including Microcaecilia, Brasilotyphlus guarantanus, Siphonops, and Luetkenotyphlus. In addition, Microcaecilia and Siphonops were recovered as paraphyletic with respect to Brasilotyphlus guarantanus and Luetkenotyphlus brasiliensis, respectively (Fig. 2). Recently, Correia et al. (2018) also presented evidence that Microcaecilia is paraphyletic with respect to Brasilotyphlus. The placement of Luetkenotyphlus brasiliensis within Siphonops contrasts with results of Pyron and Wiens' (2011) and Maciel et al.'s (2019) analyses that found Luetkenotyphlus and Siphonops to be sister taxa. Although analyses by San Mauro et al. (2006), San Mauro et al. (2014) and Correia et al. (2018) also recovered Luetkenotyphlus and Siphonops as sister groups, these studies only included one species of Siphonops (S. annulatus). Therefore, additional molecular data are needed to clarify the delimitation of these clades.

Consistent with previous findings (i.e., Correia et al. 2018), our phylogenetic analysis recovers Microcaecilia as non-monophyletic. Previously, based on evidence from dentition (relationship between VPs and rows of PM) and orbit (open versus closed orbit), Wilkinson et al. (2013) suggested that some Microcaecilia, including the type species of the genus (Dermophis albiceps Boulenger, 1882; not included herein), are more closely related to M. nicefori (Gymnophis nicefori Barbour, 1925, the type species of Parvicaecilia, currently in the synonymy of Microcaecilia; analyzed here for the first time) than to other species of Microcaecilia. That is, the position of trans-Andean Microcaecilia nicefori compared to other cis-Andean members of the genus suggests the revalidation of the genus Parvicaecilia. However, our analysis does not represent solid evidence due to several aspects, such as the low number of genes used, the low support values (a bootstrap value of only 45\%), and the absence of key terminals, such as the type species of the Amazonian Microcaecilia (M. albiceps (Boulenger, 1882). Thus, inclusion of relevant taxa, such as M. albiceps, in future phylogenetic analyses is key to guiding taxonomic changes. At the interfamilial level, our results provide evidence for the first time that Shiphonopidae is paraphyletic with respect to Dermophiidae due to the placement of $M$. nicefori (Fig. 2). Additional, large scale phylogenetic studies are required to rigorously test this finding.

## Status of the trans-Andean populations of Caecilia degenerata

Lynch (1999) suggested that Caecilia degenerata is restricted to the Cordillera Oriental of Colombia (Departments of Boyacá, Cundinamarca and Santander). However, morphological and biogeographical evidence suggests that the cis- and trans-Andean populations are not conspecific. The type series was collected at two cis-Andean localities: Garagoa (Boyacá Department), the type locality, and Choachí (Cundinamarca Department), ca. 90 km southwest of the type locality (Dunn 1942). Later, Ruiz-Carranza et al. (1996) and Lynch (1999) examined a series of trans-Andean specimens collected at Muzo (Boyacá Department), Tena and Sasaima (Cundinamarca Department), and Charalá (Santander Department), and referred them to C. degenerata, based on morphological similarity and (presumably) relative geographical proximity. Although the absence of secondary annular grooves, the number of primary annular grooves (127-138 in the cis-Andean populations vs. 123-137 in the trans-Andean populations), and the ratio of length/diameter (3260 in the cis-Andean populations vs $48-58$ in the trans-Andean populations; RuizCarranza et al. 1996, Lynch 1999) are consistent with the hypothesis of conspecific populations. The cis- and trans-Andean populations are isolated by biogeographic barriers that includes high and steep mountains, xerophytic areas, and rainy environments, factors that usually play a fundamental role in the speciation of Andean amphibians (Lynch et al. 1997). To test the conspecificity of the populations of $C$. degenerata, a more extensive sampling of specimens, populations, and additional
molecular data are required. Finally, although Taylor (1968) recorded specimens of C. degenerata in Tomaque (probably in Colombia or Peru) and Río Pache (probably in Peru), we agree with Lynch (1999) that C. degenerata is restricted to the (eastern) Cordillera Oriental of Colombia.

## Conclusions

Caecilia pulchraserrana sp. nov. is described as an endemic species from the Serranía de los Yariguies. The species is similar to C. degenerata, from which it can be distinguished using morphological characters. According to their morphology, we hypothesize there is a group of closely related species that comprises C. caribea, C. corpulenta, C. degenerata, C. orientalis, and C. subdermalis. The trans-Andean Microcaecilia nicefori is an endemic and poorly known species from Colombia. We provide here the first analysis of molecular data that tests its phylogenetic position. Our results address the need to evaluate with more evidence the status of the genus Parvicaecilia Taylor, 1968 (currently under the synonymy of Microcaecilia), and the potential non-monophyly of the family Siphonopidae. Further analyses sampling additional taxa and molecular markers are required to establish a more robust classification for Gymnophiona.

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## References

Acevedo-Rincón AA, Franco R, Silva-Pérez K (2014) Geographic Distribution: Caecilia subnigricans (Magdalena Valley Caecilian). Herpetological Review 45(3): 456.
Acosta-Galvis AR (2000) Ranas, Salamandras y Caecilias (Tetrapoda: Amphibia) de Colombia. Biota Colombiana 1(3): 289-319. http://revistas.humboldt.org.co/index.php/biota/ article/view/80/80
Acosta-Galvis AR, Huertas-Salgado C, Rada MA (2006) Aproximación al Conocimiento de los Anfibios en una localidad del Magdalena Medio (Departamento De Caldas, Colombia). Revista de la Academia Colombiana de Ciencias Exactas Físicas y Naturales 30(115): 291-303. http://www.accefyn.com/revista/Vol_30/115/115_291_303.pdf
Angarita-M O, Montes-Correa AC, Renjifo JM (2015) Amphibians and reptiles of an agroforestry system in the Colombian Caribbean. Amphibian \& Reptile Conservation 8(2) (Special Section): 19-38. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_8_ no_1/ARC_8_1_[General_Section]_33-52_e92_high_res.pdf
Bernal MH, Páez CA, Vejarano MA (2005) Composición y distribución de los anfibios de la cuenca del río Coello (Tolima), Colombia. Actualidades Biológicas 27(82): 87-92.
Boulenger GA (1902) Descriptions of new batrachians and reptiles from north western Ecuador. Annals and Magazine of Natural History, Series 7(9): 51-57. https://biodiversitylibrary.org/page/19331328
Boulenger GA (1902b) Descriptions of two new South-American apodal batrachians. Annals and Magazine of Natural History, Series 7(10): 152-153. https://biodiversitylibrary.org/ page/19338953
Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN (2011) A Tri-Oceanic Perspective: DNA Barcoding Reveals Geographic Structure and Cryptic Diversity in Canadian Polychaetes. PLoS ONE 6(7): e22232. https://doi.org/10.1371/journal.pone. 0022232
Castro-Herrera F, Bolívar-García W, Herrera-Montes MI (2007) Guía de Anfibios y Reptiles del Bosque de Yotoco, Valle del Cauca, Colombia. Grupo de Investigación Laboratorio de Herpetología, Universidad del Valle, Cali, Colombia, 70 pp.
Chernomor O, von Haeseler A, Minh BQ (2016) Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. Systematic Biology 65(6): 997-1008. https://doi. org/10.1093/sysbio/syw037
Cope ED (1877) Tenth contribution to the herpetology of tropical America. Proceedings of the American Philosophical Society 17: 85-98. https://biodiversitylibrary.org/page/29847644
Correia LL, Nunes PMS, Gamble T, Maciel AO, Marques-Souza S, Fouquet A, Rodrigues MT, Mott T (2018) A new species of Brasilotyphlus (Gymnophiona: Siphonopidae) and a contribution to the knowledge of the relationship between Microcaecilia and Brasilotyphlus. Zootaxa 4527(2): 186-196. https://doi.org/10.11646/zootaxa.4527.2.2
Crawford AJ, Lips KR, Bermingham E (2010) Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. Proceedings of the National Academy of Sciences, 200914115. https://doi.org/10.1073/ pnas. 0914115107

Chen M, Combs C (1999) An alternative anesthesia for amphibians: ventral application of benzocaine. Herpetological Review 30: 34-34.
Dinerstein E, Olson DM, Graham DJ, Webster AL, Primm SA, Bookbinder MP, Ledec G, Young KR (1995) A conservation assessment of the terrestrial ecoregions of Latin America and the Caribbean, World Bank, Washington, DC, 143 pp. https://doi.org/10.1596/0-8213-3295-3
Donnelly MA, Wake MH (2013) A new Microcaecilia (Amphibia: Gymnophiona) from Guyana with comments on Epicrionops niger. Copeia 2013(2): 223-231. https://doi.org/10.1643/ CH-12-094
Dunn ER (1942) The American caecilians. Bulletin of the Museum of Comparative Zoology. Cambridge, Massachusetts 91: 437-540. https://biodiversitylibrary.org/page/4788854
Dunn ER (1944) Notes in Colombian herpetology, III. Caldasia 2: 473-474. https://revistas. unal.edu.co/index.php/cal/article/view/32041/32071
Frost DR (2018) Amphibian Species of the World: An Online Reference. Version 6.0. http:// research.amnh.org/herpetology/amphibia/index.html [accessed 11 October 2018]
Gower DJ, Wilkinson M (2002) Phallus morphology in caecilians (Amphibia, Gymnophiona) and its systematic utility. Bulletin of the Natural History Museum: Zoology 68: 143-154. https://doi.org/10.1017/S096804700200016X
Günther ACLG (1859) Second list of cold-blooded vertebrata collected by Mr. Fraser in the Andes of western Ecuador. Proceedings of the Zoological Society of London, 1859, 402420. https://biodiversitylibrary.org/page/32274816

Hoang DT, Chernomor O (2017) UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35(2): 518-522. https://doi.org/10.1093/molbev/ msx 281
Ivanova NV, Dewaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA: Technical note. Molecular Ecology Notes 6: 998-1002. https://doi.org/10.1111/j.1471-8286.2006.01428.x
Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587-589. https://doi.org/10.1038/nmeth. 4285
Kamei RG, Wilkinson M, Gower DJ, Biju SD (2009) Three new species of striped Ichthyophis (Amphibia: Gymnophiona: Ichthyophiidae) from the northeast Indian states of Manipur and Nagaland. Zootaxa 2267(1): 26-42. https://doi.org/10.11646/zootaxa.2267.1.2
Kamei RG, San Mauro D, Gower DJ, Van Bocxlaer I, Sherratt E, Thomas A, Babu S, Bossuyt F, Wilkinson M, Biju SD (2012) Discovery of a new family of amphibians from Northeast India with ancient links to Africa. Proceedings of the Royal Society of London B 279(2): 396-2401. https://doi.org/10.1098/rspb.2012.0150
Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649. https://doi.org/10.1093/bioinformatics/bts199

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. Molecular Biology and Evolution 29(6): 1695-1701. https://doi.org/10.1093/molbev/mss020
Linnaeus C (1758) Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis, Vol. 1 ( $10^{\text {th }}$ edn). L. Salvii, Stockholm. https://doi.org/10.5962/bhl.title. 37256
Lynch JD (1999) Una aproximación a las culebras ciegas de Colombia (Amphibia: Gymnophiona). Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 23(Suplemento especial): 317-337. http://www.accefyn.com/revista/Vol_23/supl/317-337.pdf
Lynch JD, Romero H (2012) Anfibios de la Región Caribe. In: Rangel-Ch JO (Ed.) Colombia Diversidad Biótica XII, La región Caribe de Colombia, Universidad Nacional de Colom-bia-Instituto de Ciencias Naturales, Bogotá DC, 677-701. http://www.uneditorial.net/ pdf/TomoXII.pdf
Lynch JD, Ruiz-Carranza PM, Ardila-Robayo MC (1997) Biogeographic patterns of Colombian frogs and toads. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 21(80): 237-248. http://www.accefyn.com/ranas/frogs/Rev237.html\#tex80(237)
Lynch JD, Acosta-Galvis AR (2004) Discovery of Oscaecilia polyzona (Amphibia: Gymnophiona: Caeciliaidae) in the Middle Magdalena with notes on its abundance and Habitat Revista de la Academia Colombiana de Ciencias Exáctas Físicas y Naturales 28(109): 585-589. http://www.accefyn.com/revista/Vol_28/109/13_585_589.pdf
Lyra ML, Haddad CFB, de Azeredo-Espin AML (2017) Meeting the challenge of DNA barcoding Neotropical amphibians: polymerase chain reaction optimization and new COI primers. Molecular Ecology Resources 17(5): 966-980. https://doi.org/10.1111/1755-0998.12648
Maciel AO, Mott T, Hoogmoed MS (2009) A second Brasilotyphlus (Amphibia: Gymnophiona: Caeciliidae) from Brazilian Amazonia. Zootaxa 2226: 19-27.
Maciel AO, Hoogmoed MS (2011) Taxonomy and distribution of caecilian amphibians (Gymnophiona) of Brazilian Amazonia, with a key to their identification. Zootaxa 2984: 1-53. https://doi.org/10.11646/zootaxa.2984.1.1
Maciel AO, Hoogmoed MS (2013) A new species of Microcaecilia (Amphibia: Gymnophiona: Siphonopidae) from the Guianan region of Brazil. Zootaxa 3693: 387-394. https://doi. org/10.11646/zootaxa.3693.3.9
Maciel AO, Sampaio MI, Hoogmoed MS, Schneider H (2017) Phylogenetic relationships of the largest lungless tetrapod (Gymnophiona, Atretochoana) and the evolution of lunglessness in caecilians. Zoologica Scripta 46(3): 255-263. https://doi.org/10.1111/zsc. 12206
Maciel AO, Hoogmoed MS (2018) A new species of Caecilia Linnaeus, 1758 (Amphibia: Gymnophiona: Caecilidae) from French Guiana. Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais 13: 13-18.
Maciel AO, Sampaio MI, Hoogmoed MS, Schneider H (2018) Description of Two New Species of Rhinatrema (Amphibia: Gymnophiona) from Brazil and the Return of Epicrionops niger to Rhinatrema. South American Journal of Herpetology 13(3): 287-299. https://doi. org/10.2994/SAJH-D-17-00054.1
Maciel AO, de Castro TM, Sturaro MJ, Silva IEC, Ferreira JG, dos Santos R, Risse-Quaioto B, Barboza BA, de Oliveira JCF, Sampaio I, Schneider H (2019) Phylogenetic systematics
of the Neotropical caecilian amphibian Luetkenotyphlus (Gymnophiona: Siphonopidae) including the description of a new species from the vulnerable Brazilian Atlantic Forest. Zoologischer Anzeiger 281: 76-83. https://doi.org/10.1016/j.jcz.2019.07.001
Mueses-Cisneros JJ, Moreno-Quintero V (2012) Fauna Amphibia de la Reserva Natural Biotopo Selva Húmeda, Barbacoas, Nariño, Colombia. Herpetotropicos 7(1-2): 39-54. http://erevistas.saber.ula.ve/index.php/herpetotropicos/article/viewFile/4020/3835
Nguyen L, Schmidt HA, von Haeseler Arndt, Minh BQ (2015) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies, Molecular Biology and Evolution 32(1): 268-274. https://doi.org/10.1093/molbev/msu300
Nussbaum RA, Hoogmoed MS (1979) Surinam caecilians, with notes on Rhinatrema bivittatum and the description of a new species of Microcaecilia (Amphibia, Gymnophiona). Zoologische Mededelingen Leiden 54: 217-235. https://www.repository.naturalis.nl/document/150047
Olson DM, Dinerstein E (2002) The Global 200: priority ecoregions for global conservation. Annals of the Missouri Botanical Garden 89: 199-224. https://doi.org/10.2307/3298564
Palumbi S (1996) Nucleic acids II: the polymerase chain reaction.In: Hillis DM, Moritz C, Mable BK (Eds) Molecular Systematic. Sinauer and Associates Inc., Sunderland, 205-247.
Paternina-H A, Carvajal-Cogollo JE, Medina-Rangel G (2013) Anfibios De Las Ciénagas del Departamento del Cesar. In: Rangel-Ch JO (Ed.) Colombia: diversidad biótica XIII. Complejo cenagoso de Zapatosa y ciénagas del sur del Cesar. Universidad Nacional de Colombia. Bogotá, 499-509.
Pyron RA, Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution 61: 543-583. https://doi.org/10.1016/j.ympev.2011.06.012
Restrepo A, Molina-Zuluaga C, Hurtado JP, Marín CM, Daza JM (2017) Amphibians and reptiles from two localities in the northern Andes of Colombia. Check List 13(4): 203-23. https://doi.org/10.15560/13.4.203
Rivera-Correa M (2006) Geographic Distribution: Caecilia caribea (Pensilvania Caecilian). Herpetological Review 37(4): 491
Ruiz-Carranza PM, Ardila-Robayo MC, Lynch JD (1996) Lista actualizada de la fauna de Amphibia de Colombia. Revista de la Academia Colombiana de Ciencias Exactas Físicas y Naturales 20(77): 365-415.
San Mauro D, Gower DJ, Müller H, Loader SP, Zardoya R, Nussbaum RA, Wilkinson M (2014) Life-history evolution and mitogenomic phylogeny of caecilian amphibians. Molecular Phylogenetics and Evolution 73: 177-179. https://doi.org/10.1016/j.ympev.2014.01.009
Taylor EH (1968) The Caecilians of the World: A Taxonomic Review. University of Kansas Press, Lawrence, 848 pp .
Taylor EH (1969) A new caecilian from Brasil. University of Kansas Science Bulletin 48: 307313. https://doi.org/10.5962/bhl.part. 11225

Taylor EH (1973) A caecilian miscellany. University of Kansas Science Bulletin 50: 187-231. https://biodiversitylibrary.org/page/3278573
Wake MH, Donnelly MA (2010) A new lungless caecilian (Amphibia: Gymnophiona) from Guyana. Proceedings of the Royal Society of London. Series B, Biological Sciences 277: 915-922. https://doi.org/10.1098/rspb.2009.1662

Wilkinson M, Nussbaum RA, Hoogmoed MS (2009) A new species of Microcaecilia (Amphibia: Gymnophiona: Caeciliidae) from Surinam. Herpetologica 65: 413-418. https:// doi.org/10.1655/08-030.1
Wilkinson M, Kok PJR (2010) A new species of Microcaecilia (Amphibia: Gymnophiona: Caeciliidae) from Guyana. Zootaxa 2719: 35-40. https://doi.org/10.11646/zootaxa.2719.1.3
Wilkinson M, San Mauro D, Sherrat E, Gower DJ (2011) A nine-family classification of caecilians (Amphibia: Gymnophiona). Zootaxa 2874: 41-64. https://doi.org/10.11646/ zootaxa.2874.1.3
Wilkinson M, Sherratt E, Starace F, Gower DJ (2013) A new species of skin-feeding caecilian and the first report of reproductive mode in Microcaecilia (Amphibia: Gymnophiona: Siphonopidae). PLoS One 8(3): e57756. https://doi.org/10.1371/journal.pone. 0057756
Wilkinson M, O'Connor A, Nussbaum RA (2013a) Taxonomic status of the neotropical caecilian genera Brasilotyphlus Taylor, 1968, Microcaecilia Taylor, 1968 and Parvicaecilia Taylor, 1968 (Amphibia: Gymnophiona: Siphonopidae). Occasional Papers of the Museum of Zoology University of Michigan 2013(744): 1-12. https://deepblue.lib.umich.edu/bitstream/handle/2027.42/122718/OP\ 744.pdf?sequence=1\&isAllowed=y
Wilkinson M, Antoniazzi MM, Jared C (2015) A new species of Microcaecilia Taylor, 1968 (Amphibia: Gymnophiona: Siphonopidae) from Amazonian Brazil. Zootaxa 3905(3): 425-431. https://doi.org/10.11646/zootaxa.3905.3.8
Zardoya R, Meyer A (2000) Mitochondrial evidence on the phylogenetic position of caecilians (Amphibia: Gymnophiona). Genetics 155(2): 765-775. http://www.genetics.org/content/ genetics/155/2/765.full.pdf
Zhang P, Wake MH (2009) A mitogenomic perspective on the phylogeny and biogeography of living caecilians (Amphibia: Gymnophiona). Molecular Phylogenetics and Evolution 53(2): 479-491. https://doi.org/10.1016/j.ympev.2009.06.018

## Appendix I

Additional specimens examined in this study. Number of specimens examined of each species in parenthesis.

Caecilia guntheri (2): COLOMBIA: NARINO: La Planada Natural Reserve, 7 km South of Chucunes, 1780 m above sea level; IAvH-Am-1396; RISARALDA: Pueblo Rico Municipality, Vereda Montebello, Montezuma Reserve, 4³3'40.5"N, $74^{\circ} 21^{\prime} 4.9^{\prime \prime} \mathrm{W}, 1650 \mathrm{~m}$ above sea level, IAvH-Am-8872.
Caecilia isthmica (1): COLOMBIA: SUCRE: San Benito Abad Municipality, Vereda La Caimanera, site La Caimanera, $9^{\circ} 2^{\prime} 33.7^{\prime \prime} \mathrm{N}, 74^{\circ} 54^{\prime} 17.6^{\prime \prime} \mathrm{W}, 26 \mathrm{~m}$ above sea level, IAvH-Am-8246 (tissue IAvH-CT-22982).
Caecilia subdermalis (10): COLOMBIA: CALDAS: Norcasia Municipality, Hidromiel camp, $5^{\circ} 34^{\prime} 16.4^{\prime \prime} \mathrm{N}, 74^{\circ} 53^{\prime} 24.8^{\prime \prime} \mathrm{W}, 850 \mathrm{~m}$ above sea level. IAvH-Am-9663; HUILA, Acevedo Municipality, Cueva de los Guácharos National Natural Park,

1820 m above sea level. IAvH-Am-0687, IAvH-Am-3541, IAvH-Am-3549, IA-vH-Am-4316-7, IAvH-Am-4322-23, IAvH-Am-4708, IAvH-Am-5388.
Caecilia thompsoni (1): COLOMBIA: CUNDINAMARCA: La Mesa Municipality, site Payacal, La Gran Via, Tacarcuna Farm, $04^{\circ} 39^{\prime} 6,77^{\prime \prime} \mathrm{N}, 74^{\circ} 25^{\prime} 1.0^{\prime \prime} \mathrm{W} ; 1100 \mathrm{~m}$ above sea level, MUJ 3713 (tissue IAvH-CT-22986).
Epicrionops aff.parkeri (2): COLOMBIA: ANTIOQUIA: municipality of El Carmen de Viboral, vereda El Porvenir, creek afferent to the Melcocho River, $5^{\circ} 54^{\prime} 7.9^{\prime \prime} \mathrm{N}$, $75^{\circ} 10^{\prime} 25.6^{\prime \prime W}, 898 \mathrm{~m}$ above sea level, IAvH-Am-14608, IAvH-Am-14609 (tissue IAvH-CT-21477).
Microcaecilia nicefori (1): COLOMBIA: TOLIMA: municipality of Coello, El Neme farm (outside of town), $4^{\circ} 7^{\prime} 12.50^{\prime \prime} \mathrm{N}, 74^{\circ} 55^{\prime} 21.10^{\prime \prime} \mathrm{W}, 327 \mathrm{~m}$ above sea level, IAvH-Am-14879 (tissue IAvH-CT-22985).
Typhlonectes natans (2): COLOMBIA: SUCRE: San Benito Abad Municipality, Vereda La Caimanera, site La Caimanera, $9^{\circ} 27^{\prime} 1^{\prime \prime} \mathrm{N}, 74^{\circ} 54^{\prime} 26.7^{\prime \prime} \mathrm{W}, 25 \mathrm{~m}$ above sea level, IAvH-Am-8275 (tissue IAvH-CT-22983). NORTE DE SANTANDER: San José de Cúcuta Municipality, Aguasal Creek, Footbridge about 1.2 km northeast of the community of Aguasal, $08^{\circ} 13^{\prime} 05^{\prime \prime} \mathrm{N}, 072^{\circ} 32^{\prime} 31.2^{\prime \prime} \mathrm{W}, 62 \mathrm{~m}$ above sea level, IAvH-Am-14559 (tissue IAvH-CT-22984).


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[^2]:    * From original description

[^3]:    * from original description

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