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Colobopsis explodens sp. n., model species for studies on "exploding ants" (Hymenoptera, Formicidae), with biological notes and first illustrations of males of the *Colobopsis cylindrica* group

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Abstract

A taxonomic description of all castes of *Colobopsis explodens* Laciny & Zettel, **sp. n.** from Borneo, Thailand, and Malaysia is provided, which serves as a model species for biological studies on "exploding ants" in Southeast Asia. The new species is a member of the *Colobopsis cylindrica* (COCY) group and falls into a species complex that has been repeatedly summarized under the name *Colobopsis saundersi* (Emery, 1889) (formerly *Camponotus saundersi*). The COCY species group is known under its vernacular name "exploding ants" for a unique behaviour: during territorial combat, workers of some species sacrifice themselves by

rupturing their gaster and releasing sticky and irritant contents of their hypertrophied mandibular gland reservoirs to kill or repel rivals. This study includes first illustrations and morphometric characterizations of males of the COCY group: *Colobopsis explodens* Laciny & Zettel, **sp. n.** and *Colobopsis badia* (Smith, 1857). Characters of male genitalia and external morphology are compared with other selected taxa of Camponotini. Preliminary notes on the biology of *C. explodens* Laciny & Zettel, **sp. n.** are provided. To fix the species identity of the closely related *C. badia*, a lectotype from Singapore is designated. The following taxonomic changes within the *C. saundersi* complex are proposed: *Colobopsis solenobia* (Menozzi, 1926), **syn. n.** and *Colobopsis trieterica* (Menozzi, 1926), **syn. n.** are synonymized with *Colobopsis corallina* Roger, 1863, a common endemic species of the Philippines. *Colobopsis saginata* Stitz, 1925, **stat. n.**, hitherto a subspecies of *C. badia*, is raised to species level.

Keywords

autothysis, behavioural ecology, Camponotini, *Colobopsis*, Formicidae, integrative taxonomy, male morphology, molecular biology, morphometry, new species, new status, new synonymy, phylogeny, Southeast Asia, taxonomy

Introduction

The *Colobopsis cylindrica* (COCY) group likely represents a monophyletic clade containing Southeast Asian ant species with distinctive hypertrophied mandibular gland reservoirs. In territorial combat, minor workers use the sticky and irritant contents of their enlarged mandibular gland reservoirs to kill or repel rival arthropods. In species where this defensive behaviour is more advanced, this happens via the characteristic suicidal "exploding" by voluntary rupture of the gastral integument (autothysis) (Cook 2008). This behaviour was first mentioned by Viehmeyer as early as 1916, and subsequently described in detail by Maschwitz and Maschwitz (1974), as well as Davidson et al. (2012), and Shorter and Rueppell (2012).

The Bornean members of the COCY group have been the subject of various ecological (e.g., Cook 2008, Davidson et al. 2007, 2009, 2016), morphological (Davidson et al. 2012, Laciny et al. 2017) and chemical (Jones et al. 2004, Hoenigsberger et al. in prep.) studies in the past. Based on the results of previous investigations, in 2014 an interdisciplinary research project started to explore the evolution and ecological significance of autothysis in the COCY group. From the surroundings of the Kuala Belalong Field Studies Centre (KBFSC) in Brunei, at least 15 species are known (Davidson et al. 2007), most of which are probably new to science. One species, previously referred to as "yellow goo" (Davidson et al. 2007) or "YG" (Davidson et al. 2016) for the bright yellow colour of its mandibular gland secretion, was found to have a large colony just at the KBFSC. As this abundant species frequently exhibits characteristic autothysis behaviour and can be observed *in situ* and *in vitro*, it became the main object of behavioural and chemical experiments, and a model species for biological studies on "exploding ants". Preliminary taxonomic and molecular analyses revealed that this morphospecies is in fact an undescribed species. As the revision of the COCY group is still ongoing (I. Druzhinina et al. in prep.), the aim of this paper is to provide a

valid name, *Colobopsis explodens* Laciny & Zettel, sp. n., for subsequent use in the various behavioural, chemical, microbiological, and evolutionary publications currently in preparation. Within this study, we employ the multidisciplinary concept of integrative taxonomy (*sensu* Schlick-Steiner et al. 2010) by combining morphometric, ecological, and molecular data. We provide a taxonomic description of all castes of *Colobopsis explodens* sp. n. including males. Illustrations and morphometric characterizations of males of the COCY group had not been previously published. We compare males of *Colobopsis explodens* sp. n. with the newly illustrated male of *C. badia* (Smith, 1857) to highlight species-specific characters in the complex. Morphological characters of the male, including genitalia, are also compared with other selected taxa of Camponotini. Based on field observations, the first records on the natural history and biology of *Colobopsis explodens* sp. n. are provided.

Materials and methods

Sampling-sites and imaging of living ants

The primary field research took place in the lowland dipterocarp rainforest at the Kuala Belalong Field Studies Centre (KBFSC), Temburong District, Brunei Darussalam (4°32'48.2"N, 115°09'27.9"E), where *Colobopsis explodens* sp. n. was sampled during five collecting trips (each of 30 days duration) encompassing different seasons from 2014 to 2016.

The behaviour of *C. explodens* sp. n. was observed at multiple nesting sites on several height-levels, starting from the forest floor and understory up to the canopy and emergent layer. The activity of ants was recorded *in situ* and *in vitro* using a CANON 70D Digital SLR Camera with a CANON EF 100 mm macro lens and a Tamron AF 28–200 mm F/3.8–5.6 XR Di aspherical (IF) macro zoom lens (Suppl. material 2: S2a). For macro and close-up filming the Neewer adjustable LED light with LCD display was used. When necessary, the camera was mounted with the use of a Manfrotto Gorillapod 494RC2 tripod. The movie (Suppl. material 7) was annotated and cut using Corel VideoStudio X10 Software.

Sampling of *Colobopsis badia* in southern Thailand was conducted by H. Zettel and W. Jaitrong in June 2016. The sampling site was located in the Khao Chong Botanical Garden, near the Ton Pliw Waterfall (07°32'34"N, 99°47'33"E); a single male specimen was caught at a light at the Botanical Garden headquarters.

Host trees and activity assessment

Nesting habits of *C. explodens* sp. n. were observed based on the model colony occupying several trees and an artificial nest (nest #38, Fig. 9) in direct vicinity to the kitchen facility at KBFSC. The artificial nest consisted of a 100 cm tall and 6 cm wide square wooden stake,

with a cavity of approximately 15 mm in diameter drilled into the centre and a 4 mm wide entrance hole in the top third of the stake. The nest was painted with green acrylic paint and fastened to a small tree with rope (for detailed method of construction, see Davidson et al. 2009 and Laciny et al. 2017). The host trees were identified by comparison with type samples preserved in the herbarium of Universiti Brunei Darussalam, Brunei. The main host tree was DNA barcoded (see Suppl. material 6 "accession numbers").

The activity of *C. explodens* sp. n. occupying artificial nest #38 was observed from 14th to 30th November of 2015 at different times during the day, for 30 minutes each by counting the ants entering and leaving the nest. Temperature, barometric pressure, and weather conditions were recorded, as well as any observed noteworthy behaviour (see Fig. 9; Suppl. material 6 "activity").

DNA Extraction, PCR amplification, and Sanger sequencing

DNA extraction, gene fragment amplification, and sequencing were performed for minor worker ants of five different taxa (*C. explodens* sp. n., *C. badia*, *C.* nr. *saundersi*, *C. aruensis* Karawajew, 1933, and *C. cylindrica* (Fabricius, 1798)), as well as for mandibular gland reservoir content of *C. explodens* sp. n., one symbiotic cricket (*Camponophilus* sp.) from artificial nest #38, and the host plant of *C. explodens* sp. n. (*Shorea johorensis*).

For DNA barcoding of ant specimens, DNA was extracted from ant legs using Qiagen's tissue QIAamp DNA Micro kit following the manufacturer's protocol (Qiagen, Venlo, Netherlands). To obtain sufficient DNA quantity for further processing, the amount of legs used per sample varied. For the DNA extraction of queens, a minimum of three legs of one individual were transferred into one 1.5 ml microcentrifuge tube and frozen with liquid nitrogen. Three legs of one individual was also the minimum amount for males and major workers. For minor workers, all legs from two to four individuals were pooled (see Suppl. material 6 "accession numbers"). The frozen legs were ground into small pieces with disposable pestles attached to a pestle motor (Kimble, Vineland, NJ, USA). Subsequent steps were performed according to manufacturer's instructions with the following exceptions: sample lysis for 20 hours, final elution step with 25–50 µl elution buffer. To assess the purity of the extraction, DNA concentration and 260/280 nm ratio were measured with a NanoDrop ND-1000 Spectrophotometer (Software Version ND-1000 v.3.8.1, Thermo Fisher Scientific, MA, USA).

For DNA barcoding of symbiotic crickets, DNA was extracted from one whole specimen applying the same procedure as used for ants legs, but with a pretreatment with an enzymatic lysis buffer (Tris·Cl 20 mM, pH 8.0, sodium EDTA 2 mM, Triton X-100 1.2%, add lysozyme to 20 mg/ml for 60 min).

For DNA barcoding of the host plant, 100 mg of a leaf were ground with mortar and pestle under the use of liquid nitrogen and DNA was extracted using Qiagen's DNeasy Plant Mini Kit according to manufacturer's instructions.

For the ants, the gene fragments cytochrome C oxidase subunit I and II (COI, COII), cytochrome B (cytB), and carbamoyl-phosphate synthase II (cad) were am-

plified, for the cricket only COI. Additionally, a fragment of 16S rRNA was amplified from the DNA extracted from the mandibular gland content of *C. explodens* sp. n. minor workers, to assess the presence of bacteria. For the plant, the gene fragment maturase K (matK) was amplified. Primer sequences and specific annealing temperatures are given in Tab. 1. Final concentrations for PCR were 1× GoTaq Flexi Buffer (Promega, Madison, Wisconsin, USA), 0.16 mM dNTP's, 3 mM $MgCl_2$ (Promega), 0.4 µM forward and reverse primer (Microsynth, Balgach, Switzerland), 0.8 Units GoTaqG2 Flexi polymerase (Promega) and 2–50 ng sample (diluted with HPLC water, ROTH), in a final volume of 50 µl. PCR was performed with a Biometra T3 Thermocycler (Biometra, Göttingen, Germany) with the following conditions: 2 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at primer specific annealing temperature, 90 sec at 72 °C and finally 7 min at 72 °C. PCR products were separated by 1.5% agarose gel electrophoresis. PCR products were purified using mi-PCR Purification Kit (Metabion, Planegg, Germany) and one direction sequencing was performed at Microsynth (Austria).

Sequences are deposited in NCBI GenBank. Accession numbers for ant specimens are given in Table 2; see Suppl. material 6 "accession numbers" for additional details.

Table 1. Primers used in this study.

Gene	Name	Sequence 5'–3'	Length [bp]	GC content [%]	Fragment Length [bp]	Annealing Temp [°C]	Reference
COI	LCO1490-F	GGTCAACAAATCAT-AAAGATATTGG	25	32	709	45	Chen et al. 2013
	HCO2198-R	TAAACTTCAGGGT-GACCAAAAAATCA	26	35			
COII	J2791-F	ATACCHCGDCGA-TAYTCAGA	20	40–55	858	51	Chen et al. 2013
	H3665-R	CCACARATTTCW-GAACATTG	20	35–40			
cytB	CB11400-F	TATGTACTACCHT-GAGGDCAAATATC	26	35–42	485	45	Chen et al. 2013
	CB11884-R	ATTACACCNCCTAA-TTTATTAGGRAT	26	27–35			
cad	CD1423EF	AGGTRATACRATCG-GARAGRCCDGA	25	40–60	800	55	Ward et al. 2010
	CD1910R	CCGAGRGGRTCRAC-RTTYTCCATRTTR-CAYAC	32	38–63			
matK	472F	CCCRTYCATCTG-GAAATCTTGGTTC	25	44–52	750	47	Yu et al. 2011
	1248R	GCTRTRATAATGA-GAAAGATTTCTGC	26	31–38			
16S rRNA	fD1	AGAGTTTGATCCTG-GCTCAG	20	50	1500	56	Weisburg et al. 1991
	rP1	ACGGTTACCTTGT-TACGACTT	21	43			

Table 2. List of sequence accession numbers in NCBI GenBank. * Nucleotide sequences from NCBI GenBank.

TUCIM	Other IDs	Organism	ng/µl	COI	COII	cytB	cad
5053		*C. explodens* sp. n.	14.7	MF993252	MF993269	MF993286	MF993304
5056		*C. explodens* sp. n.	19.7	MF993253	MF993270	MF993287	MF993305
5080		*C. explodens* sp. n.	10.4	MF993254	MF993271	MF993288	MF993306
5098		*C. explodens* sp. n.	8.6	MF993256	MF993273	MF993290	MF993308
5104		*C. explodens* sp. n.	16.6	MF993257	MF993274	MF993291	MF993309
5148		*C. explodens* sp. n.	6	MF993258	MF993275	MF993292	MF993310
5185		*C. explodens* sp. n.	7.3	MF993259	MF993276	MF993293	–
5205		*C. explodens* sp. n.	29.8	MF993260	MF993277	MF993294	MF993311
6600		*C. explodens* sp. n.	8	–	MF993284	–	–
5855		*C. explodens* sp. n.	16.9	MF993262	MF993278	MF993297	MF993314
5856		*C. explodens* sp. n.	28.2	MF993263	MF993279	MF993298	MF993315
5942		*C. explodens* sp. n.	34.3	MF993264	MF993280	MF993299	MF993316
5943		*C. explodens* sp. n.	142.1	MF993265	MF993281	MF993300	–
	YG*	*C. explodens* sp. n.	n.a.	EF634201		–	–
6461		*C. badia*	21.3	MF993266	MF993282	MF993301	MF993317
6463		*C. badia*	5.4	MF993267	MF993283	MF993302	MF993318
6601		*C. badia*	17.91	MF993268	MF993285	MF993303	MF993319
5698		*C.* nr. *saundersi*	50.1	KU975365.1	KU975366.1	MF993296	MF993313
	CH*	*C.* cf. *cylindrica*	n.a.	EF634198		–	–
5086		*C. cylindrica*	26	MF993255	MF993272	MF993289	MF993307
5300	CAMP004	*C. aruensis*	169.1	MF993261	–	MF993295	MF993312
	Cflor36*	*Camponotus floridanus*	n.a.	AY334397	–	–	–

Sequences of non-ant material are deposited under MG582639 for COI of myrmecophilous crickets (*Camponophilus* sp.), MF993320 for matK of *Shorea johorensis* and MF996752 for 16S rRNA of cf. *Blochmannia* (Enterobacteriales).

Phylogenetic analysis

GapStreeze v. 2.1.0 (https://www.hiv.lanl.gov/content/sequence/GAPSTREEZE/gap.html) was used for COI gene alignment with 95 % gap tolerance in order to retain only the conserved region. The individual gene alignments were subjected to best substitution model selection using the BIC criterion in SMS (Lefort et al. 2017). Consecutively, HKY85, HKY85+I, HKY85+G, and GTR+G were chosen as best substitution models for genes cad, cytB, COI, and COII respectively. The concatenated alignment was partitioned for each locus using MrBayes v. 3.2.5 (Ronquist et al. 2012), and the respective substitution models were assigned to each partition. The substitution and branch length estimates were allowed to vary independently between each partition. Priors for an exponential distribution with mean 1 to all branch lengths and to all shape parameters were assigned for all four partitions. Metropolis-coupled Markov

chain Monte Carlo (MCMCMC) sampling was performed using MrBayes v. 3.0B4 (Ronquist et al. 2012) with two simultaneous runs of four incrementally heated chains that performed 1 million generations. Bayesian posterior probabilities (PP) were obtained from the 50 % majority rule consensus of trees sampled every 100 generations after removing the first 25 % of trees using the "burnin" command. According to the protocol of Leache and Reeder (2002), PP values higher than 0.94 were considered significant. The phylogenetic trees were visualized in FigTree v. 1.4.3 (Rambaut 2016) and then annotated using vector graphic software.

Morphological methods

All specimens used for morphometry were card-mounted, individually numbered, and measured at magnifications from 25.6× up to 256× with a Nikon SMZ1500 binocular microscope. Genital structures of two male specimens were dissected and mounted separately. Results represent minimum and maximum values for each morph; in cases where a character could not be measured in all individuals, the number of measured specimens is given in parentheses. The complete dataset of measurements is provided in Suppl. material 6 "measurements".

Measurements and indices (* = only gynes and males)

EL Eye length. Maximum diameter of compound eye, measured in lateral view.

FeL Femur length. Maximum length of metafemur, measured from base to apex.

FWL* Forewing length. Length of forewing, measured from tegula to distal tip.

HaL Hair length. Length of the longest standing hair on first gastral tergite, measured from base to apex.

HL Head length. Maximum length of head in full-face view, excluding mandibles, measured from anteriormost point of clypeus to posterior-most point of head vertex, parallel to midline.

HS Head size. (HW + HL) / 2.

HW Head width. Maximum width of head in full-face view (including eyes if protruding; only in gynes).

ML Mesosoma length. Measured laterally from anterior surface of pronotum proper (excluding collar) to posterior extension of propodeal lobes.

MSW* Mesoscutum width. Maximum diameter of mesoscutum, measured dorsally.

NH Node height. Height of petiolar node, measured laterally, from the intersection point of the axes of maximum height and length to dorsal apex

OcD* Ocellar distance. Minimum distance between lateral ocelli, measured between median borders.

OcW* Ocellus width. Maximum diameter of median ocellus.

OED* Ocellar eye distance. Minimum distance between lateral ocellus and outer border of compound eye.

PH Petiole height. Maximum height of petiole in lateral view, measured from ventral-most point of petiolar sternum to dorsal apex.
PL Petiole length. Maximum length of petiole in lateral view, measured from inflexion point of anterior constriction to posterior margin, perpendicular to axis of maximum height.
PS5 Length of maxillary palp segment V, measured from base to apex.
PS6 Length of maxillary palp segment VI, measured from base to apex.
SL Scape length. Maximum length of antennal scape in dorsal view excluding basal neck and condyle.
SW Scape width. Maximum width of antennal scape, measured dorsally.
TL Total length. The added lengths of head (excluding mandibles), mesosoma, petiole, and gaster.
2r* Maximum length of 2nd radial crossvein (see Figs 5e, 6b).
4Rs+M* Length of 4th radial sector fused with median (see Figs 5e, 6b).
CI Cephalic index. HW / HL × 100
EI Eye Index. EL / HW × 100
FeI Femur Index. FeL / HW × 100
OI* Ocellar Index: OED / OcD × 100
PI Petiole Index. PH / PL × 100
PSI Palp Segment Index. (PS5 + PS6) / HS × 100
SI Scape index. SL / HW × 100
SWI Scape width index. SW / SL × 100
WVI* Wing Vein Index. 4RsM / 2r × 100

Digital stacked images of most specimens (Figs 2–6) were acquired with a Leica DFC camera attached to a Leica MZ16 binocular microscope with Leica Application Suite v3 and stacked with Zerene-Stacker 64-bit. Images of labels were taken with a Nikon D60 camera with an AF-S Micro Nikkor 105 mm objective and an EM-140 DG macro ring flash. Photographs of genital structures of males (Figs 7, 10c–f) as well as of the male *C. badia* specimen (Fig. 10 a, b) were created with the help of Leica Application Suite v3.8, using a Leica DFC450 camera attached to a Leica Z16APO optics carrier. All images were processed with Adobe Photoshop 7.0.

Material examined

Type material of *C. explodens*: **Holotype** (minor worker): Brunei, Temburong, Kuala Belalong Field Studies Centre, 04°33'N, 115°09'E, 60 m a.s.l., 10.XI.–5.XII.2015, leg. A. Laciny & A. Kopchinskiy ("YG Vienna Colony", specimen number COCY 01565).

Paratypes (59 minor workers, 8 major workers, 16 gynes, and 6 males dry mounted; > 500 imagines stored in 96 % ethanol): 19 minor workers, 2 major workers, 12 alate gynes, 4 dealate gynes, 6 males (including allotype) (all dry mounted), as well as 8 males, 2 alate gynes, ca. 500 minor workers (in alcohol) from the same nest sam-

ple as holotype; 1 major worker, same locality and collector as holotype, 17.IV.2015, "YG 373 main natural nest"; 1 major worker, same data as holotype ("YG doorkeeper #19"); 2 major workers, same data as holotype except 20.IV.2015, leg. A. Kopchinskiy ("cf. YG 39 (351) artificial nest"); 8 minor workers, 2 major workers, same locality as holotype, 2002, leg. D.W. Davidson ("YG KB02-108"); 4 minor workers, same locality and collector as previous, no collection date, "YG 2025"; 5 minor workers, same locality and collector as previous, I.2012, "YG T-trail (202)"; 5 minor workers, same locality and collector as previous, 15.V.2014, "YG-2 (73)"; 7 minor workers, same locality and collector as previous, 15.V.2014, "YG-2 (49)"; 2 minor workers, same data as previous except Batu Apoi Forest Reserve, N04°32', E115°10', 200 m a.s.l., 25.XI.2004, ("CAYG A-370"); 15 minor workers (on 5 pins), same data as previous, except N04°55', E115°19', 60 m, 3.VII.2002, ("YG KB02-108 voucher"); 4 minor workers, Thailand, Chumphon Province, Krom Luang Chumphon W.S, 3.II.2002, leg. W. Jaitrong ("WJT02-TH-0116"); 5 minor workers, West Malaysia, Kelantan, 60 km NE Tanah Rata, Tanah Kerajaan, 1000 m a.s.l., 12.–30.IV2007, leg. P. Cechovský.

Additional material: 3 pupae (Suppl. material 5) and 6 myrmecophilous crickets (*Camponophilus* sp., det. S. Ingrisch), from the same nest sample as the holotype.

For unique identification numbers of all 90 dry mounted specimens (60 minor workers, 8 major workers, 16 gynes, and 6 males), as well as information on caste and colony affiliation, see Suppl. material 6 "measurements".

The holotype and a portion of the paratypes will be deposited at the Brunei Museum; additional paratypes will be housed in the Universiti Brunei Darussalam, the Natural History Museum Vienna, the University of California (Davis, USA), the Natural History Museum of Los Angeles County (Los Angeles, USA), the Thailand Natural History Museum (Technopolis, Thailand), and the collection of H. Zettel (Vienna, Austria).

Molecular results

The topology of the phylogram based on the concatenated alignment of 2757 bp was concordant with topologies of COI and COII and not contradicted by the topology of cytB. The phylogram based on cad was statistically unresolved (data not shown). The obtained Bayesian consensus tree (Fig. 1) shows conspecificity of the newly obtained *C. explodens* sp. n. specimens from Brunei and Thailand (TUCIM 6600) with a sequence previously deposited under "*Colobopsis cylindrica* s.l. YG". While there is some intraspecific variation within the analysed *C. explodens* sp. n. specimens, they form a clade distinctly separate from the closely related *C. badia*. The male of *C. badia* (TUCIM 6463) is clearly grouped with its conspecific workers from a nearby locality, thus confirming species identity. The herein examined representatives of the *C. saundersi* subclade, *C. explodens* sp. n., *C. badia* and the undescribed *C.* nrSA (see Laciny et al. 2017), are clearly distinct from other members of the COCY group (e.g., *C. cylindrica*) and selected outgroup taxa of *Colobopsis* and *Camponotus*.

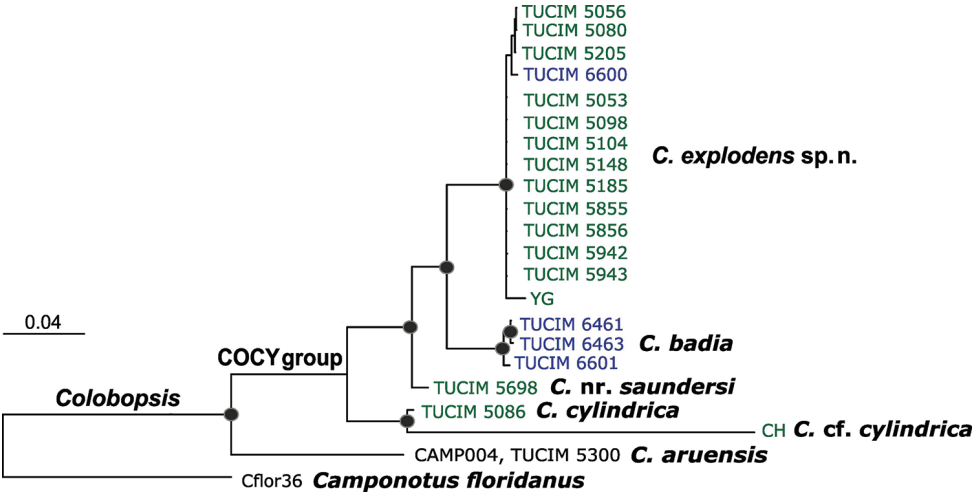

Figure 1. Bayesian consensus phylogram of *C. explodens* sp. n. and related taxa based on the concatenated alignments (2757 bp) of the three mitochondrial (COI, COII, cytB) and one nuclear (cad) phylogenetic markers. Nodes with posterior probabilities above 0.94 are marked with black dots. Specimens from Borneo are shown in green, from Thailand in blue. TUCIM 6463 corresponds to a male specimen.

Taxonomic results

Colobopsis explodens **Laciny & Zettel, sp. n.**
http://zoobank.org/DB4767B0-C745-4843-BE3F-B17DBCEB3A96
Figs 2–9; Suppl. materials 1–7

Camponotus (*Colobopsis*) sp. Yellow Goo: Davidson et al. 2007: 470.
Camponotus (*Colobopsis*) sp. YG: Cook 2008. Davidson et al. 2012: 488.
Colobopsis sp. YG: Davidson et al. 2016: 518. Laciny et al. 2017: 95.

Etymology. Present participle of Latin *explodere*, referring to the "exploding"-like autothysis behaviour.

Description of phenotypes.

Minor worker (Figs 2, 4b–d; Suppl. material 1: S1a).

Measurements of holotype minor worker: TL 6.78; HW 1.48; HL 1.67; HS 1.58; PS5 0.23; PS6 0.25; EL 0.42; SL 1.33; SW 0.14; ML 2.05; HaL 0.15; PH 0.55; PL 0.47; NH 0.33; FeL 2.05. Indices: CI 88; SI 90; SWI 11; EI 29; PI 116; FeI 139; PSI 30.

Measurements of paratype minor workers: (n = 59): TL 4.74–7.21; HW 1.22–1.57; HL 1.30–1.78; HS 1.27–1.67; PS5 0.21–0.25 (20); PS6 0.20–0.26 (21); EL 0.33–0.43; SL 1.21–1.39; SW 0.11–0.16; ML 1.50–2.22; HaL 0.08–0.19; PH 0.41–0.56 (44); PL 0.33–0.49 (47); NH 0.24–0.38 (52); FeL 1.73–2.10. Indices: CI 85–94; SI 87–104; SWI 9–12; EI 27–29; PI 112–133 (41); FeI 123–151; PSI 28–35 (20).

Structures: Head (Fig. 2a) subovate, longer than wide, narrower anteriorly; sides posteriorly convex, posterior cephalic margin roundly convex; microstructure consisting of

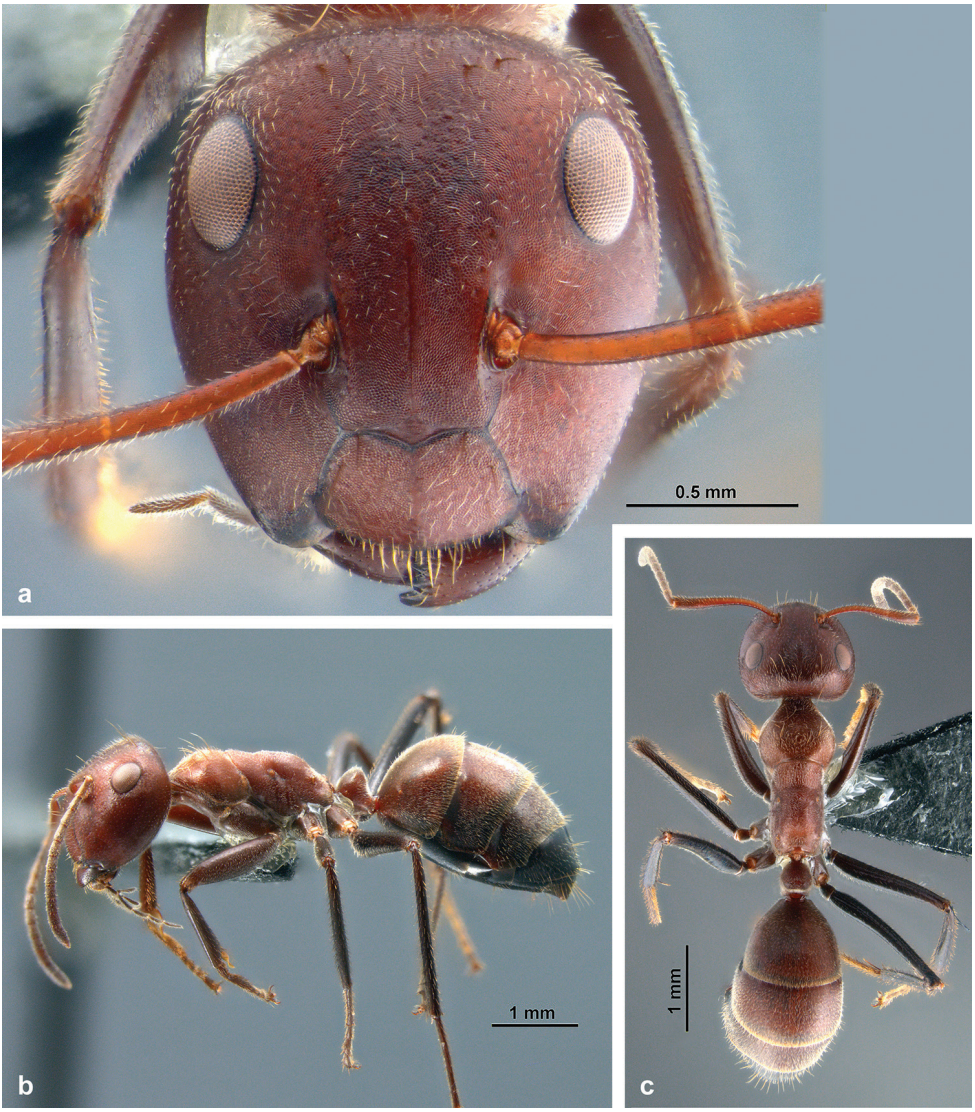

Figure 2. Habitus of *C. explodens* sp. n., holotype, minor worker; **a** full-face **b** lateral, and **c** dorsal view.

very fine, isodiametric or transverse mesh-like reticules; intermixed punctures very fine and inconspicuous on face, larger but shallow laterally and ventrally. Eyes small compared to other castes (EI 27–29, vs. 28–31 in major workers and 35–37 in gynes), flat, positioned dorsolaterally. Ocelli lacking, in some larger specimens position of median ocellus indicated by shallow impression. Frons with very fine impressed midline; frontal carinae slightly converging anteriorly, not elevated. Median carina of clypeus not reaching anterior clypeal margin, especially in small specimens. Mandibles mostly smooth, with rather dense punctures; masticatory margin with five teeth. Maxillary palpi long (PSI 28–35). Antennal scape long, its length roughly equal to head width (SI 87–104), moderately

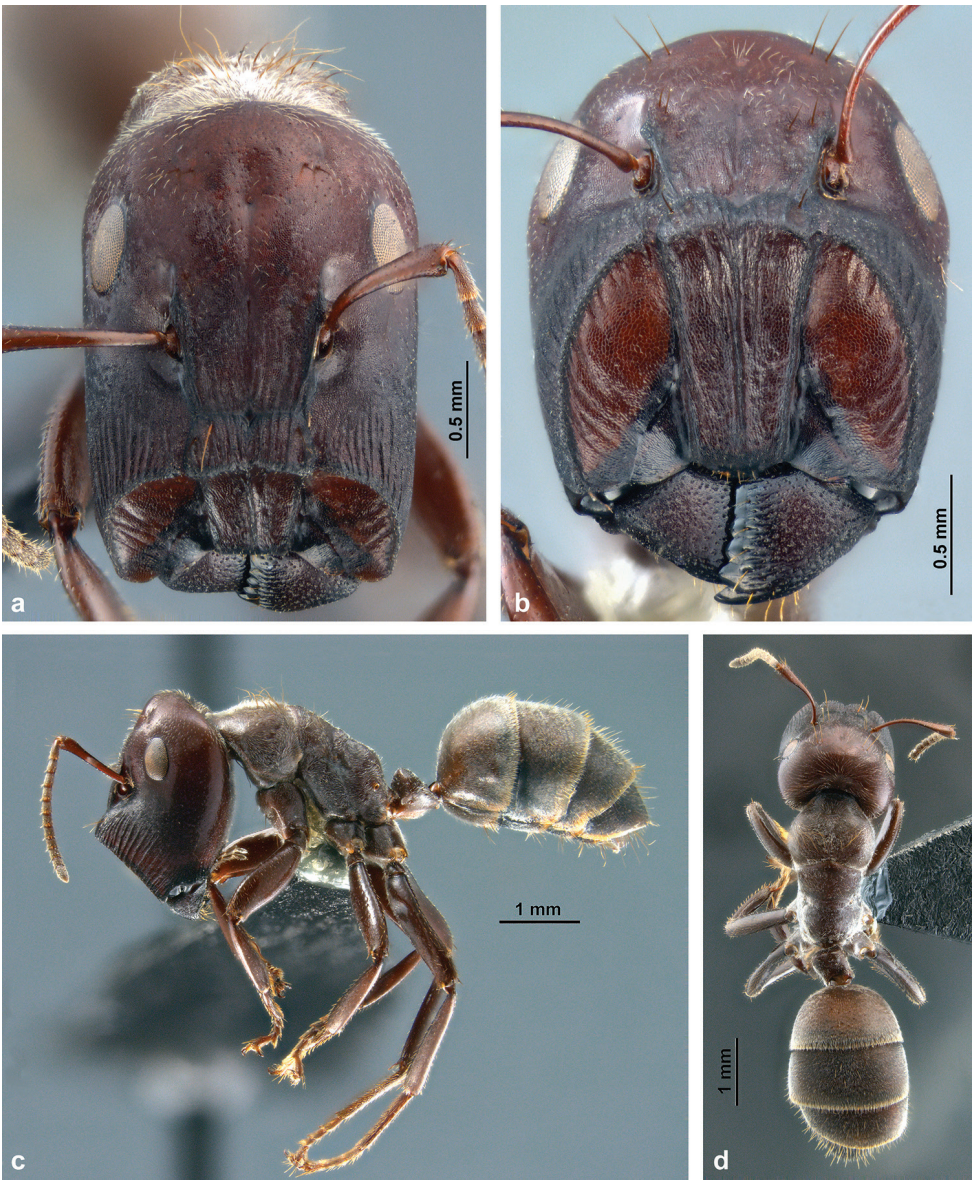

Figure 3. Habitus of *C. explodens* sp. n., paratype, major worker; **a** full-face view **b** frontal shield **c** lateral, and **d** dorsal view.

flattened, slightly widened towards apex, integument punctate. Antenna 12-segmented; antennal segment III approx. 1/5 shorter than each IV and V, and approx. 2/5 shorter than II. Mesosoma slender, moderately low. Microreticulation isodiametric or slightly transverse, dorsally denser than laterally. Metanotal region delimited from mesonotum by a shallow groove; groove delimiting metanotum from propodeum indistinct or missing. Dorsal and posterior outline of propodeum rounded in lateral view, or meeting at

an obtuse angle, dorsal face slightly convex, posterior face flat to shallowly concave. Legs slender. Petiole with isodiametric reticulation; petiolar node moderately high, its short, slightly convex anterior and its rather straight posterior face forming a triangular shape in lateral view, its apex not truncated, rather rounded; node narrow in dorsal view, a crest indistinct; a medial depression indicated in most specimens. Gaster: dorsum of tergites I–III with extremely fine, dense, transverse microreticulation, slightly shiny (Fig. 4b); mesh-like reticulation wider on lateral areas of tergites I–III , tergite IV, and sternites , therefore meshes appearing not so strongly transverse, and the integument shinier (Fig. 4c). Exposed parts of tergite V and sternite V with dense, almost isodiametric reticulation, dull; base of tergite V (usually covered by tergite IV) sculptured as tergite IV.

Colour: Body mainly reddish brown. Vertex of head, margins of clypeus, masticatory and lateral margins of mandibles, dorsum and ventral margins of mesosoma, mid portion of gastral tergites I–III, and legs slightly darker brown in most specimens; some specimens with darker area extending medially from head vertex to frons. Gastral tergites and sternites with very narrow hyaline margins. All gastral sternites, lateral fourths and posterior margins of tergites I–III, as well as entire tergites IV and V black.

Pilosity: Dorsum of head with very short, inconspicuous, appressed and subdecumbent setae; a few very long, standing setae on frons near declivity to vertex, medial of frontal carinae, and on lateral portions of clypeus. Mesosoma and petiole with fine and short, whitish, velvety pilosity; long, standing, slightly undulated setae restricted to pronotum; declivity of propodeum and node of petiole with few very short standing setae. Gastral tergites with moderately dense, short whitish, decumbent setae and few slightly darker, longer standing setae, most of them in transverse rows near hind margins. Longest setae in transverse rows near hind margins of sternites and at base of gastral tergite V.

Notes: Minor workers of *Colobopsis explodens* sp. n. show a continuous size variation across a remarkably wide range, similar to that found in the undescribed *Colobopsis* sp. nrSA (Fig. 8; compare with Laciny et al. 2017).

Phragmotic major worker (Figs 3, 4a; Suppl. material 1: S1b).

Measurements of paratype major workers (n = 8): TL 7.30–8.71; HW 1.72–1.89; HL 2.25–2.58; HS 1.99–2.20; PS5 0.15–0.17 (6); PS6 0.15–0.17 (6); EL 0.50–0.56; SL 1.15–1.26; SW 0.17–0.20; ML 2.22–2.74; HaL 0.11–0.20; PH 0.59–0.69 (6); PL 0.45–0.51 (6); NH 0.40–0.45 (6); FeL 1.50–1.70. Indices: CI 71–77; SI 64–69; SWI 14–17; EI 28–31; PI 125–143 (6); FeI 87–95; PSI 14–17 (6).

Structures: Integument mostly dull, only head and legs shiny. Head (Fig. 3a) large, subcylindrical, anteriorly truncated. On posterior areas of face punctation slightly stronger than in minor worker. Eyes somewhat larger and more distant from vertex compared to minor worker. Ocelli lacking, their positions often indicated by shallow grooves (Fig. 4a). Anterior part of head forming a large shield (Fig. 3a, b) formed by clypeal and genal components, limited by a sharp and elevated crest so that the shield surface appears concave in lateral view. Shield with fine isodiametric reticulation and rather variable, mostly longitudinal rugae; most prominent are a pair of rugae along sides of clypeus and a single median carina that does not reach the anterior margin,

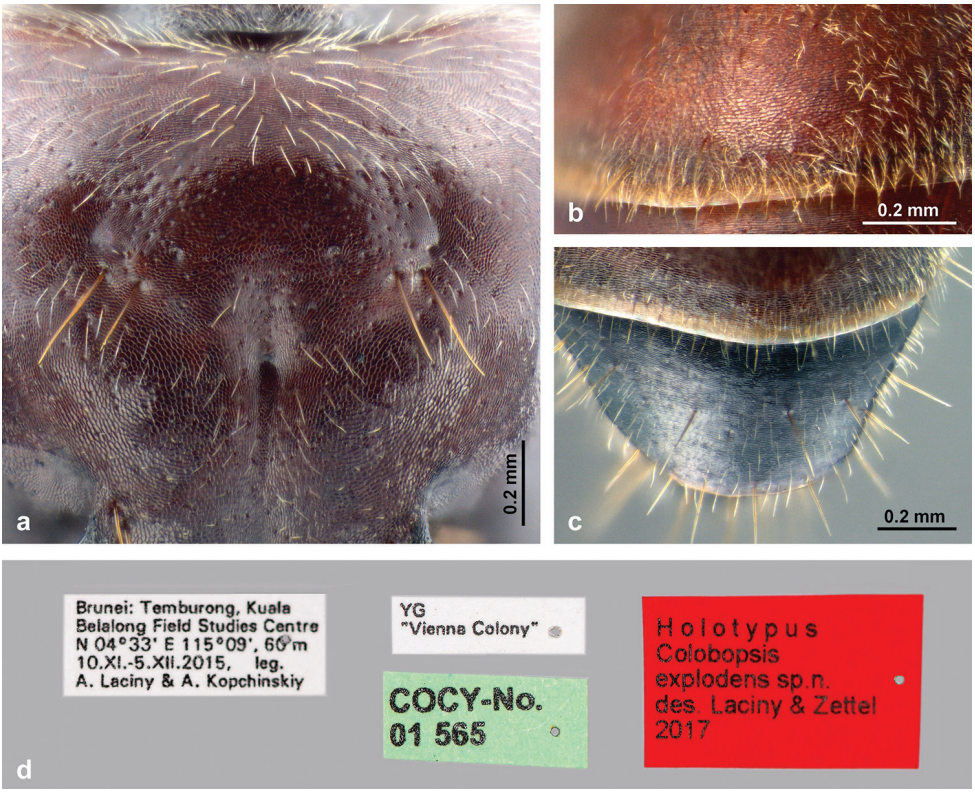

Figure 4. Cuticular microsculpture of *C. explodens* sp. n.; **a** vertex of major worker (paratype) **b** gastral tergite I, and **c** gastral tergite IV of minor worker (holotype) **d** labels of holotype, minor worker.

often reduced towards base. Genal part with curved rugae of variable number, length, and distinctiveness, but only exceptionally reaching onto the anteromedial triangle. Additional longitudinal rugae on clypeus often present, including a usually distinct pair of paramedian rugae running from base of clypeus over the crest anteriorly towards middle of shield; in specimens with short median carina, the area between these carinae more or less grooved. Longitudinal striation more regular and pronounced on frons and genae up to level of antennal insertions, laterally on genae similarly long and strong. Mandible with sharp and high ventrolateral ridge, coarsely punctate, its lateral face weakly rugose-striate; masticatory margin with acute apex and few (1–3) more or less distinct, very blunt teeth in distal half (Fig. 3b). Maxillary palpi very short (PSI 14–16). Antenna considerably shorter than head width (SI 64–69) and stouter than in all other morphs (Figs 3a, 8b); antennal scape distinctly widened towards apex. Mesosoma stouter and higher than in minors, especially promesonotum expanded; in lateral view dorsal and posterior face of propodeum forming an obtuse angle, somewhat less rounded than in minor workers, dorsally without concavity. Legs much shorter and stouter than in minors (Fig. 3c). Shape of petiole similar to minor workers, somewhat wider in dorsal view. Structures of gaster similar as in minor worker.

Colour: Overall slightly darker than minor worker; head, legs and mesosoma reddish brown; gaster slightly darker chocolate-brown, becoming darker towards caudal apex, hyaline margins yellowish; elevated crest of frontal shield, anterior clypeal margin, frontal carinae, and masticatory and lateral margin of mandibles blackish brown.

Pilosity: As in minor worker, except long setae on clypeus sides restricted to the area behind clypeal shield; mesonotum with standing setae which are approx. half the length of those on pronotum.

Notes: The head shield with a sharp, elevated crest is typical for majors of the *Colobopsis saundersi* complex (Fig. 3b).

Gyne (Fig. 5, Suppl. material 3: S3d).

Measurements of paratype gynes (n = 16): TL 10.50–12.16; HW 1.74–1.83; HL 2.28–2.45; HS 2.02–2.14; PS5 0.19–0.21 (13); PS6 0.19–0.23 (13); EL 0.62–0.66; SL 1.33–1.45; SW 0.20–0.22; ML 4.11–4.63; HaL 0.14–0.29 (15); PH 0.77–0.92 (11); PL 0.54–0.67 (10); NH 0.40–0.54 (11); FeL 2.25–2.35; OcW 0.13–0.16; OED 0.34–0.38; OcD 0.54–0.64; FWL 9.72–10.50 (11); MSW 1.68–2.15; 2r 0.50–0.64 (12); 4Rs+M 0.14–0.32 (12). Indices: CI 73–77; SI 75–80; SWI 15–16; EI 35–37; PI 123–150 (8); FeI 125–132; PSI 18–20 (13); OI 54–69; WVI 26–58 (12).

Structures: Head (Fig. 5a) large, subcylindrical, anteriorly truncated, similar to that in major worker with the following exceptions: eyes larger than in workers (EI 35–37) and breaking outline of head in full-face view. Ocelli fully developed, their colour ranging from almost clear to reddish amber. Head shield sharply delimited, but slightly smaller than in major worker, distinctly narrower than head width. Striation of clypeus, frons, and genae similar as in major, though somewhat more strongly developed on lateral parts of shield. Mandible with sharp ventrolateral ridge; its lateral face weakly rugose-striate, narrower than in major; dorsal-anterior face punctured; masticatory margin with acute apex and 3–4 blunt teeth in distal half, mandible basally with blunt ridges (Fig. 5b). Maxillary palpi moderately long (PSI 18–20). Antennal scape moderately long, slightly shorter than head width (SI 75–80), somewhat widened towards apex (Figs 5a, 8b). Mesosoma large, structures as typical for caste; propodeum large and evenly convex in lateral view. Cuticular microstructures dorsally consisting of very fine punctation, with intermixed larger punctures, laterally finely reticulated. Legs stout, but not as short as in major (Fig. 5c). Forewing venation strongly reduced, as in most Camponotini; M-Cu absent; Mf2+ interstitial (Fig. 5e). Petiole distinctly wider than in workers; node more rounded in lateral view, in some specimens its apex shallowly impressed medially, in others with two shallow lateral impressions forming a trilobed outline. Gastral tergites I–IV and sternites I–IV with extremely fine and dense microstructures consisting of strongly transverse meshes; only sides of tergites with wide mesh-like reticulation and shiny; tergite V with dense isodiametric reticulation.

Colour: Chiefly as in major worker. Head and pronotum reddish brown; ventral and posterior mesosoma, petiole, legs and gaster somewhat darker chocolate-brown; mandibles and ridges of clypeal shield blackish brown. Pronotum and mesonotum with very narrow yellow margins. Gastral tergites medially with very narrow hyaline margins; sternites with relatively broad posterior margins. Wings hyaline, but forewing

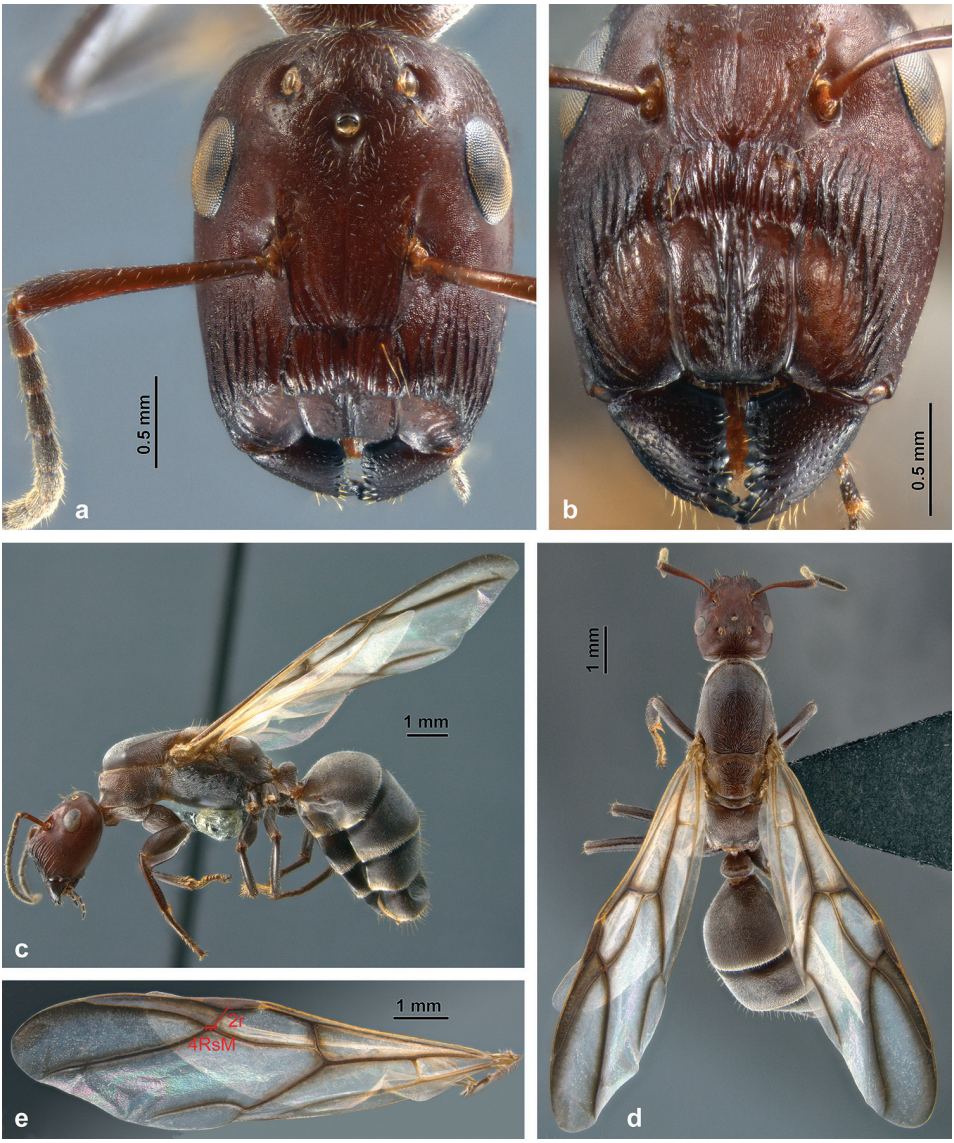

Figure 5. Habitus of *C. explodens* sp. n., paratype, alate gyne; **a** full-face view **b** frontal shield **c** lateral **d** dorsal view **e** forewing with indicated measurements 2r and 4RsM.

cells along veins, as well as pterostigma darkened to brownish. On hind wing all veins pale yellow (Fig. 5c–e).

Pilosity: Short pilosity and distribution of long setae on head, petiole, and gaster similar as in major worker, but that of mesosoma different; pronotum with few long, undulated setae. Medial part of mesonotum (between parapsidal furrows) with numerous long erect setae, scutellum with few long erect setae; lateral part of mesonotum in front of tegulae without setae.

Notes: The head shield with a sharp, elevated crest is typical for gynes of the *Colobopsis saundersi* complex (Fig. 5b).

Male (Figs 6, 7). This is the first detailed description and illustration of males from the *C. cylindrica* group.

Measurements of allotype male: TL 7.11; HW 1.26; HL 1.20; HS 1.23; PS5 0.20; PS6 0.15; EL 0.44; SL 0.84; SW 0.11; ML 2.54; HaL n.a.; PH 0.46; PL.40; NH 0.29; FeL 1.83; OcW 0.18; OED 0.27; OcD 0.43; FWL 6.33; MSW 1.37; 2r 0.38; 4Rs+M 0.27. Indices: CI 105; SI 66; SWI 13; EI 35; PI 116; FeI 145; PSI 28; OI 62; WVI 70.

Measurements of paratype males (n = 5): TL 6.46–6.85; HW 1.24–1.29 (4); HL 1.14–1.24; HS 1.20–1.27; PS5 0.17–0.21 (4); PS6 0.13–0.17 (4); EL 0.43–0.46; SL 0.80–0.85; SW 0.10–0.12; ML 2.38–2.87; HaL n.a.; PH 0.45–0.49 (4); PL 0.38–0.40 (4); NH 0.26–0.33 (4); FeL 1.71–1.86; OcW 0.18–0.19; OED 0.25–0.27; OcD 0.43–0.46; FWL 5.87–6.33; MSW 1.17–1.50; 2r 0.38–0.47; 4Rs+M 0.14–0.22. Indices: CI 104–110 (4); SI 64–67 (4); SWI 12–15; EI 35–36 (4); PI 113–123 (4); FeI 136–151 (4); PSI 27–30 (4); OI 53–62; WVI 31–53.

Structures: Head (Fig. 6a) small, subtrapezoidal, eyes very large, round and protruding, EL more than one third of HL (EI 35–36). Ocelli very large, diameters larger than in gynes. Integument of head rather matt. Frons and genae finely reticulated, genae additionally finely punctured. Clypeus with some stronger punctures at margins (at base of setae), median carina weakly developed, present in proximal third of clypeus or entirely obsolete. Frons with impressed midline from median ocellus to level of antennal insertions. Frontal carinae weakly developed, converging more strongly than in minor worker. Mandible short with reduced dentition, masticatory margin with 2–3 blunt teeth; dorsal surface finely punctate. Maxillary palpi long (PSI 27–30). Antenna 13-segmented; scapes short (SI 64–67) and relatively slender (Fig. 8b). First funicular segment conspicuously enlarged distally, pear-shaped, 30–50% wider and ca. 20% longer than the following segment (Fig. 6a); all other funicular segments cylindrical, without modifications. Mesosoma large, structures as typical for alate ants. Mesoscutum anteriorly strongly convex with narrow impressed midline in posterior tenth. Scutellum moderately elevated; propodeum evenly convex. Cuticular microstructures of mesosoma consisting of a very fine reticulation with intermixed minute punctures at bases of short hairs, additionally with larger punctures dorsally at bases of erect setae. Legs very long and slender (FeI 136–151). Forewing venation strongly reduced, as in most Camponotini. M-Cu absent; 4Rs+M shortly developed or (more rarely) Mf2+ interstitial (Fig. 6b). Petiole small; in lateral view node more bluntly rounded than in female castes, anterior and posterior faces straight, not convex, apex not impressed medially in dorsal view. Gastral tergites I–IV and sternites I–IV with fine and dense microreticulation consisting of moderately transverse meshes; only sides of tergites with wide meshes and shiny; tergite V with almost isodiametric reticulation. Sternite VI posteriorly emarginated, sternite VII truncated.

Genital structures (Fig. 7): Genital capsule (Fig. 7a–c) approx. as long as wide in dorsal aspect (Fig. 7a), ventrally longer than dorsally, protruding from apex of gaster. Gonopod high, distally broadly rounded. Gonostylus (Fig. 7c) elongated and acuminated, with reticulated microstructure (only visible at very high magnification) and

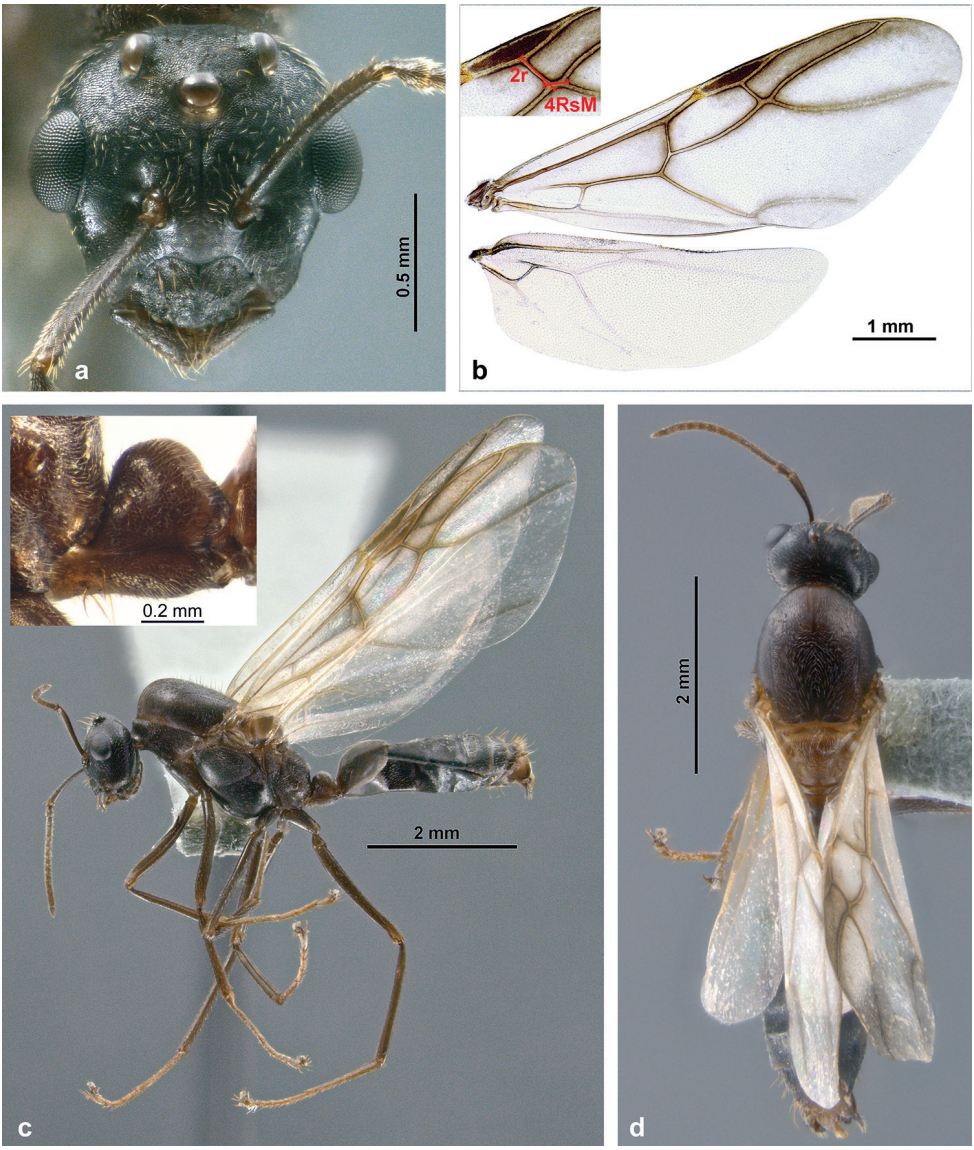

Figure 6. Habitus of *C. explodens* sp. n. allotype, male; **a** full-face view **b** wings (see insert for illustration of measurements 2r and 4RsM) **c** lateral (see insert for detailed view of petiole), and **d** dorsal view.

some long setae. Basivolsella (Fig. 7e) dorsally with roundish structure, ventrally with evenly distributed, comparatively short setae. Digitus (Fig. 7e) large, evenly widened towards apex; apex rounded but with rectangular corner ventrally. Penis valvae (Fig. 7d) in dorsal aspect broad at base, but very narrow distally. Valviceps leaf-shaped in lateral view, apically rounded; surface smooth; ventral margin with very fine serration.

Colour: Mainly dark chocolate-brown. Head somewhat darker; eyes pale grey to blackish; ocelli translucent, ranging from almost clear to reddish amber. Antennae

Figure 7. Genital structures of *C. explodens* sp. n. paratype, male; genital capsule in **a** dorsal **b** ventral, and **c** lateral view **d** left penis valve **e** right volsella and gonostylus.

and legs lighter brown, fading into yellowish towards apices. Margins of mesoscutum, scutellum, and metanotum lighter yellowish brown. Gastral tergites medially with very narrow hyaline margins; sternites with relatively broad, indistinctly separated posterior margins. Wings almost hyaline, with a slight whitish tinge, but forewing cells along veins, as well as pterostigma darkened to brownish, all veins pale yellowish brown. On hind wing all veins pale yellow.

Pilosity: On head setae sparsely distributed, short, inconspicuous, appressed, subdecumbent; a few very long standing setae on frons near vertex, and on anterior and posterior clypeal margins. Mandibles with dense short pilosity on lateral face, and few moderately long setae on anterolateral margin. Short pilosity and distribution of long

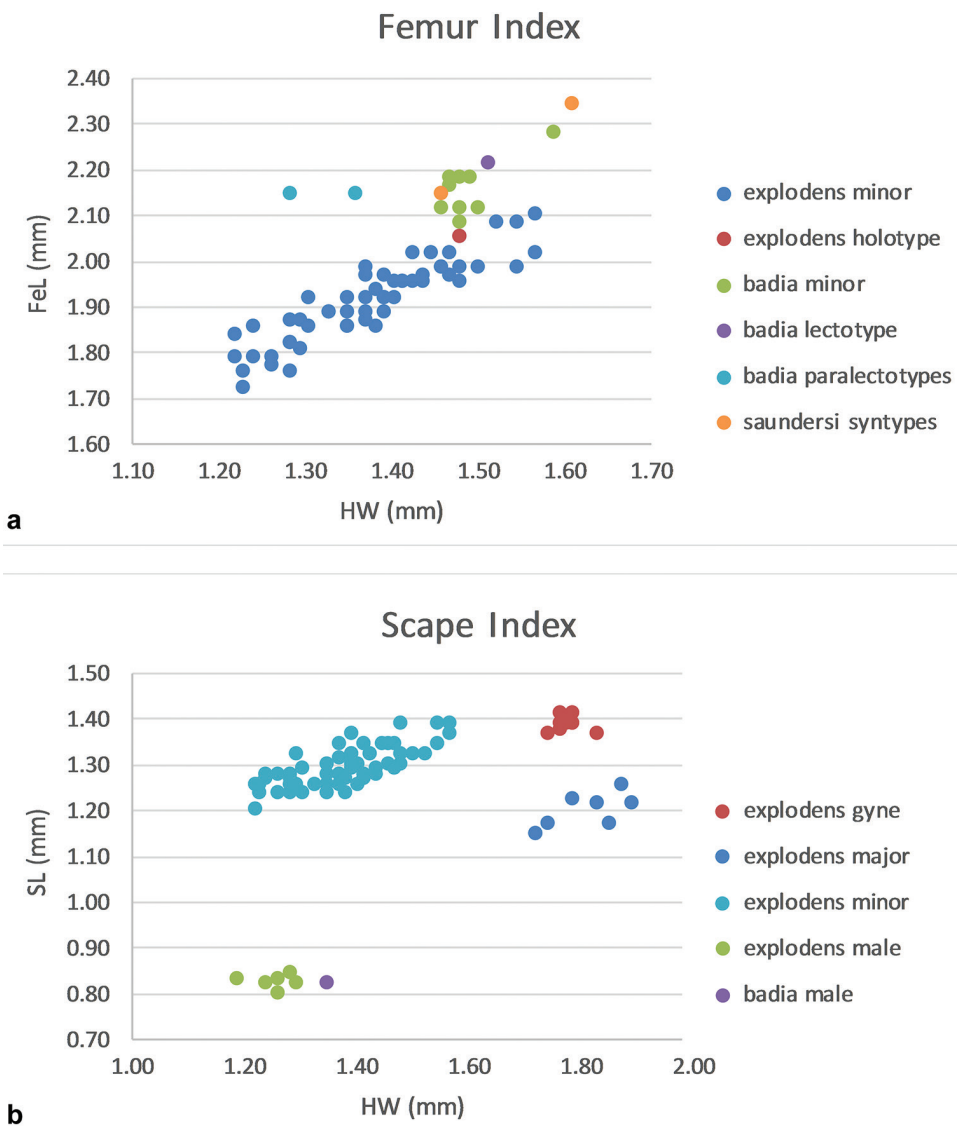

Figure 8. Variation of relevant morphometric measurements for the distinction of castes and species; **a** Metafemur length (FeL) in relation to head width (HW) for minor workers of *C. explodens* sp. n., as well as minor workers of *C. badia* and *C. saundersi* (type specimens plotted separately) **b** Scape length (SL) in relation to head width (HW) for minor workers, major workers, gynes and males of *C. explodens* sp. n., as well as male of *C. badia.*

setae on mesosoma, petiole, and gaster similar as in gyne, but pronotum lacking long, undulated setae. Medial part of mesonotum (between parapsidal furrows) with numerous long erect setae, scutellum with few long erect setae; lateral part of mesonotum in front of tegulae without setae. Tegulae with dense brush of setae. Petiole with a few stout setae anteroventrally (see insert Fig. 6c). Petiolar node lacking any standing setae;

gastral tergite I without or with few subdecumbent, moderately long setae. Posterior gastral tergites and sternites (segments II and following) with sparse, relatively long, obliquely standing setae.

Biological notes on *Colobopsis explodens* sp. n.

Colonies of *C. explodens* sp. n. observed in the Ulu Temburong National Park are commonly polydomous and polygynous. This species was selected as a model for the study of the "exploding ants" because among the species with advanced autothysis behaviour it was the most abundant COCY taxon in the vicinity of KBFSC.

Colobopsis explodens sp. n. frequently nests on dipterocarp trees and its colonies can contain thousands of individuals. The largest part of the studied colony lived on a 60 m tall *Shorea johorensis* Sym. (Dipterocarpaceae) tree identified morphologically and by DNA barcoding (matK, identical to GenBank accession number KY973022, E-value is zero; Heckenhauer et al. 2017). The colony's foraging ground included the canopy of the main tree, its direct vicinity, and also covered canopies of a 25 m tall *Horsfieldia wallichii* (Hook.f. & Th.) Warb. (Myristicaceae) tree and a smaller tree of *Shorea maxwelliana* King (9 m). Colony fragments on all trees were connected by ant trails either through the canopy or on the forest floor in the litter layer. The total area occupied by the colony was estimated to be at least 2500 m^2.

The colonies are distributed three-dimensionally, occupying any suitable nesting ground within the colony boundaries. On the main tree, we found four nesting sites of the examined colony in dead branches at heights ranging between 35 and 55 m above ground and two nesting sites in the living stem 50–60 m above ground. No nests in living branches were observed. At least five nest entrances were also seen in the stem of *S. maxwelliana*. No signs of necrosis of the plant tissue were observed around stem entrances.

The translocation of a nest fragment in a fallen branch to the laboratory's terrace, 30 m away from its original location, resulted in the expansion of the colony's foraging ground to a neighbouring *Shorea* sp. tree where these ants were not previously present, while the connection to the colony on the original host tree was maintained.

If provided with an appropriate artificial nest (Fig. 9c–d), *C. explodens* sp. n. ants will inhabit it within several weeks up to several months and even use it to rear brood. One artificial nest, mounted on the main host tree, was colonized one week after it was installed. For the activity assessment, the easily accessible artificial nest #38 was observed. During behavioural monitoring, *C. explodens* sp. n. was observed to be mainly diurnal, foraging between 6:00 and 18:00 hrs, with peak activity around 9:00 and 16:00 hrs (Fig. 9a). The activity correlated positively with the temperature with lowest values at 24.2 °C and highest at 28.6 °C (Fig. 9b). The atmospheric pressure and clouds did not influence the activity of *C. explodens* sp. n. (Suppl. material 6 "activity"); humidity was constant over the period of observations ranging from 86 to 88%. A slight rain on a warm day did not reduce the activity of ants near the nest but no activity was observed during heavy rains. However, if a shelter was provided, *C. explodens* sp. n. remained active

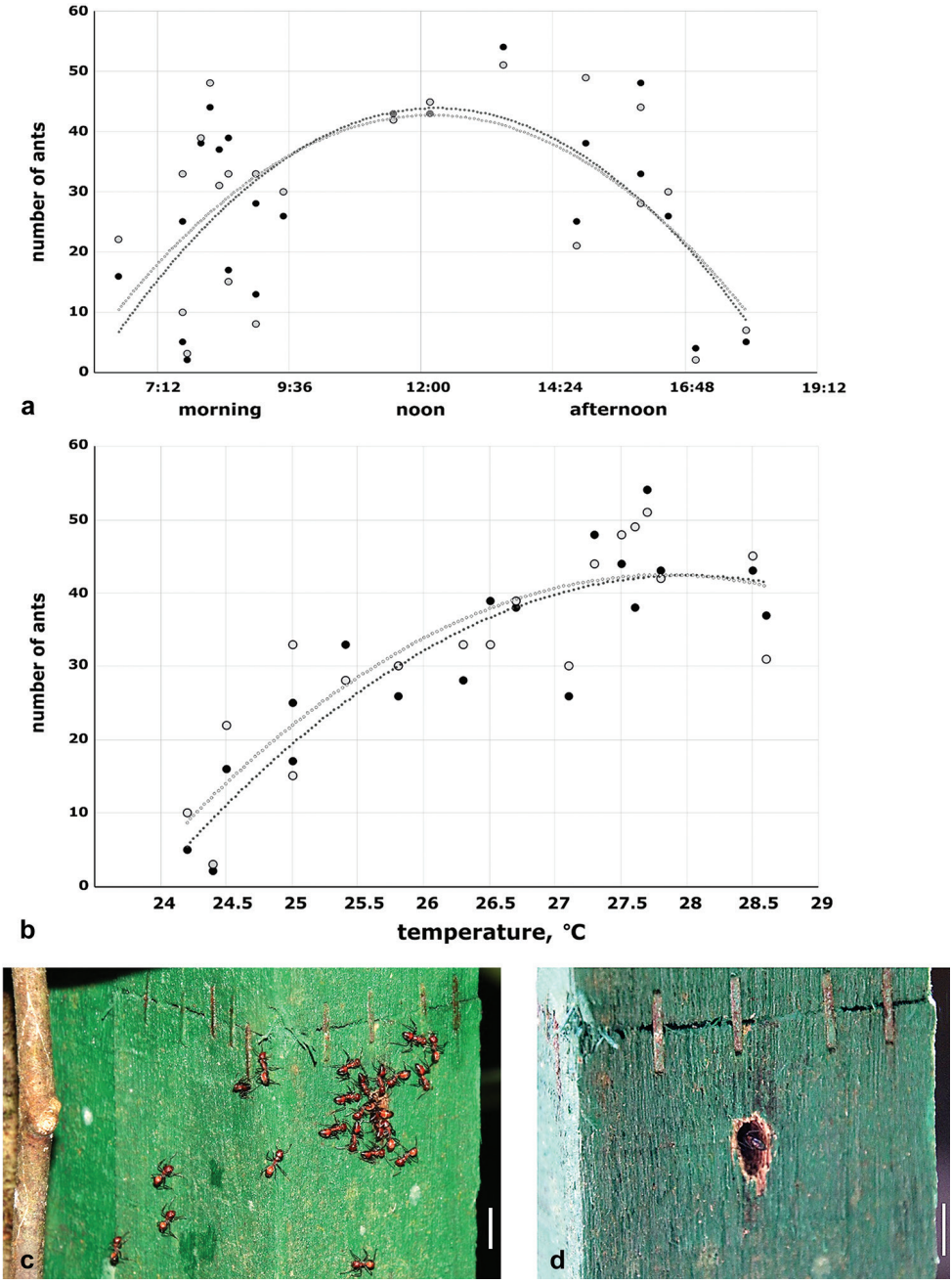

Figure 9. Activity of *C. explodens* sp. n. at the entrance to the artificial nest #38. **a** Scatter plot of the number of minor workers entering (black dots) and leaving (open dots) the nest depending on the time of day **b** Scatter plot of the number of minor worker ants entering (black dots) and leaving (open dots) the nest depending on the air temperature. Polynomial trend lines on a and b are shown for the numbers of entering (black) and exiting (grey) minor workers **c** and **d** show high and low worker activity near the entrance of nest #38, respectively; white bars denote 1 cm.

also during the rain and even after sunset. A drastic reduction in the number of minor workers at the nest entrance was observed on the days of nuptial flight, when several alate gynes and males left the nest in the early evening (Suppl. material 6 "activity"). Between one and six minor workers ("guards") were frequently positioned at the nest entrance, touching all incoming and outgoing workers with their antennae and seemingly monitoring the activity of foragers. In the early afternoon of the day with the highest activity, larvae were carried out of the nest. No carrying of larvae into the nest was ever observed.

Remarkably, during all observations the numbers of the minor workers leaving and entering the nest were almost equal. The fact that this proportion did not change over the day (Fig. 9a) suggests a tendency to maintain a constant number of individuals present inside the nest.

After dusk, other species of Camponotini such as *Polyrhachis* spp., *Camponotus* spp., and *Dinomyrmex gigas* (Latreille, 1802) were observed on the trees in the vicinity of the artificial nest.

Within the colonies, minor workers were by far the most abundant caste of *C. explodens* sp. n., whereas major workers (soldiers) were rare and almost never seen outside the nest. Alate gynes and males were observed leaving the nest during nuptial flight after dusk on two occasions during our field observations (Suppl. material 6 "activity"). Several more alate sexuals were found inside a detached nest fragment (Suppl. material 3). The same nest fragment also contained symbiotic ant crickets of the genus *Camponophilus* Ingrisch, 1995. DNA barcoding of the cricket based on COI sequence resulted in the highest value of 80 % similarity to COI sequences of insects from several groups including Mann's ant cricket *Myrmecophilus manni* (Schimmer, 1911) (EU938370, Fenn et al. 2008). Thus, the molecular identification of these crickets is currently not possible due to lack of reference sequences.

Observations have shown that minor workers of *C. explodens* sp. n. display a characteristic, possibly defensive pose with raised gaster (Suppl. material 1: S1a) (compare with Davidson et al. 2007) and are extremely prone to self-sacrifice when threatened. The mandibular gland content is released during autothysis by contracting the gaster until the integument ruptures, leading to the death of the ant (Suppl. material 4). The secretion is slightly viscous, sticky, and has a species-specific bright yellow colour and a distinctive spice-like odour (Hoenigsberger et al., in prep.).

Minor workers of *C. explodens* sp. n. spend significant time on leaves, which has previously been hypothesized to contribute to their nutrition (compare with Davidson et al. 2007, 2016). However, the exact purpose of their activity on leaves is yet to be understood. Observations suggest a patrolling or monitoring behaviour aiming at the removal of debris from the phyllosphere (mainly adaxial leaf surface but also abaxial leaf and petiole surfaces) and possible deterrence of intruding arthropods. Similar "cleaning" behaviour was observed *in vitro*, as well.

Another very specific behaviour was exhibited on the tree bark: Minor workers "graze" on the layers of epiphytes (mosses, lichens, algae, filamentous fungi, and yeasts) with their mandibles, often for up to 60 minutes. This behaviour differs from the cleaning behaviour on leaves and presumably contributes to the ants' nutrition.

Preliminary feeding experiments using cultures of filamentous fungi isolated from the phyllosphere of the host trees remained unsuccessful, no fungal feeding was observed. Only a suspension of yeast in water was accepted *in vitro* (M. Rahimi, pers. obs.). However, minor workers of *C. explodens* sp. n. have been observed to feed on small dead insects, fruit, and fish when offered on the foraging grounds (A. Kopchinskiy, A. Laciny & M. Hoenigsberger pers. obs.).

Commonly observed modes of behaviour of *C. explodens* sp. n. *in situ* and *in vitro* as well as a variety of nesting sites are documented in the Suppl. material 7 (Video S7).

The molecular analysis of the mandibular gland (MG) content of *C. explodens* sp. n. resulted in PCR amplification and sequencing of the 16S rRNA fragment of the bacteria *Blochmannia* sp. (Gammaproteobacteria), a genus of obligate symbiotic bacteria found in carpenter ants (Williams and Wernegreen 2015). We revealed four identical mOTUs originating from two different DNA extracts from samples composed of five pooled MG reservoirs of the minor workers each. The sequences of 728 nt were 99 % similar (11 SNP sites) to the "uncultured bacterium clone 193-11" KC136854 from *Camponotus* sp. voucher KC-A017-01 defined as *Blochmannia* sp. in Russell et al. (2012).

More detailed data on autothysis, composition of mandibular gland secretion, biodiversity of the COCY-associated microorganisms, and experimental assessment of nutrition will be presented in future publications.

Colobopsis badia **(Smith, 1857)**

Figs 8, 10

Formica badia: Smith 1857: 54.
Camponotus badius: Roger 1863: 3.
Colobopsis badia: Ward et al. 2016: 350. Bolton 2017.

Type material examined. 1 lectotype minor worker (Oxford University Museum of Natural History, present designation), Singapore, "Formica badia", "Syntype", CASENT 0901897, "Lectotypus Formica badia Smith, 1857 des. Laciny & Zettel, 2017", 2 paralectotype minor workers (Oxford University Museum of Natural History) mounted on the same card, Sarawak ("Sar 32"), "Formica badia", "Syntype", "Paralectotypes Formica badia Smith, 1857".

Additional material examined. 1 male (Natural History Museum Vienna), Thailand, Trang Province, Nayong District, Khao Chong Botanical Garden, at light of head quarter, 7°33'N, 99°46'E, 60 m a.s.l., 1–7.VI.2016, leg. H. Zettel (68); 10 minor workers (Natural History Museum Vienna), Thailand, Trang Province, Nayong District, Khao Chong Botanical Garden, trail to Ton Pliw Waterfall, N07°32'34", E99°47'33", 150 m a.s.l., 1–7.VI.2016, leg. H. Zettel (66-4).

Description notes on the type specimens. *Lectotype*: Minor worker glued to a square cardboard, in relatively good condition; right hind leg missing; tarsi of middle

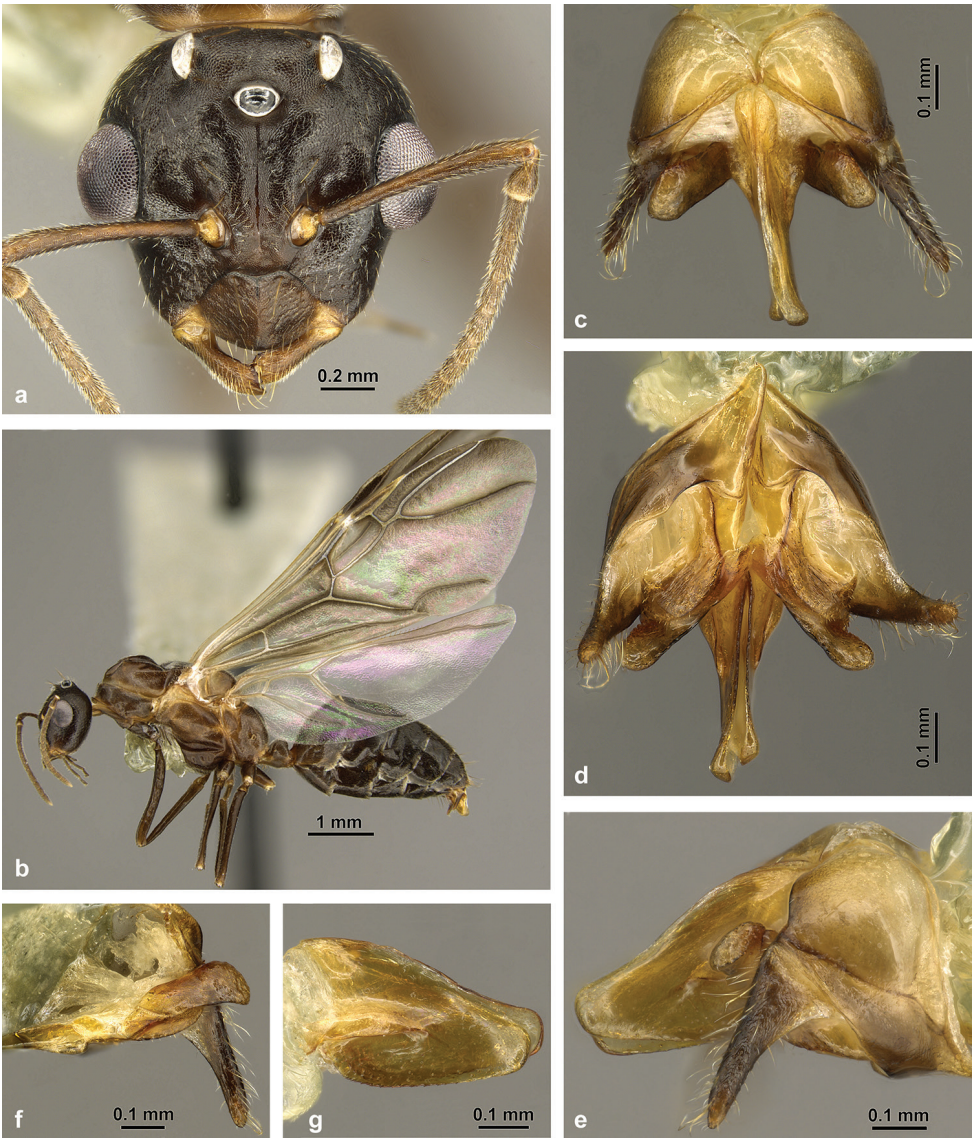

Figure 10. Habitus of *C. badia*, male; **a** frontal **b** lateral; genital capsule in **c** dorsal **d** ventral, and **e** lateral view **f** right volsella and gonostylus **g** left penis valve.

legs and left hind leg broken; erect setae on dorsum probably lost. Structures agree well with other species of the *C. saundersi* complex, a few characteristic features are given: Setae on scape more decumbent than in *C. explodens* sp. n. Dorsal outline of mesosoma almost straight, only with weak indentation at meso-metanotal suture. Propodeum forming a distinct obtuse angle in lateral view. Petiolar node relatively short, apex acute in lateral view, its crest slightly indented in middle. Tergites I–III with very fine, strongly transverse microsculpture (lateral parts not visible). Colour relative-

ly dark brown; appendages strongly infuscate; antennal segments III–XII, meso- and metafemora almost black.

Paralectotypes: Two minor workers glued to the same square cardboard, in relatively poor condition. Left specimen with damaged head and gaster, lacking right middle leg; major parts of body covered by dirt or glue; most erect setae probably lost. Right specimen with slightly damaged head, lacking gaster and right hind leg; some parts of body covered by dirt or glue; most erect setae probably lost. The two specimens are probably conspecific, but conspecificity with the lectotype is uncertain. The combination of morphological features is intermediate between *C. badia* and *C. explodens* sp. n.: setae on scape similar to *C. explodens* sp. n., more erect than in the lectotype; dorsal outline of mesosoma intermediate, more structured than in the lectotype, but propodeum with angle; shape of node intermediate, apex more acute than in *C. explodens* sp. n. Colour almost as dark as in the lectotype.

Measurements of lectotype minor worker: TL 6.13; HW 1.51; HL 1.63; HS 1.57; PS5 n.a.; PS6 n.a.; EL 0.40; SL 1.43; SW 0.15; ML 1.96; HaL 0.17; PH 0.54; PL 0.36; NH 0.32; FeL 2.22. Indices: CI 93; SI 95; SWI 10; EI 26; PI 150; FeI 147; PSI n.a.

Measurements of paralectotype minor workers* (n = 2): TL 6.13, n.a.; HW n.a., 1.36; HL n.a., 1.52; HS n.a., 1.44; PS5 n.a., 0.21; PS6 n.a., 0.25; EL 0.36, 0.37; SL 1.39, 1.40; SW 0.12, 0.13; ML 1.89; HaL 0.13, n.a.; PH n.a., 0.51; PL 0.37, 0.42; NH 0.27; FeL 2.15. Indices: CI n.a., 89; SI n.a., 103; SWI 8, 9; EI n.a., 27; PI n.a., 121; FeI n.a., 158; PSI n.a., 32. *One specimen with strongly damaged head, one with missing gaster.

Measurements of non-type minor workers (n = 10): TL 5.64–6.23; HW 1.46–1.59; HL 1.63–1.72; HS 1.54–1.65; PS5 0.24–0.25 (3); PS6 0.24 (3); EL 0.38–0.40; SL 1.37–1.43; SW 0.13–0.14; ML 1.96–2.22; HaL 0.13–0.19; PH 0.51–0.56 (7); PL 0.41–0.45; NH 0.31–0.37 (9); FeL 2.09–2.28. Indices: CI 88–92; SI 90–96; SWI 9–10; EI 25–27; PI 118–130 (7); FeI 141–148; PSI 30–31 (3).

Male. *Notes on collecting and identification*: A single male collected at light was identified as a specimen of the *C. cylindrica* group. DNA barcoding revealed specific identity with a nest series of *C. badia* from the same botanical garden. The morphological identification of this nest series (Col. 66-4) was carried out by direct comparison to the lectotype of *C. badia*.

Description (Fig. 10): Overall very similar to *C. explodens* sp. n. and differing by the following characters:

Measurements of male (n = 1): TL 8.28; HW 1.35; HL 1.26; HS 1.30; PS5 0.20; PS6 0.15; EL 0.48; SL 0.83; SW 0.10; ML 3.07; HaL n.a.; PH 0.47; PL 0.41; NH 0.31; FeL 1.96; OcW 0.19; OED 0.29; OcD 0.46; FWL 7.43; MSW 1.37; 2r 0.41; 4Rs+M 0.37. Indices: CI 107; SI 61; SWI 13; EI 36; PI 116; FeI 145; PSI 26; OI 61; WVI 91.

Structures: Size larger (TL ca. 8.3 mm). Integument rather shiny (Fig. 10a, b), especially on mesosoma. Clypeus with distinctly developed median carina, almost reaching anterior margin. Maxillary palpi (PSI 26) and antennal scapes (SI 61) relatively short. First funicular segment slightly more enlarged (30% wider than the following segment, Fig. 10a). Vein 4Rs+M of forewing long. Petiolar node slightly more widely rounded in lateral aspect.

Genital structures (Fig. 10c–g) very similar to *C. explodens* sp. n., with the following exceptions: Gonostylus very narrow, with weaker reticulation of lateral surface (Fig. 10e). Basivolsella with extremely short ventral setae (Fig. 10f). Digitus with rounded apex, without ventroapical corner (Fig. 10f). Valviceps with slightly coarser ventral serration (Fig. 10g).

Colour: Head chiefly dark brown, with lighter area comprising frons between antennal insertions and clypeus. Eyes grey. Ocelli clear, almost colourless. Posterior and anterior clypeal margins, as well as proximal fourth of clypeal carina black. Gaster dark brown. Mesosoma, petiole, mandibles, antennae, and legs lighter brown, appendages becoming yellowish towards apices. Antennal insertions, mandibular bases, margins of thoracic sclerites (especially below tegulae) creamy yellow. Gastral tergites medially with very narrow hyaline margins; sternites with relatively broad posterior margins. Wings hyaline, forewing with a slight brownish tinge and cells along veins, as well as pterostigma darker brownish, all veins pale brown. On hind wing all veins pale yellow.

Pilosity: Appressed and subdecumbent setae comparatively shorter and sparser, but difference less obvious on gaster. Standing setae on mesonotum and gaster shorter, on mesonotum less numerous.

Comparative notes: The male of *C. badia* can be distinguished from males of *C. explodens* sp. n. by larger body size, differing colour pattern, more shiny integument, well-developed clypeal carina, differing proportions of wing venation, and relatively shorter scapes (Fig. 8b). In the genitalia, the most striking differences are in the narrower gonostylus and the more rounded digitus apex (compare Figs 7e, 10f).

Discussion

Molecular results

In this study, three mitochondrial DNA loci and one nuclear DNA fragment were applied for the construction of a molecular phylogenetic tree (Fig. 1). The evolutionary analysis based on four loci showed that *C. explodens* sp. n. is clearly genetically distinct from morphologically similar species. A minor level of infraspecific polymorphism within the specimens from Brunei was observed for COI, COII, and in particular for cytB marker. While tree topologies based on single mitochondrial loci were concordant, the cad tree (nuclear locus) was not resolved for the entire COCY group. The comparison of COI sequences with several hundred COCY sequences available in our local database and 13 sequences deposited in NCBI (Nov. 2017) suggests that this marker can be used for the reliable molecular identification (DNA barcoding) of *C. explodens* sp. n., as COI sequences of the nearest COCY taxa share only 91% similarity (*C. badia*), and the similarity to the selected non-COCY species *C. aruensis* is 83%.

The DNA extraction from the gastral parts of the mandibular gland reservoirs of *C. explodens* sp. n. minor workers resulted in drastically low yields indicating no abundant microbial symbionts present there. The successful 16S rRNA PCR amplification

gave a sharp band that was sequenced with high reproducibility. The 16S rRNA fragment corresponding to the whole genome sequenced *Blochmannia* endosymbiont of North American *Colobopsis obliquus* strain 757 (NCBI GenBank accession number CP010049, Williams and Wernegreen 2015) was 92 % similar to mOTU revealed in this study resulting in 56 polymorphic sites. This confirms that *C. explodens* sp. n. also harbours these bacteria that usually colonize the midgut of Camponotini workers (Sauer et al. 2002) and are considered to be beneficial for N-nutrition of these ants; they may also contribute to the general health of the workers and gynes. Thus, the detection of cf. *Blochmannia* bacteria rather indicated the contamination of the MG sample by fragments of the digestive system. In this respect, it is interesting to note that no *Wolbachia* (Alphaproteobacteria) mOTUs were recovered, but neither digestive tract nor ovaries were specifically investigated.

Taxonomy

The treatment of *Colobopsis* as a genus separate from *Camponotus* is supported by molecular, morphological, and biological data (Blaimer et al. 2015, Ward et al. 2016). Naked pupae (Wheeler 1904; see Suppl. material 5) and presence of phragmotic soldiers and gynes are important features of *Colobopsis*, although unknown in many of the 94 valid species assigned to this genus by Ward et al. (2016). The morphological separation of minor workers of *Colobopsis* and *Camponotus* is chiefly based on head morphology, but complicated by extensive evolutionary changes within each group (Ward et al. 2016); however, the phylogeny of *Colobopsis* species has not been studied to date. The molecular data published by Blaimer et al. (2015), obtained from only four species, do not allow an interpretation of the relationships of intrageneric clades. Attempts to classify the species by morphological characters (Emery 1925, McArthur 2012), although useful for a rough sorting of species, probably hardly reflect their evolutionary relationships.

A first attempt of a comprehensive classification of the species of *Colobopsis* (as a subgenus of *Camponotus*) was done by Carlo Emery. In his outstanding treatment of Formicinae (Emery 1925) he treated 58 species and established six groups to hold 49 of them (nine remained unclassified). He defined the [*Camponotus* (*Colobopsis*)] *cylindricus* group by a gradual variation between worker and soldier, interspecific variation of head in soldiers and females (from concave and marginate to oblique and obtuse), and generally large size. Emery included eight species presently classified as *Colobopsis* (Ward et al. 2016), of which *Colobopsis calva* Emery, 1920, *C. quadriceps* (Smith, 1859), and *C. smithiana* (Wheeler, 1919) are not presently assigned to this group (see below), whereas *C. badia* and *C. corallina* were not included (listed under incertae sedis). Although Emery (1925) correctly recognized the size variation of workers, he failed to recognize the unique characteristics of the soldier caste (see Laciny et al. 2017).

More species of the COCY group were subsequently described by Stitz (1925), Menozzi (1926) and Karawajew (1929, 1935). A second attempt at classification was

made by McArthur (2012): His *Camponotus* (*Colobopsis*) *cylindricus* group consists of species with "neck attached to head well below vertex" and is broader than Emery's (1925) *cylindricus* group. It includes the following species (according to current classification) that do not fit the characteristics of the COCY group in the present sense: *Colobopsis anderseni* (McArthur & Shattuck, 2001), *C. brachycephala* Santschi, 1920, *C. cotesii* (Forel, 1893), *C. desecta* (Smith, 1860), *C. excavata* (Donisthorpe, 1948), *C. hosei* (Forel, 1911a), *C. mutilata* (Smith, 1859), and *C. quadriceps*, as well as *Camponotus dedalus* Forel, 1911b, and *Camponotus kutteri* Forel, 1915.

According to our morphological studies the COCY group can be defined as such: polymorphic *Colobopsis* with at least three distinct female castes: (i) winged, phragmotic gynes, (ii) phragmotic soldiers (doorkeepers), and (iii) minor workers with a considerable size variation; intermorphic workers may occur in addition (Laciny et al. 2017). Minor workers: Vertex highly raised above foramen. Eyes of minor worker small and flat, not breaking head sides in full-face view. Entire trunk with dense, reticulated microstructures; punctures of integument often reduced. Head with moderate, mesosoma with dense pubescence. Mesosoma (at least the pronotum, except in *C. clerodendri* Emery, 1887) dorsally with long undulated setae, never arranged in distinct rows. Gaster with appressed pubescence and two or three types of setae of different lengths (not arranged in rows, except at hind margin). Soldiers (not known of all taxa): differing from minor workers by enlarged heads and short appendages (antennae, palpi, legs); in most species with a clearly circumscribed head shield for phragmosis. Microsculpture and pilosity similar to minor worker.

Following this definition, the COCY group presently comprises 17 names in the rank of species, subspecies or variations, which are partly in synonymy to each other. The strong intraspecific variation of minor workers, the frequently lacking knowledge on soldiers (or gynes), and the historical descriptions of taxa from different morphs (either workers or gynes) make the species taxonomy a true challenge. A preliminary analysis of morphological and molecular data (unpublished) supports the division of the group into four species complexes (molecular data of one complex not available). We restrict the following analysis to the species of the *C. saundersi* complex, which includes *C. explodens* sp. n. and can be defined by the following combination of characters observable in minor workers and soldiers: head always red or brown (not black); mesosoma moderately elongated and dorsally with some long undulated setae, at least on pronotum; node of petiole without long setae; gastral tergites with dense (in most species strongly transverse) micro-reticulation and with small hair pits (without large grooves). Soldiers and gynes (not known of all species) have a strongly truncated head, in most species with a well-defined (crested) head shield. The following names are available in this group (listed chronologically): *Colobopsis badia* (Smith, 1857), *C. corallina* Roger, 1863, *C. saundersi* Emery, 1889, *C. badia* var. *krama* Forel, 1912, *C. badia saginata* Stitz, 1925, *C. solenobia* (Menozzi, 1926), and *Colobopsis trieterica* (Menozzi, 1926), comb. n.

Colobopsis corallina (=*C. solenobia* syn. n., =*C. trieterica* syn. n.) is an endemic species from the Philippines. Soldiers and gynes differ strongly from *C. explodens* sp. n.

and other taxa of the complex (as far as such morphs are known) by a very obtusely margined, not crested head shield. Minors have a bright orange colour on head, mesosoma, and petiole, often extending to gastral tergite I. Morphometrically, the examined minor workers of *C. corallina* (n = 31) mainly differ from those of *C. explodens* sp. n. by a greater average length of appendages (SI 92–109 vs. 87–104; FeI 136–159 vs. 123–151; PSI 30–39 vs. 28–35).

The greatest similarity is observed between *C. explodens* sp. n. and *C. saginata* (stat. n.), a taxon only known from a single alate gyne from Northern Borneo. The important structures of the head shield are almost identical. Although strongly different from *C. explodens* sp. n. by pale orange brown colour, this gyne differs only by subtle morphometric characters of which the long and distally wide scape seems to be the most reliable (SI 83 vs. 78–80). The length of vein 4Rs+M is considerably longer in *C. saginata* than in *C. explodens* sp. n. (WVI 65 vs. 26–58).

Colobopsis badia var. *krama*, described from a soldier from Java (Forel 1912) is a very poorly known taxon. We have not been able to study any further material from Java yet. The type (illustrated by AntWeb.org under CASENT0910610) differs from *C. explodens* sp. n. by a pale red head that strongly contrasts with the dark brown mesosoma, by a well-developed median carina of the head shield that reaches the foremargin of the clypeus, and by a stronger punctation of the preocellar area.

Colobopsis badia was described by Smith (1857) from Singapore and Sarawak (Borneo). However, the original description is too brief to draw any meaningful taxonomic conclusions. Viehmeyer (1916) describes workers of this species in more detail, also noting the secretion of a sticky liquid upon capture. He mentions a strong variability in colouration (from red to almost black with reddish head) and propodeal shape. This raises the question whether all examined specimens were truly members of the same species or perhaps belonged to one of the other, similar species of the *C. saundersi* complex. We examined the three syntype minor workers of *C. badia* in the Oxford University Museum of Natural History. To fix the identity of this taxon, we have chosen the syntype from Singapore (imaged by AntWeb.org under CASENT0901897) as the lectotype of *Formica badia*. The two syntypes from Sarawak are in a relatively poor condition, which does not allow a complete morphometric analysis, and therefore the conspecificity with the *C. badia* type remains doubtful. We were not successful in obtaining fresh material of *C. badia* from Singapore, but a nest sample (minors only) from southern Thailand (Trang Province) which agrees well with the lectotype in morphology, especially morphometry, was available for a molecular analysis. It shows that *C. badia* and *C. explodens* sp. n. are closely related, but distinct (Fig. 1). Although very similar to *C. explodens* sp. n. in overall habitus and colouration, the examined *C. badia* minor workers are on average somewhat larger with less size variation (HW 1.22–1.57 vs. 1.46–1.59) and possess longer appendages (e.g., FeI 123–151 vs. 141–168; see Fig. 8a).

We examined two syntype minor workers of *Colobopsis saundersi* from Myanmar ("Tenasserim, Thagata", one illustrated by AntWeb.org under CASENT0905463). Morphometric analysis revealed no differences between the types of *C. saundersi* and *C. badia*, suggesting that the two species should be synonymized. *Colobopsis saundersi*

was considered a junior synonym of *C. badia* by Carlo Emery himself (Emery 1896) but revived from synonymy by Bingham (1903) without providing a reason. The large geographic distance of the type locality of *C. saundersi* and some minor differences in morphology led us to the decision to refrain from a formal synonymization at this time. A comparative molecular analysis of specimens from the type localities (Myanmar, Singapore) would most likely be necessary to corroborate this synonymy.

Morphology of males

The morphology of males of the tribe Camponotini is insufficiently studied, so that a complete comparison at generic level is not possible. The modified (enlarged) first funicular segment is presumably characteristic for males of *Colobopsis*. This characteristic has been described in the type species, *Colobopsis truncata* (Spinola, 1808), by Kutter (1977) and has been equally observed in several species of the *C. cylindrica* group.

Males of the COCY group have previously been described for three species (see below). However, these descriptions largely lack the necessary details to meaningfully compare taxa. No previous accounts of genital morphology or illustrations of male specimens have been found in the literature.

Colobopsis badia: Viehmeyer (1916) gives a brief description of a male from Singapore. Colouration, size, proportions of head and ocelli, as well as the enlarged first funicular segment correspond well to the examined male from Thailand.

Colobopsis severini (Forel, 1909): The extremely brief description of a male from the island Labuan (near Borneo) is not sufficient to draw any meaningful taxonomic conclusions.

Colobopsis leonardi (Emery, 1889): Karawajew (1929) gives a rather detailed description of males collected within a nest-series on Sumatra. The correct species identification by Karawajew is uncertain; the series may belong to another species of the *C. leonardi* complex as well. The pattern of pilosity on the gaster, with standing setae only present on the posterior half, also corresponds to our observations in males of the *C. saundersi* complex.

According to our knowledge, males of the *C. cylindrica* group can be distinguished from other Southeast Asian *Colobopsis* species by the relatively rich subdecumbent pilosity and the dense microreticulation of gastral tergites.

Biology

The behavioural observations on *C. explodens* sp. n. conducted at KBFSC revealed multiple modes of behaviour that are either poorly studied or new to science.

The diurnal activity pattern, as well as the positive influence of high temperatures correspond to the results of previous studies in related taxa (see Hamdan et al. 2013). Similarly large colonies containing several thousand individuals and extend-

ing to multiple trees and / or artificial nesting structures have also been described for other members of the genus (Federle et al. 1998, Laciny et al. 2017). However, it is still unclear whether individual workers are linked to certain parts of the colony or whether all foragers can move freely through the entire territory of the colony. An interesting and hitherto undescribed phenomenon in this regard is the presence of one or multiple "guards" at the artificial nest's entrance: These minor workers were frequently observed to touch any incoming or leaving workers with their antennae. In some instances, returning foragers were delayed or altogether denied entrance by the guarding ants. One reason for this may be that some workers are linked to different parts of the colony. Alternatively, the observed guarding behaviour may be related to the limited capacity of the artificial nest, which is also suggested by the conspicuously balanced numbers of workers entering and leaving the nest during times of foraging activity. These behavioural patterns are hitherto undescribed and must be investigated in future studies.

A further noteworthy activity observed in foraging workers was so-called "grazing" behaviour, in which minor workers were frequently seen using their mandibles to pluck and chew at various mosses, lichens, and other epiphytes on the bark of trees or other surfaces. While this activity can last for up to one hour at a time, its exact purpose remains unclear. It is possible that minor workers cut and consume parts of the plants and microorganisms or merely ingest fluids. As previous analyses of nitrogen isotopes (Davidson et al. 2016) suggest a largely plant-based diet for COCY ants, it seems likely that "grazing" contributes to their nutrition. However, other previously hypothesized modes of nutrition, such as tending of scale insects (Davidson et al. 2016) were not observed, and recent investigations on *Colobopsis leonardi* (Emery, 1889) (Zettel et al., ms submitted to Asian Myrmecology) even suggest a higher prevalence of carnivory in COCY ants than previously suspected.

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Supplementary material 1

Figure S1. Living workers of *C. explodens* sp. n. on a detached branch containing a nest fragment

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: **a** Minor worker with characteristically raised gaster **b** Major worker with phragmotic head to close nest entrances. Photo: H. Wiesbauer

Link: https://doi.org/10.3897/zookeys.751.22661.suppl1

Supplementary material 2

Figure S2. Natural nest of *C. explodens* sp. n. in a dead branch in the high canopy of *S. johorensis* (the main host tree for the model colony)

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: **a** Camera setup in the canopy, white arrow marks the nest entrance **b, c** Entrance and minor workers.

Link: https://doi.org/10.3897/zookeys.751.22661.suppl2

Supplementary material 3

Figure S3. Interior of the natural nest of *C. explodens* sp. n. found in a dead tree branch of *S. johorensis* on the forest floor

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: **a** Longitudinal section of the nest **b** Nest cavity. Sawdust is an artefact due to cutting. **c** Enlarged view of the nest cavity with a chamber made of dark carton **d** Dealate gyne and eggs found inside the chamber shown on **c**. The nest contained at least three such chambers.

Link: https://doi.org/10.3897/zookeys.751.22661.suppl3

Supplementary material 4

Figure S4. Autothysis as defensive behaviour in an experimental setting

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: A worker of the predatory species *Oecophylla smaragdina* is attacked by three minor workers of *C. explodens* sp. n.; all four animals died after the encounter. Black arrows indicate the yellow secretion expelled by rupture of the mandibular gland reservoirs.

Link: https://doi.org/10.3897/zookeys.751.22661.suppl4

Supplementary material 5

Figure S5. Pupa of *C. explodens* sp. n. found inside the opened natural nest

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: Note the absence of a pupal cocoon which is diagnostic for *Colobopsis*.

Link: https://doi.org/10.3897/zookeys.751.22661.suppl5

Supplementary material 6

Table S6.

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: specimens data

Explanation note: "measurements": Complete dataset of measurements, indices, locality data and type status of all measured specimens of *C. explodens* sp. n.

"activity": Activity of minor workers of *C. explodens* sp. n. at the entrance of the artificial nest #38.

"accession numbers": Complete list of accession numbers at NCBI GenBank for all organisms treated in this study.

Link: https://doi.org/10.3897/zookeys.751.22661.suppl6

Supplementary material 7

Video S7. Video depicting habitat, nesting sites and behaviour of *C. explodens* sp. n.

Authors: Laciny A, Zettel H, Kopchinskiy A, Pretzer C, Pal A, Salim KA, Rahimi MJ, Hoenigsberger M, Lim L, Jaitrong W, Druzhinina IS

Data type: multimedia

Explanation note: Video depicting habitat, nesting sites and behaviour of *C. explodens* sp. n. accessible under http://explodingants.com/index.php/publications/colobopsis-explodens

Link: https://doi.org/10.3897/zookeys.751.22661.suppl7

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Madicolous Chironomidae from the Brazilian Atlantic Forest: a checklist with notes on altitudinal distributions (Diptera, Insecta)

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Abstract

Thin layers of water running over rocky surfaces are characteristic of madicolous habitats, which harbor a peculiar Chironomidae community. However, information on the identity, distribution, and ecology of madicolous chironomids in the Neotropical region are still sparse. The main purpose of this research is to reveal and contribute to the ecology of madicolous Chironomidae species, especially regarding their altitudinal distribution in the Atlantic Forest. Sampling was performed using our own designed emergence traps deployed from 0 to 2700 m a.s.l. in 70 sites in three mountains in southeastern Brazil. Sixty taxa of chironomids were collected and identified, of which only 22 are known to science. Most of the species showed a wider distribution than previously known, both in terms of geographic and altitudinal ranges, while others showed significant association with particular altitudinal bands (as evidenced by the indicator species analysis). Atlantic Forest mountainous regions are known to harbor one of the richest fauna in the world and have been suffering from several types of environmental impacts, including climate change, which will especially affect taxa living in specialized habitats. The narrow range of tolerance to environmental conditions verified for mountain species, and the fact that many of them are rare and endemic, make the conservation efforts in these areas indispensable.

Keywords

hygropetric habitats, mountains, non-biting midges, semi-aquatic habitats, tropical forest

Introduction

Madicolous habitats are characterized by a thin layer of water that frequently flows over rocky surfaces, and for this reason they are also known as hygropetric habitats. The first to use the term "hygropetrischen" was Thienemann in 1909, when studying the biology of trichopterans from Central Europe. Throughout the twentieth century, some catalogues of madicolous fauna were done in North America (Sinclair and Marshall 1987) and Europe (Bertrand 1948, Vaillant 1956). More recently, most of the progress done on the study of madicolous organisms came out of taxonomic works (Sinclair 1988, Cranston 1998, Roque and Trivinho-Strixino 2004, Short 2009, Short et al. 2013, Bilton 2015, Pinho and Andersen 2015, Trivinho-Strixino and Shimabukuro 2017, Shimabukuro et al. 2017a, b, Pinho and Shimabukuro 2018), emphasizing the potential of this habitat in harboring a rich and endemic overlooked fauna. In South America, madicolous habitats have recently provided remarkable discoveries on the occurrence of insects, from new records (Roque and Trivinho-Strixino 2004, Short et al. 2013, Pinho and Andersen 2015) to several new species (Pepinelli et al. 2009, Silva et al. 2012, Trivinho-Strixino et al. 2012, Miller and Montano 2014, Shimabukuro et al. 2017a, b, Pinho and Shimabukuro 2018).

In natural ecosystems, madicolous insects can live in a wide range of habitats, such as shoreline of streams or in isolated overflowing groundwater. Additionally, when robust water bodies like streams and lakes are scarce, for example on mountaintops, madicolous biotopes can be the only source of permanent water allowing aquatic and semi-aquatic insects to establish themselves and survive, contributing to the maintenance of biodiversity in natural systems. The true madicolous inhabitants (eumadicoles) present morphological and physiological adaptations favoring their survival in such a specific environmental condition, as seen by the presence of strong locomotor appendages to hang on the rocky substrate in larval stages (Trivinho-Strixino et al. 2012), presence of strong hooks on the pupal abdomen and the production of silk by the larvae (Boothroyd 2005) or living inside portable cases to avoid water carrying (Fittkau and Reiss 1998).

The Chironomidae family is one of the most diverse within Diptera. Species numbers reach an estimated 20,000 (Coffman 1995), though only 6,000 approximately have been described. This remarkable evolutionary success allowed them to occur in all zoogeographic regions, including Antarctica, tolerating even the harshest environmental conditions (Sugg et al. 1983, Linevich 1971, Watanabe et al. 2006, Andersen et al. 2016a). Although the immature stages of known species show high dependence on water (Ferrington 2008), some are semi-aquatic or terrestrial, and researchers have recorded some in artificial madicolous systems (Cranston 1984, Boothroyd 2005, Hamerlík et al. 2010).

A high diversity of chironomids is expected to occur in natural madicolous habitats from tropical regions, but this biotope has so far been neglected in freshwater researches, making it difficult to have an estimate on the diversity of insects living in such habitats. Furthermore, concerning the taxonomy of chironomids, most of the descriptions are based exclusively on adult males, making it difficult to obtain the information on the habitats, behavior, and other ecological information related to aquatic stages.

Despite significant progress on Chironomidae research in the last decade (Trivinho-Strixino 2011, Mendes et al. 2007a), most registered species are still concentrated in Nearctic and Palearctic regions, which emphasize the urgent need for studies in Tropical regions that present potentially higher diversity. In this research, the first checklist is provided of madicolous Chironomidae from the Atlantic Forest, which is one of the richest hotspots in the world, and still the most affected by habitat loss (Myers et al. 2000). In addition, notes on distribution in the altitudinal gradients and other ecological features are included.

Methods

Study area

The exceptional biodiversity verified in the Brazilian Atlantic Forest is mainly due to the environmental heterogeneity owed to its singular geographical characteristics. Specifically, the region is characterized by a high variation in latitudinal (originally from parallels 3° to 30°) and altitudinal ranges (0 to 2892 m a.s.l.). These generate a diverse forest composition and a wide variety of habitats (Ribeiro et al. 2009). Also, the high precipitation rates recorded annually (about 1400 mm) (Forti et al. 2005) that are allied to the abundant and easily found overflowing groundwater, provide a perfect condition for maintaining different kinds of water bodies. More specifically, madicolous habitats thrive even in the upmost sites.

This study was conducted inside three conservation units from the Atlantic Forest in southeastern Brazil: Serra do Mar State Park (PESM), Serra dos Órgãos National Park (PARNASO), and Serra da Mantiqueira Environmental Protection Area (APASM) (mean distance between those areas is 185 km) (Figure 1). These specific locations were chosen because they present the utmost variation in topographic profiles in the Atlantic Forest, which allowed us to properly explore madicolous Chironomidae communities in a wide range of altitudes (from 0 to 2700 m a.s.l.).

PESM is the widest continuous protected area inside the Atlantic Forest (332,000 ha). It encompasses the whole coast of São Paulo State, including stretches at the sea level and some peaks that elevate the altitudinal range up to 1270 m a.s.l. The vegetation present in the region includes: mangroves, "restinga" (costal dunes), costal vegetation, ombrophilous dense forest, and "campos de altitude" (high altitude-vegetation composed predominantly of grass, shrub, and herbaceous vegetation). In this locality, sites between 0–1100 m a.s.l. were explored (Figure 1C).

PARNASO is in the mountainous region of Rio de Janeiro State, where it occupies 20,024 ha. The region's relief is marked with slopes, which generates a high altitudinal gradient. The upmost site in the park reaches 2263 m a.s.l., and the vegetation changes alongside variations in elevation: submontane forest, montane forest, misty forest, and campos de altitude. In this park, intermediate altitudes were explored, between 1200–2100 m a.s.l. (Figure 1A).

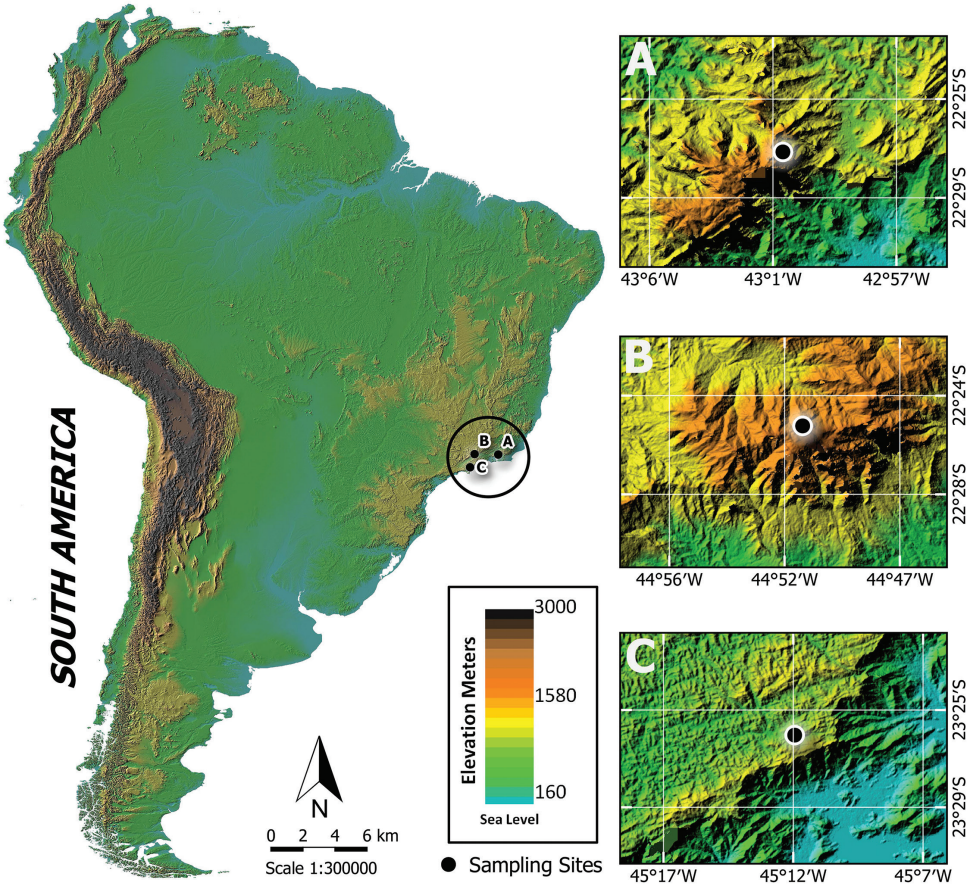

Figure 1. Localization of the study area comprehending the complete altitudinal range in the Atlantic Forest (Brazil). **A** Maximum altitudinal range in PARNASO **B** Maximum altitudinal range in APASM **C** Maximum altitudinal range in PESM.

APASM includes three states from the Southeast: São Paulo, Rio de Janeiro, and Minas Gerais, comprising an area of 421,804 ha. It harbors two of the five highest mountains in Brazil, including the culminant site at 2798 m a.s.l. (Pedra da Mina Mountain). The vegetation in the region creates a mosaic of phytophysiognomies, composed by upper-montane forests, araucaria forests and campos de altitude. In this locality, sites from 1700 to 2700 m a.s.l. were sampled (Figure 1B).

Sampling

Madicolous habitats were sampled every 200–300 m along the gradient. At least three replicates were obtained in each altitudinal band.

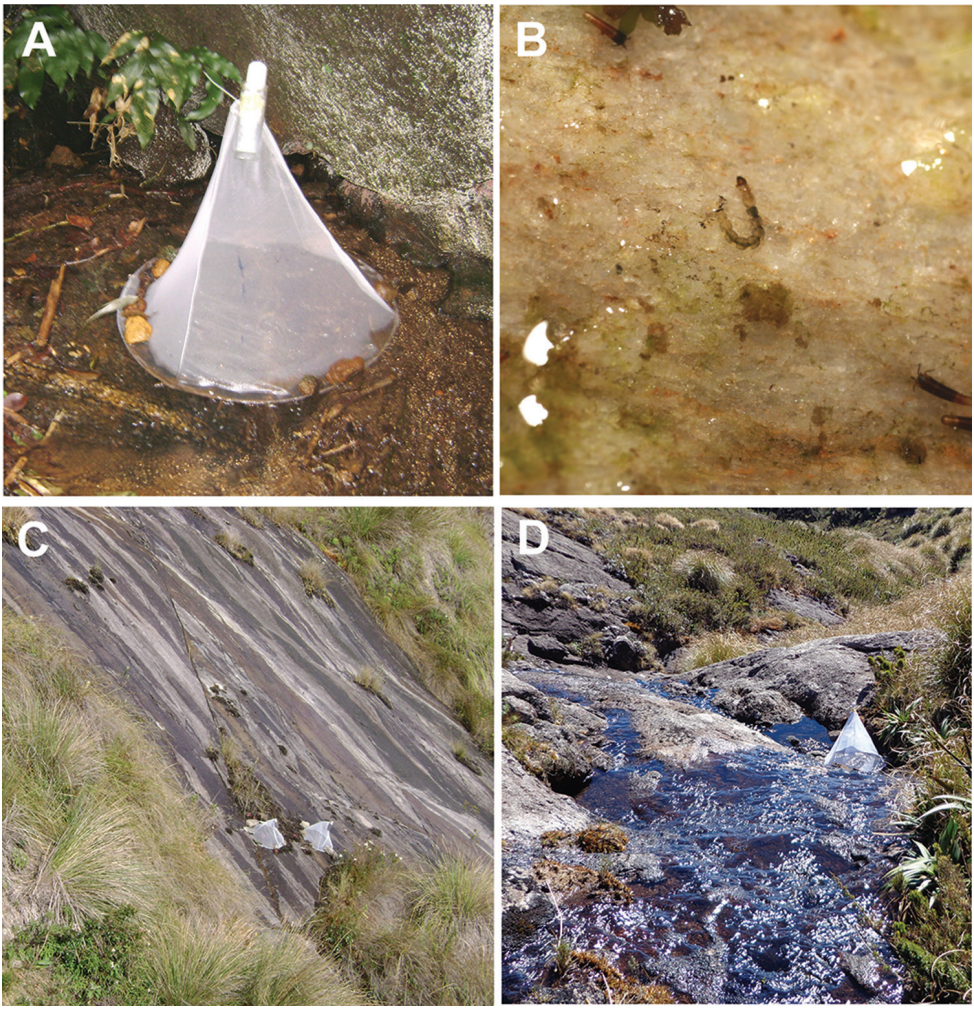

Figure 2. Field images. **A** The emergence trap installed above a madicolous system **B** Madicolous larva in natural habitat (*Podonomus* sp.) **C** Example of madicolous system characterized by seepage on rocky substrate **D** Example of madicolous system created at the stream bank.

The adult sampling was performed with emergence traps (Shimabukuro et al. 2015), that was left in the field for 7 days (Figure 2A). The advantage of using this type of trap is that it guarantees that the emergent adults really belong to the interested habitat, once the immature have completed their development at that specific site. In addition, the isolation provided by this trap prevents invasion by foreign specimens. From the substrate below the traps, larvae, pupae, and exuviae (Figure 2B) were also collected with a hand net. Organisms were preserved in absolute ethanol and slide mounted with Euparal before analyzing them in optic microscopy. Only male adults were identified to species level. When the specimens did not match any species'description, probably representing new species, they were designated as morphotypes.

Data analysis

For each species found, a set of data from the literature was added, including previous information on their geographical distribution, altitudinal records, habitats and development stages and sexes so far known. After the literature data, the "Remarks" include the data obtained by us: 1. type of madicolous habitat where the species was found, such as stream edge (Figure 2C) or isolated rocky outflow (Figure 2D); 2. latitudinal and altitudinal records; 3. environmental data (water temperature, pH, dissolved oxygen, and canopy cover); 4. locality where the species was recorded (PESM, PARNASO, and APASM); and 5. altitudinal specificity depicted by the species. In addition, some information about the morphospecies (probably new species) found was included, hereafter denominated as "unknown species".

Information on the development stages, habitat, and locality of each species and morphospecies recorded in our study is summarized in Table 1 (supplementary material). When the development stage consists only of adults (A), it does not mean that immature specimens were not collected; rather, that the association between adult and immature stages were not yet established. In addition, some taxa were recorded only from its immature instars (I).

In order to test the degree of each species'affinity within the altitude in which they occurred, an indicator species analysis (Dufrêne and Legendre 1997) was applied and the "indicator value" (IndVal) and "p-value" obtained for each species; the IndVal varies from 0 to 1.0, and higher values indicate a more expressive altitudinal representation. Significant indicator species presented $p<0.05$, although $p = 0.06$ species were considered as high altitudinal specificity, $0.06<p<0.2$ species with median altitudinal specificity, and $p>0.2$ species with low altitudinal specificity. This analysis was performed in R Cran Project 3.0.3 (R Core Development) software using the "labdsv" package (Roberts 2007).

Results

A total of 60 species, including 22 known species and 38 morphospecies, was recorded, as follows:

SUBFAMILY CHIRONOMINAE
TRIBE CHIRONOMINI

***Claudiotendipes* Andersen, Mendes & Pinho, 2017**

Two valid species; Neotropical; running water.

***C. froehlichi* Andersen, Mendes & Pinho, 2017**

Distribution. BRAZIL – Camacan, Bahia State (15°23'28"S, 39°33'56"W; 15°23'02"S, 39°34'10"W; 15°23'10"S, 39°34'03"W) Rio de Janeiro State (11°53'40"S, 45°36'06"W),

Salesópolis, São Paulo State; Campos do Jordão, São Paulo State (22°41'40"S, 45°27'36"W); Grão-Pará, Santa Catarina State (28°11'28"S, 49°23'30"W); Urubici, Santa Catarina State (28°01'41"S, 49°22'36"W).

Elevation. 508–1014 m a.s.l.

Habitats. Low-order streams.

Known stages. L, P, M.

References. Andersen et al. (2017).

Remarks. *C. froehlichi* was found on marginal rocks of small streams, at 206 m a.s.l. (23°27'52"S, 45°11'55"W) and 1444 m a.s.l. (22°26'51"S, 43°00'48"W), extending the altitudinal range of the species. Environmental characterization: Water temperature 10–17 °C; dissolved oxygen 9.0–9.2 mg.l^{-1}; pH 5.0–5.5; dense canopy cover. The species was found in PESM (São Paulo State) and PARNASO (Rio de Janeiro State). Low altitudinal specificity (IndVal: 0.17; p = 0.84).

Lauterborniella Thienemann & Bause, 1913

One valid species; Holartic, Nearctic, and Neotropical; standing water.

Unknown species. *Lauterborniella* sp. 1. Locality: PESM. Altitudinal record: 1080 m a.s.l. Significant altitudinal specificity (IndVal: 0.5; **p = 0.02**).

Nilothauma Kieffer, 1921

52 valid species; worldwide; running and stagnant water.

Unknown species. *Nilothauma* sp. 1 Locality: APASM. Altitudinal record: 1570 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p = 1.00).

Oukuriella Epler, 1986

24 valid species; Neotropical, associated with freshwater sponges and submerged wood.

O. sublettei Messias & Oliveira, 1998

Distribution. BRAZIL – Paru do Oeste River, missão Cururu, Amazonas State; Parque Estadual de Campos do Jordão, São Paulo State (22°41'40"S, 45°27'36"W).

Elevation. 20–1600 m a.s.l.

Habitats. Larvae found in submerged wood in a rocky first-order stream.

Known stages. L, P, M.

References. Messias and Oliveira (1998); Fusari et al. (2013); Bellodi et al. (2016).

Remarks. *O. sublettei* was found on marginal rocks of small streams, at 745 m a.s.l. Environmental characterization: Water temperature 17 °C; dissolved oxygen 8.2 mg.l^{-1}; pH 5.5; fast flowing; dense vegetal canopy (more than 80% covered). The species was found in PESM (São Paulo State). Median altitudinal specificity (IndVal: 0.33; p = 0.14).

Polypedilum Kieffer, 1912

More than 440 valid species; worldwide; standing and running water.

P. solimoes Bidawid-Kafka, 1996

Distribution. BRAZIL – Tarumã River, Amazonas State; Florianópolis, Santa Catarina State (27°28'05"S, 48°22'58"W), UCAD, Santa Catarina State (27°31'51"S, 48°30'44"W), Santinho Beach, Santa Catarina State (27°27'S, 48°23'W).

Elevation. 20–80 m a.s.l.

Habitats. Adults collected close to a large Amazonian river. Larva found in leaf packs and detritus associated with the following bromeliad species: *Aechmea lindeni* (E. Morren) Baker, *Canistrum lindenii* (Regel) Mez, *Neoregelia laevis* (Mez) L.B. Smith, *Nidularium innocentii* Lem., *Vriesea philippocoburgii* Wawra, and *V. vagans* (L.B. Smith) L.B. Smith.

Known stages. L, P, F, M.

References. Bidawid-Kafka (1996); Pinho et al. (2013).

Remarks. *P. solimoes* was found on marginal rocks of small stream, at 1570 m a.s.l., extending the elevation records of this species. Environmental characterization: Water temperature 16.3 °C; dissolved oxygen 8.4 mg.l^{-1}; pH 6.4; slow flowing; vegetal canopy absent. The species was found in APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.14; p = 1.0).

Unknown species. *Polypedilum* (s. str.) sp. 1. Locality: APASM. Altitudinal record: 1570 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p = 1.00); *Polypedilum* (s. str.) sp. 2 Locality: PESM. Altitudinal record: 1080 m a.s.l. High altitudinal specificity (IndVal: 0.33; p = 0.06); *Polypedilum* (s. str.) sp. 3. Locality: PARNASO. Altitudinal record: 2125 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p = 0.84); *Polypedilum* (*Pentapedilum*) sp. 1; Locality: PESM. Altitudinal record: 200 m a.s.l. Median altitudinal specificity (IndVal: 0.33; p = 0.15). *Polypedilum* (*Pentapedilum*) sp. 2. Locality: PARNASO. Altitudinal record: 2125 m a.s.l. Low altitudinal specificity (IndVal: 0.17; 6 p = 0.85). *Polypedilum* (*Tripodura*) sp. 1. Locality: PESM. Altitudinal record: 25 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p = 0.33).

Stenochironomus Kieffer, 1919

101 valid species; worldwide; miners of living or dead vegetal tissue.

Unknown species. *Stenochironomus* sp. 1. Locality: PESM. Altitudinal record: 1075 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p = 0.84); *Stenochironomus* sp. 2. Locality: PESM. Altitudinal record: 70 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p = 0.32).

TRIBE PSEUDOCHIRONOMINI

Pseudochironomus Thienemann, 1934

13 valid species; Nearctic, Palearctic, Neotropical; running and standing water.

P. ruah Shimabukuro & Trivinho-Strixino, 2017

Remarks. *P. ruah* was recorded for the first time on marginal rocks of a small stream, at 2575 m a.s.l. (22°25'41"S, 44°50'38"W), in APASM (Minas Gerais State). Larva, pupa, female and male have been described by Shimabukuro et al. (2017a). Environmental characterization: Water temperature 22 °C; dissolved oxygen 7.4 mg.l^{-1}; pH 5.5; fast flowing; vegetal canopy absent. High altitudinal specificity (IndVal: 0.67; **p = 0.003**).

TRIBE TANYTARSINI

Paratanytarsus Thienemann & Bause, 1913

45 valid species; worldwide; running and stagnant water

P. silentii Trivinho-Strixino, 2010

Distribution. BRAZIL – Parque Estadual do Jaraguá, São Paulo State (23°24'S, 45°44'W).

Elevation. 800 m a.s.l.

Habitats. Stream.

Known stages. M.

References. Trivinho-Strixino (2010).

Remarks. *P. silentii* was found on marginal rocks of small streams, from 200–1445 m a.s.l., extending the altitudinal range of the species. Environmental characterization: Water temperature varied from 10–21 °C; dissolved oxygen 7.9–9.9 mg.l^{-1}; pH 5–5.5; slow to fast flowing; occurring in scarce vegetal canopy to dense coverage (30–70% covered). The species was found in PARNASO (Rio de Janeiro State) and PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.24; p = 0.25).

Tanytarsus van der Wulp, 1874

More than 300 valid species; worldwide; aquatic and terrestrial

T. alaidae Trivinho-Strixino & Shimabukuro, 2017

Remarks. *T. alaidae* was recorded for the first time on marginal rocks of a small stream, at 2575 m a.s.l. (22°25'41"S, 44°50'38"W), in APASM (Minas Gerais State). At present, only adult males have been described (Trivinho-Strixino and Shimabukuro 2017). Environmental characterization: Water temperature 22 °C; dissolved oxygen 7.4 mg.l^{-1}; pH 5.5; fast flowing; vegetal canopy absent. Low altitudinal specificity (IndVal: 0.17; p = 0.82).

T. alienus Trivinho-Strixino & Shimabukuro, 2017

Remarks. *T. alienus* was recorded for the first time on marginal rocks of a small stream, at 2575 m a.s.l. (22°25'41"S, 44°50'38"W), in APASM (Minas Gerais State). At present, only adult males have been described (Trivinho-Strixino and Shimabukuro 2017). Environmental characterization: Water temperature 22 °C; dissolved oxygen 7.4 mg.l^{-1}; pH 5.5; fast flowing; vegetal canopy absent. Low altitudinal specificity (IndVal: 0.17; p = 0.85).

T. angelae Trivinho-Strixino & Shimabukuro, 2017

Remarks. *T. angelae* male adults were recorded in a temporary pool at 2218 m a.s.l. (22°24'697"S, 44°50'93"W) in APASM (Minas Gerais State), in a rocky fountain at 2126 m a.s.l. (22°27'60.5'S, 43°01'68.9"W) in PARNASO (Rio de Janeiro State), and in the same locality at 1444 (rocky substrate marginal to a stream – 22°26'84.8'S, 43°00'79.8"W). At present, only adult males have been described (Trivinho-Strixino and Shimabukuro 2017). Environmental characterization: Water temperature 10–12 °C; dissolved oxygen 8.8–9.0 mg.l^{-1}; pH 5.0; partial to absent canopy cover. Low altitudinal specificity (IndVal: 0.17; p = 0.85).

T. digitatus Sanseverino & Fittkau, 2006

Distribution. BRAZIL – Paquequer River, Teresópolis, Rio de Janeiro State.
Elevation. 1100 m a.s.l.
Habitats. Adults collected in light trap close to a first-order stream.
Known stages. M.
References. Sanseverino and Fittkau (2006).

Remarks. *T. digitatus* was found on marginal rocks of small streams, at 25 m (23°28'20.72"S, 45°11'38.43"W) and 1445 m a.s.l (22°26'84.8"S, 43°00'79.8"W), extending the altitudinal range of the species. Environmental characterization: Water temperature 10–19.5 °C; dissolved oxygen 8.1–9.0 mg.l^{-1}; pH 5.0; slow flowing; dense canopy cover. The species was found in PESM (São Paulo State) and PARNASO (Rio de Janeiro State). Low altitudinal specificity (IndVal: 0.5; **p = 0.02).**

T. giovannii Sanseverino & Trivinho-Strixino, 2010

Distribution. BRAZIL – São Carlos, São Paulo State (21°59'10"S, 47°52'32"W; 21°58'16"S, 47°53'14"W; 21°59'21.4"S, 47°51'14.2"W); Corumbá, Mato grosso do Sul State (19°34'30.06"S, 57°00'52.4"W).

Elevation. 90–850 m a.s.l.

Habitats. Low-order streams and eutrophic lake.

Known stages. L, P, M.

References. Sanseverino and Trivinho-Strixino (2010); Trivinho-Strixino et al. (2015).

Remarks. *T. giovannii* was found on marginal rocks of small streams, at 2575 m a.s.l., extending the altitudinal range of the species. Environmental characterization: Water temperature 22 °C; dissolved oxygen 7.4 mg.l^{-1}; pH 5.5; fast flowing; vegetal canopy absent. The species was found in APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.17; p = 0.84).

SUBFAMILY ORTHOCLADIINAE

Corynoneura Winertz, 1846

Approximately 96 valid species; worldwide; running and stagnant waters.

C. unicapsulata Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL – Parque Estadual do Jaraguá, São Paulo State (23°27'S, 46° 45'W) Serra do Japi, Jundiaí, São Paulo State (23°14'30"S, 46°57'16"W); Paraisópolis, Minas Gerais State (22°39'54.81"S, 45°55'38.29"W); São Luís do Purunã, Paraná State (25°27.180'S, 49°43.435'W); BRAZIL, Serra da Bodoquena, Mato Grosso do Sul State (20°41'49"S, 56°52'54"W); Alto Paraíso de Goiás, Goiás State (14°9'34.92"S, 47°35'37.32W); COSTA RICA, Caccao, Guanacaste.

Elevation. 750–1370 m a.s.l.

Habitats. Litter in stones marginal to small streams.

Known stages. L, P, F, M.

References. Wiedenbrug and Trivinho-Strixino (2011); Wiedenbrug et al. (2012).

Remarks. *C. unicapsulata* was found on marginal rocks of small streams, at 2575 m a.s.l., extending its altitudinal occurrence. Environmental characterization: Water temperature 22 °C; dissolved oxygen 7.4 mg.l^{-1}; pH 5.5; slow flow to stagnant; vegetal canopy absent (shrub-herbaceous vegetation. The species was found in APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.17; p = 0.85).

C. hermanni Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL – Ubatuba, São Paulo State (23°30.468'S, 45°11.923'W and 23°30.789'S, 45°14.442'W)

Elevation. 0–60 m a.s.l.

Habitats. Litter standing near the water surface from small streams.

Known stages. L, P, F, M.

References. Wiedenbrug and Trivinho-Strixino (2011); Wiedenbrug et al. (2012).

Remarks. *C. hermanni* was found on marginal rocks of small streams and rocky seepages (large exposed rock in the middle of the forest), at 1570–1580 m a.s.l., extending the altitudinal occurrence of this species. Environmental characterization: Water temperature 15–16.3 °C; dissolved oxygen 8.4–10.2 mg.l^{-1}; pH 5.0–6.4; slow to fast flowing; vegetal canopy completely absent. The species was found in APASM (Minas Gerais State) and PARNASO (Rio de Janeiro State), extending slightly the geographical distribution of this species. Low altitudinal specificity (IndVal: 0.29; p = 0.23).

C. septadentata Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL – Parque Estadual do Jaraguá, São Paulo State (23°27'S, 46°45'W), Nova Friburgo, Rio de janeiro State, Rio Boa Vista; Bocaina de Minas, Minas Gerais State (22°19'S, 44°34'W); Serra do Japi, Jundiaí, São Paulo State (23°14'30"S, 46°57'16"W); Serra da Bodoquena, Mato Grosso do Sul State (20°41'49"S, 56°52'54"W).

Elevation. 700–1200 m a.s.l.

Habitats. Litter near the water surface of a small shallow stream.

Known stages. L, P, F, M.

References. Wiedenbrug and Trivinho-Strixino (2011); Wiedenbrug et al. (2012).

Remarks. *C. septadentata* was found on marginal rocks of small streams and on rocky seepages (large exposed rock in the middle of the forest), from 1045–1580 m a.s.l., extending slightly the altitudinal range of this species. Environmental characterization: Water temperature 15–17 °C; dissolved oxygen 8.6–10.2 mg.l^{-1}; pH 5; moderate to fast flowing; vegetal canopy reduced or absent (less than 30% covered). The species was found in PARNASO (Rio de Janeiro State) and PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.09; p = 0.88).

C. sertaodaquina Wiedenbrug & Trivinho-Strixino, 2011

Distribution. BRAZIL – PESN, Ubatuba, São Paulo State (23°30.789'S, 45°14.442'W; 23°31.068'S, 45°14.845'W; 23°31.231'S, 45°14.625'W); Alto Paraíso de Goiás, Goiás State (14°9'34.92"S, 47°35'37.32"W); Serra da Bodoquena, Mato Grosso do Sul State (20°41'49"S, 56°52'54"W), São Simão, São Paulo State; São Luís do Purunha, Paraná State (25°27.180'S, 49°43.435'W).

Elevation. 20–1020 m a.s.l.

Habitats. Surface of stones of shallow fast flowing waters and also in litter near the water surface of streams.

Known stages. L, P, F, M.

References. Wiedenbrug and Trivinho-Strixino (2011); Wiedenbrug et al. (2012).

Remarks. *C. sertaodaquina* was found on marginal rocks of small streams, at 70 m a.s.l. Environmental characterization: Water temperature 17 °C; dissolved oxygen 9.2 $mg.l^{-1}$; pH 5.5; wet substrate, without any flow; reduced vegetal canopy (about 20% covered). The species was found in PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.25; p = 0.32).

Onconeura Andersen & Seather, 2005

Seven valid species; Neotropical and Nearctic; running and stagnant water.

O. japi Wiedenbrug, Mendes, Pepinelli & Trivinho-Strixino, 2009

Distribution. BRAZIL, Serra do Japi, Jundiaí, São Paulo State (23°14'38"S, 46°57'02"W); PESM, Ubatuba, São Paulo State (23°30.46'S, 45°11.923'W and 23°30.789'S, 45°14.442'W).

Elevation. 1058 m a.s.l.

Habitats. Litter below a waterfall of a first-order stream.

Known stages. L, P, F, M.

References. Wiedenbrug et al. (2009).

Remarks. *O. japi* was found on marginal rocks of small streams, at 1570 m a.s.l., extending the altitudinal record of the species. Environmental characterization: Water temperature 16.3 °C; dissolved oxygen 8.4 $mg.l^{-1}$; pH 6.4; slow flowing; vegetal canopy completely absent. The species was found in APASM (Minas Gerais State), extending the geographical distribution of the species. Low altitudinal specificity (IndVal: 0.14; p = 1.0).

O. oncovolsella Wiedenbrug, Mendes, Pepinelli & Trivinho-Strixino, 2009

Distribution. BRAZIL – PESM, Ubatuba, São Paulo State (23°31.068'S, 45°14.845'W; 23°31.087'S, 45°14.621'W; 23°30.789'S, 45°14.442'W); São Francisco de Paula, Rio Grande do Sul State.

Elevation. 23–61 m a.s.l.

Habitats. Surface of stones in fast flowing waters.

Known stages. L, P, F, M.

References. Wiedenbrug et al. (2009).

Remarks. *O. oncovolsella* was found on marginal rocks of small streams, at 1445 m a.s.l., extending the altitudinal record of the species. Environmental characterization: Water temperature 10 °C; dissolved oxygen 9 mg.l^{-1}; pH 5; fast flowing; partial vegetal canopy (50% covered). The species was found in PARNASO (Rio de Janeiro State). Low altitudinal specificity (IndVal: 0.25; p = 0.31).

Unknown species. *Onconeura* sp. 1. Locality: PARNASO. Altitudinal record: 1445 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p = 0.30); *Onconeura* sp. 2 Locality: PARNASO. Altitudinal record: 1445 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p = 0.32); *Onconeura* sp. 3 Locality: PESM. Altitudinal record: 1085 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p = 0.83).

Thienemanniella Kieffer, 1911

53 valid species; worldwide; running and stagnant water.

Unknown species. *Thienemanniella* sp.1. Locality: APASM. Altitudinal record: 1570 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p = 1.00).

Bryophaenocladius Thienemann, 1934

115 valid species; worldwide; terrestrial and semi-terrestrial, few aquatic.

B. carus (Roback, 1962)

Distribution. BRAZIL – Parque Estadual Intervales, Iporanga, São Paulo State (24°30'S, 48°37'W); PANAMA, Canal Zone, Curundu, Holbrook Air Force Base.

Elevation. 20–100 m a.s.l.

Habitats. Mosses on tree barks.

Known stages. L, P, F, M.

References. Roback (1962); Sæther (1976); Spies and Reiss (1996); Sæther (1981); Wang et al. (2006); Donato (2011).

Remarks. *B. carus* was found on marginal rocks of small streams, at 1075 m a.s.l., extending the altitudinal range of the species. Environmental characterization: Water temperature 15.8 °C; dissolved oxygen 8.1 mg.l^{-1}; pH 5; moderate to fast flowing; dense vegetal canopy (more than 70% covered). The species was found in PESM (São Paulo State). Low altitudinal specificity (IndVal: 0.17; p = 0.85).

Unknown species. *Bryophaenocladius* sp. 1. Locality: PESM. Altitudinal record: 1075 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p = 0.87).

Caaporangonbera Andersen, Pinho & Mendes, 2015

Four valid species; Brazil, Atlantic Forest; unknown habitat, but possibly terrestrial or semi-terrestrial.

C. intervales Andersen, Pinho & Mendes, 2015

Distribution. BRAZIL – Parque Estadual Intervales, Ribeirão Grande, São Paulo State (24°15'S, 48°10'W).

Elevation. 500 m a.s.l.

Habitats. Unknown, but possibly terrestrial or semi-terrestrial.

Known stages. M.

References. Andersen et al. (2015b).

Remarks. *C. intervales* was found on marginal rocks of small streams, at 740 m a.s.l., extending slightly the altitudinal range of this species. Environmental characterization: Water temperature 15.7 °C; dissolved oxygen 9.4 $mg.l^{-1}$; pH 5.5; very slow flowing; partial vegetal canopy (50% covered). The species was found in PESM (São Paulo State). Median altitudinal specificity (IndVal: 0.33; p = 0.14).

Cricotopus van der Wulp, 1874

218 valid species; worldwide; running and standing water.

Unknown species. *Cricotopus* sp. 1. Locality: APASM. Altitudinal record: 2575 m a.s.l.. Low altitudinal specificity (IndVal: 0.17; p = 0.85); *Cricotopus* sp. 2 Locality: APASM and PARNASO. Altitudinal range: 1445–1570 m a.s.l. Low altitudinal specificity (IndVal: 0.15; p = 0.47); *Cricotopus* sp. 3 Locality: PESM. Altitudinal range: 70–1075 m a.s.l. Low altitudinal specificity (IndVal: 0.13; p = 0.58); *Cricotopus* sp. 4 Locality: PESM. Altitudinal range: 70–200 m a.s.l.. Significant altitudinal specificity (**IndVal: 0.71; p = 0.003**); *Cricotopus* sp. 5 Locality: APASM. Altitudinal range: 1750 m a.s.l. Low altitudinal specificity (IndVal: 0.14; p = 1.0).

Limnophyes Eaton, 1875

91 valid species; worldwide, except for Oceania and Antarctic; aquatic, terrestrial and semi-terrestrial habitats.

L. guarani Pinho & Andersen, 2015

Distribution. BRAZIL – Serra do Corvo Branco, Grão-Pará, Santa Catarina State (28°03'21"S, 49°22'00"W).

Elevation. 1241 m a.s.l.

Habitats. Madicolous habitat.

Known stages. L, P, F, M.

References. Pinho and Andersen (2015).

Remarks. *L. guarani* was found on rocky seepages and also on marginal rocks of first order streams, from 1570–2700 m a.s.l., extending the altitudinal range of the species. Environmental characterization: Water temperature 16.3–22 °C; dissolved oxygen 6.4–8.4 $mg.l^{-1}$; pH 5.5–6.4; slow to fast flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in APASM (Minas Gerais State), extending the geographical records to northernmost. Median altitudinal specificity (IndVal: 0.19; p = 0.18).

L. gercinoi (Oliveira, Messias & Santos, 1995)

Distribution. BRAZIL – Parque João Paulo II, Curitiba, Paraná State; UCAD, Florianópolis, Santa Catarina State; Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07'32"S, 49°29'38"W); Nova Teutônia, Santa Catarina State (27°11'S, 52°23'W).

Elevation. 300–1822 m a.s.l.

Habitats. Adults collected with entomological net and malaise trap close to stream.

Known stages. F, M.

References. Oliveira et al. (1995); Spies and Reiss (1996); Mendes et al. (2007b); Roque et al. (2007).

Remarks. *L. gercinoi* was found on marginal rocks of small streams, from 1080–1445 m a.s.l. Environmental characterization: Water temperature 10.0–16.1 °C; dissolved oxygen 8.1–9.9 $mg.l^{-1}$; pH 5; slow to fast flowing; partial canopy (about 50–70% covered). The species was found in PARNASO (Rio de Janeiro State), PESM (São Paulo State) extending the geographical records to northernmost. Low altitudinal specificity (IndVal: 0.19; p = 0.2).

Unknown species. *Limnophyes* sp. 1. Locality: PARNASO. Altitudinal range: 1445–2125 m a.s.l. Low altitudinal specificity (IndVal: 0.11; p = 0.87).

Lipurometriocnemus Saether, 1981

Four valid species; Nearctic and Neotropical; unknown habitat, probably semi-aquatic and terrestrial.

L. biancae Andersen, Pinho & Mendes, 2016

Distribution. BRAZIL – Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07'37"S, 49°28'47"W).

Elevation. 1670 m a.s.l.

Habitats. Male collected in malaise trap in cloud forest.

Known stages. M.

References. Andersen et al. (2016b).

Remarks. *L. biancae* was found on marginal rocks of low order streams and small waterfalls, extending the altitudinal records from 1570–2575 m a.s.l. Environmental characterization: Water temperature varied from 11–22 °C; dissolved oxygen 7.4–10.0 $mg.l^{-1}$; pH 4.5–6.4; slow to fast flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in PARNASO (Rio de Janeiro State) and APASM (Minas Gerais State), the northernmost records. Low altitudinal specificity (IndVal: 0.16; p = 0.39).

Unknown species. *Lipurometriocnemus* sp. Locality: APASM. Altitudinal record: 2700 m a.s.l. Low altitudinal specificity (IndVal: 0.2; p = 0.45).

Metriocnemus van der Wulp, 1874

67 valid species, worldwide, except for Oceania and Antarctic; mosses, Phytotelmata, springs, ditches, streams, lakes, and rock pools.

Unknown species. *Metriocnemus* sp. 1 Locality: APASM. Altitudinal record: 2200 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p = 0.84).

Parakiefferiella Thienemann, 1936

44 valid species; worldwide; running and standing waters.

P. strixinorum Wiedenbrug & Andersen, 2002

Distribution. BRAZIL – Taquara, Rio Grande do Sul State (29°46'S, 50°53'W); São Francisco de Paula, Rio Grande do Sul State (29°26'S, 50°35'W); Bom Jesus, Rio Grande do Sul State (28°40'S, 50°26'W).

Elevation. 600–1000 m a.s.l.

Habitats. Stream.

Known stages. P, M.

References. Wiedenbrug and Andersen (2002).

Remarks. *P. strixinorum* was found on marginal rocks of small streams, at 1045 m a.s.l., extending slightly the altitudinal range of the species. Environmental characterization: Water temperature 17 °C; dissolved oxygen 8.6 $mg.l^{-1}$; pH 5; fast flowing; reduced vegetal canopy (less than 20% covered). The species was found in PESM (São Paulo State) extending the geographical records to northernmost. Low altitudinal specificity (IndVal: 0.17; p = 0.85).

Unknown species. *Parakiefferiella* sp. 1. Locality: PESM. Altitudinal record: 70 m a.s.l. Low altitudinal specificity (IndVal: 0.25; p = 0.33).

Parametriocnemus Goetgebuer, 1932

34 valid species; worldwide; springs, streams and rivers.

Unknown species. *Parametriocnemus* sp. 1. Locality: APASM. Altitudinal record: 2575 m a.s.l. High altitudinal specificity (IndVal: 0.33; p = 0.06); *Parametriocnemus* sp. 2. Locality: PESM and APASM. Altitudinal range: 25–1570 m a.s.l. Low altitudinal specificity (IndVal: 0.12; p = 0.70); *Parametriocnemus* sp. 3. Locality: PESM and APASM. Altitudinal range: 25–1445 m a.s.l. Low altitudinal specificity (IndVal: 0.11; p = 0.76).

Pseudosmittia Edwards, 1932

98 valid species; worldwide; aquatic, terrestrial and semi-terrestrial habitats.

P. catarinense Andersen, Saether & Mendes, 2010

Distribution. BRAZIL – Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07'32"S, 49°29'38"W).

Elevation. 1822 m a.s.l.

Habitats. Male collected in malaise trap in cloud forest, close to small stream.

Known stages. M.

References. Andersen et al. (2010).

Remarks. *P. catarinense* was found on rocky seepages, at 2200 m a.s.l., extending slightly it altitudinal occurrence. Environmental characterization: water temperature 27.6 °C; dissolved oxygen 7.0 $mg.l^{-1}$; pH 6; slow flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in APASM (Minas Gerais State), extending the geographical records to northernmost. Low altitudinal specificity (IndVal: 0.17; p = 0.84).

Rheocricotopus Brundin, 1956

69 valid species; worldwide except Antarctica and Oceania; mostly rheophilic.

Unknown species. *Rheoricotopus* sp. 1 Locality: APASM. Altitudinal record: 2200 m. a.s.l. Low altitudinal specificity (IndVal: 0.17; p = 0.85); *Rheoricotopus* sp. 2. Locality: PARNASO. Altitudinal range: 1580–1670 m a.s.l. Median altitudinal specificity (IndVal: 0.29; p = 0.11).

Urubicimbera Andersen, Mendes & Pinho, 2015

One valid species; Brazil, Atlantic Forest; unknown habitats.

U. montana Andersen, Mendes & Pinho, 2015

Distribution. BRAZIL – Parque Nacional de São Joaquim, Urubici, Santa Catarina State (28°07'37"S, 49°28'47"W).

Elevation. 1670 m a.s.l.

Habitats. Male collected in malaise trap in cloud forest.

Known stages. M.

References. Andersen et al. (2015a).

Remarks. *U. montana* was found on rocky seepages, from 2200–2700 m a.s.l., expanding its altitudinal range. Environmental characterization: Water temperature varied from 21.3–27.6 °C; dissolved oxygen 6.4–7.0 mg.l^{-1}; pH 6; slow flowing; vegetal coverage completely absent (shrub-herbaceous vegetation). The species was found in APASM (Minas Gerais State), extending the geographical records to northernmost. Median altitudinal specificity (IndVal: 0.26; p = 0.13).

Unknown species. *Urubicimbera* sp. 1. Locality: APASM. Altitudinal range: 2575–2700 m a.s.l. Significant altitudinal specificity (IndVal: 0.91; **p = 0.002).**

SUBFAMILY PODONOMINAE
TRIBE PODONOMINI

Podonomus Philippi, 1865

40 valid species; Neotropical and Australasian; running water and tarn inhabitants.

P. mina Shimabukuro, Pepinelli & Trivinho-Strixino, 2017

Remarks. *P. mina* was recorded for the first time on marginal bedrock of a mountain stream, at 1270 m a.s.l. (20°25'12"S, 41°50'45.6"W), in Serra do Caparaó (Espírito Santo State) (Trivinho-Strixino et al. 2012), but only larvae have been evidenced and the description was only possible after the molecular association with the adults (Shimabukuro et al. 2017b). *P. mina* was recorded in isolated seepages and rocky substrate in the stream edges from 2575–2700 m a.s.l. in APASM (Minas Gerais State) (Shimabukuro et al. 2017b). Water temperature varied from 15–22 °C; dissolved oxygen 6.4–10.2 mg.l^{-1}; pH 5.0–5.5; fast water flow; vegetal coverage completely absent Significant altitudinal specificity have been found (IndVal: 0.41; **p = 0.04).**

P. pepinellii Roque & Trivinho-Strixino, 2004

Distribution. BRAZIL – Mantiqueira and Espinhaço mountain ranges: Monte Verde, Minas Gerais State (22°53'9.6"S, 46°1'55.2"W); Campos do Jordão, São Paulo

State (22°46'1.2"S, 45°31'15.6"W); Teresópolis, Rio de Janeiro State (22°27'3.6"S, 43°0'50.4" W); Alto Caparaó, Minas Gerais State (20°25'12"S, 41°50'45.6"W).

Elevation. 1275–1815 m a.s.l.

Habitats. Pupae found in a first-order stream; larvae found in madicolous habitats.

Known stages. L, P, M, F.

References. Roque and Trivinho-Strixino (2004); Trivinho-Strixino et al. (2012).

Remarks. In this study larvae was found living on marginal rocks of a low order stream and in isolated rocky seepages, extending the altitudinal records up to 2700 m a.s.l. Environmental characterization: Water temperature varied from 10–22 °C; dissolved oxygen 6.4–9.0 mg.l^{-1}; pH 5.0–6.0; very slow water flow; vegetal coverage completely absent (shrub-herbaceous vegetation). *P. pepinellii* was found in PARNASO (Rio de Janeiro State) and APASM (Minas Gerais State). Low altitudinal specificity (IndVal: 0.25; p = 0.32).

SUBFAMILY TANYPODINAE
TRIBE PENTANEURINI

Hudsonimyia Roback, 1979

Four valid species; Nearctic and Neotropical; madicolous.

H. caissara Silva, Wiedenbrug, Trivinho-Strixino, Oliveira & Pepinelli, 2012

Distribution. BRAZIL, Ubatuba, São Paulo State, (23°30.468'S, 45°11.923'W)

Elevation. 0 m a.s.l.

Habitats. Few larvae found on leaf litter in shallow-water streams flowing over granite outcrops.

Known stages. L, P, M.

References. Silva et al. (2012).

Remarks. *H. caissara* was found on marginal rocks of a small stream, at 200 m a.s.l. This slightly extended the altitudinal records of the species. Environmental characterization: Water temperature 21 °C; dissolved oxygen 7.9 mg.l^{-1}; pH 5.5; fast flowing; sparse vegetal canopy (less than 30% covered). The species was found in PESM (São Paulo State). Median altitudinal specificity (IndVal: 0.33; p = 0.13).

Unknown species. *Hudsonimyia* sp.1. Locality: PESM. Altitudinal record: 1080 m a.s.l. Low altitudinal specificity (IndVal: 0.17; p = 0.85).

Notes on altitudinal distribution

A summarized list of the species, morphospecies and the genera of immature found, along with respective ecological and geographical information, is presented in sup-

plementary material (Table 1). In this study, the chironomid community was predominantly composed of species belonging to the subfamily Orthocladiinae (35 spp.), followed by Chironominae (21 spp), Podonominae and Tanypodinae (2 spp each). Among the 60 species recorded, a higher percentage has been found at APASM (45%), of which 85% were exclusive from this locality. Further, 31% of the possible new species occurred above 2100 m a.s.l. Only five from the 60 species recorded were significant indicators of specific altitudes, they are: *Urubicimbera* sp. 1, *Cricotopus* sp. 4, *Pseudochironomus ruah*, *Lauterborniella* sp. 1 and *Podonomus mina* (Figure 3). *Urubicimbera* sp. 1, and *P. mina*, represented the highest sites in this study (> 2600 m a.s.l.); *P. ruah* was a significant indicator of the 2500 m–altitudinal–band; *Lauterborniella* sp. 1 and *Cricotopus* sp. 4 were significant indicators of 1100 and 200 m–altitudinal–band, respectively (Figure 3). Furthermore, these five species were all unknown to science previous to this investigation in madicolous habitats of the Atlantic Forest.

Regarding the 22 recognized species, 17 of them had spread the altitudinal distribution (Figure 4). Even those species that have previously been found in mountain regions, such as *Podonomus pepinellii*, *Lipurometriocnemus biancae*, *Urubicimbera montana*, *Pseudosmittia catarinense* and *Limnophyes guarani,* were recorded at higher altitudes in this study, and, except for *P. catarinense,* the altitudinal distribution increased more than 1000 m for each of these species (Figure 4).

For those species that have so far been verified at low altitudes, such as *Tanytarsus giovannii*, *Polypedilum solimoes*, *Onconeura oncovolsella*, *Corynoneura hermanni* and

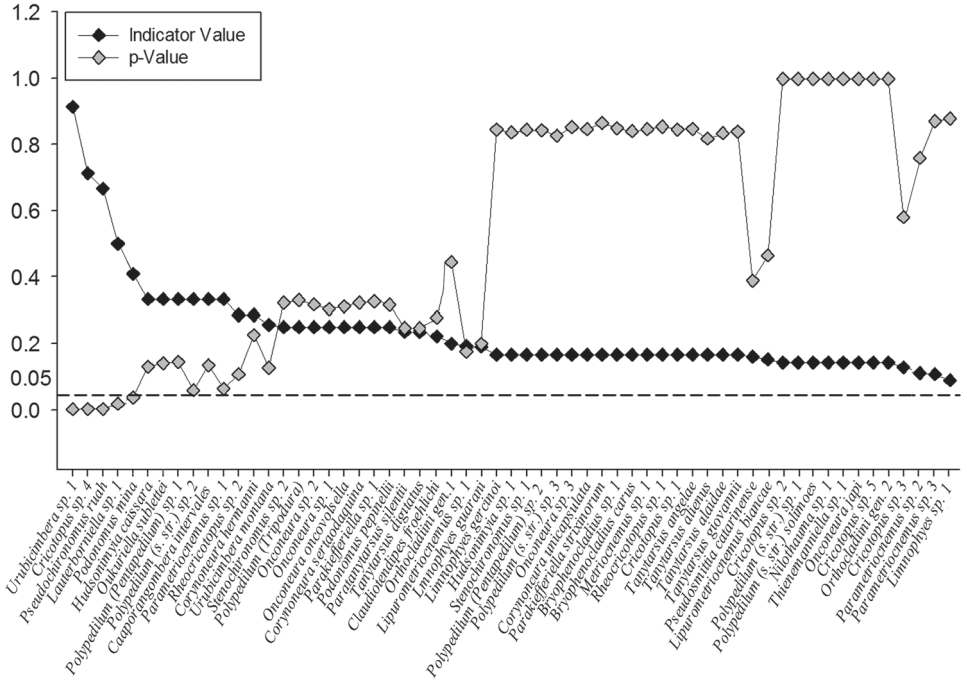

Figure 3. Indicator species of altitudinal range. Indicator values in black and significance value (p) at 0.05 level in gray obtained for each species and morphospecies found in the present study.

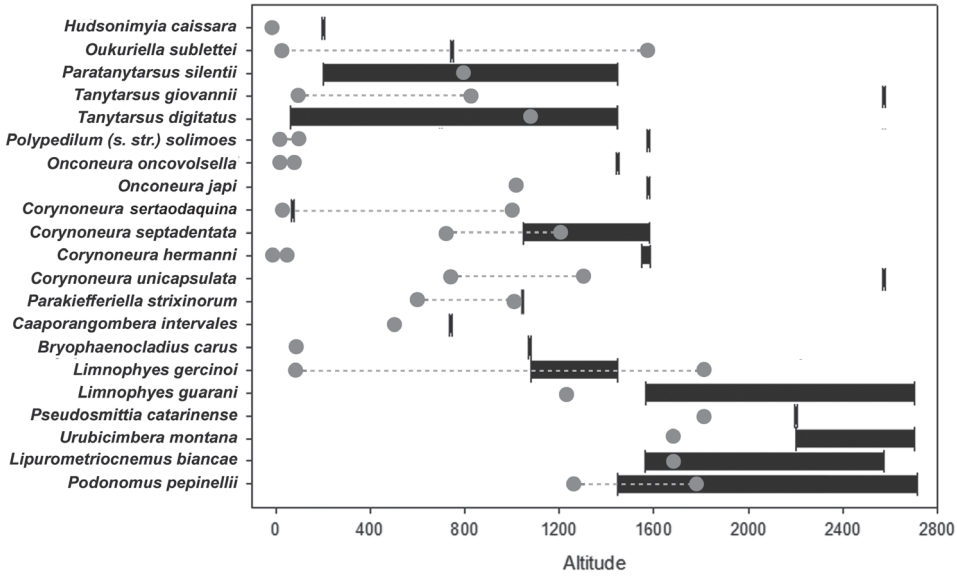

Figure 4. Altitudinal record of each species found in madicolous habitats in the present study. Previous altitudinal records (from literature) in gray and altitudinal records from this research in black.

Bryophaenocladius carus, the amplitude of the altitudinal distribution was even more remarkable, adding more than 1500 m to the altitudinal range in some cases. The only exception was *Hudsonimyia caissara,* that have been firstly reported at the sea level, and here it was found at 200 m a.s.l., slightly increasing the altitudinal range of the species (Figure 4). *Paratanytarsus silentii*, *Tanytarsus digitatus*, *Onconeura japi*, *Corynoneura septadentata*, *Corynoneura unicapsulata* and *Caaporangombera intervales* also had the altitudinal distribution extended, while the remaining five species were recorded within their typical altitudinal ranges (Figure 4).

Discussion

Compositional changing in chironomid assemblages along altitudinal gradients have been verified by many researchers worldwide (McKie et al. 2005, Tejerina and Malizia 2012, Henriques-Oliveira and Nessimian 2010, Scheibler et al. 2014, Robinson et al. 2016, Matthews-Bird et al. 2016). In mountain ecosystems the occurrence of chironomid species can be influenced by environmental changes related to altitude variation, such as temperature and oxygen availability (Oliver 1971, Pinder 1986, Eggermont and Heiri 2012), dispersal capacity (Ashe et al. 1987), historical events (McKie et al. 2005, Allegrucci et al. 2006, Krosch et al. 2011) or other regional particularities (Körner 2007). Mountains are therefore important objects to biogeography studies, revealing rich communities and many endemic species (Lods-Crozet et al. 2001, Gar-

cia and Suaréz 2007; Brundin 1966). In our study, the locality with higher altitudes (APASM) yielded a higher number of species, most of them are unknown to science and were exclusively found in this place, especially above 2,100 m a.s.l.. These evidences are essential in view of the conservation perspective, once the majority of the species found are possibly endemic.

A clear gap on the taxonomic knowledge of mountain fauna can be observed. This gap is likely due to the low accessibility of these areas, thereby hampering sampling strategies. Studies in mountain regions are urgently needed, especially when dealing with one of the most threatened biomes in the world (Ribeiro et al. 2009) whose geomorphological characteristics are so heterogeneous. Mountains have been suffering from several types of environmental impacts, but the most alarming today is climate change (Burke 2003, Catalan et al., 2017). Current forecasts suggest that rainfall will be less constant and temperature will raise 2.0 to 6.0 °C by 2100 (Garcia et al. 2014), strongly affecting the flora and fauna. The climate changes will especially affect those living in small water bodies with high exposure to environmental pressure. The narrow range of tolerance to environmental conditions verified for mountain species, and the fact that many of them are rare and endemic, make the conservation efforts in these areas indispensable.

The indicator's analysis evidenced that all species significantly associated with their respective altitudinal band were previously unknown to science. All of them, except for *Cricotopus* sp. 4, recorded from 70–200 m a.s.l. were found exclusively at mountaintops. *Lauterborniella* sp. 1 was recorded at the highest sites in PESM and the remaining taxa were recorded at the summit of APASM mountains. Regarding the geophysical characteristics of mountaintops (shape, size, insulation value), also known as Inselbergs (Porembski 2007); some species, especially those with limited dispersal capacity, are more likely to deal with speciation process and local extinctions (MacArthur and Wilson, 1967). The narrow altitudinal range expressed by these unknown species, make us believe that they should present a high endemicity degree.

Our new records extend the altitudinal range of 17 known species. Most species seem to tolerate a wide altitudinal range, such as *Tanytarsus giovannii* and *Limnophyes gercinoi,* while others presented a narrow range, such as *Hudsonimyia caissara.* The altitudinal range is related to the extent of the geographical distribution of each species; species that are widely distributed are expected to occur in a wider range of altitudes compared to those that have limited distribution (Stevens 1992). Brundin (1966), analyzing the distributional patterns of Podonominae in South America concluded that species found in Patagonian region could be recorded at the highest sites of tropical Andean mountains. Similarly, in this study, species that have previously been recorded further south such as *Lipurometriocnemus biancae*, *Urubicimbera montana*, *Pseudosmittia catarinense,* and *Limnophyes guarani*, were found at higher altitudes, and may be related to temperature requirements.

Madicolous habitats have never been formally studied in Brazilian mountainous regions, in contrast to other Atlantic Forest water bodies in which the Chironomidae fauna have already been extensively investigated (Henriques-Oliveira et al. 2003, Roque

et al. 2007, Silveira et al. 2015). Taxonomists, and especially ecologists, have paid little attention to semi-aquatic and terrestrial Chironomidae, and therefore, madicolous species were completely overlooked. In our current study, a remarkable diversity of Chironomidae living in madicolous habitats was revealed, and most of the species (about 64%) were probably new before this project. However, from the 38-unknown species collected, five have recently been described: *T. alaidae, T. alienus, T. angelae* (Trivinho-Strixino and Shimabukuro 2017), *P. mina* (Shimabukuro et al. 2017b), and *P. ruah* (Shimabukuro et al. 2017a), increasing the number of madicolous chironomid species.

Despite the low knowledge on semi-aquatic forms, evidences from chironomids fossils preserved in amber reveals that terrestrial life-styles have been common since the late Eocene (about 40 million years ago) (Zelentsov et al. 2012), raising the importance of madicolous and other semi-aquatic habitats on the evolutionary history of many Chironomidae taxa. Within them, Orthocladiinae harbors the majority of semi-aquatic species (Andersen et al. 2010), what might explain their notable richness in madicolous habitats. Sinclair and Marshall (1987) also noted the remarkable dominance of Orthocladiinae among madicolous chironomids in Southern Ontario, Canada. In their study, ten of 14 genera recorded were Orthocladiinae, including *Parakiefferiella*, *Metriocnemus*, *Parametriocnemus*, *Thienemanniella,* and *Limnophyes*, also verified in this study. One more evidence that these genera can adapt well to this habitat.

Only two of the species verified here were previously known to occur in madicolous habitats (*Podonomus pepinellii* and *Limnophyes guarani*). Although *Podonomus* larvae can be found in streams and other fast flowing running waters, they are also common on the edge of streams (Brundin 1966). *Podonomus pepinellii* and all *Podonomus* morphotypes in the Atlantic Forest highlands are associated with madicolous habitats (Trivinho-Strixino et al. 2012), and as such they occur in rocky outflows and stream shorelines. The remaining species identified in this study were previously considered stream-dweller, although some have been found in habitats close to madicolous ones, such as those from the Corynoneurini tribe and *Hudsonimyia caissara.*

The larvae of *Hudsonimyia caissara* were originally found in low abundance (two specimens) in leaf litter of a mountain stream (Silva et al. 2012), possibly an inhabitant of the stones in the stream's edge. Further, it is very plausible that some stream-dweller species can tolerate both conditions (Vaillant 1956, Sinclair and Marshall 1987). Thus, a richer fauna is expected to occur in marginal stream rocks compared to isolate seepages.

It is not surprising that members of *Hudsonimyia*, *Bryophaenocladius*, *Metriocnemus*, *Limnophyes* and *Pseudosmittia* have been found in madicolous habitats during this study. These genera are known to have larval instars associated with semi-aquatic and terrestrial conditions. Roback (1979) was the first to verify *Hudsonimyia* larvae living on a thin layer of current water with periphyton and moss. *Metriocnemus* species are adapted to an extremely broad range of habitats within Chironomidae (Cranston and Judd 1987), including madicolous, as exemplified by the species *M. hygropetricus* (Kieffer 1911), whose name was given after their type locality habitat – natural rock seepages and artificial madicoulous habitats. Most *Limnophyes* larvae are semi-aquatic

(Saether 1990), and recently a new species of this genus, *Limnophyes guarani*, has been recorded on madicolous habitats in the south of Brazil (Pinho and Andersen 2015). *Pseudosmittia* and *Bryophaenocladius* larvae are largely terrestrial or semi-terrestrial; however, this was the first time that Neotropical species of these genera have been recorded in a madicolous habitat.

Although many genera were expected to occur, some were particularly intriguing, such as *Stenochironomus* and *Oukuriella*. Both are known to be highly habitat-specialized in larval phase. The first is a vegetal miner (Epler et al. 2013) while the second is typically associated with sponge or wood detritus, although the habitat of basal groups in the phylogeny of *Oukuriella* could not be defined yet (Fusari et al. 2014). *Oukuriella sublettei* recorded in this study was reported in association with submerged wood found in first order streams with bedrock (Bellodi et al. 2016). Their presence in the marginal rocks of the stream might have been accidental, considering that only one specimen was found. The same is expected for both *Stenochironomus* species. Their reduced size suggests that adults emerged from leaf detritus or small fragments of wood, possibly inside rock fissures. However, the emergence of these taxa in such conditions was interesting, since in this case they completed their development in a thin layer of water, a complement to previous observations of immature submerged in the streams (Fusari et al. 2014, Bellodi et al. 2016; Dantas et al. 2016).

Far from being semi-aquatic, most of the *Rheotanytarsus* species require flowing water conditions to survive and emerge (Coffman and Ferrington 1996). However, the capacity to live in madicolous habitats may not be disregarded as some species, such as *R. gloveri*, demonstrated tolerance to drying rock faces of streams and survived in thin layers of current water (Cranston 1997). The strict definition given by Vaillant, in 1956, considers hygropetric habitats all flowing water with less than 2 mm thick. However, this delimitation is hard to establish when dealing with microhabitats constantly susceptible to water flow oscillations due to climatic conditions. In some occasions, our sampling sites, especially those at the margins of the streams, had the water flux modified as a consequence of the contraction or expansion of the main channel. Probably, *Rheotanytarsus* species were favored when stronger currents rose, although the intense dark coloration of the cephalic capsule of the larvae may indicate that they are truly madicolous inhabitants (Brundin 1966, Sinclair 1988, Trivinho-Strixino et al. 2012).

The procedure of rearing immature specimens to obtain the adults is most of the time unsuccessful due to their environmental requirements (Ekrem et al. 2007). Therefore, descriptions are frequently based only on adults, whose sampling methods often preclude the knowledge of immature habitat and other aspects of their ecology. Some species recorded have only been known by the adults, previously sampled with malaise or light traps. This was the case of *Lipurometriocnemus biancae*, *Urubicimbera montana*, *Pseudosmittia catarinense*, *Caaporangombera intervales*, and *Paratanytarsus silentii*.

Using our modified emergence traps allowed us to assure that the immature organisms and the adults from the species sampled in this study were madicolous inhabitants. The association and description of the immature is a fundamental task when studying chironomids, best accomplished with the help of molecular tools, such as

DNA barcode. The larva and the pupa of *P. silentii* have been successfully associated with adult males after this investigation (Trivinho-Strixino and Shimabukuro 2017). Furthermore, even for those species whose immature forms are known, the first record of them in madicolous habitats represents a remarkable note on their success in colonizing a wide range of habitats.

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Supplementary material 1

Table S1. Taxa recorded in madicolous habitats from Atlantic Forest in Southeastern Brazil

Authors: Erika Mayumi Shimabukuro, Susana Trivinho-Strixino

Data type: species data

Explanation note: DS: development stage; A: adults; I: immature; SE: stream edges; RS: rocky seepages; LAB: low altitudinal band, sampling sites in PESM; IAB: intermediate altitudinal band, sampling sites in PARNASO; HAB: high altitudinal band, sampling sites in APASM.

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RESEARCH ARTICLE

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New species of the genus *Mesocletodes* Sars, 1909 from the deep Gulf of California (Copepoda, Harpacticoida)

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Abstract

Investigations on the effects of the oxygen minimum zone on the distribution, abundance, and diversity of deep-sea benthic and pelagic fauna of the Gulf of California and Eastern Tropical Pacific has received attention recently. However, very little is known about the diversity of deep-sea benthic harpacticoids from this region, and only three species, *Ancorabolus hendrickxi* Gómez & Conroy-Dalton, 2002, *Ceratonotus elongatus* Gómez & Díaz, 2017 and *Dendropsyllus californiensis* Gómez & Díaz, 2017, have been described so far. The genus *Mesocletodes* Sars, 1909 is one of the most common and abundant genera of deep-sea harpacticoids. This genus has been traditionally subdivided into two species groups, the *abyssicola* and the *inermis* groups, based on the presence/absence of a dorsal cuticular process on the cephalothorax and anal somite, but some species have been reported to deviate from this scheme. As a result of their investigations, other researchers proposed the monophyly of the *abyssicola* group, and suggested the probable monophyly of two other species-groups. In this paper, the descriptions of three new species of the genus *Mesocletodes* from the deep sea of the Gulf of California are presented with some notes on their relationships with other species. Some comments on the monophyly of the genus are given.

Keywords

Argestidae, *Mesocletodes*, deep sea, new species, taxonomy

Introduction

Great effort has been deployed since the late 1980's to study the biodiversity of the deep sea of the Gulf of California and Eastern Tropical Pacific (a complete list of contributions is available upon request). Extensive oceanographic campaigns (Talud I-XIII cruises) on board the research vessel "El Puma" of the Universidad Nacional Autónoma de México (UNAM) have been carried out from 1989 to 2009 in the frame of several research projects to better understand the effects of the oxygen minimum zone on the distribution, abundance and diversity of deep-sea benthic and pelagic fauna (crustaceans, molluscs, echinoderms, fishes and polychaetes among others) of the Gulf of California and Eastern Tropical Pacific (Hendrickx 2012). One of the components studied during Talud IV- Talud XIII cruises was the meiofauna, with especial attention to benthic harpacticoid copepods. Preliminary personal observations revealed a high diversity of harpacticoids. However, only three species of the family Ancorabolidae, *Ancorabolus hendrickxi* Gómez & Conroy-Dalton, 2002, *Ceratonotus elongatus* Gómez & Díaz, 2017 and *Dendropsyllus californiensis* Gómez & Díaz, 2017 have been described so far from the deep sea of the Gulf of California.

The genus *Mesocletodes* Sars, 1909 is one of the most common and abundant genera of the family Argestidae in deep-sea samples (Menzel 2011). Traditionally, two species-groups are recognized within the genus, and Bodin (1968, 1997) proposed the subdivision of the genus *Mesocletodes* into the *abyssicola* group and the *inermis* group, based on the presence/absence of a simple dorsal cuticular process on the cephalothorax and anal somite, but pointed out that that such subdivision has no taxonomic value. However, with the discovery of some new species, it was found that some deviate from Bodin's (1968, 1997) scheme. For example, Por (1986: 95) suggested that *M. opoteros* Por, 1986, described without a dorsal process on the cephalothorax but with a dorsal process on the anal somite, could well belong to a different species-group within the genus, and suggested that Bodin's (1968, 1997) division of this genus into species-groups could change with the discovery of new species. A similar condition has been observed for *M. fladensis* Wells, 1965 and *M. angolaensis* Menzel & George, 2009. However, Menzel and George (2009) did not follow Bodin's (1968, 1997) and Por's (1986) views. Instead, they suggested the monophyly of the *abyssicola* group based on the elongation of the caudal rami, and on the presence of a dorsal process on the cephalothorax and on the anal somite, and pooled all the species of *Mesocletodes*, with a dorsal cuticular process either on the cephalothorax or on the anal somite, or both, and with long or short caudal rami, in the *abyssicola* group, arguing that the deviation of Bodin's (1968, 1997) scheme (presence of a dorsal process on the cephalothorax or on the anal somite only), and the shorter caudal rami observed in some species could eventually be regarded as secondary reductions (Menzel and George 2009: 253). Additionally, Menzel and George (2009) suggested the monophyly of, at least, two other groups of species, viz. those in which the males lack mouth parts (Menzel and George 2009: 253–254), and those with bifid dorsal processes on the P3-P5-bearing somites, and on the posterior half of the female double genital-somite (Menzel and George 2009: 254).

In this paper three new species of *Mesocletodes* from the deep sea of the Gulf of California are proposed. Additionally, some comments on the monophyly of *Mesocletodes* are provided.

Materials and methods

Sediment samples for meiofaunal analyses were taken in August 2000 in the Southern Gulf of California from Carmen basin to off Nayarit State, and in February 2007 in the Southern Trough of Guaymas Basin, during Talud IV and Talud X cruises, respectively, on board the research vessel "El Puma" of the Universidad Nacional Autónoma de México (UNAM). Sediment samples were collected at depths ranging from about 520 m to 2120 m during Talud IV cruise using a multiple sediment corer equipped with six cores of 30 cm in length and sampling surface of 3.9 cm^2, and from about 379 m to 1902 m during Talud X cruise using a box corer from which triplicate sub-samples were taken with 69 cm^2 cores of 20 cm in length. The upper 3 cm layer of sediment was preserved in 70% alcohol, sieved through 500 and 38 µm sieves to separate macro- and meiofauna, and stained with Rose Bengal. Meiofauna was sorted at a magnification of 40× using an Olympus SZX12 stereomicroscope equipped with DF PLAPO 1× objective and WHS10× eyepieces, and harpacticoid copepods were stored separately in 1 ml vials with 70% ethanol. Illustrations and figures were made from whole individuals and its dissected parts using a Leica DMLB microscope equipped with L PLAN 10× eyepieces, N PLAN 100× oil immersion objective, and drawing tube. The dissected parts were mounted on separate slides using lactophenol as mounting medium. Huys and Boxshall (1991) and Menzel and George (2009) were followed for general terminology. Huys (1996) was followed for the subapical tubulate extensions (STE).

Abbreviations used in the text:

acro acrotheke;
ae aesthetasc;
ENP endopod;
EXP exopod;
EXP (ENP)1 (2,3) first (second, third) exopodal (endopodal) segment;
P1–P6 first to sixth legs;
se pinnate, naked, setiform;
sp spinose, spiniform;
STE subapical tubulate extension.

The type material was deposited in the Copepoda collection of the Instituto de Ciencias del Mar y Limnología, Unidad Académica Mazatlán (**ICML-EMUCOP**). The map showing the sampling locations where the new species were found were prepared with GeoMapApp (http://www.geomapapp.org/) and the Global Multi-Resolution Topography (GMRT) default basemap (Ryan et al. 2009).

Taxonomy

Family Argestidae Por, 1986a
Genus *Mesocletodes* Sars, 1909

***Mesocletodes brevisetosus* sp. n.**
http://zoobank.org/8E54143A-D029-4D06-BC1E-A5EBFFF9847E

Material examined. One female holotype as follows: habitus, left antennule and right antenna left intact and preserved in alcohol (ICML-EMUCOP-270800-02), right antennule, left antenna, mandibles, maxillules, maxillae and maxillipeds dissected and mounted onto four slides (ICML-EMUCOP-270800-01); Talud IV cruise; August 27, 2000; coll. S. Gómez.

Type locality. Southern Carmen Basin, Gulf of California, México (25°54.7'N, 110°11'W), 2018 m depth (see Fig. 1); coll. S. Gómez.

Diagnosis (based on female only). Body subcylindrical. Cuticula of body surface reticulated. Free thoracic somites and urosomites, except for anal somite, with posterior margin coarsely serrated. Cephalothorax with dorsal process curved posteriorly. P3–P5-bearing somites and second half of genital-double somite with bifid cuticular processes dorsally. Genital somite and third urosomite incompletely fused dorsolaterally. Anal somite with upward bifid cuticular process dorsally. Caudal rami 17 times as long as wide in lateral view, with seven setae. Antennule octa-segmented; second segment with strong protrusion bearing one strong element. Antenna with basis, with uni-segmented exopod bearing two setae subequal in length. Gnathobase of mandible with grinding face, palp uni-segmented, endopodal lobe with four setae. P2–P4 ENP1 with inner seta. P1–P4 ENP2 with 3, 3, 2, 2, setae respectively. P5 endopodal lobe represented by two setae; inner seta of the P5 EXP very small, issuing subapically.

Description of female. *Body*: total length 1420 µm measured from anterior margin of rostrum to posterior margin of caudal rami, subcylindrical, tapering slightly posteriorly, without clear demarcation between prosome and urosome, cuticula of body surface reticulated (Fig. 2A); P2–P5-bearing somites (Fig. 2D), both halves of genital double-somite and fourth and fifth urosomites (Fig. 2E) with posterior margin coarsely serrated; lateral margin of cephalothorax less coarsely serrated (Fig. 2B). Rostrum (Figs 2A–B, 3A) fused to cephalothorax, with two sensilla. Cephalothorax (Fig. 2A–B) 0.2 times as long as entire body length; with small sensilla as shown, and with exceedingly long lateral sensilla (Fig. 2B); with dorsal process curved posteriorly (Fig. 2A, B), the latter with sensilla as shown (Fig. 2C). Body somites with posterior transverse longitudinal row of spinules (Fig. 2A, D, E). P2-bearing somite without, P3–P5-bearing somites with bifid cuticular processes dorsally, of P4-bearing somite smallest, of P3- and P5-bearing somites subequal (Fig. 2D). Genital somite and third urosomite (genital double-somite) incompletely fused dorsolaterally (Fig. 2E), posterior margin of genital somite indicated by suture with few spinules and sensilla, and serrated posterior margin, completely fused ventrally (Fig. 3B); first half of genital

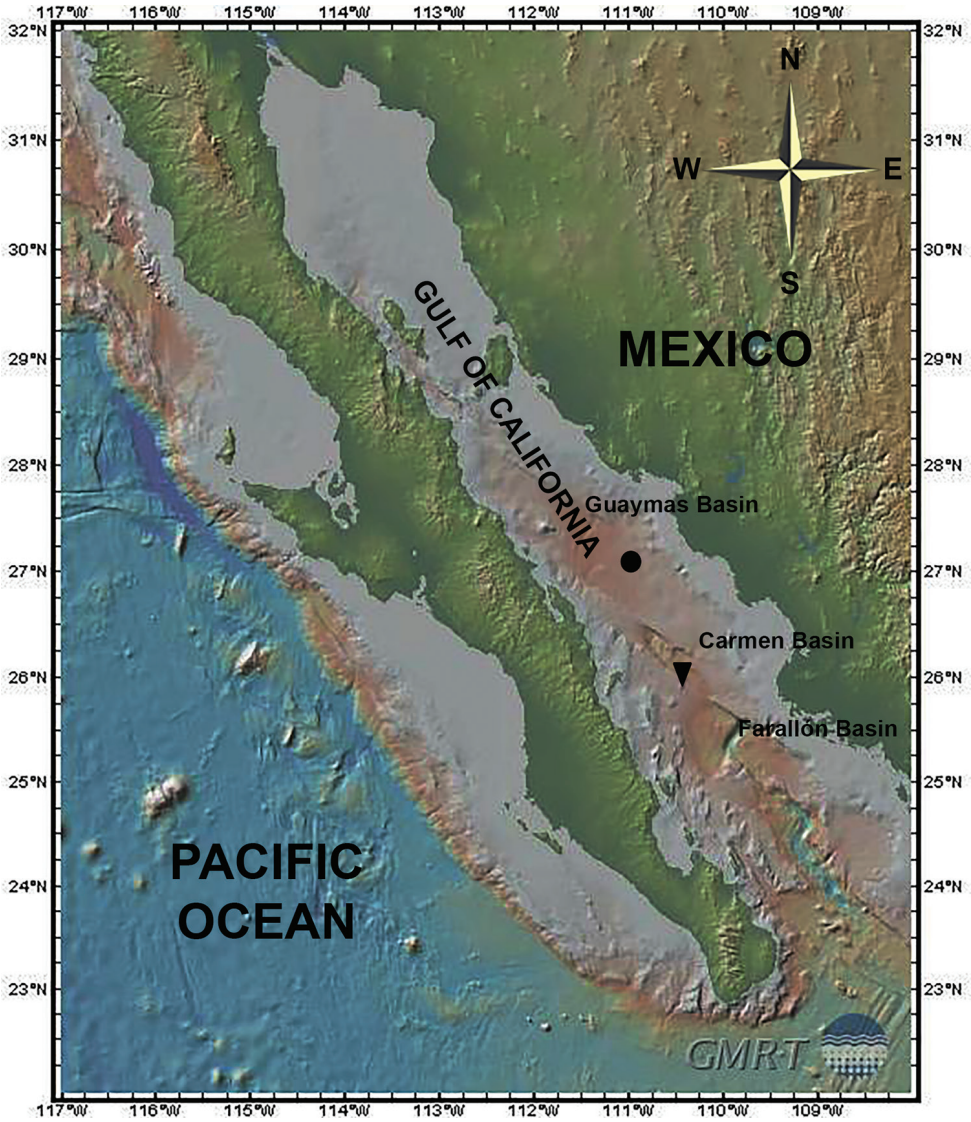

Figure 1. Sampling sites and type localities of *Mesocletodes simplex* sp. n. (full circle), and *M. brevisetosus* sp. n. and *M. unisetosus* sp. n. (full triangle). Figure prepared with GeoMapApp (http://www.geomapapp.org/) and the Global Multi-Resolution Topography (GMRT) default basemap (Ryan et al. 2009).

double-somite without cuticular process dorsally (Fig. 2A, E), with few lateral spinules close to posterior margin (Fig. 2E), ventrally with medial genital field (Fig. 3B) and with some spinules close to lateral margins, second half with bifid cuticular process dorsally (Fig. 2A, E), with lateral spinules along posterior margin, serrated posterior margin between pair of ventral sensilla less pronounced (Figs 2E, 3B). Dorsal and lateral surface of fourth urosomite with short row of small spinules, with few sensilla, posterior margin serrated (Fig. 2A, E), ventrally with posterior serrated margin slightly less

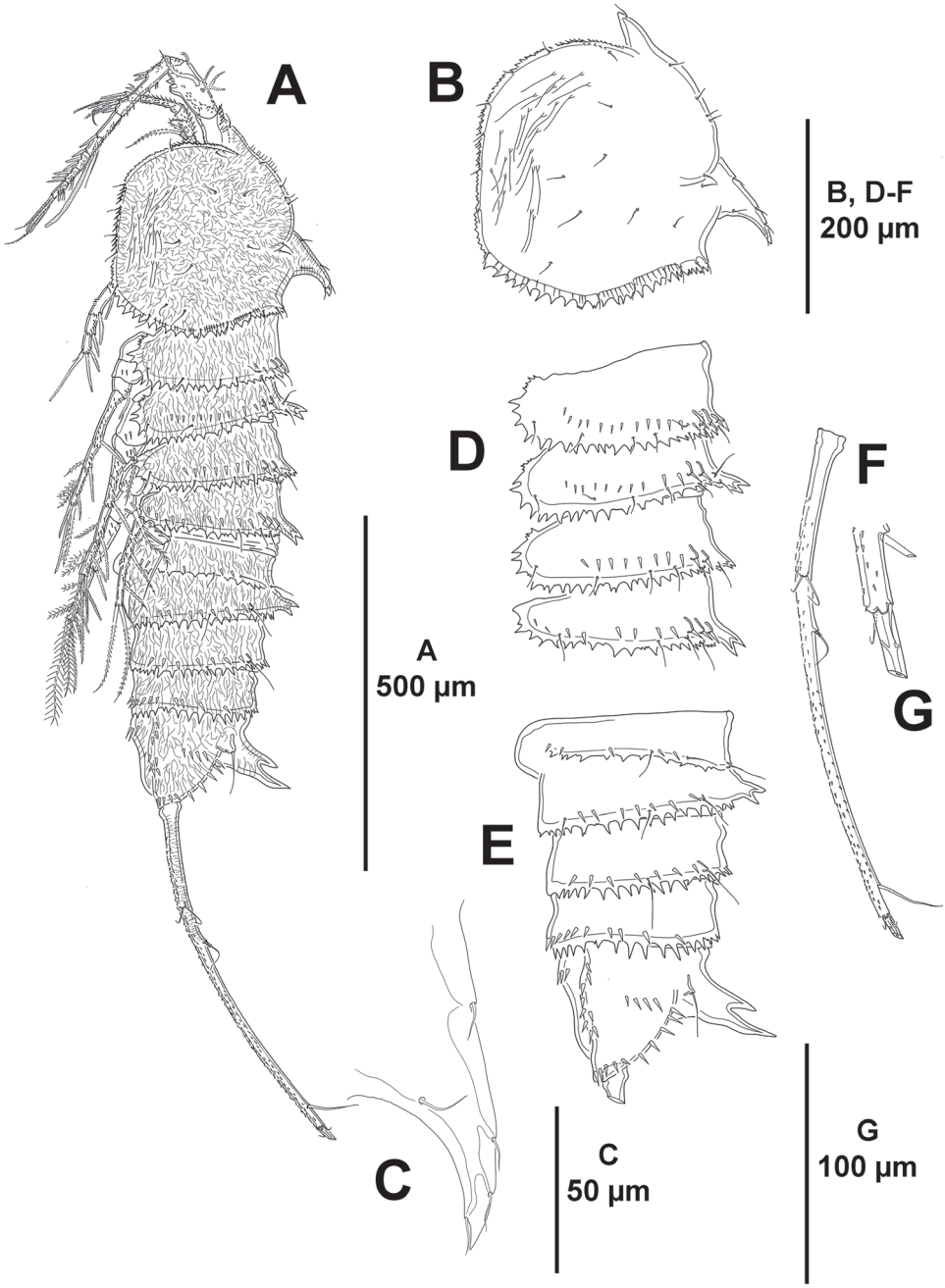

Figure 2. *Mesocletodes brevisetosus* sp. n., female holotype. **A** habitus, lateral **B** cephalothorax, lateral, reticulated pattern omitted **C** dorsal cuticular process of cephalothorax showing situation of sensilla, reticulated pattern omitted **D** P2–P5-bearing somites, lateral, reticulated pattern omitted **E** genital double-somite, fourth and fifth urosomites, and anal somite, lateral, reticulated pattern omitted **F** left caudal ramus, lateral, reticulated pattern omitted **G** distal part of left caudal ramus, lateral, reticulated pattern omitted.

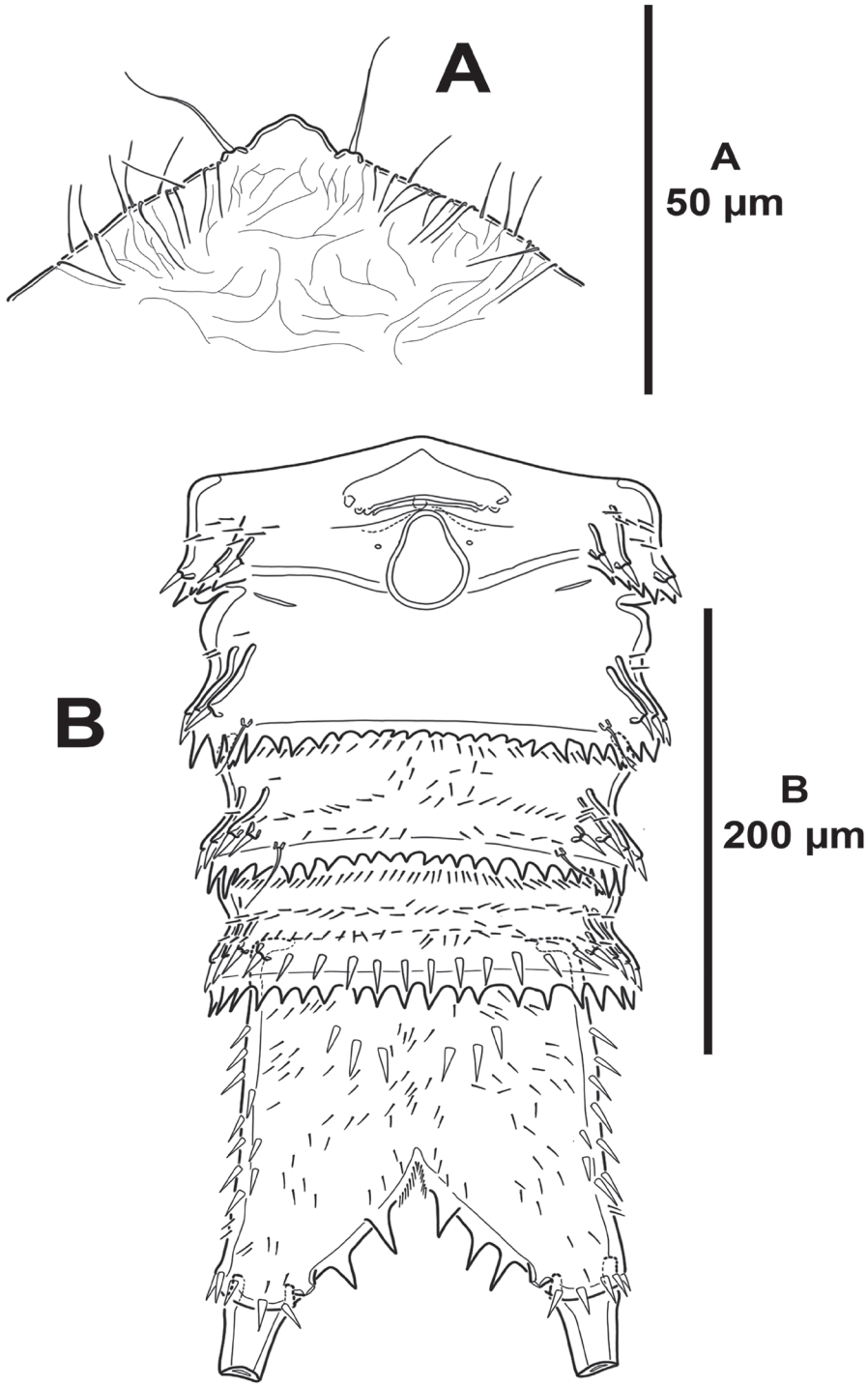

Figure 3. *Mesocletodes brevisetosus* sp. n., female holotype. **A** rostrum, dorsal **B** urosome, ventral, P5-bearing somite omitted.

pronounced between pair of ventral sensilla, with minute spinules as shown (Fig. 3B). Fifth urosomite (Fig. 2E) with less spinules than in preceding somite, without sensilla, posterior margin equally serrated along entire margin, ventrally (Fig. 3B) with continuous spinular row close to posterior margin, with minute spinules as shown.

Anal somite (Figs 2A, E, 3B) quadrate, as long as two preceding somites combined; with lateral (Fig. 2A, E) and ventral (Fig. 3B) spinules, with upward bifid cuticular process dorsally, flanked by pair of sensilla, posterior tip of cuticular process longer than anterior (Fig. 2A, E).

Caudal rami slender, exceedingly elongated, 17 times as long as wide in lateral view, as long as P4-bearing somite and entire urosome combined, gently curved upwards from lateral view, covered with small spinules (Fig. 2A, F); with seven elements as follows: seta I and II in distal part of first third of ramus, the former ventral to and smaller than the latter (Fig. 2F); seta III situated subdistally on dorsal surface (Fig. 2G); seta IV and VI small, subequal in length, arising at outer and inner distal corners, respectively; seta V longest; dorsal seta VII tri-articulated, situated on anterior part of second third of ramus.

Antennule (Fig. 4A, B) octa-segmented; all segments with small slender spinules; first segment without armature, with one medial and one distal row of strong spinules; second segment with strong protrusion bearing one strong element (arrowed in Fig. 4B), and two strong spinules; third segment elongated, as long as fourth–seventh segments combined; eighth segment smallest. Armature formula as follows: 1-[0], 2-[5sp+3se], 3-[5sp], 4-[1sp+(1se+ae)], 5-[1sp], 6[3sp], 7-[2sp+2se], 8-[5se+acro]. Spinulose, spiniform elements (sp) with STE.

Antenna (Fig. 4C). Basis elongated, covered with small spinules. Exopod uni-segmented, with two apical setae. Endopod bi-segmented; first segment with strong inner spinules, covered with smaller spinules, shorter than basis; second segment covered with small spinules, inner margin with stronger spinules and with two thin subdistal spines with STE, and six apical elements (one inner strong spinulose spine, one spinulose spine, two geniculate spinulose elements, and two outer elements fused basally of which innermost longer and with STE).

Mandible (Fig. 5A). Mandibular gnathobase with some surface spinules, with two distal blunt teeth, and with broad grinding face. Palp uni-segmented (exopod and endopod fused to basis), with one basal, one exopodal, and one subdistal and three apical endopodal setae.

Maxillule (Fig. 5B, C). Praecoxal arthrite with some very long spinules, two surface setae, and eight apical spines (Fig. 5C). Coxa with five elements, strongest fused to coxa. Basis with subapical row of spinules and five setae.

Maxilla (Fig. 5D). Syncoxa with outer and inner spinules, with smaller spinules close to allobasis; with two endites; proximal endite with one slender seta, distal endite with one strong spinulose element, one pinnate and one bare seta. Allobasis with longitudinal row of outer spinules, with one strong spinulose spine fused to allobasis, one slender seta and one spinulose spine. Endopod uni-segmented, very small, with two setae.

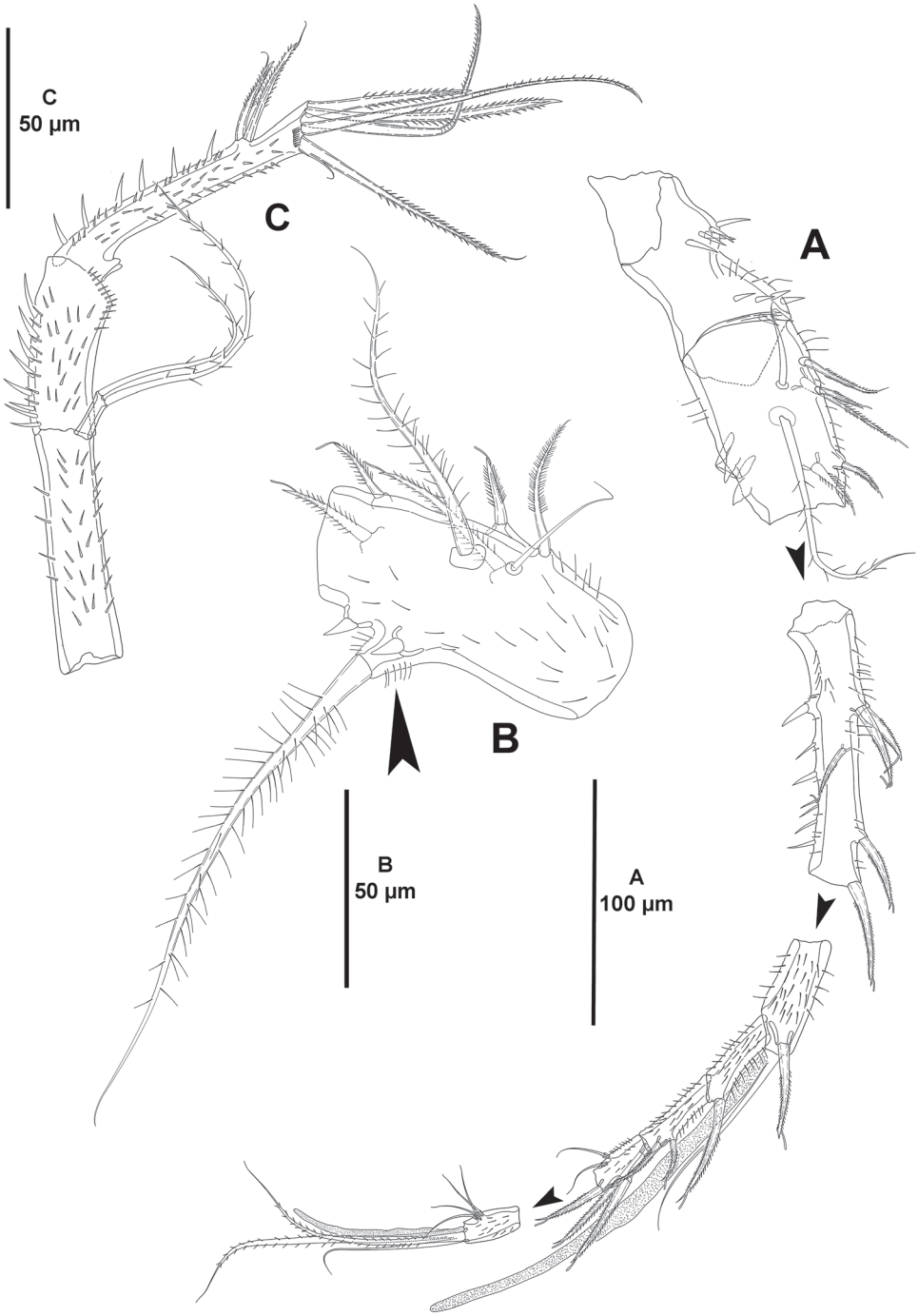

Figure 4. *Mesocletodes brevisetosus* sp. n., female holotype. **A** antennule **B** second segment of antennule, showing strong protrusion with strong seta **C** antenna.

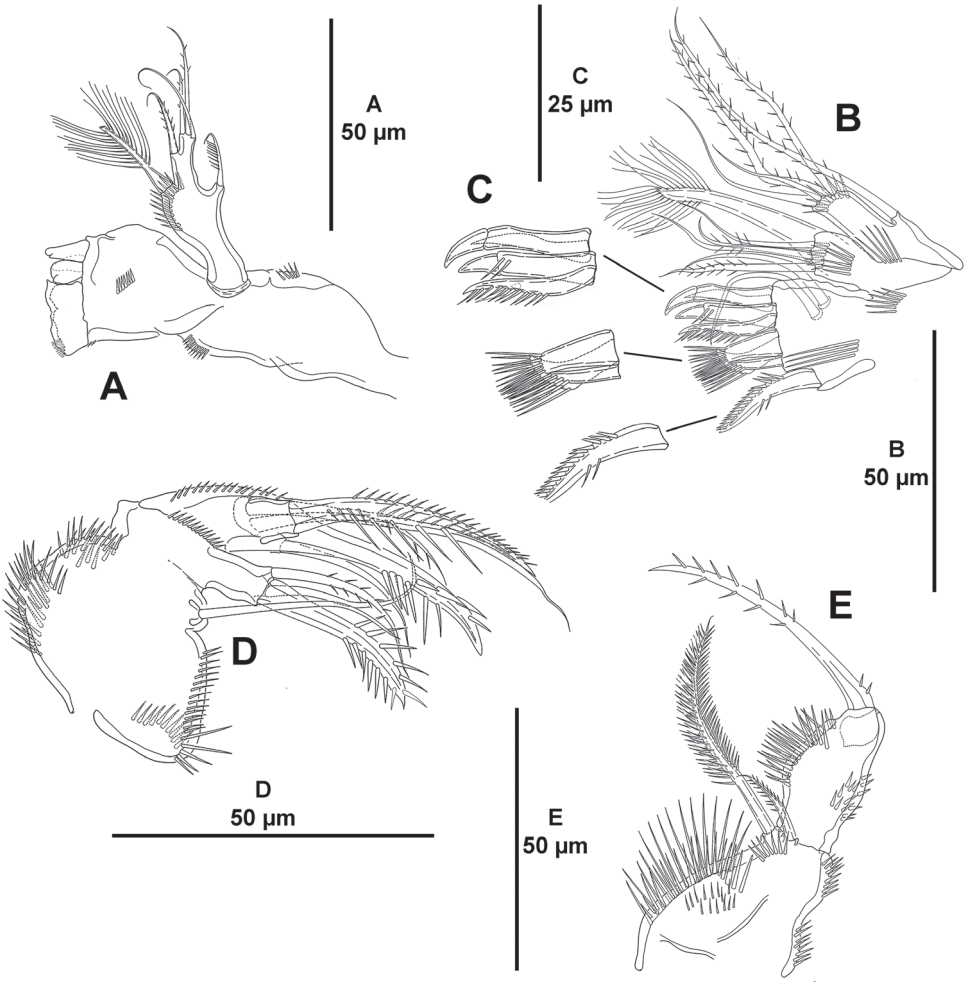

Figure 5. *Mesocletodes brevisetosus* sp. n., female holotype. **A** mandible **B** maxillule **C** distal armature of the arthrite of the maxillule **D** maxilla **E** maxilliped.

Maxilliped (Fig. 5E) subchelate, strong. Syncoxa with inner long and outer small spinules as shown, with two setae, one of which strong and longer than syncoxa. Basis unarmed, with inner long and outer small spinules. Endopod uni-segmented, fused to strong spinulose claw.

P1 (Fig. 6A). Coxa with outer and inner slender spinules, with stronger spinules medially. Basis seemingly without spinular ornamentation, with outer and inner spine, the former longer. Exopod tri-segmented; EXP1 as long as following segments combined; with spinules as depicted; EXP1 and EXP2 without inner armature; EXP3 with four elements, of which outer and apical elements with STE, inner seta slender and reduced. Endopod bi-segmented, small; ENP1 with few inner spinules and one inner seta; ENP2 with few inner spinules, two apical and one outer seta.

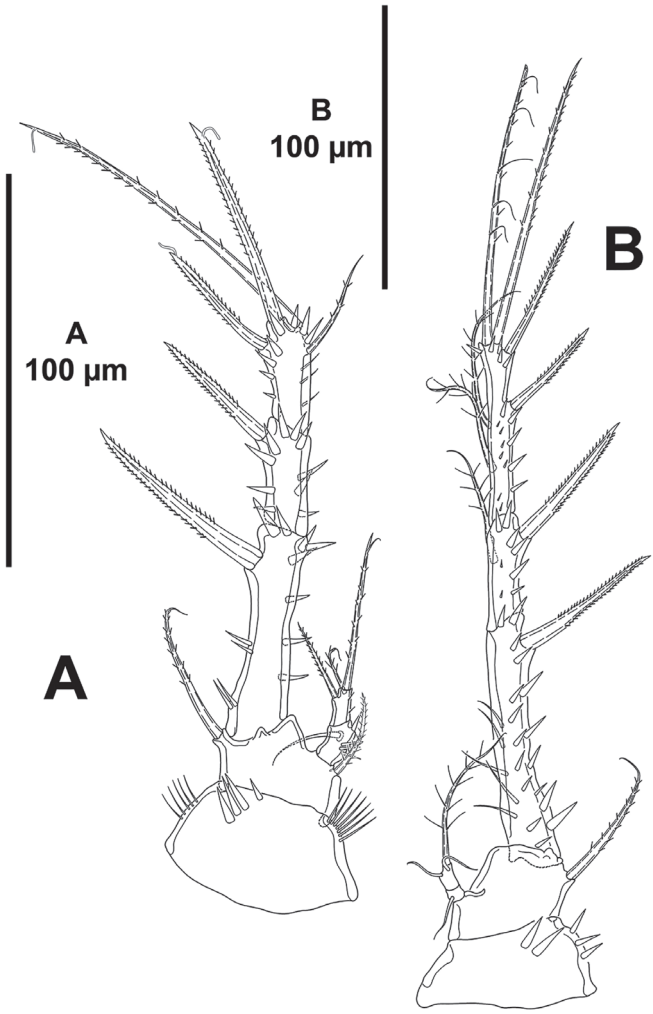

Figure 6. *Mesocletodes brevisetosus* sp. n., female holotype. **A** P1, anterior **B** P2, anterior.

P2–P4 (Figs 6B, 7A–B). Praecoxa as in P3 and P4, small (see Fig. 7A, B). Coxa with few strong spinules close to outer distal corner. Basis seemingly without spinules (but two small spinules were observed at the base of the basal setophore of P3); outer basal seta of P2 spiniform, without setophore (Fig. 6B), of P3 and P4 setiform, long, with well-developed setophore (Fig. 7A, B). Exopod tri-segmented; EXP1 and EXP3 elongated, subequal in length, EXP2 shortest; EXP1 without, EXP2 with inner seta; P2 EXP3 and P3 EXP3 with two outer spines, two apical elements, and two inner setae (Fig. 6A–B), P4 EXP3 with two outer spines, two apical elements and one inner seta (Fig. 7B). Endopod bi-segmented; first segment with one inner seta; second segment of P2 with one inner and two apical setae (Fig. 6B), of P3 (Fig. 7A) and P4 (Fig. 7B) with one inner and one apical seta.

P5 (Fig. 8A–C) with some spinules on baseoendopodal setophore. Endopodal lobe poorly developed, with two setae (innermost lost during dissection), of which outermost very small (Fig. 8A, B). Exopod distinct, long, slender, 7.8 times as long as wide (maximum width measured at its base), with spinules as figured, with three outer, one apical and one inner seta, and one subdistal tube pore (arrowed in Fig. 8C).

Armature formula as follows:

	EXP	ENP
P1	0.0.121	1.021
P2	0.1.222	1.120
P3	0.1.222	1.020
P4	0.1.122	1.020
P5	320+subdistal tube pore	2

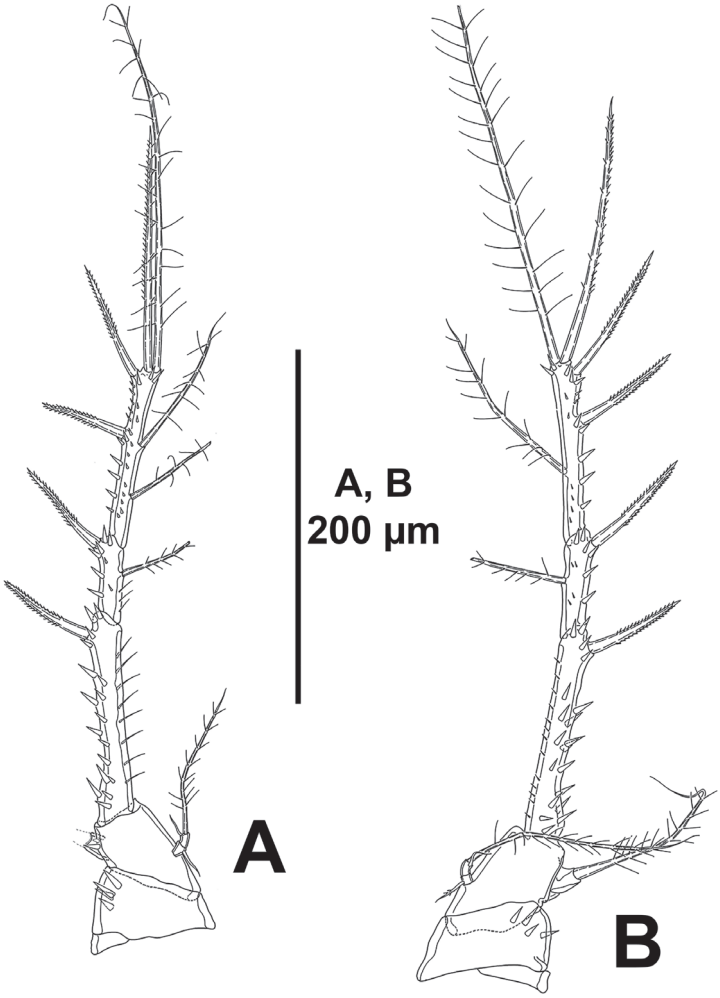

Figure 7. *Mesocletodes brevisetosus* sp. n., female holotype. **A** P3, anterior **B** P4, anterior.

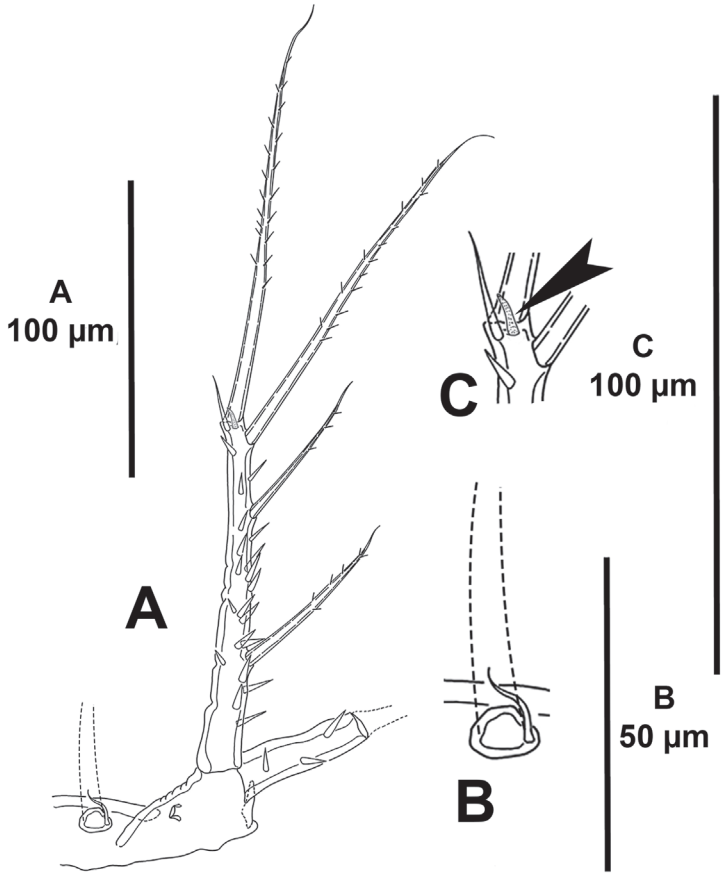

Figure 8. *Mesocletodes brevisetosus* sp. n., female holotype. **A** P5, anterior **B** endopodal setae **C** distal part of exopod, showing apical tube-pore.

Description of male. Unknown.

Etymology. The specific epithet is derived from the Latin adjective *brevis*, meaning short, and the Latin noun *seta*, meaning hair, and refers to the reduced innermost seta of the female P5 EXP. The name is a noun in the nominative singular.

Remarks. *Mesocletodes brevisetosus* sp. n. seems to be more closely related to *M. dorsiprocessus* than to *M. bicornis*, both from the Angola Basin, by the combination of several characters: 1) serrated posterior margin of the cephalothorax and P2-bearing somite to penultimate urosomite (serrated posterior margin on cephalothorax, P2-bearing somite to first half of genital double-somite in *M. bicornis*), 2) relative length of the setae of the antennary exopod (subequal in *M. dorsiprocessus* and in *M. brevisetosus* sp. n., but one of them noticeably reduced in *M. bicornis*), 3) presence of an inner seta on P2–P4 ENP1 in *M. dorsiprocessus* and *M. brevisetosus* sp. n., but absent in *M. bicornis*, 4) number of setal complements on P1–P4 ENP2 (3, 3, 2, 2 in *M. dorsiprocessus* and *M. brevisetosus* sp. n., but 2, 4, 4, 4 in *M. bicornis*), 5) number of setae on the female P5 endopodal lobe (two setae in *M. dorsiprocessus* and *M. brevisetosus* sp. n.,

but three in *M. bicornis*), 6) position of the inner seta of the female P5 EXP (issuing subapically in *M. dorsiprocessus* and *M. brevisetosus* sp. n., but situated in distal third of inner margin of ramus in *M. bicornis*), and 7) degree of development of the endopodal lobe of the female P5 (without any trace of endopodal lobe in *M. dorsiprocessus* and *M. brevisetosus* sp. n., but endopodal lobe discernible in *M. bicornis*). *Mesocletodes dorsiprocessus* and *M. brevisetosus* sp. n. can be separated based on the number of setae on the endopodal lobe of the mandibular palp (one seta in *M. dorsiprocessus*, but four elements in *M. brevisetosus* sp. n.), the relative length of the innermost seta of the female P5 EXP (well-developed in *M. dorsiprocessus*, but very reduced in *M. brevisetosus* sp. n.), appearance of the cuticula (plain in *M. dorsiprocessus*, but reticulated in *M. brevisetosus* sp. n.), armature complement of the seventh antennular segment (six in *M. dorsiprocessus*, but four in *M. brevisetosus* sp. n.), and number of setae on the syncoxa of the maxilliped (one in *M. dorsiprocessus*, but two in *M. brevisetosus* sp. n.).

Mesocletodes simplex sp. n.

http://zoobank.org/536B7890-8AB1-4FA5-884C-34A5C5895D5E

Material examined. One dissected female holotype mounted onto five slides (ICML-EMUCOP-130207-01); Talud X cruise; February 13, 2007; coll. S. Gómez.

Type locality. Southern Trough of Guaymas Basin, Gulf of California, México (27°01'N, 110°53'04"W), 1642 m depth (see Fig. 1); coll. S. Gómez.

Diagnosis (based on the female only). Body subcylindrical. Cephalothorax with dorsal cuticular process curved posteriorly. Genital somite and third urosomite incompletely fused dorsolaterally. Anal somite quadrate from dorsal view, with simple dorsal cuticular process curved posteriorly. Caudal rami subcylindrical, 2.5 times as long as wide, with seven setae. Antennule octa-segmented, second segment with protrusion bearing a long seta, but not as pronounced as in other species of the genus. Antenna with basis and uni-segmented exopod. Gnathobase of mandible with grinding face, and tri-segmented palp. Maxillary syncoxa with two endites, proximal endite with one, distal endite with three elements; endopod uni-segmented, with two setae. Syncoxa of maxilliped with one seta. P4 ENP2 with four setae. Outer setae of the P5 EXP issuing close to each other.

Description of female. *Body*: total length 725 µm measured from anterior margin of rostrum to posterior margin of caudal rami, subcylindrical, tapering slightly posteriorly, without clear demarcation between prosome and urosome (Fig. 9A, B). Rostrum fused to cephalothorax, with two sensilla. Cephalothorax 0.24 times as long as entire body length; ornamented with sensilla and spinular patches as shown; with dorsal cuticular process curved posteriorly, the latter as in Fig. 9C. P2–P5-bearing somites with sensilla and small spinules along posterior margin, with minute spinules laterally. Genital somite and third urosomite (genital double-somite) incompletely fused dorsolaterally (Fig. 9A, B) (i.e. posterior margin of genital somite indicated by suture with transverse row of spinules and few sensilla dorsolaterally), completely fused ven-

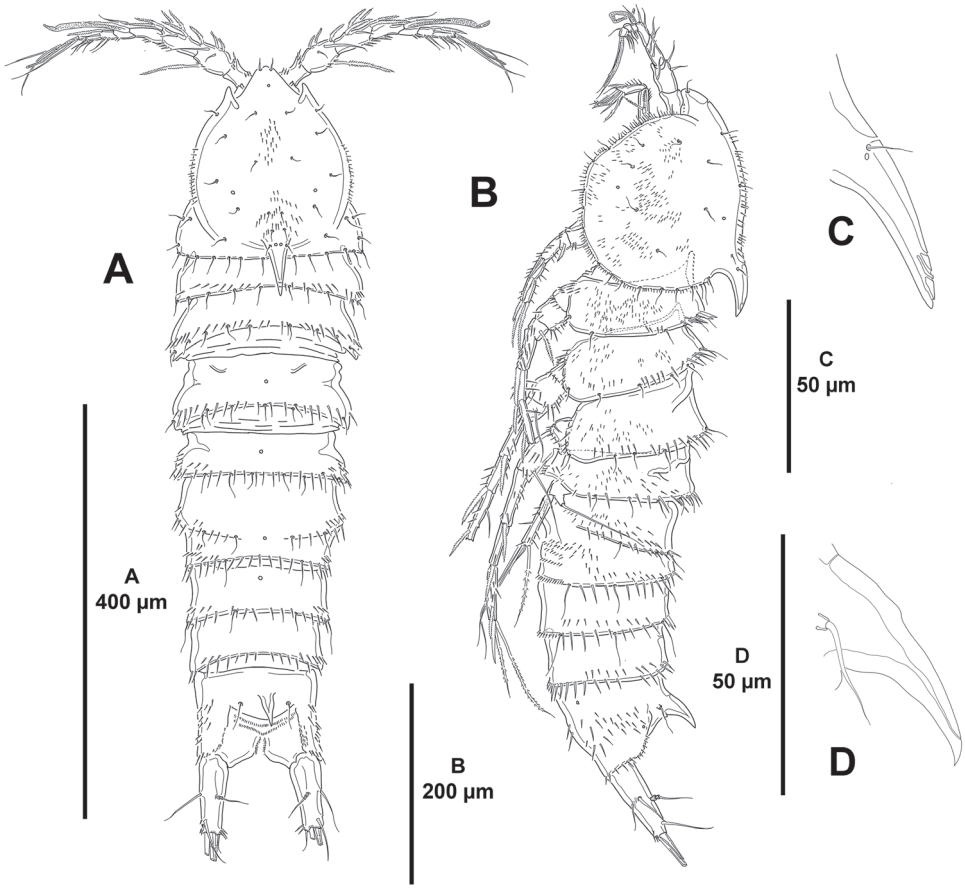

Figure 9. *Mesocletodes simplex* sp. n., female holotype. **A** habitus, dorsal **B** habitus, lateral **C** dorsal cuticular process of cephalothorax showing cuticular ornamentation **D** dorsal cuticular process of anal somite showing cuticular ornamentation.

trally (Fig. 10A); first half of genital double-somite with medial genital field proximally (Fig. 10A), with few minute spinules close to lateral margins, second half with more dense spinular patches ventrally as shown and with minute spinules along posterior margin between pair of long ventral sensilla. Fourth urosomite with short row of small spinules laterally (Fig. 9B), ventrally with transverse row of larger spinules along posterior margin interrupted by longitudinal row of minute spinules along posterior margin flanked by two long sensilla (Fig. 10A). Fifth urosomite as preceding somite except for shorter transverse row of minute spinules ventrally, without sensilla (Fig. 10A).

Anal somite (Figs 9A, B, 10A, 11A, B) quadrate from dorsal view, nearly as long as two preceding somites combined; posterior margin cleft medially; anal operculum rounded and smooth, flanked by two sensilla; with dorsal (Figs 9A, 11A), lateral (Figs 9B, 11B) and ventral (Fig. 10A) spinules as figured; with simple dorsal cuticular process curved posteriorly (Figs 9A, B, D, 11A, B), the latter with a tiny aperture (Figs 9D, 11B).

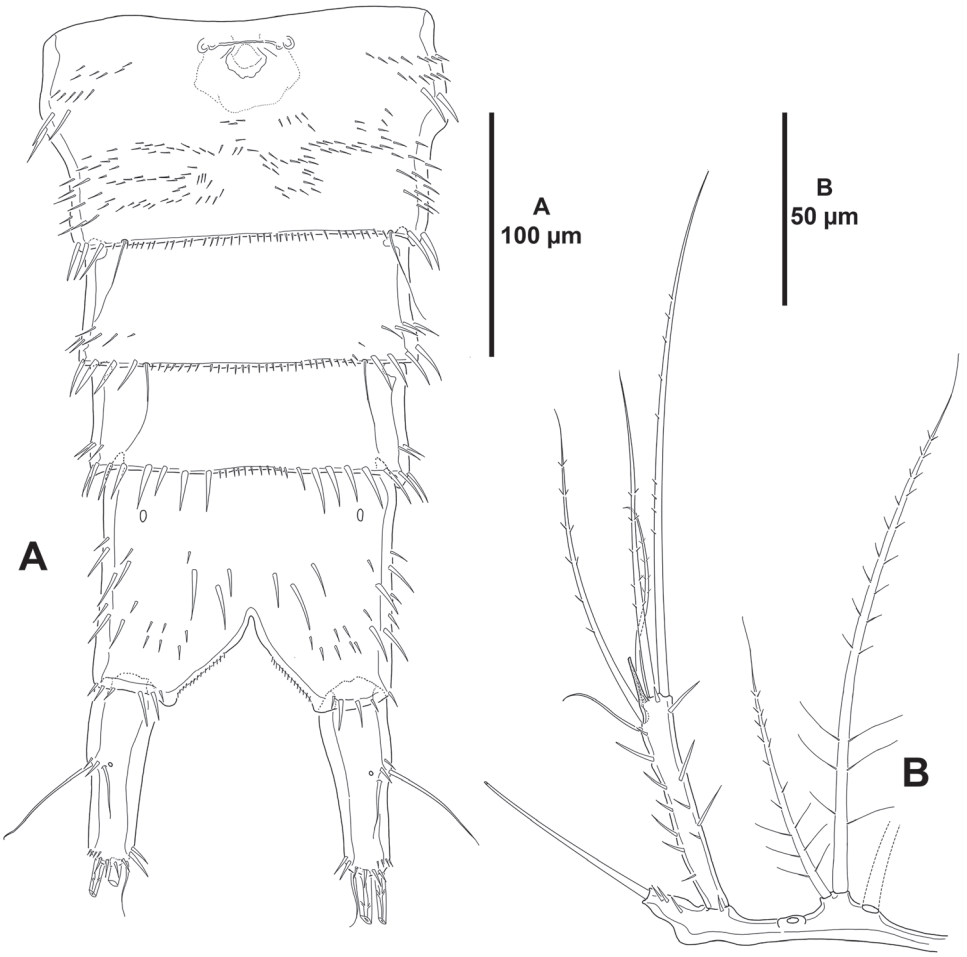

Figure 10. *Mesocletodes simplex* sp. n., female holotype. **A** urosome, ventral, P5-bearing somite omitted **B** P5, anterior.

Caudal rami (Figs 9A, B, 10A, 11A, B) subcylindrical, slightly tapering posteriorly, nearly as long as anal somite and 2.5 times as long as wide; with seven setae as follows: setae I and II located midway lateral margin, the former ventral to the latter and shorter; seta III as long as seta II, arising close to outer distal corner; setae IV and V situated distally; seta VI smallest, arising at inner distal corner; dorsal seta VII bi-articulated, somewhat shorter than seta II.

Antennule (Fig. 12A) octa-segmented; first segment with one proximal and one subdistal row of spinules; second and third segments with longitudinal short row of strong spinules; second segment somewhat globose and with protrusion bearing a long seta not as pronounced as in other species of the genus (indicated by an asterisk in Fig. 12A); third segment two times as long as wide; fourth segment with one, fifth segment without spinules; sixth segment with short transverse row of smaller spinules;

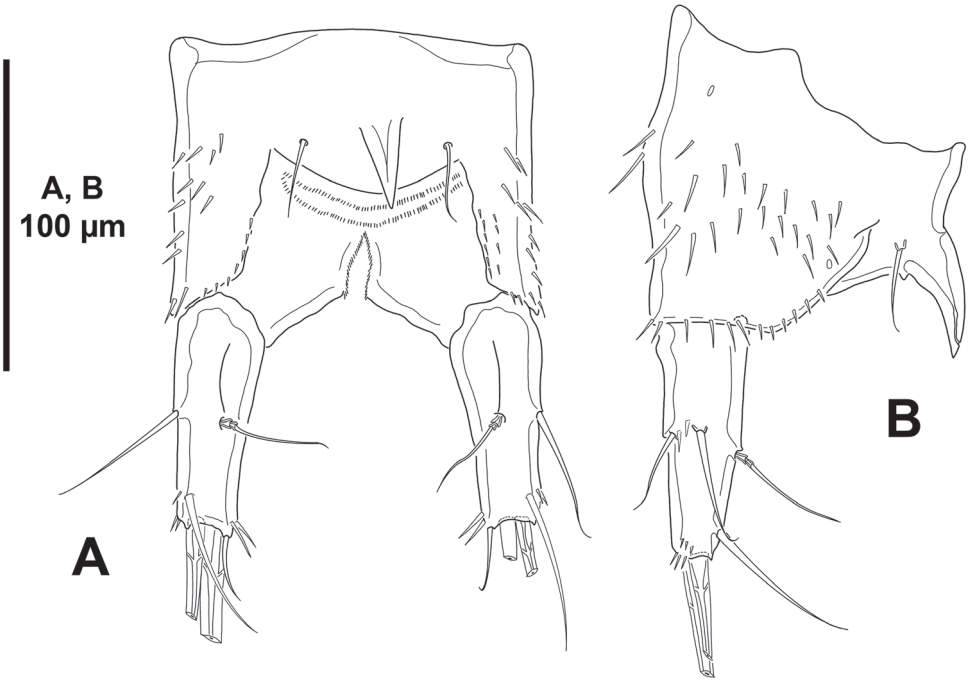

Figure 11. *Mesocletodes simplex* sp. n., female holotype. **A** anal somite, dorsal **B** anal somite, lateral.

seventh segment with one, eighth segment without spinules. Armature formula as follows: 1-[0], 2-[5sp+3se], 3-[5sp+1se], 4-[1sp+(1sp+ae)], 5-[1sp], 6[2sp], 7-[1sp+3se], 8-[5se+acro]. Spinose, spiniform elements (sp) with STE.

Antenna (Fig. 12B). Coxa small, with few strong spinules. Basis with inner spinules. Exopod uni-segmented, with two setae. Endopod bi-segmented; first segment with strong inner spinules; second segment with some outer small spinules, inner margin with strong spinules and two thin lateral spines with STE, and six apical elements (one inner strong spinulose spine, two geniculate spinulose and one bare element, and two outer elements fused basally of which innermost longer and with STE).

Mandible (Fig. 12C, D) with robust coxa. Gnathobase with row of surface spinules, three distal single teeth as shown and several fused tooth-like elements forming a broad grinding face (Fig. 12C). Palp tri-segmented (Fig. 12D); basis with medial and distal small spinules, with one strong seta; exopod uni-segmented, small, with two setae of which innermost smaller; endopod quadrate, with six setae as shown.

Maxillule (Fig. 13A, B, C). Praecoxal arthrite with some very long spinules, two surface setae, and seven apical spines as shown (Fig. 13A). Coxa with five elements, strongest fused to coxa (Fig. 13B). Basis with seven setae (Fig. 13C).

Maxilla (Fig. 13D). Syncoxa with slender outer spinules, and with comparatively stronger spinules close to allobasis; with two endites; proximal endite with one slender seta, distal endite with one strong spinulose element and two slender smooth setae. Allobasis with few outer spinules, with one strong spinulose spine fused to

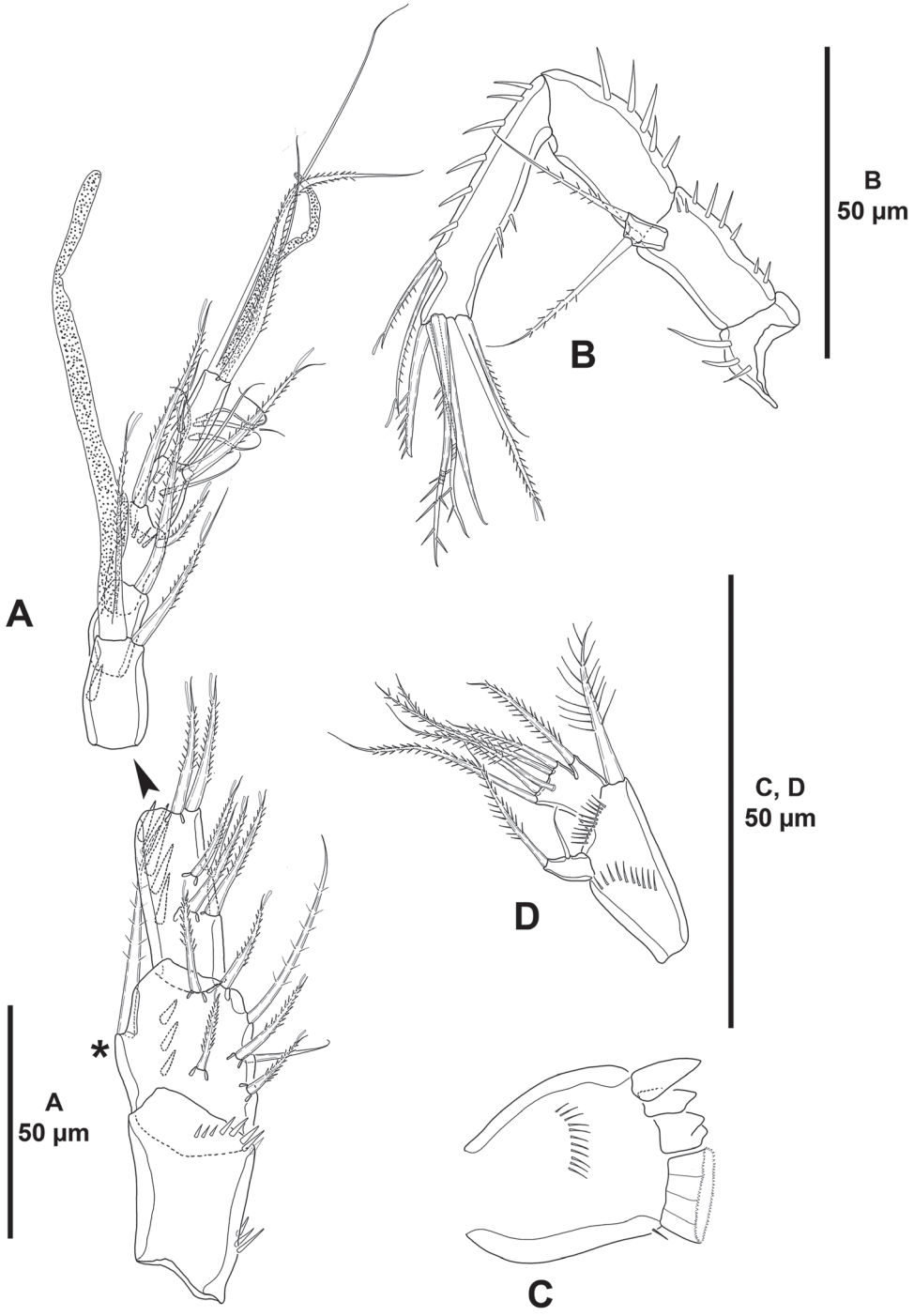

Figure 12. *Mesocletodes simplex* sp. n., female holotype. **A** antennule, protrusion on second segment bearing a strong seta indicated by an asterisk **B** antenna **C** mandibular gnathobase **D** mandibular palp.

allobasis, one slender seta and one spinulose spine. Endopod uni-segmented, very small; with two setae.

Maxilliped (Fig. 13E) subchelate, strong. Syncoxa with one spinulose strong seta slightly longer than basis; the latter with outer and inner spinules as shown. Endopod uni-segmented, fused to strong spinulose claw.

P1 (Fig. 14A). Coxa with sets of outer, medial, and inner strong spinules, and with outer long slender spinules. Basis with strong spinules at base of outer and inner spine. Exopod tri-segmented; exopodal segments subequal in length, with spinules as depicted; EXP1 and EXP2 without inner armature; EXP3 with four elements, of which two outermost spines with STE, innermost slender and reduced. Endopod bi-segmented, not reaching tip of EXP1; ENP1 with few outer spinules and one inner seta, shorter than ENP2; the latter with few outer spinules and three elements.

P2–P4 (Figs 14B, 15A, B). Praecoxa as in P4 (see Fig. 15B), small, with transverse row of small spinules close to coxa. Coxa with small spinules close to basis, and with stronger outer spinules on anterior face and some medial strong spinules on posterior face. Basis with strong spinules at base of outer element, between rami, and at base of endopod, and with slender long inner spinules; basis of P2 (Fig. 14B) with outer spiniform element, of P3 and P4 (Fig. 15A, B) with outer slender bare seta. Exopod tri-segmented; segments slender and elongate, ornamented as shown; EXP1 without, EXP2 with inner seta; P2 EXP3 and P3 EXP3 with two outer spines, two apical elements, and two inner setae (Figs 14B, 15A), P4 EXP3 (Fig. 15B) with two outer spines, two apical elements and one inner seta.

P5 (Fig. 10B) with some spinules on baseoendopodal setophore; with long outer basal seta. Endopodal lobe poorly developed, with three setae (innermost lost during dissection), of which outermost and medial elements close to each other, innermost separated from the former two elements. Exopod distinct, long, slender, 6.7 times as long as wide (maximum width at distal part), with outer and inner spinules as figured, with three outer and two apical setae, and one outer distal tube pore.

Armature formula as follows:

	EXP	**ENP**
P1	0.0.121	1.120
P2	0.1.222	1.121
P3	0.1.222	1.121
P4	0.1.122	1.121
P5	320+distal tube pore	3

Description of male. Unknown.

Etymology. The specific epithet is derived from the Latin adjective *simplex*, meaning simple, and refers to the simple (not bifurcated) dorsal process on the anal somite. The name is an adjective in the nominative singular.

Remarks. *Mesocletodes simplex* sp. n. is attributed here to Bodin's (1968) *abyssicola* group. The dorsal process on the anal somite is simple in most species within this genus, but it is bifurcated in *M. brevifurca*, *M. katharinae* Soyer, 1964, *M. meteorensis*,

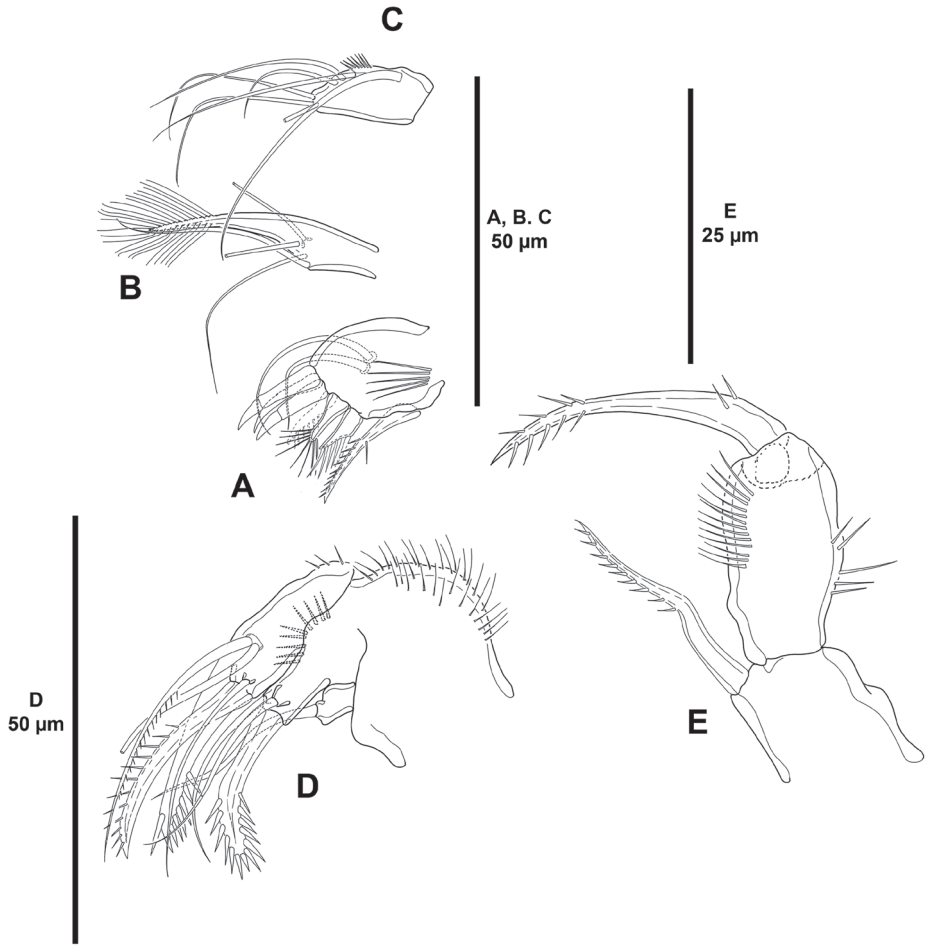

Figure 13. *Mesocletodes simplex* sp. n., female holotype. **A** praecoxal arthrite of maxillule **B** coxal endite of maxillule **C** basis of maxillule **D** maxilla **E** maxilliped.

M. monensis, and *M. soyeri* Bodin, 1968. The appendages of the species of this group exhibit an amalgam of conditions, most of which are shared with some species of the *inermis* group *sensu* Bodin (1968). The antenna of most species of Bodin's (1968) *abyssicola* group possesses a basis, but the condition of the antenna is inconclusive for *M. bathybia* Por, 1964a and *M. brevifurca*. Also, the antennary exopod of most species of this group is uni-segmented with two setae, but *M. abyssicola* seems to be unique among these species in that it is represented by one seta only (the condition of the antennary exopod of *M. monensis* is inconclusive, and the exopod of *M. bathybia* remains unknown). The mandibular palp of most species of this group is bi-segmented (exopod incorporated to basis, endopod uni-segmented), but uniramous in *M. soyeri*, *M. bathybia* and *M. katharinae*, and tri-segmented (with basis, uni-segmented exopod, and uni-segmented endopod) in *M. simplex* sp. n. The palp of the maxillule is

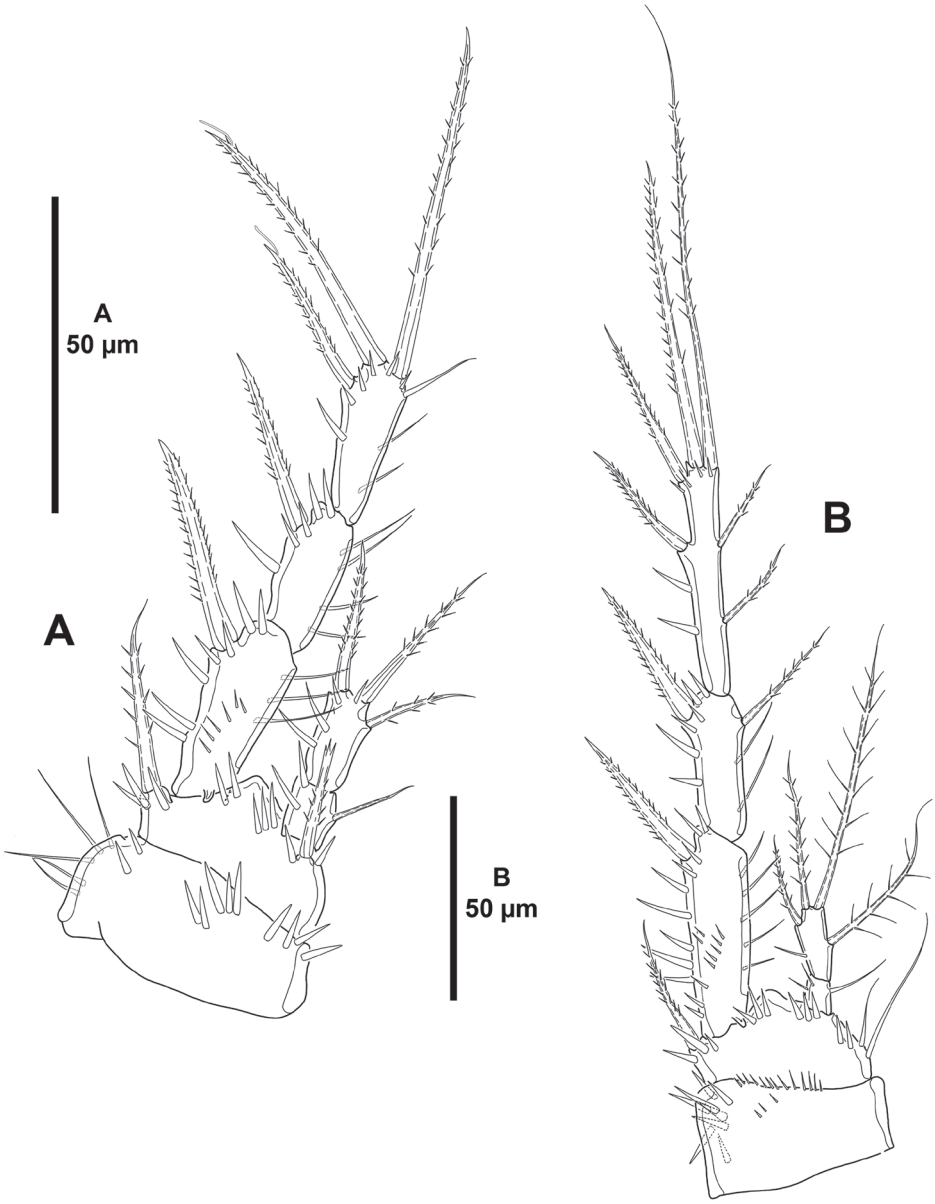

Figure 14. *Mesocletodes simplex* sp. n., female holotype. **A** P1, anterior **B** P2, anterior.

uni-segmented in *M. abyssicola*, *M. katharinae*, *M. meteorensis*, *M. robustus* Por, 1965, *M. simplex* sp. n., and *M. soyeri*. Also, the proximal and distal endites of the maxilla possess one and three setae, respectively, in *M. katharinae*, *M. meteorensis*, *M. simplex* sp. n., and *M. robustus*, but two setae only in *M. soyeri*. The syncoxa of the maxilliped possesses two setae in most species, but one seta only in *M. brevifurca*, *M. simplex* sp. n., and *M. abyssicola* (the maxilliped of *M. monensis*, *M. bathybia*, and *M. dolichurus*

Smirnov, 1946 remains unknown). The P1 ENP is uni-segmented with three setae in *M. abyssicola*, *M. robustus*, and *M. soyeri*, but uni-segmented with one seta only in *M. bathybia*. A uni-segmented P1 ENP is present also in the species of Bodin's (1968) *inermis* group (e.g. *M. makarovi* Smirnov, 1946, *M. guillei* Soyer, 1964, *M. inermis*, *M. trisetosa* Schriever, 1983, and *M. quadrispinosa*). The P1 ENP is bi-segmented with an armature complement of 0,3 in the first and second segment, respectively, in *M. brevifurca*, *M. dolichurus* and *M. katharinae*, and 1,3 in *M. meteorensis* and *M. simplex* sp. n. The P2–P4 ENP is uni-segmented in *M. monensis*, *M. dolichurus*, *M. robustus* and *M. soyeri*, and probably also in *M. abyssicola* and *M. bathybia*, but bi-segmented in the other species of the group of which, only *M. katharinae* lack the inner seta on P2 ENP1. The female P5 EXP and endopodal lobe possess five and three setae, respectively, in all the species of this group, except for *M. abyssicola* and *M. soyeri* (EXP with four, endopodal lobe with two setae). Similar armature complements and structure of P1–P5 is present in several species of the *inermis* group *sensu* Bodin (1968). The caudal rami are more than 10 times as long as wide in most species, but 2.5 to 3 times as long as wide in *M. brevifurca*, *M. meteorensis*, and *M. simplex* sp. n., and 6 times as long as wide in *M. katharinae*. *Mesocletodes simplex* sp. n. shares the relatively short caudal rami with *M. brevifurca* and *M. meteorensis*. The former can be easily separated from the latter two species by the dorsal process on the anal somite (simple in *M. simplex* sp. n., but bifurcated in the other two species). Additionally, *M. simplex* sp. n. seems to be more closely related to *M. meteorensis* than to *M. brevifurca* by the relative position of the outer setae of the female P5 EXP (both setae separated by a wide gap in *M. brevifurca*, but both setae issuing close one of each other in *M. simplex* sp. n. and *M. meteorensis*), and number of setae on the P4 ENP2 (three setae in *M. brevifurca*, but four setae in *M. simplex* sp. n. and *M. meteorensis*).

Mesocletodes unisetosus sp. n.

http://zoobank.org/CB4BE678-C76F-4D35-A3BD-300B3324F960

Material examined. One male holotype preserved in alcohol (ICML-EMU-COP-270800-04), one male paratype preserved in alcohol (ICML-EMU-COP-270800-03), and one male paratype dissected and mounted onto seven slides (ICML-EMUCOP-270800-05); Talud IV cruise; August 27, 2000; coll. S. Gómez.

Type locality. Southern Carmen Basin, Gulf of California, México (25°54.7'N, 110°11'W), 2018 m depth (see Fig. 1); coll. S. Gómez.

Dignosis (based on the male only). Body subcylindrical. Cephalothorax, free prosomites and urosomites, except for anal somite, with posterior margin serrated. Cephalothorax dorsoventrally flattened, without dorsal cuticular process. Anal somite quadrate, with dorsal cuticular process. Caudal rami 14 times as long as wide, with seven elements. Antennule octa-segmented, haplocer, second segment with strong protrusion bearing one strong seta and two strong spinules. Antenna with basis, without exopod. Mandibles, maxillules, maxillae and maxillipeds

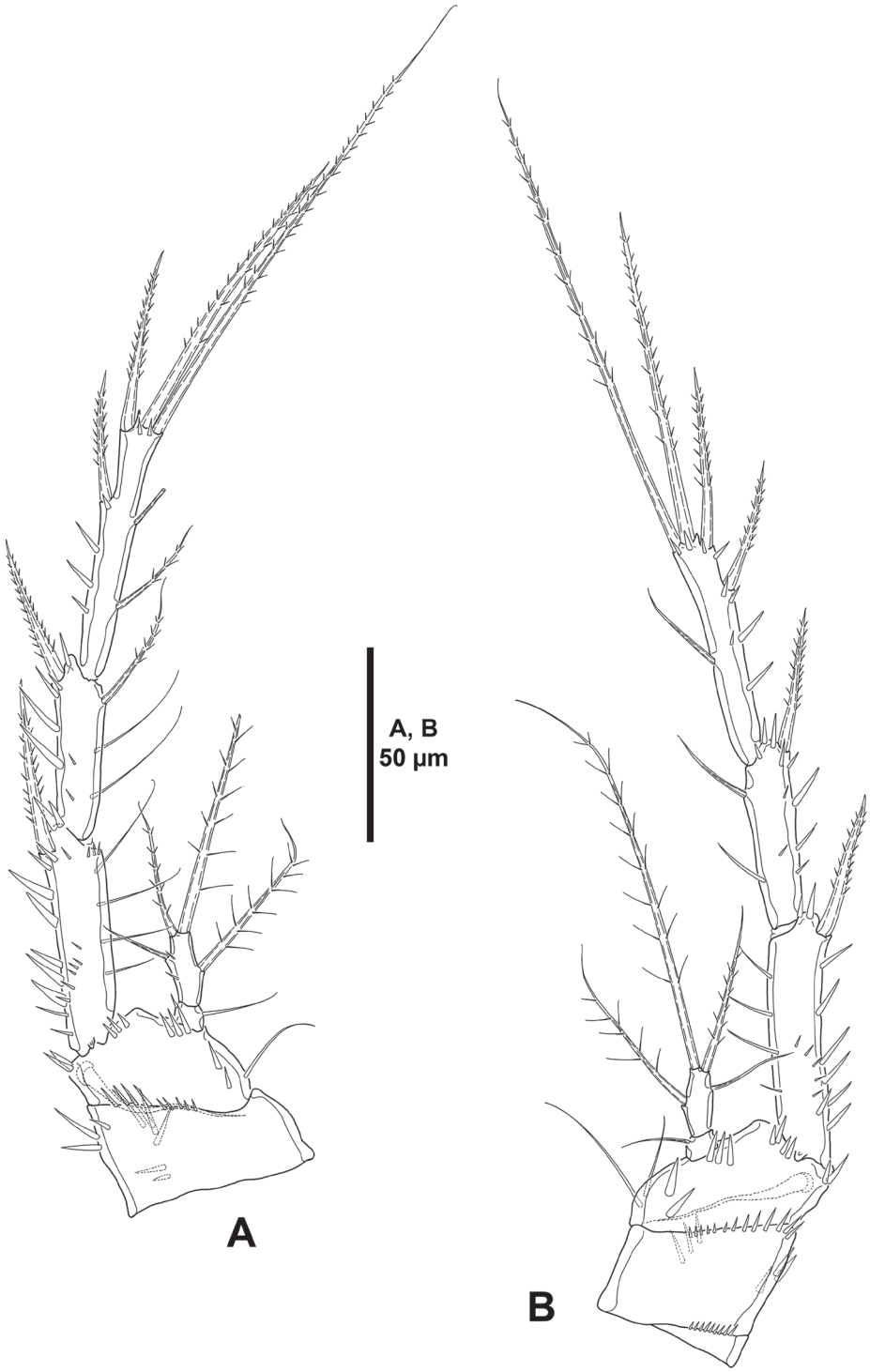

Figure 15. *Mesocletodes simplex* sp. n., female holotype. **A** P3, anterior **B** P4, anterior.

strongly atrophied, nontraceable. P2 and P3 ENP1 with one inner seta. P5 endopodal lobe with one seta.

Description of male. *Body*: total length ranging from 655 µm to 695 µm (mean= 670 µm; n= 3) measured from anterior margin of rostrum to posterior margin of caudal rami, subcylindrical (Fig. 16A, B), tapering slightly posteriorly, without clear demarcation between prosome and urosome. Cephalothorax, free prosomites and urosomites, except for anal somite, with posterior margin serrated (Fig. 16A, B), of cephalothorax and free prosomites less pronounced, of urosomites comparatively coarser; lateral margin of cephalothorax plain (Fig. 16B). Rostrum fused to cephalothorax. The latter dorsoventrally flattened, without dorsal cuticular process, with few sensilla on surface and along posterior margin. P2–P4-bearing somites without spinular ornamentation, with sensilla along posterior margins. P5-bearing somite with four medial spinules dorsally (Fig. 16A) and some spinules laterally (Fig. 16B). Genital somite as preceding somite dorsally (Fig. 16A) and laterally (Fig. 16B); ventrally (Fig. 17A) without spinular ornamentation and with two sensilla, sixth leg represented by asymmetrical plate. Third and fourth urosomites as preceding somites dorsally and laterally (Fig. 16A, B), ventrally (Fig. 17A) with serrated posterior margin and long posterior spinules. Fifth urosomite with two medial spinules dorsally (Fig. 16A), laterally (Fig. 16B) and ventrally (Fig. 17A) as two preceding somites, without sensilla.

Anal somite (Figs 16A, B, 17A) quadrate; dorsally without (Fig. 16A), laterally (Fig. 16B) and ventrally (Fig. 17A) with spinules as shown; with dorsal cuticular process flanked by pair of sensilla (Fig. 16A, B).

Caudal rami (Figs 16A, B, 17B–F) slender, exceedingly elongated, 14 times as long as wide (maximum width measured at the base of ramus), as long as urosome, almost straight in lateral view (Figs 16B, 17D), covered with small spinules; with seven elements as follows: seta I and II issuing laterally in distal part of first third of ramus, the former ventral to and smaller than the latter (Fig. 17D, F); seta III situated subdistally on dorsal surface (Fig. 17B–E); seta IV reduced, fused basally to seta V (Fig. 17B, C, E), the latter longest; seta VI reduced, somewhat smaller than seta IV, arising at distal inner corner (Fig. 17B, C); dorsal seta VII bi-articulated, situated subdistally on posterior part of first half of ramus (Fig. 17B, D, F); with large outer pore distally (Fig. 17B–E).

Antennule (Fig. 18A) octa-segmented, haplocer; first segment without armature, with some medial and some distal spinules; second segment with strong protrusion bearing one strong element (arrowed in Fig. 18A), with two strong spinules; third and fourth segments smallest; fifth segment somewhat swollen, with aesthetasc fused basally to slender seta, with four spiniform elements, two of which modified; sixth segment with one normal and one modified element; sixth to eight segments elongated, subequal in length. Armature formula as follows: 1-[0], 2-[6sp+2se], 3-[2sp+1se], 4-(1sp), 5-[4sp+ (1se+ae)], 6[1sp+1se], 7-[3se], 8-[2sp+7se+acro]. Spinose, spiniform elements (sp) with STE.

Antenna (Fig. 18B). Basis elongate, with few inner spinules on distal corner. Exopod absent. Endopod bi-segmented; first segment with strong inner spinules, as long as

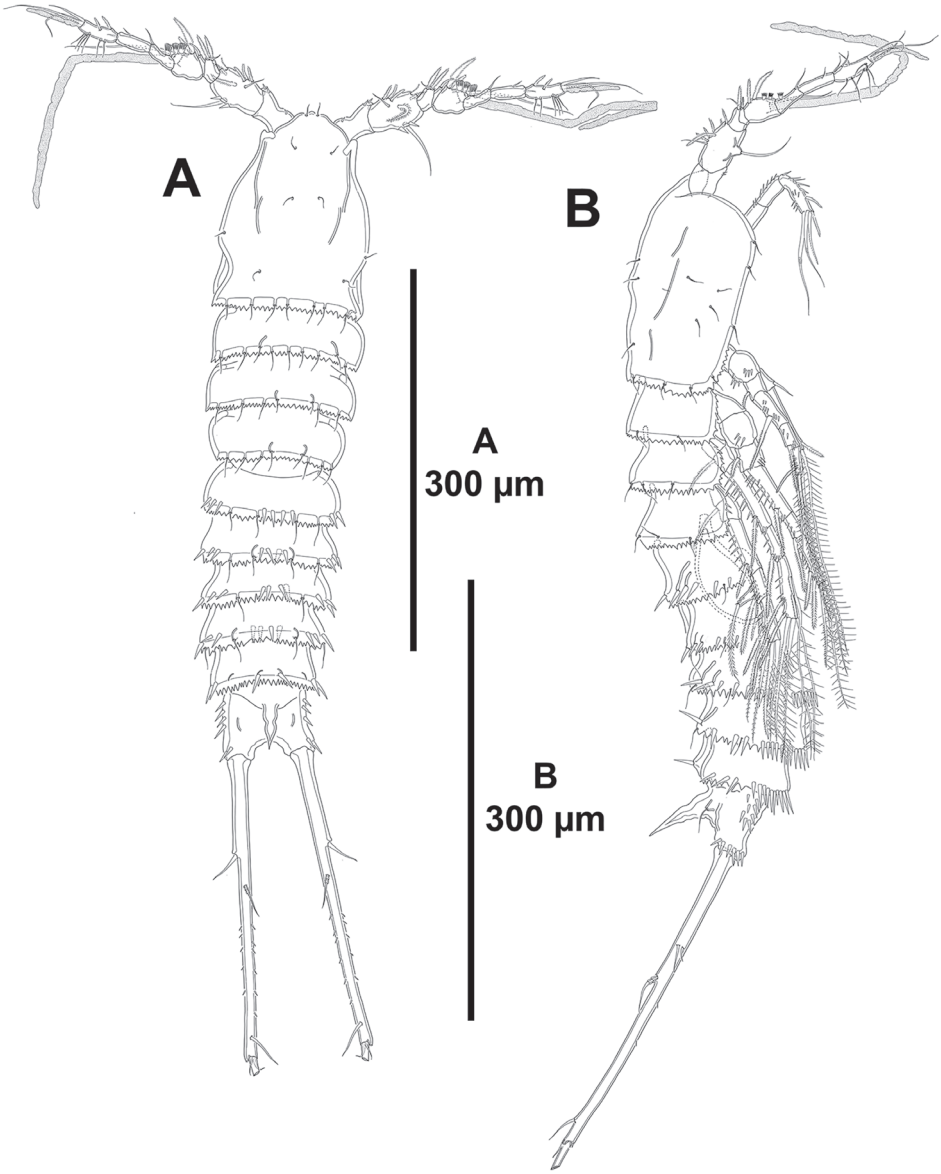

Figure 16. *Mesocletodes unisetosus* sp. n., male holotype. **A** habitus, dorsal **B** habitus, lateral.

basis; second segment with inner strong spinules proximally and medially, with outer spinules on distal half of segment, laterally with one well-developed lateral spine with STE, and one very reduced element (the latter indicated in Fig. 18B, C), and with four apical elements (two spinulose spines, of which innermost smaller, and two geniculate elements).

Mandibles, maxillules, maxillae and maxillipeds strongly atrophied, non-traceable.

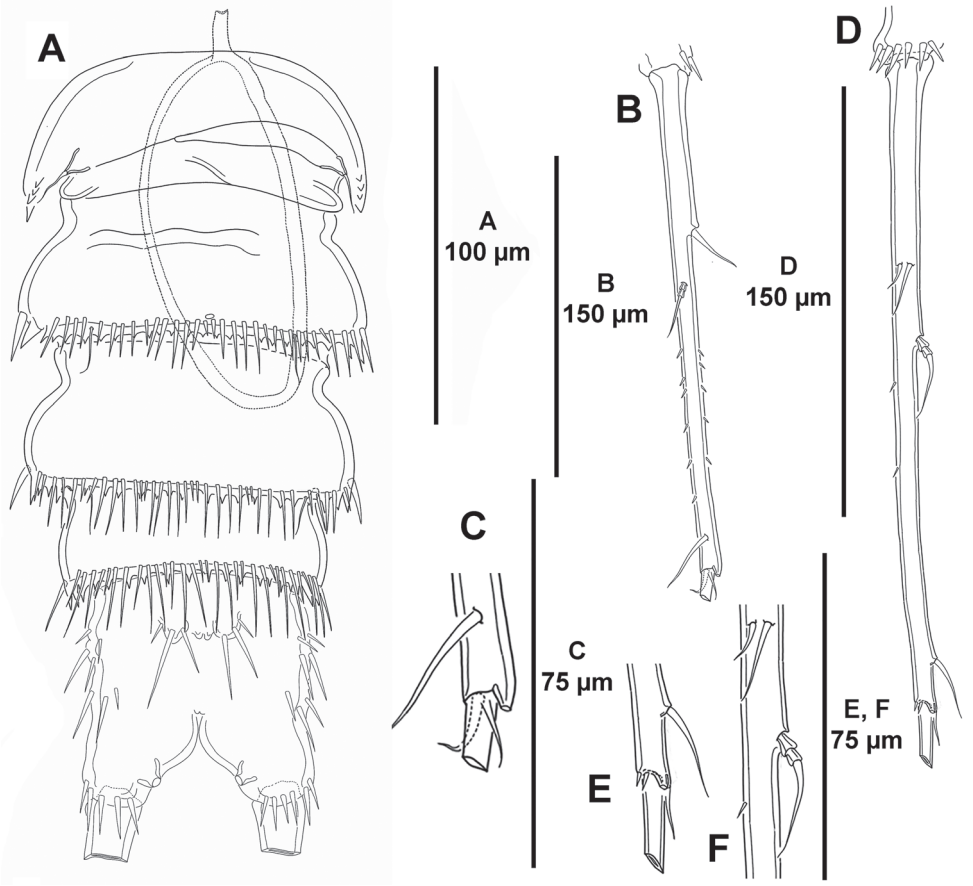

Figure 17. *Mesocletodes unisetosus* sp. n., male paratype. **A** urosome, ventral, P5-bearing somite omitted **B** right caudal ramus, dorsal **C** distal part of right caudal ramus, dorsal, sowing seta III, IV, V, and VI **D** left caudal ramus, lateral **E** distal part of left caudal ramus, lateral, showing seta III, IV and V **F** medial part of left caudal ramus, lateral, showing seta I, II and VII.

P1 (Fig. 19A). Praecoxa small, with spinular row as shown. Coxa with small median and longer outer spinules. Basis with inner spinules, with outer and inner spines, the former somewhat longer. Exopod tri-segmented; EXP1 as long as EXP3, EXP2 shortest; with spinules as depicted; EXP1 and EXP2 without inner armature; EXP3 with four elements, of which outer and apical element with STE. Endopod bi-segmented, reaching proximal fourth of EXP3; ENP1 seemingly without spinular ornamentation, with one inner seta; ENP2 with few inner spinules, with one outer, one apical and one inner element.

P2–P4 (Figs 19B, 20A, B). Praecoxa small, with few spinules as depicted. Coxa with outer spinules. Basis with inner spinules; outer element of P2 spiniform (Fig. 19B), of P3 and P4 setiform (Fig. 20A, B). Exopod tri-segmented; EXP1 and EXP3 elongated, subequal in length, EXP2 shortest; EXP1 without, EXP2 with inner seta; P2 EXP3 and P3

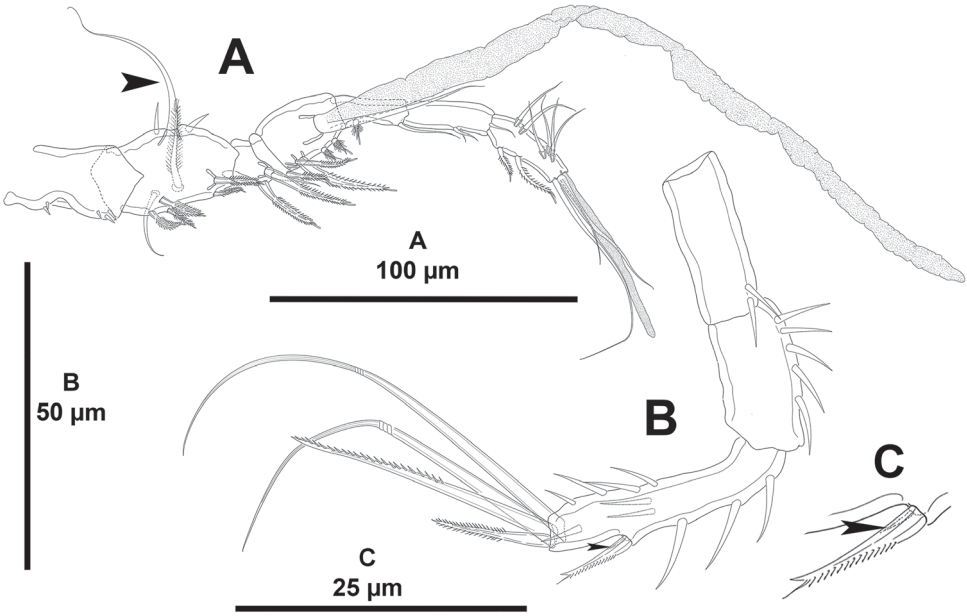

Figure 18. *Mesocletodes unisetosus* sp. n., male paratype. **A** antennule **B** antenna, reduced lateral spine arrowed **C** lateral armature of the antenna, reduced spine arrowed.

EXP3 with two outer spines, two apical elements, and two inner setae (Figs 19B, 20A), of P4 EXP3 with two outer spines, two apical elements and one inner seta (Fig. 20B). Endopod of P2 reaching tip of EXP2 (Fig. 19B), of P3 and P4 reaching slightly beyond EXP2 (Fig. 20A, B); ENP1 with one inner small seta (Figs 19B, 20A, B); ENP2 with three (P2; Fig. 19B) and four (P3 and P4; Fig. 20A, B) setae.

P5 (Fig. 21A, B) with few strong spinules on baseoendopodal setophore. Endopodal lobe poorly developed, with one seta (Fig. 21A). Exopod distinct, long, slender, 3.5 times as long as wide (maximum width measured at its base), with few inner spinules subapically, with two outer, one apical and one inner seta, and one subdistal tube pore.

Armature formula as follows:

	EXP	ENP
P1	0.0.121	1.111
P2	0.1.222	1.120
P3	0.1.222	1.121
P4	0.1.122	1.121
P5	211+subdistal tube pore	1

Description of female. Unknown.

Etymology. The specific epithet is derived from the Latin prefix *ūni*, meaning one, and the Latin noun *seta*, meaning hair, and refers to the presence of one seta only on the endopodal lobe of the male P5. The name is a noun in the nominative singular.

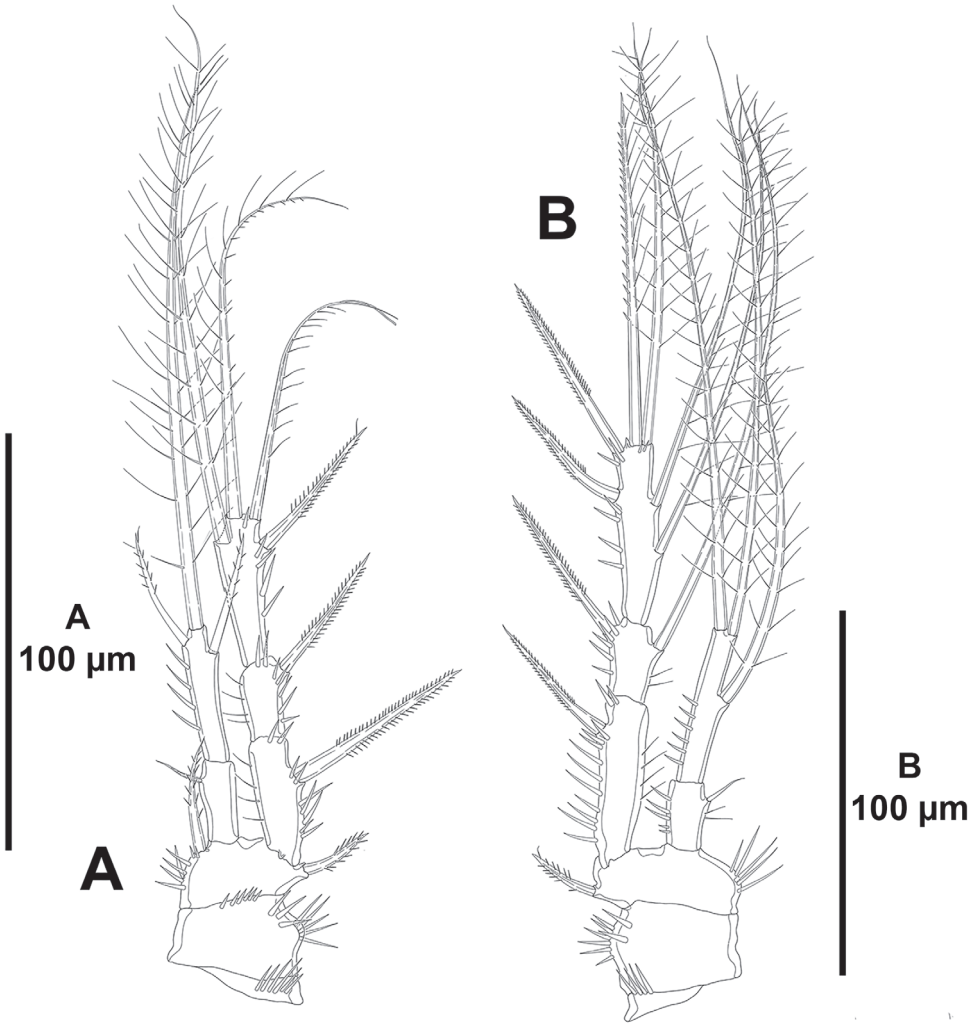

Figure 19. *Mesocletodes unisetosus* sp. n., male paratype. **A** P1, anterior **B** P2, anterior.

Remarks. The only species for which the male is known are *M. angolaensis*, *M. elmari* Menzel, 2011, *M. fladensis*, *M. nudus* and *M. unisetosus* sp. n. Of these, the female is known only for *M. elmari*. These species are attributed to Bodin's (1968) *inermis* group. The males of *M. fladensis*, *M. angolaensis*, and *M. unisetosus* sp. n. possess a dorsal process on the anal somite only and lack mouth parts. *Mesocletodes nudus* and *M. elmari*, lack the dorsal process on the cephalothorax and anal somite, and of these, only the male of *M. nudus* lacks mouth parts. Menzel and George (2009) suggested that the lack of mouth parts in the males of *M. fladensis* and *M. angolaensis*, and consequently in *M. unisetosus* sp. n. and *M. nudus*, might support a monophylum of derived Argestidae (but see below). The same trend has been observed, for example, in the families Aegisthidae (e.g. *Nudivorax* Lee & Huys, 2000, *Scabrantenna* Lee & Huys, 2000, *Andromastax* Conroy-Dalton &

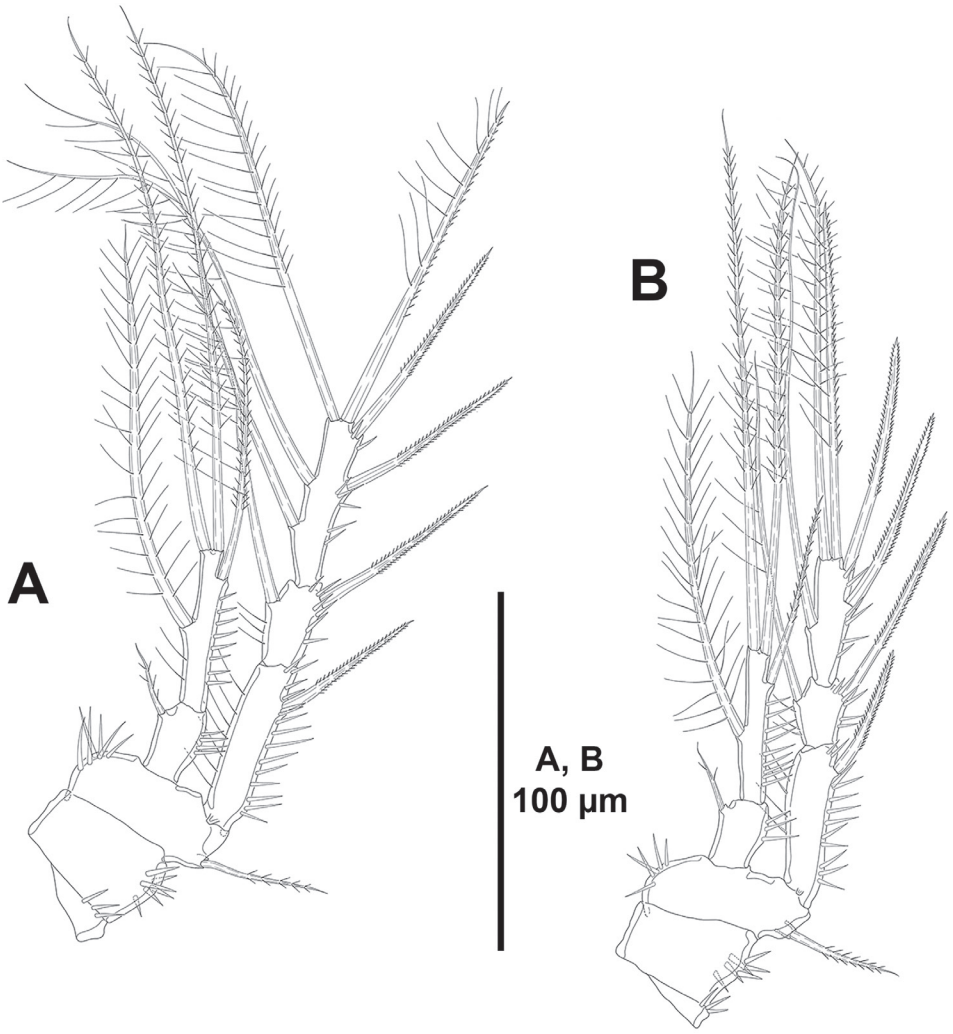

Figure 20. *Mesocletodes unisetosus* sp. n., male paratype. **A** P3, anterior **B** P4, anterior.

Huys, 1999) and Pseudotachidiidae (e.g. Paranannopus Huys, 2009; see Willen 2005). Menzel (2011) suggested that, regardless the developmental stage of the mouth parts (well-developed, strongly reduced or absent), the males of *Mesocletodes* become non-feeding during the last moult as an adaptation to the sparsely populated and oligotrophic deep-sea environments, and could represent a derived character. Also, Menzel (2011) noted that the sexual dimorphism in *M. elmari* (attributed to the *inermis* group), the only species for which both sexes have been described, is expressed, among other characters, in the antennule, P5, P6 and most interestingly, in the armature formula of the P1–P4 ENP2 (but the bi-segmented condition of the rami of the swimming legs is the same in both sexes), and that the mouth parts are present in both sexes regardless of whether the male is non-feeding or not. The males of *M. fladensis*, *M. angolaensis*, and *M. unisetosus* sp. n. are non-feeding

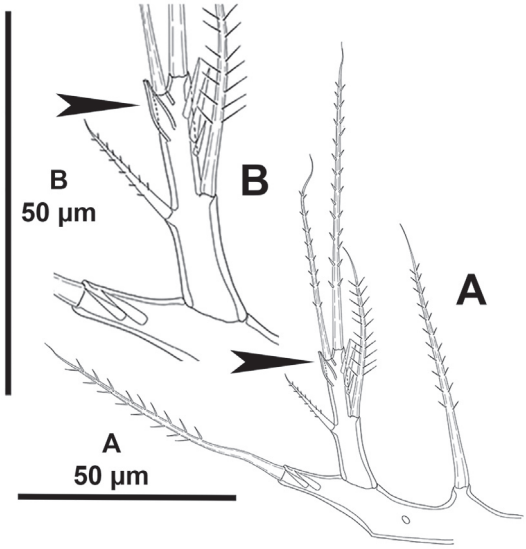

Figure 21. *Mesocletodes unisetosus* sp. n., male paratype. **A** P5, anterior, subdistal tube arrowed **B** exopod of P5 showing distal tube-pore.

and lack mouth parts. Therefore, the option for comparing the armature formula of P1–P4 of the males of these three species only was chosen, viz. with a dorsal process on the anal somite only. Among these species, *M. angolaensis* and *M. unisetosus* sp. n. possess a serrated posterior margin of the cephalothorax, and on the posterior margin of P2-bearing somite to penultimate urosomite, and only *M. fladensis* exhibits posterior spinules instead. An antennary basis is present in *M. angolaensis* and *M. unisetosus* sp. n., and only *M. fladensis* possesses an allobasis. *Mesocletodes angolaensis*, *M. unisetosus* sp. n., and probably *M. fladensis* share the lack of the exopod of the antenna. The Mexican species, *M. unisetosus* sp. n., seems to be more closely related to *M. angolaensis* than to *M. fladensis*. In addition to the characters above shared between these two species, they also share the presence of an inner seta on the P2 and P3 ENP1 (this seta is missing in *M. fladensis*) and the exceedingly elongated caudal rami more than 13 times as long as wide. Briefly, the male of the Mexican species, *M. unisetosus* sp. n., is unique and can be separated from the male of *M. angolaensis*, and *M. fladensis*, by the number of setae on the P5 endopodal lobe (two setae in *M. angolaensis*, and *M. fladensis*, but one seta only in the Mexican *M. unisetosus* sp. n.).

Discussion

Sars (1909: 290–291) created the genus *Mesocletodes* within the Cletodidae, and presented the diagnosis for the genus based solely on his redescription of the type species, *M. irrasus* (T. Scott & A. Scott, 1894) (Sars 1909: 291–292). Subsequently, Por (1986a) created and diagnosed the family Argestidae to reallocate, among other genera, the ge-

nus *Mesocletodes*. For a general historical background on the genus *Mesocletodes* see also Menzel and George (2009) and Vakati et al. (2017).

Sars (1921) described *M. inermis* Sars, 1921, and redescribed *M. monensis* (Thompson, 1893) and *M. abyssicola* (T. Scott & A Scott, 1901), and recognized the relationship between the latter two species (with a dorsal process on the cephalothorax and on the anal somite). He also noted the lack of dorsal processes on the cephalothorax and anal somite of *M. inermis*, but omitted any comment on the relationship of this species and *M. irrasus*. In his key to the species of *Mesocletodes*, Lang (1936) used the presence/absence of a curved dorsal process on the cephalothorax and on the anal somite to separate the species of *Mesocletodes* into two groups, *M. irrasus* and *M. inermis* without dorsal process on the cephalothorax and anal somite *vs. M. brevifurca* Lang, 1936, *M. monensis* and *M. abyssicola* with dorsal process on the cephalothorax and anal somite. Subsequent authors also recognized the relationships amongst those species with a dorsal process on the cephalothorax and on the anal somite (e.g. Soyer 1964, Por 1964a), and amongst those species without such processes (e.g. Por 1964b). It was Bodin (1968) who formally suggested that the species of *Mesocletodes* can be subdivided into two groups, the *abyssicola* group with a dorsal process on the cephalothorax and on the anal somite, and the *inermis* group without a dorsal process on the cephalothorax and anal somite, but noted that such subdivision has no taxonomic value. That Bodin (1968) included in his *abyssicola* group only those species with a dorsal process on the cephalothorax and on the anal somite is evident in his key to the species of *Mesocletodes* where he, for example, included *M. fladensis* Wells, 1965 with a dorsal process on the anal somite only, as part of his *inermis* group. Bodin's (1968) subdivision was accepted and used by subsequent authors (e.g. Coull 1973, Soyer 1975, Schriever 1983, 1985, Bodin 1997).

Wells (1965) described *M. fladensis* from Fladen (in the Scottish sector of the North Sea) based on the male only. This species was described without dorsal process on the cephalothorax, but with a dorsal process on the anal somite, and without mouth parts (i.e. mandibles, maxillules, maxillae and maxillipeds strongly reduced and nontraceable). Later, in his report on five new species of *Mesocletodes* from the North Atlantic Ocean, Schriever (1985) described *M. quadrispinosa* Schriever, 1985, based on four females from the Iceland-Faroe Ridge. *Mesocletodes quadrispinosa* was described with a dorsal process on the cephalothorax, but without a dorsal process on the anal somite. Note that Menzel and George (2009: 252) and Menzel (2011: 47) diagnosed the genus *Mesocletodes* with four setae/spines on P1 EXP3, casting doubts about the relationships and position of *M. quadrispinosa* which was described with three setae only on P1 EXP3.

Por (1986b) presented the description of *M. opoteros* based on five females from the Mozambique Channel between Mozambique and Madagascar. He described *M. opoteros* without a dorsal process on the cephalothorax, but with a dorsal process on the anal somite, and suggested that this species could well belong to a different species-group within *Mesocletodes*, and noted that Bodin's (1968) division of the genus could change with the discovery of new species. However, upon re-inspection of the type

material of *M. opoteros*, Menzel et al. (2011: 862) confirmed the presence of a cuticular dorsal process on the cephalothorax.

Menzel and George (2009) presented the description of *M. angolaensis* from the Angola Basin (Southeastern Atlantic). The only male specimen of this species was described without dorsal process on the cephalothorax, but with a dorsal process on the anal somite, and without mouth parts, similar to what was reported for *M. fladensis* some years earlier. Note that Menzel and George (2009: 253, 254 table 6) erroneously commented on the lack of a dorsal process on the anal somite of *M. fladensis* (see the written description of the species in Wells (1965: 23–24, fig. 77)). They also presented the description of *M. bicornis* Menzel & George, 2009 and *M. dorsiprocessus* Menzel & George, 2009, based on six and two females, respectively, from the Angola Basin (Southeastern Atlantic). They described the females of these two species with a dorsal process on the cephalothorax and anal somite, but also with small dorsal bifid cuticular processes on P3–P5-bearing somites and on the second half of the genital double-somite. In the same paper, Menzel and George (2009) presented the description of *M. meteorensis* Menzel & George, 2009 based on two females from the Angola Basin. This species was described with a dorsal process on the cephalothorax and anal somite, but contrary to the other species of the *abyssicola* group, with the caudal rami barely three times as long as wide. As a result of their investigations, Menzel and George (2009) gave an amended diagnosis of *Mesocletodes* for which they presented four synapomorphies/plesiomorphies, viz. 1) the presence of a strong protrusion with a strong, bipinnate seta pointing backwards on the second antennular segment/without such protrusion and corresponding seta normal, 2) the proximal outer spine of P1 EXP3 reduced/proximal outer spine well developed, 3) the presence of STE's on the spines of P1 EXP3/without STE, and 4) the mandibular gnathobase with a strong, grinding tooth/gnathobase of normal shape. They did not follow Bodin's (1968) and Por's (1986) views regarding the division of the genus, and pooled all the species of *Mesocletodes* with a dorsal cuticular process either on the cephalothorax or on the anal somite, or on both, and with long or short caudal rami, in the *abyssicola* group, arguing that the deviation of Bodin's (1968) scheme could eventually be regarded as secondary reductions (Menzel and George 2009: 253). Following this reasoning, they suggested the monophyly of the *abyssicola* group as defined by them, for which they proposed three synapomorphies/plesiomorphies: 1) presence of a dorsal cuticular process on the cephalothorax/cephalothorax without dorsal process, 2) presence of a dorsal cuticular process on the anal somite/anal somite without dorsal process, and 3) caudal rami remarkably elongated between seta III and VII/caudal rami not remarkably elongate.

Additionally, Menzel and George (2009) suggested the probable monophyly of, at least, two other groups of species. On one hand, Menzel and George (2009: 254) suggested that the presence of bifid dorsal processes on the P3–P5-bearing somites and on the second half of the genital double-somite (not on the first urosomite as in Menzel and George 2009: 254) of *M. bicornis* and *M. dorsiprocessus* might be of high phylogenetic value to establish a monophylum. On the other hand, Menzel and George (2009: 253–254) suggested that the lack of mouth parts, as observed in *M. fladensis*

and *M. angolaensis*, both of the *abyssicola* group *sensu* Menzel and George (2009), can be regarded as derived and therefore, might support a monophylum.

Some years later, Menzel (2011) presented a new and corrected diagnosis for *Mesocletodes* (in their generic diagnosis, Menzel and George (2009) omitted the maxillule, and they described the maxilliped of *Mesocletodes* as stenopodial), presented the sexually dimorphic modifications for the genus with *M. elmari* Menzel, 2011 (without dorsal process on the cephalothorax and anal somite, and with well-developed mouth parts in the male) as model of study, and relegated *M. faroerensis* Schriever, 1985 and *M. thieli* Schriever, 1985 (not *M. thielei* as in Menzel (2011)) as *incertae sedis* within Argestidae due to the presence of an inner seta on P1 EXP2 in the former, and five setae on P1 EXP3 in the latter, and questioned the belonging of *M. arenicola* Noodt, 1952 to this family by the shape and armature of the caudal rami and armature complement of the P1 EXP3. This view is followed here and *M. faroerensis*, *M. thieli* and *M. arenicola* are relegated to *species incertae sedis* within Argestidae. The species presented herein matches the diagnosis of the genus *Mesocletodes* and exhibit the four synapomorphies for the genus.

More recently, Vakati et al. (2017) presented the description of two new species attributable to the *inermis* group, *M. tetrasetosus* Vakati, Thistle & Lee, 2017 and *M. nudus* Vakati, Thistle & Lee, 2017, based on one female and three males, respectively, from the San Diego Trough. Vakati et al. (2017) described the female of *M. tetrasetosus* without dorsal cuticular process on the cephalothorax and anal somite, and with mouth parts. On the other hand, they described the male of *M. nudus* without dorsal processes on the cephalothorax and anal somite, but also, as in the males of *M. fladensis* and *M. angolaensis*, which belong to the *abyssicola* group *sensu* Menzel and George (2009), with atrophied mouth parts. Vakati et al. (2017) presented an amended key to the species of *Mesocletodes* based on Schriever's (1985) key that, in turn, followed Bodin's (1968) scheme.

No unequivocal apomorphies have been detected so far to justify the monophyly of the family Argestidae, and its monophyletic status has not yet been demonstrated (George 2004, 2008, 2011). Corgosinho and Martínez Arbizu (2010) suggested that the shape and armature of the maxilla could shed some light on the monophyly of the family. The genus *Mesocletodes* has been diagnosed based on four synapomorphies, but the phylogenetic relationships within the genus are far from clear (Menzel and George 2009). At this point, the *abyssicola* group *sensu* Menzel and George (2009) is considered monophyletic based on the synapomorphic dorsal cuticular process on the cephalothorax and anal somite and the remarkable elongation of the caudal rami between seta III and VII, and the deviations from this scheme have been tentatively attributed to secondary reductions (Menzel and George 2009). The synapomorphic condition of the dorsal process on the cephalothorax and anal somite seems to be well supported since they do not appear in the ground-pattern of Harpacticoida and Argestidae (Menzel and George 2009). Careful inspection of about 800 adult females of *Mesocletodes* with a dorsal process on the cephalothorax, but without an evident dorsal process on the anal somite, revealed the presence of a very small, inconspicu-

ous, dorsal process on the anal somite (Menzel, pers. comm., in litt.). Interestingly, Soyer (1964: 604, fig. D) described the anal operculum of *M. guillei* with a complex ornamentation and with a "crête médiane se terminant par une courte dent…." (Soyer 1964: 604, fig. D). Whether this tooth is homologous to the dorsal process on the anal somite of other species of *Mesocletodes*, remains obscure. Menzel´s observations and the probable unique condition of the dorsal process on the anal somite of *M. guillei*, supports Menzel and George's (2009) view regarding the definition and monophyly of their *abyssicola* group. Regarding the length:width ratio of the caudal rami, Menzel (2011) hypothesized that the presence of extremely elongated caudal rami in some species of the *inermis* group, viz. *M. elmari*, and in the *abyssicola* group, could be due to convergence. On the other hand, the *inermis* group does not seem to be supported by any synapomorphy.

Given all the above, there seems to be another approach towards the monophyly of the genus *Mesocletodes*. It seems plausible that this genus could eventually be attributed to a new subfamily defined by the four synapomorphies currently known for the genus, viz. the presence of a strong protrusion with a strong, bipinnate seta pointing backwards on the second antennular segment, the proximal outer spine of P1 EXP3 reduced, the presence of STE's on the spines of P1 EXP3, and the mandibular gnathobase with a strong, grinding tooth, plus the presence of a dorsal cuticular process on the cephalothorax and anal somite.

Under this scheme, the presence of a dorsal process on the cephalothorax and anal somite, and the extreme elongation of the caudal rami between seta III and VII could be regarded as plesiomorphic within the subfamily, and the lack of such processes and the reduction of the caudal rami, as secondary apomorphic reductions. However, this requires more robust, in-depth analyses, accompanied by the diagnosis of this hypothesized subfamily, the re-diagnosis of the genus *Mesocletodes*, and the proposal of a new genus to include all the remaining species. These two genera could be composed as follows:

Hypothetical genus. *M. dorsiprocessus*, *M. bicornis*, and *M. brevisetosus* sp. n.; defined by the synapomorphic bifid dorsal processes on P3–P5-bearing somites and posterior half of genital double-somite. The bifid dorsal process on the cephalothorax could be regarded as autapomorphic for *M. bicornis*. The bifid dorsal process on the anal somite would be regarded as plesiomorphic. The monophyly of this taxon was suggested earlier by Menzel and George (2009).

Mesocletodes.– *M. monensis*, *M. abyssicola*, *M. bathybia*, *M. brevifurca*, *M. dolichurus*, *M. katharinae*, *M. meteorensis*, *M. robustus*, *M. soyeri*, *M. opoteros*, *M. simplex* sp. n., *M. quadrispinosa*, *M. fladensis*, *M. angolaensis*, *M. unisetosus* sp. n., *M. irrasus*, *M. inermis*, *M. langi* Smirnov, 1946, *M. makarovi*, *M. glaber* Por, 1964b, *M. guillei*, *M. farauni* Por, 1967, *M. commixtus* Coull, 1973, *M. bodini* Soyer, 1975, *M. carpinei* Soyer, 1975, *M. ameliae* Soyer, 1975, *M. parirrasus* Becker Noodt & Schriever, 1979, *M. sarsi* Becker Noodt & Schriever, 1979, *M. parabodini* Schriever, 1983, *M. trisetosa*, *M. variabilis* Schriever, 1983, *M. kunzi* Schriever, 1985, *M. duosetosus* Schriever, 1985, *M. elmari*, *M. tetrasetosus*, and *M. nudus*; defined by the secondary synapomor-

phic loss of the dorsal process of the cephalothorax and/or anal somite and reduction of the caudal rami. The elongation of the caudal rami between seta III and VII in some of these species, and the bifid dorsal process on the anal somite of *M. opoteros* are, therefore, plesiomorphic. The presence of the latter in *M. opoteros* and in the previous genus could support a closer relationship between these two taxa. Four species groups without taxonomic value, for which no apomorphies have been detected, can be envisaged based on the presence/absence of a dorsal cuticular process on the cephalothorax and/or anal somite:

I *M. abyssicola*, *M. brevifurca*, *M. bathybia*, *M. dolichurus*, *M. katharinae*, *M. meteorensis*, *M. monensis*, *M. opoteros*, *M. robustus*, *M. simplex* sp. n., and *M. soyeri*
II *M. quadrispinosa*
III *M. angolaensis*, *M. fladensis*, and *M. unisetosus* sp. n.
IV *M. ameliae*, *M. bodini*, *M. carpinei*, *M. commixtus*, *M. duosetosus*, *M. elmari*, *M. farauni*, *M. glaber*, *M. guillei*, *M. inermis*, *M. irrasus*, *M. kunzi*, *M. langi*, *M. makarovi*, *M. nudus*, *M. parabodini*, *M. parirrasus*, *M. sarsi*, *M. tetrasetosus*, *M. trisetosa*, and *M. variabilis*.

Group I includes Bodin's (1968) *abyssicola* group. Group II contains only *M. quadrispinosa*, but as noted above, the position of this species is doubtful. Group III contains those species with a dorsal cuticular process on the anal somite only. Group IV contains Bodin's (1968) *inermis* group. No synapomorphies have been detected so far for each of these groups. On the other hand, Menzel and George (2009) suggested that the lack of mouth parts in *M. fladensis* and *M. angolaensis*, but also in *M. unisetosus* sp. n. and *M. nudus* could support a monophylum of derived Argestidae. However, if the monophyly of the above groups is confirmed, the lack of mouth parts in group III and group IV could be attributed to convergence.

Finally, it is worth mentioning the presence of some other undescribed forms related to *M. angolaensis*, *M. bicornis*, *M. brevisetosus* sp. n., *M. dorsiprocessus*, *M. meteorensis*, *M. simplex* sp. n., and *M. unisetosus* sp. n. in the Clarion-Clipperton Fracture Zone in the Pacific Ocean (Samantha Tong Jia Wen, Tropical Marine Science Institute, National University of Singapore, pers. comm.).

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RESEARCH ARTICLE

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A new species of *Pristimantis* (Anura, Craugastoridae) from the Cajas Massif, southern Ecuador

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Abstract

A new species of *Pristimantis* is described from the highland paramos on the eastern slopes of the Cajas Massif, southern Andes of Ecuador, at 3400 m. This new species is characterized by having a distinctive reddish color, cutaneous macroglands in suprascapular region and surfaces of arm and legs, and by lacking dentigerous processes of vomers. The cutaneous macroglands are similar to those exhibited by several species of the *Pristimantis orcesi* group, and may suggest a close phylogenetic relationship. The new species could be a latitudinal substitution of *Pristimantis orcesi* in the southern Andes of Ecuador.

Keywords

Andes, glandular frog, paramo, *Pristimantis erythros* sp. n., taxonomy, Terrarana

Resúmen

Describimos una nueva especie de *Pristimantis* desde las laderas orientales del macizo El Cajas en los páramos andinos del sur de Ecuador a 3400 m.s.n.m. Esta nueva especie tiene un color rojizo distintivo y se caracteriza por tener macroglándulas cutáneas en varias regiones del cuerpo, de la siguiente manera: la región supraescapular, las superficies del antebrazo, parte superior del brazo, las manos y el borde de

las piernas. Además, carece de procesos dentígeros en los vomerinos. Las macroglándulas cutáneas son similares a las presentes en el grupo de *Pristimantis orcesi*, y podrían representar una posición filogenética cercana. La nueva especie puede constituir una sustitución latitudinal de *Pristimantis orcesi* en los Andes sur de Ecuador.

Palabras clave
Andes, rana glandular, páramo, *Pristimantis erythros* sp. n., taxonomía, Terrarana

Introduction

The Andes are one of the major physiographic features on our planet. A heterogeneous mountain system, three geographical separations have been identified in the Andes, based on their different geological, geographical, climatic, and ecosystemic characteristics: northern, central, and southern Andes (Graham 2009). The paramo ecosystem is one of the most distinctive features on the northern Andes, showing remarkable and complex high-altitude flora and fauna communities (Luteyn 1999). Paramos occur on mountain tops above continuous forest line (ca. >3000 m) and below perpetual snow line, mainly in the Andes of Venezuela, Colombia and Ecuador, with outliers on the Andes of northern Peru, and the Central American Cordillera of Costa Rica and Panama. Different vegetation communities are found in paramos, but its general physiognomy is characterized by bush-grasses, rosette and cushion plants, mycrophyllous and dwarf shrubs, and geophytes, with trees usually absent (except for members of the genus *Polylepis*; Luteyn 1999).

Evolution of paramo biodiversity is strongly linked to orogeny and geomorphology, and complex and rich biotas are known to occur across the northern Andes due to their heterogeneous history and topography (Luteyn 1999, Mena and Josse 2000, Sklenář et al. 2011). Paramos show discontinuous distribution, being biogeographic continental islands—isolated one from another by lower areas with different ecologic and physiographic characteristics (Mena and Josse 2000; Mena-Vásconez 2010). Due to their insularity, paramo biota shows important levels of speciation and endemism (Vuilleumier 1970; Fjeldsa 1992; Luteyn 1999; Sklenář et al. 2011; Llambí and Cuesta 2014).

Although amphibian species richness decreases with higher altitude (Navas 2006; Wiens 2007), anurans seem to be more diverse than other ectothermic tetrapods in paramos (Navas 1997, 2006). Due to their low dispersion capacity and high ecophysiological adaptations, anurans are strongly influenced by the insularity of paramos, thus showing high levels of endemism and speciation (Duellman 1979, Lynch 1987). Anuran fauna of the Andes of Ecuador is extraordinarily rich (Duellman 1988), but most collection efforts in the paramo ecosystem have focused on its northern portion. Herein, we describe a distinctive new species of *Pristimantis* from the paramos of the Cajas massif, on the southern section of the Cordillera Occidental, Andes of Ecuador.

Materials and methods

Collections were made at Chanlud hydroelectric project (Fig. 1), managed by the CELEC hydroelectric company (near ETAPA protected area), northeast of the Macizo del Cajas, province of Azuay, Ecuador. Field work as part of amphibian inventory in the Azuay paramos, and was done across transects methodology (Heyer et al. 1994, Angulo et al. 2006), the sampling were conducted in diurnal (7:00 to 11:00 a.m.) and nocturnal periods (7:00 to 11:00 p.m.). The area has a greater coverage of paramo grassland between 3430 and 3883 meters, with small scattered fragments of forest and shrub. At lower elevation (between 3076 and 3430) the vegetation chances to montane forest, here, have great pressure for deforestation and change land use for agricultural land. Photographs of both living and preserved individuals and their habitat were taken by Juan Carlos Sánchez Nivicela (JCSN). Coordinates and elevations of localities were taken with a GPS data Garmin Etrex 10.

Definitions and terminology follows proposals by Lynch and Duellman (1997) and Duellman and Lehr (2009), except for glandular descriptions that follow Toledo and Jared (1995). Specimens were euthanized with 10% roxicaine, fixed in 10% formalin, and preserved in 75% ethanol. Measurements were taken with digital calipers and rounded to the nearest 0.1 mm, following recommendations by Watters et al (2016).

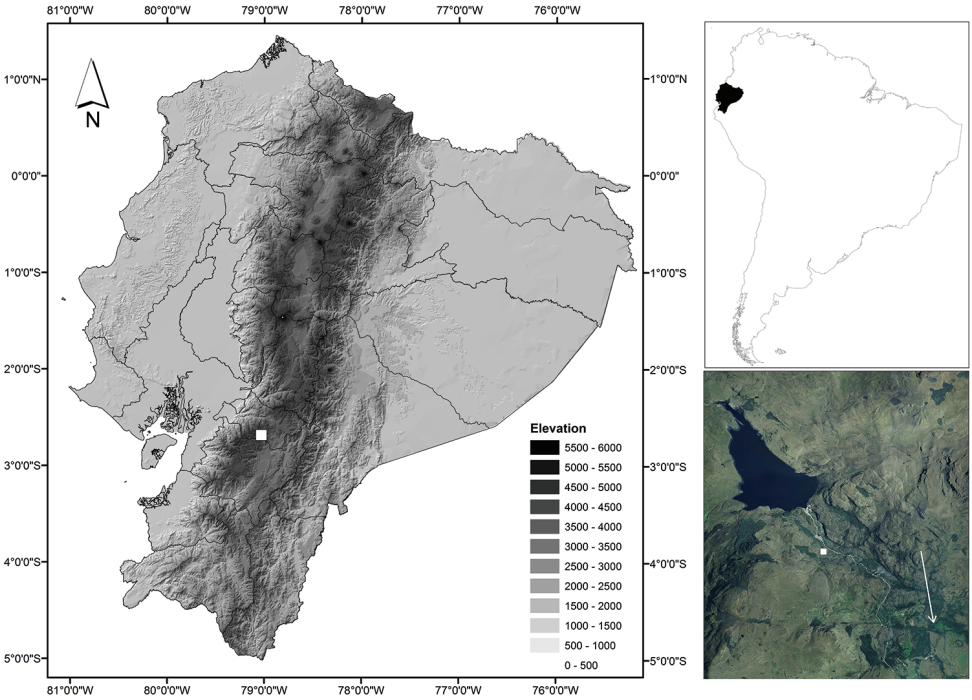

Figure 1. Map of Ecuador showing the type locality of *Pristimantis erythros*, Chanlud (white square), Cajas Massif, province of Azuay, southern Andes of Ecuador. White arrow indicates the direction of Cuenca city, in austral Ecuador.

Fingers and toes are numbered preaxially to postaxially from I to IV and I to V, respectively. Comparative lengths of Toes III and V were determined when both were adpressed against to Toe IV; lengths of Fingers I and II were compared when adpressed against each other. Sex was determined by gonadal inspection. Coloration patterns in life, activity patterns, and habitat characteristics were taken from collectors' field notes and digital photographs. Ecuadorian classification of ecosystems follows the proposal by Ministerio del Ambiente del Ecuador (2013). Examined specimens are deposited at the herpetological collections of Instituto Nacional de Biodiversidad, Quito (**DHMECN**) and Museo de Zoología, Universidad del Azuay, Cuenca (**MZUA**).

Systematic account

***Pristimantis erythros* sp. n.**
http://zoobank.org/DE8E6EBB-37C8-4342-A5F9-5F9A00C2EAC9

Common name. English: Blood Rain Frog. Spanish: Cutín de Sangre

Holotype. DHMECN 12103 (field series JCS.317); (Figs 2–4), an adult female collected at Chanlud, (02°40'57.30"S, 79°1'59.21"W, 3449 m), parroquia Chiquintad, cantón Cuenca, provincial de Azuay, República del Ecuador by JCSN, Verónica Urgilés, Elvis Celi, Valentina Posse and Cristian Nieves, in October 2014.

Paratopotypes (11 specimens). DHMECN 12102, MZUA.AN.1355 adult male; MZUA.AN.1347, MZUA.AN.1348, MZUA.AN.1351, adult females; MZUA.AN.1350, subadult male; MZUA.AN.1349, MZUA.AN.1352, MZUA.AN.1353, subadult females;

MZUA.AN.1342, MZUA.AN.1343 juveniles, collected between October and November 2014 at the type locality.

Diagnosis. *Pristimantis erythros* differs from other species of the genus by the combination of the following characters: (1) Skin on head and dorsum granular, flanks and venter areolate with low warts; dorsolateral folds absent; discoidal fold weakly defined; (2) tympanic membrane and annulus present and visible, rounded, ca. 50% of eye diameter, upper half covered by parotoid macrogland; (3) snout short, rounded in dorsal and lateral views; (4) upper eyelid without tubercles, interorbital distance wider than width of upper eyelid (40%); cranial crests absent; (5) dentigerous process of vomers absent; (6) vocal slits and sacs present in males, nuptial pads absent; (7) Finger I shorter than II; discs laterally expanded with dilated pads and narrow fringes, (8) fingers with coarse lateral cutaneous fringes; (9) low ulnar warts in ventral view; radioulnar macroglands covering the upper surfaces of forearm; (10) heel and tarsus lacking tubercles; paracnemid macroglands on upper surfaces of legs, tarsi, and Toes IV and V; (11) inner metatarsal tubercle oval, not prominent, twice as large as outer metatarsal tubercle, outer metatarsal tubercle rounded and low, supernumerary tubercles low and indistinct; Toe V longer than III, disc of Toe III reaches distal border of penultimate subarticular tubercle on Toe IV, disc on Toe V reaches distal border of distal subarticular tubercle on Toe IV; (12) toes with conspicuous lateral fringes,

Figure 2. Dorsal, ventral and lateral views of holotype of *Pristimantis erythros* sp. n. (adult female, DH-MECN 12103, SVL 39.1 mm) in preservative.

extend to base of fingers, webbing absent; toe pads as large as or slight larger than those on fingers; (13) in life, dorsum uniformly burgundy, red to orange-red (reddish brown to burgundy in preserved); flanks, posterior surfaces of legs, groin, throat and venter crimson (dark reddish brown in preserved); iris dark brown with thin golden reticulations; ventral surfaces of hands and feet pinkish cream; (14) SVL in adult females 38.8–42.6 mm ($\bar{x}$ = 40.3, n = 4), in adult males 36.8–37.1 mm ($\bar{x}$ = 36.7, n = 2).

Comparisons. (Fig. 5) *Pristimantis erythros* differs from all other *Pristimantis* by its conspicuous red coloration in life (reddish brown in preservative), areolate flanks and belly with low warts, cutaneous macroglands: parotoid, paracnemid, and radioulnar; and absence of dentigerous processes of vomers. The distinctive macroglands are also known to be present in *P. orcesi* (Lynch), *P. pycnodermis* (Lynch), and *P. loujosti* Yánez-Muñoz, Cisneros-Heredia & Reyes-Puig. It has a similar external appearance. *Pristimantis orcesi* differs from *P. erythros* by its uniform black to dark brown dorsum in life, areolate skin on dorsum and flanks, low parotoid macrogland and thin paracnemid and radioulnar macroglands on arm and thigh respectively, also *P. orcesi* inhabits paramos on the northern section of Cordillera Occidental and inter-Andean depression of the Andes of Ecuador. *Pristimantis pycnodermis* differs by having low cranial crests, the

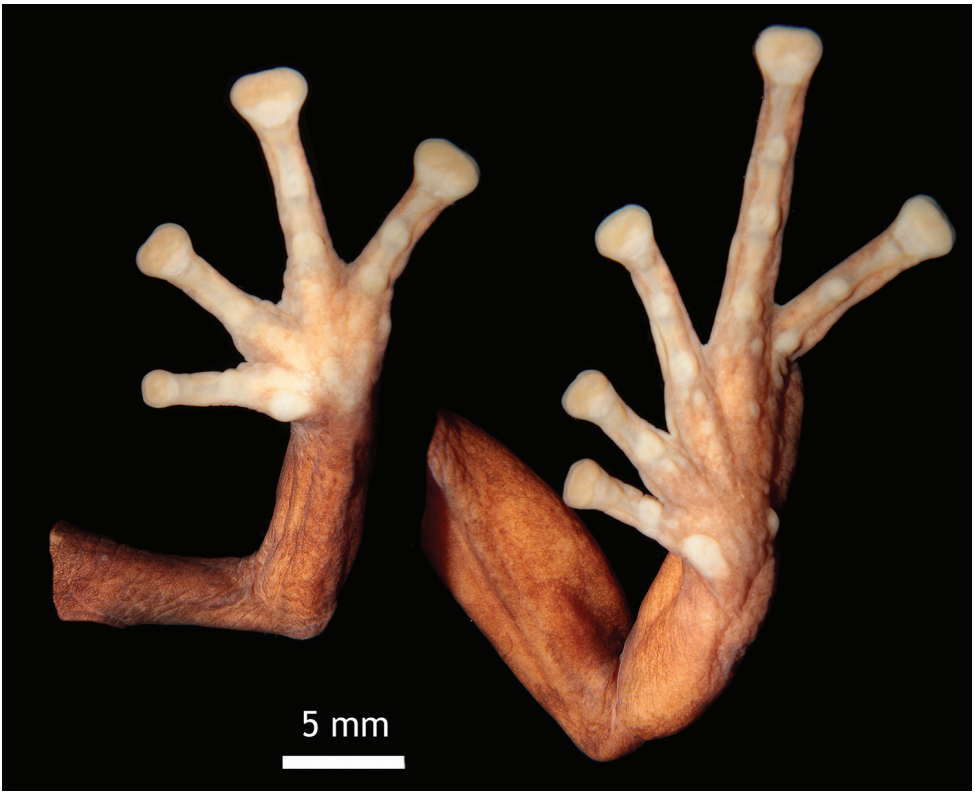

Figure 3. Detail of ventral view of hand and foot of the holotype of *Pristimantis erythros* sp. n. (DHMECN 12103).

presence of dentigerous processes of vomer, dark canthal, tympanic marks, and green or brown color with large black spots on the flanks; it inhabits paramos in the southern section of Cordillera Oriental of the Andes of Ecuador. *Pristimantis loujosti* differs by its subacuminate snout in dorsal view, large dentigerous processes of vomers, light orange dorsum, black spots on hidden surfaces of limbs, and light iris with dark reticulation.

Description of holotype. Adult female (Fig. 2), head as wide as the body, slightly wider than long, 8% of SVL; snout short, rounded in dorsal and lateral views, *canthus rostralis* rounded, loreal region concave, nostrils laterally protruding, interorbital area flat, wider than upper eyelid, upper eyelid 15% of interorbital distance; cranial crests absent; parotoid macroglands covering 65% suprascapular dorsal muscle; tympanic membrane differentiated from surrounding skin, evident and rounded ¾ tympanic annulus, laterally directed, upper quarter covered by parotoid macrogland on *cucularis* muscle, tympanum diameter 52% of eye diameter; choanae large and rounded, not covered by palatal floor or maxillary arch; dentigerous processes of vomers absent; tongue broader than long, wider in posterior region, 25% attached to mouth floor.

Skin of dorsum granular without tubercles; dorsolateral folds absent; ventral surface areolate. Discoidal fold weakly defined in ventral view; cloacal region short, and

Figure 4. Lateral, dorsal and ventral views of living specimens of *Pristimantis erythros*. Left: Male paratype (DHMECN 12102, SVL: 37.1 mm); right: Female holotype (DHMECN 12103, SVL: 39.1 mm).

covered by small and pronounced warts (Fig. 2). Ulnar warts slow, radioulnar macrogland covering dorsal surface of arm, forearm and hand; palmar tubercles large, external palmar tubercle, slightly larger than inner, inner palmar tubercle oval; super-

numerary tubercles pronounced; subarticular tubercles expanded in dorsal and lateral view; fingers with lateral cutaneous fringes, without interdigital membranes; Finger I shorter than Finger II, discs expanded laterally, all fingers with well-defined circumferential grooves (Fig. 3).

Hind limbs robust, tibia length 44% SVL; heel and external border of tarsus without tubercles, covered dorsally and ventrally by paracnemid macroglands; inner tarsal fold absent; inner metatarsal tubercles oval, twice as larger than the external metatarsal tubercle; supernumerary tubercles present, rounded and flattened; toes with lateral cutaneous fringes; basal membrane absent between toes; foot disks same size as those of hand, laterally expanded from fingers I–IV; relative length of toes 1<2<3<4>5; Toe IV larger than Toe III (Fig. 3).

Measurements of holotype (in mm). Snout-vent length 39.1; head length 10.8; head width 13.8; eye diameter 3.4; eye-nostril distance 3.5; interorbital distance 5.8; internarial distance 3.5; tympanum diameter 1.9; upper eyelid width 2.8; tibia length 17.5; foot length 20.7; hand length 14.5.

Coloration of holotype in life. Dorsum dark red with slightly lighter shades on head and limbs; dark red on venter. Tips of fingers and toes pinkish cream in dorsal view; ventral surfaces of hands and feet, creamy pink. Iris homogeneously dark brown, with thin golden reticulations (Fig. 4).

Coloration of the holotype in alcohol. Dorsum reddish brown, flanks, posterior surfaces of thighs, venter, and throat dark reddish brown. Dorsal surfaces of fingers pinkish cream; ventral surfaces of hands and feet, creamy pink (Fig. 2).

Variation. Morphometric variations of the type series are presented in Table 1. The color variation is the change of tonality that goes from dark red to clear (Fig. 4).

Etymology. The specific epithet *erythros* is derived from the Greek word for red, in allusion to the distinctive coloration of this species.

Distribution, natural history, and extinction risk. *Pristimantis erythros* is only known from its type locality in the Cajas Massif. The area is covered by paramos dominated by grassland and shrubs, between 3450 and 3500 m (Fig. 6). Specimens were collected mainly in terrestrial bromeliads (*Puya hamata*) and grasses (*Neurolepis villosa*), near to small streams. Vocalizations were heard (but unrecorded) during daytime hours from 08h00 to 11h00 and from 17h00 to 19h00. Active individuals were observed from dusk until approximately 21h00, afterwards activity decreased. The new species was recorded in sympatry with *Pristimantis* aff. *cryophilius*, *P.* aff. *orestes* and *P.* aff. *riveti*.

The Paramos on the Cajas Massif (221000 h. approx.) appear well preserved. Part of its extension includes the Cajas National Park (28544 h). However, the continued changes on land cover and land use occurring in several areas over the massif on the buffer area of the national park and not protected nearest regions are leading to habitat loss (Hofstede et al. 2002). During a period of four (4) years (2014–2017), twenty six (26) localities in suitable regions (2500–3500 m) on the Cajas Massif were surveyed, no additional records of this new *Pristimantis* were added during these excursions mentioned above. It is probable that *P. erythros* inhabit only a single locality in an area of

Figure 5. Comparison of *Pristimantis erythros* (top right) with *Pristimantis orcesi* (top left), *Pristimantis pycnodermis* (below left), and *Pristimantis loujosti* (below right).

Figure 6. Habitat of *Pristimantis erythros* in type locality.

Table 1. Measurements (in mm) of the type series of *Pristimantis erythros* sp. n. All specimens are adults, range is followed by mean ± stander deviation in parentheses. Abbreviations: SVL = snout vent length, HL = head length, HW = head width, ED = eye diameter, EN = eye-nostril distance, IOD = interorbital distance, IND = internarinal distance, UEW = upper eyelid width; TD = tympanum diameter, HAL = hand length, Finger IV disk width = Fin4DW, TL = tibia length, FL = foot length, Toe IV disk width = Toe4DW.

Measurement	Adult Female *N* = 4	Adult Male *N* = 2
SVL	38.8–42.6 (40.2 ± 1.7)	36.7–37.0 (36.9 ± 0.2)
EN	2.7–3.5 (3.2 ± 0.3)	3.1–3.4 (3.3 ± 0.2)
HL	10.6–13.7 (11.5 ± 1.4)	11.5–11.8 (3.3 ± 0.2)
HW	13.2–14.7 (13.7 ± 0.6)	12.6–13.3 (12.9 ± 0.5)
IOD	4.7–5.8 (5.1 ± 0.5)	4.2–5.4 (4.8 ± 0.9)
IND	3.1–3.5 (3.3 ± 0.9)	3.3–3.4 (3.3 ± 0.1)
TL	16.8–17.5 (17.1 ± 0.3)	15.9–16.6 (16.3 ± 0.5)
FL	19.5–21.1 (20.2 ± 0.8)	18.4–18.5 (18.4 ± 0.1)
HAL	13.0–14.4 (13.8 ± 0.6)	12.3–12.8 (12.5 ± 0.4)
TD	1.7–1.9 (1.8 ± 0.1)	1.7–1.8 (1.8 ± 0.1)
ED	3.4–4.2 (3.7 ± 0.4)	2.9–3.8 (3.3 ± 0.7)
UEW	2.6–3.5 (3.0 ± 0.4)	3.1–3.3 (3.2 ± 0.1)
Fin4DW	2.3–3.5 (2.6 ± 0.3)	2.2–2.4 (2.3 ± 0.2)
Toe4DW	2.2–2.7 (2.5 ± 0.2)	2.1–2.3 (2.2 ± 0.1)

less than 1 km^2. Finally, based on the small area of occupancy that might be restricted to the type locality which it is not under conservation in a protected area, we suggest that, it should be classified as Critically Endangered (CR) under the UICN criteria B1,B2ab(i,ii,iii,iv) (IUCN 2001).

Discussion

At least 50 species of anurans, including *Pristimantis erythros*, are currently known to inhabit the paramos of Ecuador (Table 2). The distribution of these species is fairly even along Andes, with 34 species recorded on the paramos of Cordillera Occidental (21 spp. on the northern part, 17 spp. southern part), and 36 species on the paramos of Cordillera Oriental (19 spp. on the northern section, 21 spp. southern section). Our data show that terrestrial frogs of the genus *Pristimantis* make a significant proportion of the amphibian fauna in Ecuadorian paramos (50–58% on each mountain range; slightly higher than calculations by Navarrete et al. 2016). The lowest species richness of *Pristimantis* occurs in the southern paramos (7 spp. on Cordillera Occidental and 10 spp. on Cordillera Oriental), probably referred as collection bias since several species from this section remain undescribed.

The Cajas Massif has one of the most particular landscapes in the Ecuadorian Andes. The massif was glaciated during the Pleistocene (Hastenrath 1981, Clapperton 1993), and its current physiography includes more than two hundred glacial lakes,

Table 2. Amphibians of the paramos from the Andes of Ecuador (above 3000 m). Abbreviations: N = northern section, S = southern section (sections are approximately divided by 1.5°S latitude). Nominal species that may be complexes (including more than one cryptic species) are marked with an asterisk.

Species	Cordillera Occidental		Cordillera Oriental		Source
	N	S	N	S	
Atelopus bomolochos		■		■	Peters (1973)
A. exiguus		■			Coloma et al. (2000)
A. ignescens	■		■		Coloma et al. (2000)
A. nanay		■			Coloma (2002)
A. pastuso	■				Coloma et al. (2010)
A. petersi			■	■	Coloma et al. (2007)
A. podocarpus				■	Coloma et al. (2010)
Osornophryne angel	■				Yánez-Muñoz et al. (2010)
O. antisana	■				Hoogmoed (1987)
O. talipes	■				Cannatella (1986)
Centrolene buckleyi *	■	■	■	■	Lynch and Duellman 1973
Hypodactylus brunneus			■		Lynch (1975)
H. peraccai			■		Lynch (1975)
Lynchius flavomaculatus				■	Lynch (1975)
Pristimantis buckleyi *	■		■		Lynch (1981)
P. cajamarcensis		■		■	Lynch and Duellman (1997)
P. cryophilius *		■		■	Lynch (1979)
P. cryptomelas				■	Lynch (1979)
P. curtipes *	■		■		Lynch (1981)
P. devillei *	■		■		Lynch and Duellman (1980)
P. erythros		■			This work
P. festae			■		Lynch and Duellman (1980)
P. gentryi	■				Lynch and Duellman (1997)
P. gualacenio				■	Urgilés et al. (2014)
P. huicundo			■		Guayasamin et al. (2004)
P. leoni *	■		■		Lynch and Duellman (1997)
P. lymani		■		■	Lynch and Duellman (1997)
P. mazar				■	Guayasamin and Arteaga (2013)
P. modipeplus			■		Lynch (1981)
P. myersi			■		Lynch (1981)
P. ocreatus	■				Lynch (1981)
P. orcesi *	■				Lynch (1981)
P. orestes *				■	Lynch and Duellman (1997)
P. ortizi			■		Guayasamin et al. (2004)
P. philipi		■			Lynch and Duellman (1995)
P. phoxocephalus *	■	■	■	■	Lynch and Duellman (1997)
P. pichincha	■				Reyes-Puig and Páez-Rosales (2016)
P. pycnodermis				■	Lynch and Duellman (1980)
P. riveti *		■		■	Lynch (1979)
P. thymelensis	■		■		Lynch (1981)
P. unistrigatus *	■		■		Lynch (1981)
Hyloxalus anthracinus		■		■	Coloma (1995)
H. jacobuspetersi	■				Coloma (1995)
H. vertebralis		■		■	Coloma (1995)
Gastrotheca espeletia			■		Duellman (2015)
G. litonedis *		■		■	Duellman (2015)
G. pseustes *	■	■	■	■	Duellman (2015)
Hyloscirtus larinopygion	■				Duellman and Hillis (1990)
Telmatobius niger	■	■		■	Trueb (1979)
T. vellardi		■		■	Trueb (1979)

interconnected ridges and peaks, and numerous broad hanging valleys (Coblentz and Keating 2008). The Cajas Massif holds the largest continuous paramos on the Cordillera Occidental of Ecuador. These paramos are separated from all surrounding highlands by the River Cañar basin (north), the River Jubones basin (south), and the intra-Andean basin of Paute (east). At least four species of anurans are endemic to the paramos of the Cajas Massif: *Atelopus exiguus*, *A. nanay*, *Pristimantis erythros*, and *P. philipi*. In fact, the Cajas Massif seems to be an important endemic area for biodiversity (Barnett 1997), with several endemic species of plants (incl. at last nine species of the genus *Valeriana*, Sklenář and Jørgensen 1999), birds (incl. *Metallura baroni* and *Xenodacnis* sp., Astudillo et al. 2015, and mammals (incl. *Chibchanomys orcesi*, Jenkins and Barnett 1997).

Pristimantis erythros share conspicuous cutaneous macroglands on its body and extremities with *P. orcesi*, *P. pycnodermis,* and *P. loujosti*. *Pristimantis erythros* is most similar to *P. orcesi*, from which it differs by its coloration and morphology, and has a significant biogeographic separation due to the isolation of the Cajas Massif from other paramos. Phylogenetic relationships of *P. erythros* are still uncertain, and due to the lack of additional evidence (e.g., molecular data), we refrain to assign *P. erythros* to any species group. Although *P. erythros* and *P. orcesi* may be related, the *Pristimantis orcesi* species-group is not a monophyletic group (Hedges et al. 2008, Padial et al. 2014). We do not discard the possibility that *P. erythros* replaces latitudinally *P. orcesi*.

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An audit of some processing effects in aggregated occurrence records

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Abstract

A total of ca 800,000 occurrence records from the Australian Museum (AM), Museums Victoria (MV) and the New Zealand Arthropod Collection (NZAC) were audited for changes in selected Darwin Core fields after processing by the Atlas of Living Australia (ALA; for AM and MV records) and the Global Biodiversity Information Facility (GBIF; for AM, MV and NZAC records). Formal taxon names in the genus- and species-groups were changed in 13–21% of AM and MV records, depending on dataset and aggregator. There was little agreement between the two aggregators on processed names, with names changed in two to three times as many records by one aggregator alone compared to records with names changed by both aggregators. The type status of specimen records did not change with name changes, resulting in confusion as to the name with which a type was associated. Data losses of up to 100% were found after processing in some fields, apparently due to programming errors. The taxonomic usefulness of occurrence records could be improved if aggregators included both original and the processed taxonomic data items for each record. It is recommended that end-users check original and processed records for data loss and name replacements after processing by aggregators.

Keywords

Atlas of Living Australia, GBIF, occurrence records, data errors

Introduction

Neither the Atlas of Living Australia (ALA) nor the Global Biodiversity Information Facility (GBIF) simply republishes the occurrence records it receives from data providers. Each aggregator processes incoming data in an effort to improve data quality. The processing works by adding, deleting or modifying data items, or by adding "assertions" (ALA) or "flags" (GBIF) to records that contain items identified as incorrect, incomplete, suspect or otherwise invalid.

Processing of this kind can be beneficial when data errors are corrected or flagged. Processing is not helpful when valid data items are lost and when added or modified data items are incorrect. To investigate some of the effects of processing I audited ca 345,000 occurrence records from the Australian Museum (AM) and ca 355,000 occurrence records from Museums Victoria (MV), in each case as the records appear in both ALA and GBIF. I was mainly interested in the changes made by the aggregators to taxon names, but I also checked for data losses in selected non-taxonomic fields, and for the latter purpose I examined ca 100,000 occurrence records in GBIF from the New Zealand Arthropod Collection (NZAC).

As reported below, some processing operations significantly downgraded rather than upgraded data quality, and changes in taxon names varied substantially between aggregators.

New processing routines are occasionally introduced by ALA and GBIF, and old ones improved over time (e.g., see the "Issues" section of the ALA "biocache-store" GitHub site, https://github.com/AtlasOfLivingAustralia/biocache-store). For this reason the results presented here should be seen as "date-stamped" early 2018, when I downloaded the sample data.

Methods

Data sources

From the ALA website I downloaded the "Australian Museum Malacology Collection" and "Museums Victoria provider for OZCAM" datasets. I chose Darwin Core (Wieczorek et al. 2012) downloads in TSV format with all fields in the record-class terms, occurrence, organism, event, location, identification, and taxon categories. From the ALA-MV dataset I selected all records with the *collectionCode* "Entomology". Each ALA table contains both original and processed data.

From the GBIF website I downloaded Darwin Core archives containing both original (*verbatim.txt*) and processed (*occurrence.txt*) record tables for "Australian Museum provider for OZCAM" and "Museums Victoria provider for OZCAM". From the AM dataset I selected original and processed records with the *collectionCode* "Malacology" and from the MV dataset the original and processed *collectionCode* "Entomology" records.

I also downloaded from GBIF ca 100,000 original and processed records from the New Zealand Arthropod Collection (NZAC). The NZAC dataset had internal data problems that prevented me from auditing its taxonomic content effectively, but some GBIF processing effects on non-taxonomic NZAC data are noted in Results.

Download or data citations as recommended by ALA and GBIF are as follows:

AM Malacology from ALA

Atlas of Living Australia occurrence download at https://biocache.ala.org.au/occurrences/search?&q=collection_uid%3Aco114 accessed on Wed Feb 14 18:44:13 AEDT 2018

AM from GBIF

Australian Museum (2017). Australian Museum provider for OZCAM. Occurrence Dataset https://doi.org/10.15468/e7susi accessed via GBIF.org on 2018-02-14

MV from ALA

Atlas of Living Australia occurrence download at https://biocache.ala.org.au/occurrences/search?&q=data_resource_uid%3Adr342 accessed on Wed Jan 31 06:42:40 AEDT 2018

MV from GBIF

Museums Victoria (2017). Museums Victoria provider for OZCAM. Occurrence Dataset https://doi.org/10.15468/lp1ctu accessed via GBIF.org on 2018-01-30

NZAC from GBIF

Wilton A (2018). New Zealand Arthropod Collection (NZAC). Version 1.67. Landcare Research. Occurrence Dataset https://doi.org/10.15468/lrgzz9 accessed via GBIF.org on 2018-01-08

After finding disagreements between ALA downloads and the ALA website (see Results and Discussion), I downloaded two additional record sets for checking, rather than auditing:

AM from ALA (standard download)

Atlas of Living Australia occurrence download at https://biocache.ala.org.au/occurrences/search?&q=collection_uid%3Aco114 accessed on Mon Feb 19 10:40:22 AEDT 2018

MV from ALA (standard download)

Atlas of Living Australia occurrence download at https://biocache.ala.org.au/occurrences/search?&q=collection_uid%3Aco39 accessed on Mon Feb 19 10:39:30 AEDT 2018

Data auditing and preparation

I audited the records tables on the command line with BASH and GNU text-processing tools and GNU AWK 4 (Robbins 2018). The eight original working tables (AM and MV data from ALA; AM, MV and NZAC data from GBIF) have been archived in Zenodo (https://doi.org/10.5281/zenodo.1217733; version 2 uploaded 2018-04-13).

For convenience in cross-checking the results of ALA and GBIF processing, I reduced the AM and MV datasets to records with a *catalogNumber* in common between ALA and GBIF, i.e. 345,944 AM records and 355,824 MV records.

The GBIF NZAC dataset had 1186 pairs of duplicate records, in each case with one record with *modified* date "2016-11-11" and the other with "2017-05-09" (see Results for a likely explanation). I deleted the earlier record versions, reducing the NZAC dataset to 102,092 records.

Issues with field structuring

Although the ALA and GBIF downloads both contain original and processed data, direct comparisons are not straightforward because of the way the aggregators have structured and filled their data fields. ALA, for example, has duplicated or pseudo-duplicated five of its download fields. Simple duplicates are *basisOfRecord* (two fields) and *recordedBy_raw* (three fields). *dcterms:bibliographicCitation* (two fields) is pseudo-duplicated, with different entries in the two replicates (noted in another ALA download; the fields are blank in the two downloads audited here). There are two *class* fields, and ALA explains in the *headings.csv* file included in the download archive that one *class* field contains "Class matched / The class the ALA has matched this record to in the NSL [National Species Lists] http://rs.tdwg.org/dwc/terms/class", while the other is only explained as "http://rs.tdwg.org/dwc/terms/class". A check of MV data indicates that the second *class* field contains original data items, and it is surprising that ALA does not label this field *class_raw* (as it has done with *kingdom_raw*, *phylum_raw*, *order_raw*, *family_raw* and *genus_raw*). The fifth duplicated field is more problematic. ALA generates two *specificEpithet* fields and one *specificEpithet_raw* field, with the following explanations in *headings.csv*:

specificEpithet = "Species matched / Original scientific name supplied with the record http://rs.tdwg.org/dwc/terms/scientificName"
specificEpithet = "http://rs.tdwg.org/dwc/terms/specificEpithet"
specificEpithet_raw = "http://rs.tdwg.org/dwc/terms/specificEpithet"

Contradicting the explanations, the first *specificEpithet* field is not a duplicate of *scientificName_raw* (also provided in the download), the second *specificEpithet* holds the originally supplied species name and is therefore actually *specificEpithet_raw*, and *specificEpithet_raw* is blank.

The ALA download also includes the confusingly named:

verbatimDepth = "http://rs.tdwg.org/dwc/terms/verbatimDepth"
verbatimDepth_raw = "http://rs.tdwg.org/dwc/terms/verbatimDepth"
verbatimElevation = "http://rs.tdwg.org/dwc/terms/verbatimElevation"
verbatimElevation_raw = "http://rs.tdwg.org/dwc/terms/verbatimElevation"

The two "raw" fields are empty in both the AM and MV datasets, but in another dataset I examined (National Herbarium of Victoria records, NHV; https://collections.ala.org.au/public/show/co55, accessed 2018-03-14) it is clear that the processing from *verbatimElevation_raw* to *verbatimElevation* is intended to convert elevations in units other than metres to elevations in metres. The "raw" entry "985ft", for example, was processed as "300.228". Where *verbatimElevation_raw* is already in metres in the NHV dataset, *verbatimElevation* either repeated the entry with added 0.1 m precision (e.g. "1616" becomes "1616.0") or deleted the entry if it was not simply parseable (e.g. *verbatimElevation_raw* = "1627.000 m", *verbatimElevation* blank). ALA processing also failed with range entries, e.g. "15000–17000 ft" was not converted to metres.

GBIF has not duplicated any fields or confused the field naming in its download, but *verbatim.txt* and *occurrence.txt* differ significantly in their field structure. The *associatedMedia*, *geodeticDatum*, *verbatimCoordinates*, *verbatimLatitude*, *verbatimLongitude* and *scientificNameAuthorship* fields are dropped without replacement during processing, for unknown reasons. The *country* field is dropped but its items are processed (with additions, corrections or exclusions) into *countryCode* in *occurrence.txt*. Minimum and maximum depth and elevation are recalculated by GBIF during processing. In *occurrence.txt*, *minimumDepthInMeters* and *maximumDepthInMeters* are replaced by *depth* and *depthAccuracy*, where "depth" is either the single depth value supplied, or the mean of the supplied minimum and maximum, and "depthAccuracy" is the average deviation from the mean. *minimumElevationInMeters* and *maximumElevationInMeters* are similarly replaced by *elevation* and *elevationAccuracy*.

GBIF adds *genericName* and *species* fields to its processed tables. The terms are defined by GBIF online (http://gbif.github.io/dwc-api/apidocs/org/gbif/dwc/terms/GbifTerm.html; accessed 2018-02-15) but neither term is part of the Darwin Core standard (see http://rs.tdwg.org/dwc/terms/). The first field is "The genus part of the scientific name", yet in many MV records *genericName* contains a non-genus name. The *species* field contains "The canonical name without authorship of the accepted [processed] species" and seems to be the same as the *species* field in the recommended GBIF download. I ignored the *genericName* and *species* fields in the audit.

I also found that there are ALA fields populated with data items with the corresponding GBIF fields completely blank. These are not losses due to processing, since the fields are also blank in the *verbatim.txt* file. The field contents were evidently not supplied to GBIF, either by ALA, which acts as Australia's GBIF node, or by the data provider. For example, AM *catalogNumber* C.153619.002 appears on the ALA website

(https://biocache.ala.org.au/occurrences/4b52f4f9-01e0-411c-adc5-213e40a40b0f; accessed 2018-03-15) and in the download with the *habitat* entry "On pink Aplysilla" (original and processed), but there is no *habitat* entry on the corresponding GBIF webpage (https://www.gbif.org/occurrence/1100892212; accessed 2018-03-15), and the *habitat* field in the GBIF-AM *verbatim.txt* file is blank. In the AM dataset the blanked fields are *acceptedNameUsage, associatedMedia, associatedOccurrences, class, dataGeneralizations, day, geodeticDatum, habitat, identificationRemarks, informationWithheld, month, nameAccordingTo, occurrenceStatus, otherCatalogNumbers, samplingProtocol, taxonConceptID, verbatimCoordinateSystem, verbatimEventDate, verbatimLatitude, verbatimLongitude, verbatimTaxonRank, waterBody* and *year*, and in the MV dataset *associatedMedia, class, dataGeneralizations, georeferencedBy, georeferenceProtocol, georeferenceSources, informationWithheld, nomenclaturalCode, samplingProtocol, taxonConceptID* and *waterBody*.

In all the fields I audited for processing changes, I found that the original data items ("raw" items in ALA, *verbatim.txt* items in GBIF) were identical in ALA and GBIF, i.e. there was no "cascading effect" of processing changes from ALA (as GBIF node) to GBIF.

Taxon names

In examining name changes after processing I ignored changes in taxonomic authorship. Whether attached to names or entered in the *scientificNameAuthorship* field, authorships are often incomplete or incorrect in original records. As noted above, GBIF drops the *scientificNameAuthorship* field from processed records, instead adding authorship to some, but not all names in the *scientificName* field.

I also ignored processing changes in the higher classification of taxa, such as changes in family assignments for genera. Although changes in classification can make records harder to discover in a search of aggregated data, those changes reflect differences in the classification schemes used by data providers and aggregators, and might be regarded as matters of opinion by end-users of aggregated data. However, I used higher-taxon changes as guides when looking for incorrect replacements (see Results).

The search for processing effects on names was further limited to records with genus- or species-group *scientificName* in the original, and I excluded records in which the original *scientificName* was informal, e.g. "Idiosepius _n.sp._2". Totals examined were 340,998 records in the AM dataset and 331,480 in the MV dataset.

Both ALA and GBIF attempt to match taxon names with names in reference classifications. GBIF uses a "backbone taxonomy" (https://www.gbif.org/dataset/d7dddbf4-2cf0-4f39-9b2a-bb099caae36c; accessed 18 January 2018) and ALA refers to Australian National Species Lists (https://www.ala.org.au/uncategorised/data-processing/; accessed 18 January 2018). Processing of *scientificName* could result in no change to the name supplied, or in one or more of the following outcomes, listed below with examples from ALA-processed records.

deleted. Name has no replacement; processing deletes it.

Jaffaia jaffaensis (Blochmann, 1910) (AM *catalogNumber* C.100786)

fail-match. Name replaced with *incertae sedis*, with a name from an unrelated branch of the classification, or with an incorrect name, such as a homonym.

The trichopteran *Lasiocephala basalis* (Kolenati, 1848) (MV TRI43315) was matched to the plant taxon *Drosera* sect. *Lasiocephala*

up-match. Name generalised to one at a level in the taxonomic hierarchy above the supplied or appropriate one.

Oliva parkinsoni Prior, 1975 replaced with *Oliva* (AM C.100860)

down-match. Name particularised to one at a level in the taxonomic hierarchy below the supplied or appropriate one.

Arrenurus (Arrenurus) replaced with *Arrenurus madaraszi* (MV H14890107)

swap-match. Name replaced with another at the same rank. For ALA records, this category includes species-level names differing only in subgenus.

Polyphrades brevirostris Lea (MV COL100011) replaced with *Essolithna rhombus*

subgenus. Subgenus added to or deleted from species or subspecies name; no other major changes (ALA records only).

Vexillum (Costellaria) antonelli (Dohrn, 1861) (AM C.407864) replaced with *Vexillum antonellii*

amended. Only minor change to name spelling or format.

Hasora discolor mastusia Fruhstorfer, 1911 (MV LEP11) replaced with *Hasora discolora mastusia*

For each record in which the processed *scientificName* differed from the original *scientificName*, I tabulated *catalogNumber*, original name, processed name, change type (one of the categories listed above), change detail and original *typeStatus*. An example from ALA:

T4607 | Culex (Lutzia) douglasi Dobrotworsky | Culex (Neoculex) douglasi | swap-match | species for species | Holotype

Obviously, a processed *scientificName* entry may represent more than one kind of change. For example, an up-matched taxon may also be a swap-match at the higher taxon level, as with *Anaxo cylindricus obscurus* Blackburn (MV T13669) up-matched to the synonym *Lepturidea cylindrica* by ALA. In the change tables, the ranking order for non-deleted names is fail-match > (up-match = down-match) > swap-match > subgenus > amended. Because GBIF does not usually include subgenera in processed names, the GBIF change tables do not include "subgenus"-type entries, and only a few changes involving original

subgenera could be included in other categories. It is also likely that at least some of the up-, down- and swap-matched records are actually fail-matched (see Results).

The four name change tables for AM-ALA, AM-GBIF, MV-ALA and MV-GBIF are included in the Zenodo archive with the records downloads.

Results

Name changes: *ALA*

Including all change types, ALA changed formal names in the genus- and species-groups in 72,963 records in the AM dataset (21.4%) and 46,835 (14.1%) in the MV dataset (Table 1). Ignoring the less significant "subgenus" and "amended" change types, the totals are 62,824 (AM, 18.4%) and 38,374 records (MV, 11.6%.).

Names deleted in processing are missing from the standard ALA download but still appear on the ALA website. An example is the record for the onychophoran *Planipapillus bulgensis* Reid (MV K3033) at https://biocache.ala.org.au/occurrences/e96c0cd8-79ce-43ae-82b4-90f9a2d7d6ac (accessed 28 February 2018). The webpage displays the name and classification for this museum specimen lot, but the "original vs processed values" dialog box shows that the supplied *scientificName* has been filtered out, and this webpage is not found with a search in ALA for "Planipapillus bulgensis". (GBIF did not delete or change any of the names deleted by ALA.)

The two records down-matched from genus to species (MV HET19158, HET19159) are for specimen lots of the moth *Praxis edwardsii.* MV supplied the *specificEpithet* "edwardsii" to ALA but omitted the epithet from *scientificName*. Some other down-matches are a little surprising as they occur not through replacement by a synonym, but within the same parent taxon. For example, the ant species *Pheidole bos* has three valid subspecies in Australia (https://biodiversity.org.au/afd/taxa/Pheidole/names; accessed 28 February 2018). MV records for *Pheidole bos baucis* (HYM46113) and *P. bos eubos* (HYM46138) have *taxonRank* originally specified as "subspecies" and are processed without change as subspecies. Five records for *P. bos* with *taxonRank* specified as "species" and with no subspecific name supplied are down-matched to *P. bos bos* (HYM46132-HYM46136) and re-ranked as "subspecies". The down-matching noted in the Methods section, of *Arrenurus (Arrenurus)* to *Arrenurus madaraszi* (MV H14890107), is likewise hard to understand, as MV did not specify a species, the subgenus *Arrenurus* contains numerous species and *A. madaraszi* is placed in the subgenus *Micruacarus*. (GBIF did not down-match any of the names down-matched by ALA.)

Name changes: *GBIF*

Including all change types, GBIF changed formal names in the genus- and species-groups in 50,080 records in the AM dataset (14.7%) and 44,519 (13.4%) in the MV

Table 1. Tallies of records with changes by ALA in genus- and species-group names in the AM and MV datasets. Totals of records with formal, genus- and species-group names: AM = 340998, MV = 331480.

AM:			**MV:**		
deleted	genus	116	deleted	genus	37
deleted	species	726	deleted	species	98
fail-match	genus for plant	2	fail-match	species for plant	1
fail-match	species for plant	22	down-match	genus to species	2
down-match	genus to subgenus	21	down-match	genus to subgenus	727
down-match	species to subspecies	2041	down-match	subgenus to species	2
up-match	genus to class	134	down-match	species to subspecies	1093
up-match	genus to order	1	up-match	genus to class	3
up-match	genus to family	1317	up-match	genus to order	83
up-match	subgenus to family	6	up-match	genus to family	4157
up-match	subgenus to genus	65	up-match	genus to subfamily	79
up-match	species to class	257	up-match	subgenus to genus	93
up-match	species to superfamily	2	up-match	species to class	2
up-match	species to family	8015	up-match	species to order	173
up-match	species to genus	21641	up-match	species to family	1575
up-match	species to subgenus	40	up-match	species to subfamily	159
up-match	subspecies to class	25	up-match	species to tribe	310
up-match	subspecies to family	244	up-match	species to genus	8973
up-match	subspecies to genus	397	up-match	species to subgenus	72
up-match	subspecies to species	1154	up-match	subspecies to family	68
swap-match	genus for genus	14	up-match	subspecies to genus	374
swap-match	species for species	26462	up-match	subspecies to subgenus	22
swap-match	subspecies for subspecies	122	up-match	subspecies to species	6107
subgenus	added to species	1980	swap-match	genus for genus	752
subgenus	deleted from species	338	swap-match	subgenus for subgenus	8
amended	species for species	7338	swap-match	species for species	11438
amended	subgenus for subgenus	212	swap-match	subspecies for subspecies	1966
amended	subspecies for subspecies	1	subgenus	added to species	3959
			subgenus	deleted from species	2083
			subgenus	added to subspecies	119
			subgenus	deleted from subspecies	134
			amended	genus for genus	3
			amended	species for species	1807
			amended	subspecies for subspecies	356
Total		72693	Total		46835

dataset (Table 2). Ignoring the less significant "amended" change type, the totals are 47,453 (AM, 13.9%) and 37,124 records (MV, 11.2%.).

GBIF deleted no names in processing. One fail-matched record is for AM *catalogNumber* C.479173, identified as "Aplacophora" by AM and replaced by GBIF with the bivalve genus *Aulacophora* Jeffreys, 1882. The other fail-matches are for the marine snail names *Nuculana pala* (Hedley, 1907) (12 records) and *Nuculana (Ledella) pala* (Hedley,

Table 2. Tallies of records with changes by GBIF in genus- and species-group names in the AM and MV datasets. Totals of records with formal, genus- and species-group names: AM = 340998, MV = 331480.

AM:			MV:		
fail-match	class to genus	1	swap-match	genus for genus	3021
fail-match	species for species	13	up-match	genus to family	101
swap-match	species for species	218	up-match	species to order	8
up-match	genus to family	30	up-match	species to family	169
up-match	species to phylum	2	up-match	species to genus	14957
up-match	species to family	261	up-match	subspecies to genus	368
up-match	species to genus	46900	up-match	subspecies to species	18500
up-match	species to subgenus	5	amended	genus for genus	3
up-match	subspecies to genus	1	amended	species for species	5875
up-match	subspecies to species	22	amended	subspecies for subspecies	1517
amended	genus for genus	53	Total		44519
amended	species for species	2574			
Total		50080			

Table 3. Tallies of records in which either ALA or GBIF changed formal genus- and species-group names.

AM (340998 records):		
ALA only	ALA and GBIF	GBIF only
56123	16570	33510
MV (331480 records):		
ALA only	ALA and GBIF	GBIF only
36467	10368	34151

1907) (one record), which were incorrectly matched with *Nuculana pella* (Linnaeus, 1758) (*pala*: http://www.marinespecies.org/aphia.php?p=taxdetails&id=506315, accessed 2018-03-03; *pella*: http://www.marinespecies.org/aphia.php?p=taxdetails&id=140578, accessed 2018-03-03). (ALA did not change "Aplacophora" in processing, and swap-matched the *pala* names to *Ledella pala*.)

Name changes: *ALA* vs *GBIF*

Despite the roughly comparable numbers of name changes, ALA and GBIF processed the same set of names very differently. Table 3 tallies these differences as numbers of records. The overlap (names changed by both ALA and GBIF) is remarkably low. Further, among records with names changed by both ALA and GBIF there was substantial lack of agreement on the type of change (Table 4). However, for most of the records with both ALA and GBIF up-matching the original name, the two processed names were the same, with exceptions generally limited to genus differences (species up-matched to genus) or species differences (subspecies up-matched to species).

Table 4. Tallies of name change types among records in which both ALA and GBIF changed formal genus- and species-group names.

ALA change type	GBIF change type	No. of records
AM dataset:		
deleted	up-match	34
fail-match	up-match	1
down-match	up-match	32
swap-match	swap-match	4
swap-match	fail-match	13
swap-match	up-match	5054
swap-match	amended	275
up-match	swap-match	7
up-match	up-match	8525
up-match	amended	573
subgenus	up-match	90
subgenus	amended	219
amended	up-match	1275
amended	amended	468
	Total	16570
MV dataset:		
down-match	up-match	12
down-match	amended	13
swap-match	swap-match	8
swap-match	up-match	700
swap-match	amended	330
up-match	swap-match	90
up-match	up-match	6795
up-match	amended	1076
subgenus	up-match	468
subgenus	amended	38
amended	up-match	80
amended	amended	758
	Total	10368

In the AM dataset, the four records swap-matched by both ALA and GBIF are for one species:

Gyraulus coranus (Iredale, 1943) (AM)
> *Gyraulus* (*Gyraulus*) *essingtonensis* (ALA)
> *Gyraulus corinna* (Gray, 1850) (GBIF)

The eight MV records swap-matched by both ALA and GBIF are for two species:

Lipotriches (*Hoplonomia*) (MV)
> *Nomia* (*Hoplonomia*) (ALA)
> *Hoplonomia* Ashmead, 1904 (GBIF)

Leioproctus (*Nodocolletes*) (MV)
> *Leioproctus* (*Lamprocolletes*) (ALA)
> *Nodocolletes* Rayment, 1931 (GBIF)

The 32 AM records with the same species-group name down-matched and up-matched are for:

Erronea chrysostoma Schilder, 1927
> *Erronea ovum chrysostoma* (ALA)
> *Erronea* Troschel, 1863 (GBIF)

The 12 MV records with the same name both down- and up-matched are for two species:

Palaminus australiae Fauvel, 1878
> *Palaminus australiae australiae* (ALA) [ALA here ignores a second subspecies, *P. a. hebridensis* Cameron, 1934]
> *Palaminus* Erichson, 1839 (GBIF)

Dabra termitophila Lea, 1906
> *Dabra termitophila termitophila* (ALA) [ALA here ignores a second subspecies, *D. t. victoriensis* Lea, 1910]
> *Dabra* Olliff, 1886 (GBIF)

Name changes: type status

A consequence of name changes in processing is that a type specimen can lose its association with the name it represents. The AM and MV change tables include numerous records of primary types (Table 5). Among the large number of swap-matches, especially after ALA processing, there are types listed by the aggregator which are not, in fact, types of the replacement name. An example is MV T4295, the holotype of *Amaloptila triorbis* Turner, 1903, which in ALA has the processed synonym name *Elesma subglauca* Walker, 1865 with Type status = "holotype" (https://biocache.ala.org.au/occurrences/163727ac-8ba3-4dbb-a5c0-bf79d9474f04; accessed 2018-03-03). The holotype of *E. subglauca* is actually in the Natural History Museum (London) (http://www.nhm.ac.uk/our-science/data/butmoth/search/GenusDetails.dsml?NUMBER=9539.0; accessed 2018-03-03). (GBIF did not change "*Amaloptila triorbis* Turner, 1903".)

When checking for fail-matches (see below), I noted an issue with AM types on the GBIF website. The AM specimen lot C.26622 for *Nuculana pala* (Hedley, 1907) is a holotype, as can be seen in the "Diagnostics" section of the relevant GBIF webpage (https://www.gbif.org/occurrence/1100962172, accessed 2018-03-03), but although the specimen lot has the processed *typeStatus* = "holotype" in *occurrence.txt*, "Type status" is blank on the webpage and the processed value has the remark "Excluded".

Table 5. Tallies of name change types for primary type specimen lots (holotypes, lectotypes, neotypes, syntypes) among records with formal genus- and species-group names in the AM and MV datasets.

AM-ALA		**MV-ALA**	
deleted	12	deleted	27
down-match	20	down-match	28
swap-match	397	swap-match	1637
up-match	753	up-match	560
subgenus	16	subgenus	320
amended	57	amended	46
Total	1255	Total	2618
AM-GBIF		**MV-GBIF**	
fail-match	1	swap-match	3
swap-match	1	up-match	223
up-match	1326	amended	100
amended	48	Total	326
Total	1376		

Name changes: unrecognised fail-matches

Without checking thousands of name changes individually, it was impossible to determine how many up-, down- and swap-matches resulted in a taxon name being replaced with one from another branch of biological classification, or with a non-synonym (see the GBIF *pala/pella* example, above). The 39 fail-matched records tallied in the change tables are the most obvious failures I found. I suspect there are many more, but using higher classifications in name-changed records as a guide was made impractical by unfilled higher-taxon entries (AM) and disagreements on higher taxa between data provider and aggregator (MV).

Data losses

Aggregator processing sometimes results in loss of a data item: an original record contains a data item in a particular field, but after processing that field is blank for the record concerned. I found a surprisingly high number of data losses in the audited datasets. Below I give examples of loss (see also the comment above on deleted taxon names in ALA, and on verbatim depth and elevation data in Methods: *Issues with field structuring*). For more details, see *data_notes.txt* in the Zenodo archive for this project.

identifiedBy: AM-ALA and MV-ALA, 100% loss in processing. The original *identifiedBy_raw* data item appears on the ALA webpage as "Identified by" for the record but is missing from the standard (recommended) download).

locality: MV-ALA, 100% loss in processing. The original *locality_raw* data item appears on the ALA webpage as "Locality" for the record but is missing from the standard (recommended) download). (*locality_raw* is blank in the AM dataset.)

Losses of date information were common and evidently due to processing rules written to deal with various date formats. In the *modified* field in the NZAC dataset, for example, GBIF successfully parsed 4765 entries in YYYY-MM-DDTHH:MM:SS+12:00 format, but deleted 97,327 entries in YYYY-MM-DDTHH:MM:SS.sss+12:00 format (95% data loss). This failure may explain why GBIF did not delete the earlier versions of the 1186 duplicated records (see Methods), as both the earlier and later versions of these records have *modified* entries in YYYY-MM-DDTHH:MM:SS.sss+12:00 format.

Other major losses were in the *eventDate* and *dateIdentified* fields and were sometimes inconsistent. Here are details of an example: the MV-ALA dataset contains 341,693 correctly formed entries in *eventDate_raw*. These include 13,815 interval dates. The entire interval date was excluded when the format was YYYY-MM-DD/YYYY (23 records) or YYYY-MM-DD/YYYY-MM (32). The earlier date in the interval (only) was accepted from the formats YYYY-M-D/D (1 record), YYYY-MM-DD/DD (11037 plus an exception, see below), YYYY-MM-DD/MM-DD (2317) and YYYY-MM-DD/YYYY-MM-DD (404 plus an exception). One of the two exceptional exclusions was the entry "2006-09-02/2005-11-20", which is malformed as an interval date. Its exclusion suggests that ALA tested interval dates before deleting the later date. The second exclusion was "1943-06-20/21" for *catalogNumber* C.95257 (https://biocache.ala.org.au/occurrences/5966a91c-b333-4781-924f-92f1f6f57919; accessed 2018-02-21). The same interval date ("1943-06-20/21") was accepted for four other records, e.g. C.95256 (https://biocache.ala.org.au/occurrences/b78583d0-4195-4a65-a733-6445394e7bd2; accessed 2018-02-21). Among non-interval dates, ALA excluded YYYY (98812 records) and YYYY-MM (51412), while accepting YYYY-M-D (2), YYYY-MM-D (1) and YYYY-M-DD (1). All but 258 of 177,650 valid YYYY-MM-DD dates were accepted. All 258 appear to be well-formed, and as with "1943-06-20/21" above, ALA accepted and excluded the same YYYY-MM-DD in different records. For example, "1985-10-06" was rejected in C.364665 (https://biocache.ala.org.au/occurrences/16a2f2ee-a330-4a4a-8a89-b1450f00f270; accessed 2018-02-21) but accepted in C.441797 (https://biocache.ala.org.au/occurrences/dcdc015f-363d-4d5e-a532-2a8a4988ee20; accessed 2018-02-21). In summary, ALA filtered out 150,537 of all valid *eventDate_raw* entries in the MV dataset (44 % loss) and accepted only the starting date in non-excluded interval dates.

ALA also had processing losses in fields containing names of persons and organisations. In the AM dataset, the original *recordedBy_raw* field has 236,855 entries in a range of formats. These include both "name" (e.g. "A.C. & J.E.Miller") and "reverse name" (e.g. "Abbott, E.") entries, as well as oddities such as "aborigines" and "Unknown (Sea Gypsies)". ALA excluded no *recordedBy_raw* entries and processed 66,900 entries without change. Many of the 169,955 entries changed by ALA (72% of the total) were not processed successfully. Some systematic failures are listed below. The most significant errors were the replacement of a valid string by "null", which occurred in 7,596 entries (3.2% of total), and the data losses associated with conjunction failure (see below), which I did not tally.

null A name string was replaced by the word "null", e.g. "A.Musgrave & E.LeG. Troughton" processed as "Musgrave, A.|null".

conjunction failure Conjunctions and separators were replaced with pipes ("|"). The results were variably successful, with some pipes separating two recorders correctly, e.g. "J.& D.Freeman" processed as "Freeman, J.|Freeman, D." A large number (not tallied) of entries were incorrectly piped, e.g. "J.Brazier & G.Rossiter" processed as "Rossiter, J.|Rossiter, G." and "J.Paxton & M.McGrouther" processed as "Mc, J.|Mc, M.". Conjunction failures also saw institutional affiliations become separate recorders, as with "Kessner, Mr. Vince - Australian Museum - Malacology" processed as "Kessner, V. Vince|Australian Museum|Malacology".

initial reversal The order of the first name and middle initial were reversed, e.g. "Harvey, Michael S." processed as "Harvey, S. Michael".

added initial The initial of the first name is added, e.g. "Houghton, Noel" processed as "Houghton, N. Noel".

initial comma A comma was placed at the beginning of the name(s), e.g. "B.M.R." processed as ", B.M.R." and "W.F.& J.M.Ponder & T.Habe" as ", W.F.|Ponder, J.M.|Habe, T.".

surname ending The string "and" at the end of a surname was processed as the conjunction "and", e.g. "M. Crossland" replaced by ", M. Crossl|".

GBIF processed most names without change in the *recordedBy* and *identifiedBy* fields, but excluded 443 *recordedBy* entries representing 119 unique name strings in the MV dataset. A check of several of the excluded entries shows that they were accepted by GBIF in other records. For example, "Peter K. Lillywhite - Museum Victoria" was excluded in four records but accepted in 233 others. This inconsistent processing resulted in a loss of <1% of valid data items.

Other data issues

While checking for taxon name changes and data losses, I incidentally noted several other unwelcome results of data processing, described here.

In the NZAC dataset, *verbatim.txt* has the string "not identified on slide" in the *scientificName* field for *catalogNumber* NZAC02015964, and the other taxonomic fields are empty. GBIF matched this string with the fictitious genus *Not* Chan, 2016, to which occurrence records from other datasets had also been matched, for entries such as "Not naturalised in SA sp." and "Not listed". I published this processing error in a post to the *iPhylo* blog on 24 January 2018 (http://iphylo.blogspot.com.au/2018/01/guest-

post-not-problem.html). The "*Not* Chan, 2016" page was subsequently removed from the GBIF website and the NZAC "not identified on slide" record is now processed as "incertae sedis" (https://www.gbif.org/occurrence/1315606681; accessed 2018-03-01).

In the MV dataset, ALA processed *geodeticDatum_raw* entries to *geodeticDatum* inconsistently and with errors. Of the "WGS84" entries, 341539 were correctly processed to the equivalent EPSG:4326, while 652 were deleted. Also processed to EPSG:4326 were 203 "AGD66" entries, for which the correct equivalent is EPSG:6202; the difference on the ground is ca 200 m.

ALA processes locality text fields on the basis of the supplied decimal latitude and longitude values, disregarding original values in text fields. If the supplied coordinates are incorrect, ALA's processing sometimes adds incorrect text data. In the AM dataset, *catalogNumber* C.500744 is from Roma Gorge in Australia's Northern Territory but was assigned to Mozambique ("-23.63805 32.41804" supplied instead of -23.63805 132.41804) and C.429548.002 from near Newcastle Waters in the Northern Territory was assigned to Namibia ("-17.25750 13.45444" instead of -17.25750 133.45444). In both cases *country_raw* is "Australia" and *stateProvince_raw* is "Northern Territory". In the MV dataset, ALA assigned Coral Bay (*country_raw* = "Australia", *stateProvince_raw* = "Western Australia") to Botswana ("-23.14 23.14" supplied instead of -23.14 113.77; *catalogNumber* HET5323), and Clifton Downs Station ("Australia" and "New South Wales") to South Africa ("-29.61 29.61" instead of -29.61 142.57; HET4586).

Discussion

Users can download from ALA and GBIF, as I did, sets of occurrence records containing both original and processed data items. However, both ALA and GBIF recommend smaller downloads containing only processed records. As noted above, these processed records can have blanks where data was originally provided, can feature taxon names different from the ones used by the data provider, and can contain processing errors. The user of a recommended ALA or GBIF download has no way of knowing which names have been changed or which data items lost, other than to go to the ALA and GBIF websites and look for original vs processed data differences for individual records, or investigate individual data quality "flags" and "assertions".

I do not know the extent to which data providers are informed by aggregators about changes made in the data supplied, or whether providers, under the terms of legal arrangements with aggregators, can ask that changes be reversed. Museums and herbaria might be particularly concerned at the confusion in processed records regarding the identity of type specimens. These questions were addressed in general terms in a published response to an earlier audit of mine which looked at the quality of occurrence data as provided to aggregators (Mesibov 2013):

"Some data providers encourage the ALA to make corrections to the provider's records (for provider and ALA). Other data providers would withdraw their support if similar changes were attempted on their data by the ALA. Feedback from the ALA to

a data provider may result in immediate corrections (and data propagation) while in other cases, the provider has no resources to resolve an issue. There is no single process here that will work effectively in all circumstances." (Belbin et al. 2013).

Note, however, that these comments referred to problems in data as provided, not to problems generated at aggregator level, as reported here.

It is a little surprising that GBIF asks users to cite their downloaded data as authored by the provider (See Methods, Data sources), and that ALA likewise asks (in each download's *citation.csv* file) that data be cited as records from the provider. Clearly this is not the case for processed data. It would be more correct to say that aggregated data are made available as the combined work of provider and aggregator, and that the aggregator is solely responsible for any differences between original and processed data.

Some of the processing problems noted in this paper are the result of programming errors. Given the results reported here, it seems unlikely that ALA and GBIF programming staff or contractors have systematically compared original and processed data to look for problems in selected fields, as I did. I am also aware that issues raised on the ALA GitHub site (https://github.com/AtlasOfLivingAustralia) by staff and users can remain open for long periods (e.g. https://github.com/AtlasOfLivingAustralia/ala-downloads/issues/17; accessed 2018-03-03), and that closed issues may still be open, e.g. the lack of a processed *locality* field, which was said to have been corrected two months before I downloaded ALA data for audit (https://github.com/AtlasOfLivingAustralia/ala-downloads/issues/14; accessed 2018-03-03). The apparent failure of ALA and GBIF to monitor processing output is both surprising and disappointing. It is particularly surprising that data loss in processing of dates remains a significant problem, five years after a careful analysis of GBIF date losses was published by Otegui et al. (2013a).

Data loss may be the result of programming "bugs", but both ALA and GBIF regard the replacement of originally supplied taxon names as a feature of their aggregation protocols. This view has its critics (Franz and Sterner 2018), and it is hard to understand how losing a taxon name through fail-matching or up-matching improves an occurrence record. Otegui et al. (2013b) attribute some failed matching to inadequate provision of higher-taxon information in original records. The remarkable lack of agreement between ALA and GBIF on replacement names for the same original names highlights another problem: reference classifications differ. The Catalogue of Life Plus project (https://github.com/Sp2000/colplus; accessed 2018-03-01) will attempt to replace differing reference classifications with a single "consensus" one for aggregator use, but it seems unlikely that the result will be universally adopted by data providers, let alone by the taxonomists who supply scientific names to museums and herbaria.

Three recommendations could be made to improve the taxonomic usefulness of aggregated occurrence records. A simple, easily implemented one is for aggregators to include both original and processed taxonomic data items in each record, from *scientificName* and all higher taxon fields. A second improvement would be for aggregators to employ, as Franz and Sterner (2018) propose, multiple reference classifications, so that each original taxon name could be seen in a range of taxonomic contexts. A third possible advance would be for aggregators to construct sets of related name strings, so

that each original name can be seen by the user as one element in a group of variants, synonyms and fuzzy matches – a "see also" function in searches and record selection for download.

There have been a number of recent studies which question the quality of aggregated occurrence records (see references in Franz and Sterner 2018), but Sikes et al. (2016) defend GBIF and other aggregators by saying that all data need vetting and processing and that the quality of aggregated data is the collective responsibility of the biodiversity data community. These are truisms. It is also obvious that aggregators should not lose or confuse the data they are provided with, and the present audit suggests that both ALA and GBIF could do better. End-users of aggregated occurrence records would be wise to download both the original and the processed datasets, and to check carefully for data losses and taxon name replacements.

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