

# *Nipponnemertes incainca* sp. n. Adoption of the new taxonomic proposal for nemerteans (Nemertea, Cratenemertidae)

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## Abstract

A new species *Nipponnemertes incainca* is described from the intertidal zone of Santa Marta, Colombia. A new recent approach based on both morphological and molecular characters is applied for the description. The main characteristics of the species are: red color, head shield-shaped with a mid-dorsal cephalic ridge, furrows pre-cerebral inconspicuous with few faint ridges orthogonal to furrow axis, two irregular groups of eyespots situated at lateral margins in precerebral cephalic region, proboscis provided with papillae and 12 nerves, stylet smooth supported on an oval basis, and two pouches containing 3–4 accessory stylets each. The sequence of the COI gene was analyzed as an additional support for the new species.

## Keywords

New species, Nemertea, COI, Caribbean coast of Colombia

## Introduction

Nemerteans (phylum Nemertea), commonly known as ribbon worms or Rhynchocoela, comprise a cosmopolitan group of bilateral, coelomate, and unsegmented worms (Turbeville 2002). The major synapomorphy supporting the monophyly of the phylum is the presence of an eversible proboscis housed in a fluid-filled cavity, the rhynchocoel (*ibid*). Around 1,300 species of nemerteans are recognized, most of which are found in marine environments; nevertheless, freshwater and terrestrial species are also known (Gibson 1995, Kajihara et al. 2007). Among nemerteans the genus *Nipponnemertes* contains 18 species of marine benthic worms; the interwoven muscular layers in the rhynchocoel wall, and large cerebral sense organs extending behind the brain are the two main traits that distinguish them from most other monostiliferan genera (Friedrich 1968).

Of the 36 species of nemerteans documented for the Caribbean Sea (Corrêa 1961, 1963, Kirsteuer 1973, 1974, 1977, Schwartz and Norenburg 2005, Gonzalez-Cueto et al. 2014), 13 have been reported to be present on the Colombian coast (Kirsteuer 1977, Gonzalez-Cueto et al. 2014). However, the majority of these species have been recorded from a single locality (Santa Marta Bay) despite nemerteans being an abundant component of the macrofauna communities in Colombia (Dueñas 1998, Trujillo et al. 2009). Taxonomic studies on nemerteans from Colombia are scarce. Species identifications traditionally require a detailed study of the internal anatomy, which is considered to be difficult and time consuming. This is because several morphological characters are doubtful, subjective, poorly defined, and plastic, in addition to a lack of taxon experts (Sundberg et al. 2016a).

Sundberg et al. (2016a) highlight these problems for the taxonomy of nemerteans and, to advance the study of nemerteans, they suggest transitioning from a traditional, difficult, and often unreliable taxonomy to a more integrative process of describing species based on external morphological characteristics that are easily observable combined with molecular data. Together this would facilitate more accurate species identifications, even for the non-specialist.

Herein, the method proposed by Sundberg et al. (2016a) is used to describe a new species of ribbon worm from Colombia. The external appearance of the worm and photos of the histological section of the proboscis are presented, in addition to a molecular analysis using the mitochondrial gene COI. The COI sequence of one specimen is deposited in GenBank and whole specimens fixed in formalin and other tissue pieces preserved in absolute alcohol are deposited in the “Centro de Colecciones Biológicas de la Universidad del Magdalena” for future molecular and morphological studies.

## Materials and methods

Four specimens were hand-collected on the rocky littoral from Inca-Inca Bay, Santa Marta, Colombia (11°12'30.2"N; 74°13'54.5"W). Individuals were relaxed in 7% MgCl<sub>2</sub> solution isotonic to seawater and photographed “*in vivo*” with a digital camera

Nikon D7100 with a 60 mm ED Micro-Nikkon lens. Details of morphological characters were photographed with a stereomicroscope Leica M205A with an integrated Camera Leica DFC450. Detailed images of the proboscis and stylets were obtained by pressing the specimens between a slide and a coverslip (obligating them to protrude the proboscis) and photographing them with a microscope Zeiss Axiolab A1 with an integrated camera Zeiss ERc5s. Two specimens were fixed in 100% ETOH for molecular purposes and two in 10% formalin for future morphological analysis.

Two additional specimens previously collected and deposited in the “Centro de Colecciones Biológicas de la Universidad del Magdalena, CBUMAG” (Gonzalez-Cueeto et al. 2014) were also examined. Cross sections of the proboscis were obtained from one these specimens (CBUMAG:NEM: 0049). For that, the proboscis was embedded in paraffin; sectioned at 7µm thickness with an AO 820 Spencer microtome, and stained with H&E. Coverslips were mounted with Permount®.

Total DNA was extracted from one entire worm fixed in 100% ETOH, using the DNeasy Blood & Tissue® Kit following the manufacturer’s protocol (Qiagen, Valencia, CA, USA). The partial COI gene was amplified with universal primers described in Folmer et al. (1994). The PCR was performed with 2 µL template in a 25 µL volume with final concentrations of 2 mM MgCl<sub>2</sub>, 5X buffer PCR (no MgCl<sub>2</sub> BIOLINE), 0.4 µM of each primer, 0.4 µM of each dNTP, and 2 units Taq (BIOLASE™, BIOLINE®). The PCR conditions were: 1 min at 95 °C, followed by 35 cycles of 15 s at 95 °C, 1 min at 40 °C, 1.5 min at 72 °C, and there was a final extension period of 5 min at 72 °C. The sequence was edited with ProSeq (Filatov 2009) and aligned with all the sequences from *Nipponnemertes* accessible in GenBank using the ClustalW algorithm available in MEGA (Tamura et al. 2011) with default parameters. Following the barcoding approach suggested by Hebert et al. (2003), a matrix of intraspecific and interspecific evolutionary genetic distances was made using the Kimura’s two parameter model K2P (Kimura 1980), also available in Mega (Tamura et al. 2011).

## Results

### Taxonomy

**Family:** Cratenemertidae Friedrich, 1968

**Genus:** *Nipponnemertes* Friedrich, 1968

***Nipponnemertes incainca* sp. n.**

<http://zoobank.org/942EBF8B-976E-4952-B2C5-FCFD32BF690D>

Fig. 1 A–F

**Material examined.** Holotype: COLOMBIA Santa Marta, Rodadero Inca-Inca beach (74°13'54.5"W, 11°12'30.2"N), intertidal zone under boulders, whole specimen in 70% ethanol (CBUMAG:NEM: 0056). Total body length 18.5 mm, 1 mm wide.



**Figure 1.** *Nipponnemertes incainca* sp. n. **A** Dorsal view of entire worm **B** Ventral view of entire worm. Abbreviation: *p* proboscis

Paratypes: COLOMBIA Santa Marta, Taganga (11°15'51.23"N, 74°11'31.54"W), intertidal zone under boulders covered by sponges, whole specimen in 70% ethanol (CBUMAG:NEM: 0043). Total body length 11.7 mm, 1.8 mm wide.

COLOMBIA Santa Marta, Rodadero Inca-Inca beach (11°12'30.2"N, 74°13'54.5"W), intertidal zone under boulders, transverse histological sections of the proboscis; rest of specimen in 70% ethanol (CBUMAG:NEM:0049). Total body length 22.5 mm, 2.05 mm wide.

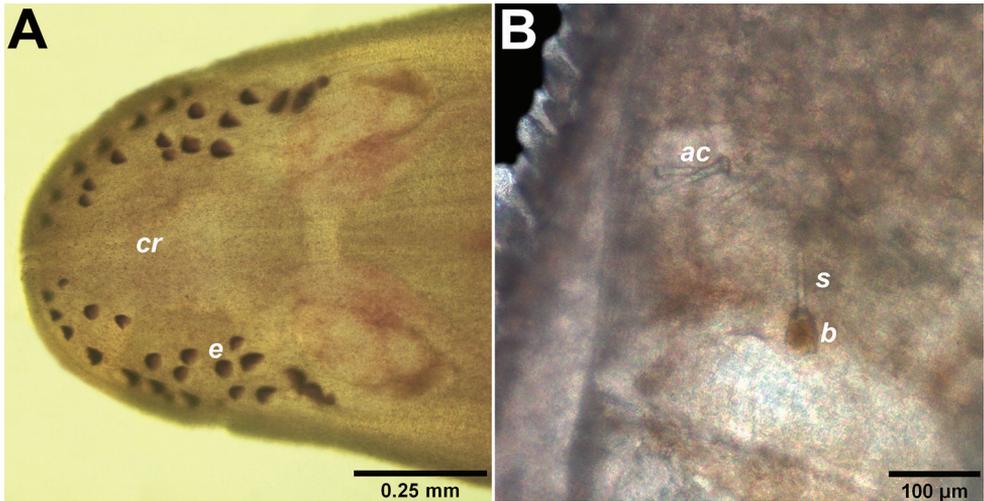
COLOMBIA Santa Marta, Rodadero Inca-Inca beach (11°12'30.2"N, 74°13'54.5"W), intertidal zone under boulders; tissue in absolute ethanol (CBUMAG:NEM:00068, CBUMAG:NEM:00069).

An entire additional worm, collected in Inca-Inca beach (11°12'30.2"N, 74°13'54.5"W) was used for DNA extraction. Sequence data for 615 bp of Cytochrome C Oxidase Subunit I deposited in GenBank under accession number KX879856 (see alignments with other congeners in supplemental information).

**Etymology.** The specific epithet refers to the “Inca-Inca beach” site from which most of the specimens were collected; this name is in apposition.

**Diagnosis.** *Nipponnemertes incainca* sp. n., like other members of *Nipponnemertes*, has a mid-dorsal cephalic ridge, is capable of retracting the head into the body when disturbed, and is capable of swimming. However, in this new species the anterior furrows and their secondary transverse grooves are faintly visible both macro- and microscopically and they are not visible in a ventral view.

**Description.** Relaxed length from 11.7 mm to 22.5 mm and width 1 to 2 mm. Dorsal side uniformly bright red color (Fig. 1A). Ventral side lighter than dorsal side (Fig. 1). Head shield-shaped, slightly demarcated from rest of body but without V-shape



**Figure 2.** *Nipponnemertes incainca* sp. n. **A** Detail of ocelli **B** Microscopic detail of stylet and accessory stylets. Abbreviations: *cr* cephalic ridge, *e* eyespot, *s* central stylet, *b* base of stylet, *ac* accessory stylets.

cephalic groove and not wider than trunk. Mid-dorsal cephalic ridge present in head (Fig. 1A, 2A). Frontal organ with small cirrus. Cerebral organ furrows pre-cerebral, inconspicuous, with few faint ridges orthogonal to furrow axis. Brain distinguishable as a pale brown bilobed structure through dorsal and ventral body wall. Two irregular groups of eyespots situated at lateral margins in precerebral cephalic region (Fig. 2A), extending beyond brain parallel to lateral nerve cords. Rhynchopore subterminal. Proboscis long and stout, with papillae (Fig. 3B), pink in color when everted (Fig. 1B). Stylet (length: 87.4 µm) smooth, supported on an oval basis (54 × 38.3 µm); two pouches containing 3–4 accessory stylets each (Fig. 2B). Twelve proboscidial nerves present (Fig. 3A–B). This species was found among sponges and brown algae underneath rocks, and in the crust formed by sediment inside the crevices of rocks in the littoral zone. Worms capable of swimming with strong undulating movements.

**Diferential diagnosis.** We compared morphological characters of *Nipponnemertes incainca* sp. n. with the 18 valid species of the genus, according to Gibson (1995) and Kajihara et al. (2007) (Table 1).

The most similar species in color, arrangement of ocelli and numbers of proboscidial nerves to *Nipponnemertes incainca* sp. n. is *N. pulchra* and it might easily represent an intraspecific variation. However, in the intraspecific and interspecific genetic distance matrix (table 2), the interspecific distance between *N. incainca* sp. n. and *N. pulchra* was 21.03%, which exceeds the highest limits given by Sundberg et al. (2016b) for the Hoplonemertea. Therefore, the fact that the new species lacks the V-shape structure formed by the cephalic grooves, and the accessory stylet in the basis of the central armature, present in *N. pulchra*, is enough to discriminate the two species.

**Table 1.** Remarks about morphological and behavioral traits useful to discriminate the species of the genus *Nipponmemertes*. Reference after authority in species column.

Species	Body Coloration,	Number of Proboscis Nerves	Mid-dorsal cephalic ridge	Shape and distinctness of posterior dorsal V-shaped cephalic groove	Other Noteworthy Characters
<i>Nipponmemertes inca</i> sp. n.	Solid bright red color pattern without designs	12	Present	Lacking	Anterior furrows and secondary transverse grooves present, but faintly visible both macro and microscopically. Inhabits rocky littoral zone
<i>Nipponmemertes africanus</i> (Wheeler, 1940) Berg, 1985	“White, pink, pinkish yellow or buff; lighter anteriorly and deeper on back” (Berg 1985). Mottled and dotted with white gonads	11	Present (McDermott 1998)	Present. Two posterior dorsal cephalic grooves, V-shaped but not joined medially (McDermott 1998, P. 252)	Faint head-glands, open close to external opening of rhynchoedeum and disappear just before brain. Found between roots of alga <i>Hypnea speciosa</i> , low on shore
<i>Nipponmemertes arenaria</i> (Uschakov, 1927) Chernyshev 1993	Margins of body lighter in color				Inhabits muddy sand (Chernyshev 1993)
<i>Nipponmemertes bimaculatus</i> (Coe, 1901) Gibson & Crandall, 1989	Head flesh in color; rest of body is deep red, brownish red, or brownish orange; lighter on ventral surface. Possesses pair scalene triangle-shaped cephalic marks and a narrow longitudinal line of dark color on dorsal surface of esophageal region	14 or 16	Present (Coe 1905, plate 18)	Lacking	Central stylet very long and slender, mounted on a remarkably tiny base
<i>Nipponmemertes danae</i> (Friedrich, 1957) Friedrich, 1968	Dorsal surface red, ventral white; color description based on Coe's description of <i>Nipponmemertes drepanophoroides</i> (Coe 1905, p. 282)				Original description is vague and lacks important information. According to Berg (1985) it is synonym of <i>N. pulchra</i>
<i>Nipponmemertes drepanophoroides</i> (Griffin, 1898) Friedrich, 1968	Red above, white beneath				Lacks intestinal caeca
<i>Nipponmemertes fernaldi</i> Iwata, 2001	Pale brown on dorsal surface and darker on the ventral side (colorless lateral margins)	14	Present	Oblique, limited to dorsal surface	

**Table 1 (continued).** Remarks about morphological and behavioral traits useful to discriminate the species of the genus *Nipponnemertes*. Reference after authority in species column.

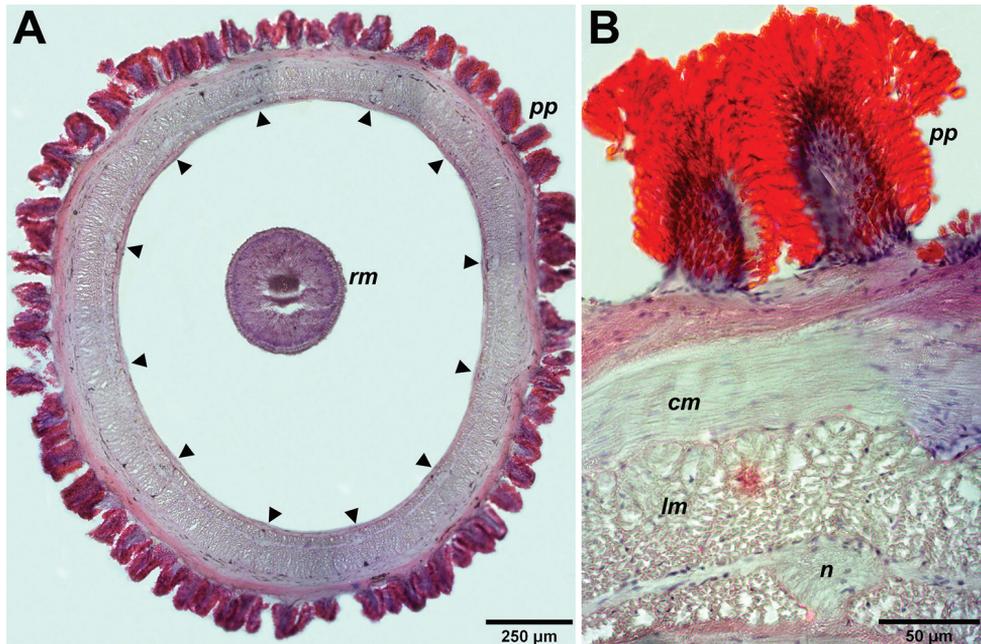
Species	Body Coloration,	Number of Proboscis Nerves	Mid-dorsal cephalic ridge	Shape and distinctness of posterior dorsal V-shaped cephalic groove	Other Noteworthy Characters
<i>Nipponnemertes mada-gascarenis</i> (Kirsteuer, 1965) Friedrich, 1968	Ochre on dorsal surface, stained with irregular reddish-brown blotches	9	Lacking	Lacking	
<i>Nipponnemertes mag-nus</i> (Punnett, 1903) Berg, 1985	Light orange-brown	20			
<i>Nipponnemertes mari-oni</i> (Hubrecht, 1887) Berg, 1985	“Dorsally blue-green, yellow-green, pale buff or light brown, and ventrally pale buff light orange-brown” (Berg 1985)	15			
<i>Nipponnemertes oc-cidentalis</i> (Coe, 1905) Friedrich, 1968	Blotchy dark reddish brown or pale ground color throughout whole dorsal surface, and “ventral surface without color” (Coe 1905)				Highly developed intestinal caecum. Caecal appendage in esophagus and one in stomach
<i>Nipponnemertes ogo-mai</i> (Yamaoka, 1947) Crandall et al., 2001	Uniformly orange (Kajihara et al. 2014) although originally described as bright vermillion (Crandall et al. 2001)	16	Present (Kajihara et al. 2014)	Present, but not significantly developed	Minute ocelli gathered as a triangle on each side of head
<i>Nipponnemertes pacifi-cus</i> (Coe, 1905) Friedrich, 1968	Reddish or brownish dorsal surface, pale beneath	14	Lacking	Lacking	Cerebral sense organs remarkably large and highly specialized. Highly developed esophageal caecum (Coe 1905)
<i>Nipponnemertes pul-chra</i> (Johnston, 1837) Berg, 1972	“Dorsal surface varying between brown, red and pink. Lateral parts of body and ventral surface always much lighter, longitudinal dor-sal swelling on head often somewhat darker” (Berg 1985)	8-14 (normally 12)	Present	Dorsally, clearly marked and darker than rest of body. Does not reach midline on ventral surface	Presence of accessory stylet in basis of central armature. This character has been highlighted as one of best criteria to recognize <i>N. pulchra</i>
<i>Nipponnemertes punc-tatulus</i> (Coe, 1905) Friedrich, 1968	Pale brown or yellowish white with numerous darker brown spots on dorsum and white ventrum (head white with two dark blotches). Proboscis transparent, with pinkish stylet basis (Iwata 2008)	15	Present	Lacking	Iwata (2008) recorded 12, 13 or 16 proboscis nerves in worms collected in United States

**Table 1 (continued).** Remarks about morphological and behavioral traits useful to discriminate the species of the genus *Nipponmemertes*. Reference after authority in species column.

Species	Body Coloration,	Number of Proboscis Nerves	Mid-dorsal cephalic ridge	Shape and distinctness of posterior dorsal V-shaped cephalic groove	Other Noteworthy Characters
<i>Nipponmemertes rubella</i> (Coe, 1905) Crandall & Norenburg, 1999	Deep flesh color, pale orange, or pale red; much paler and usually grayish beneath	14			Great development of body parenchyma and intestinal caeca
<i>Nipponmemertes sanguinea</i> Riser, 1998	Dorsum buffy white to pale yellow to orange with reddish lines (aggregation of red blood corpuscles in blood vessels), ventral side paler, brain lobes pink	12	"Not evident" (Riser 1998)	Lacking	Presence of red blood corpuscles
<i>Nipponmemertes schol-laerri</i> (Wheeler, 1934) Berg, 1985	Pale buff color	14	Lacking (Wheeler 1934, p. 265)	Lacking	
<i>Nipponmemertes variabilis</i> (Korotkevich, 1983) Chernyshev, 1993	Beige dorsal and ventrally	12-13	Lacking	Separating strongly head from rest of body	

**Table 2.** COI-based matrix of interspecific and intraspecific genetic distances, using Kimura’s two-parameter model K2P (Kimura 1980). GenBank accession numbers: *Nipponnemertes incainca* sp. n. (KX879856); *N. bimaculatus* (AJ436909); *N. pulchra* (KP697761–KP697767); *N. punctatulus* (AJ436910); *N. ogumai* (AB920907); *Nipponnemertes* sp. 1 (HQ848598); *Nipponnemertes* sp. 2 (HQ848599); *Nipponnemertes* sp. 3 (KU230295).

	<i>Nipponnemertes incainca</i> sp. n.	<i>Nipponnemertes bimaculatus</i>	<i>Nipponnemertes pulchra</i>	<i>Nipponnemertes punctatulus</i>	<i>Nipponnemertes ogumai</i>	<i>Nipponnemertes</i> sp. 1	<i>Nipponnemertes</i> sp. 2	<i>Nipponnemertes</i> sp. 3
<i>Nipponnemertes incainca</i> sp. n.	×	×	×	×	×	×	×	×
<i>Nipponnemertes bimaculatus</i>	15.62	×	×	×	×	×	×	×
<i>Nipponnemertes pulchra</i>	21.03	20.12	0.09	×	×	×	×	×
<i>Nipponnemertes punctatulus</i>	17.13	8.61	18.39	×	×	×	×	×
<i>Nipponnemertes ogumai</i>	56.60	53.33	48.44	55.31	×	×	×	×
<i>Nipponnemertes</i> sp. 1	21.44	19.14	4.50	18.31	52.26	×	×	×
<i>Nipponnemertes</i> sp. 2	16.00	18.82	10.32	18.91	47.30	10.92	×	×
<i>Nipponnemertes</i> sp. 3	17.13	8.61	18.39	0.00	55.31	18.31	21.00	×



**Figure 3.** *Nipponnemertes incainca* sp. n. **A** Transverse sections of the proboscis; nerves are highlighted by arrowheads **B** Microscopic detail of transverse section showing the proboscis papillae. Abbreviations: *pp* proboscis papillae, *lm* longitudinal muscles, *cm* circular muscles, *rm* retractor muscles of the proboscis, *n* nerve.

## Discussion

Approximately, 2.2 million ( $\sigma$  0.18) species inhabit the marine ecosystems, yet 91% of these still await description (Tittensor et al. 2010; Mora et al. 2011). The rate at which these species become extinct has reached an unprecedented degree that is much higher than the rate of new species discovered (Dirzo and Raven 2003; Schefers et al. 2012). The new taxonomic approach of Sundberg et al. (2016a) might help facilitate the description of new species of nemerteans, which otherwise would be underestimated or overlooked. With this approach some morphological characters and molecular data of the new species will be available to scientists in order to have a more integrative assessment of biodiversity. However this approach should be interpreted cautiously because some species, such as the one described here, might require the revision of some internal features (*i.e.* the number of nerves in the proboscis).

*Nipponnemertes incainca* sp. n. was recorded as Cratenemertidae sp. by Gonzalez-Cueto et al. (2014) and probably it is also the same species recorded as Cratenemertidae spp. by Collin et al. (2005) from “Bocas del Toro (Panama)”. Misidentification of nemerteans is common in the environmental assessments of marine ecosystem around the world (Sundberg et al. 2016a). In fact, in Colombia, many specimens remain named as *Nemertea* sp. even in biological collections such as the “*Museo de Historia Natural Marina de Colombia (INVEMAR)*” and the “*Centro de Colecciones Biológicas de la Universidad del Magdalena*”. The standardization of the taxonomic and behavior-based character matrix proposed by Sundberg et al. (2016a), applied in this survey (Table 3), and the use of molecular markers (e.g. COI) increase the value of taxonomic identifications in the future. Our study expands the known number of nemertean species of the Caribbean coast of Colombia from 12 to 13. In addition, it encourages a new generation of taxonomists to begin or to continue working on this neglected group of animals.

**Table 3.** Character checklist. List of external characters that could be checked in order to provide a species description with comparable characters. Modified from Sundberg et al. (2016a).

	Character	Character state	Code
1.	Biology	Free-living	0
2.	Habitat	Marine	0
3.	Benthic divisions	Littoral	1
5.	Habitat	Epibenthic	2
6.	Substratum	Rock/boulders	3
7.	Behavior when mechanically disturbed	Contracts without coiling into a spiral	0
	External morphology		
8.	Cephalic furrows/slits	One pair	1
9.	Distribution of anterior cephalic furrows/slits	Dorsal	1
10.	Shape of anterior (dorsal) cephalic furrows (viewed with tip of head directing forwards)	Ventral transversal	2
12.	Head clearly demarcated from body	Head not wider than trunk	2
13.	Position of cephalic furrows	If single pair in front of brain lobes	1

**Table 3 (continued).** Character checklist. List of external characters that could be checked in order to provide a species description with comparable characters. Modified from Sundberg et al. (2016a).

	Character	Character state	Code
14.	Shape of head/cephalic lobe	Shield-shaped	10
15.	Head viewed laterally	Without extensions	0
16.	Cross section shape of body	Rounded cylindrical	0
17.	Shape of posterior tip	Bluntly rounded	3
18.	Eyes	Eyes arranged in lateral rows or groups on each side of head	7
19.	Eye distinctiveness	Eyes visible from ventral side	0
20.	Eye morphology	Simple	0
21.	Relative eye size	All eyes more or less of equal size	0
22.	Eye position relative to brain lobes	Confined principally or entirely to precerebral cephalic region but may extend back to above brain	0
23.	General body color	No obvious color	0
24.	Primary dorsal body color	Red	0
25.	Color pattern	Absent	0
26.	Color of blood	Red	0
27.	Proboscis armature	With central and accessory stylets	2
28.	Number of accessory stylet pouches	Two	0
29.	Number of stylets in each accessory stylet pouch	Three or four	1
30.	Stylet : basis/stylet ratio	1.5:1	1
31.	Stylet shaft	Smooth and straight	0
32.	Shape of stylet basis	Oval (rounded)	0
33.	Median waist of stylet basis	Absent	0
34.	Proboscis used for locomotion	Yes	1
35.	Proboscis pore	Subterminal, ventral	1
38.	Lateral margins	No distinction in color	1
39.	Distribution of bristles/cirri	Only on head	1

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## References

- Berg G (1972) Studies on *Nipponnemertes* Friedrich, 1968 (Nemertini, Hoplonemertini). Zoologica Scripta 1(4): 211–225. <https://doi.org/10.1111/j.1463-6409.1972.tb00580.x>
- Berg G (1985) Studies on *Nipponnemertes* Friedrich (Nemertini, Hoplonemertini). II. Taxonomy of *Nipponnemertes pulcher* (Johnston) and some other species. Zoologica Scripta 14(4): 239–246. <https://doi.org/10.1111/j.1463-6409.1985.tb00194.x>
- Chernyshev AV (1993) Novuie svedeniya o sistematike nemertin semeistva Cratenemertidae (Enopla, Monostilifera). Vestnik Zoologii 1: 72–75.
- Coe WR (1901) Papers from the Harriman Alaska Expedition. XX. The nemerteans. Proceedings of the Washington Academy of Sciences 3: 1–110.
- Coe WR (1905) Nemerteans of the west and northwest coasts of America. Bulletin of the Museum of Comparative Zoology at Harvard College 47: 1–318. <https://catalog.hathitrust.org/Record/007161124>
- Collin R, Diaz MC, Norenburg J, Rocha RD, Sanchez JA, Schulze A, Schwartz M, Valdes A (2005) Photographic identification guide to some common marine invertebrates of Bocas Del Toro, Panama. Caribbean Journal of Science 41(3): 638–707. <https://repository.si.edu/handle/10088/11180>
- Corrêa DD (1961) Nemerteans from Florida and Virgin Islands. Bulletin of Marine Science 11(1): 1–44. <http://www.ingentaconnect.com/content/umrsmas/bull-mar/1961/00000011/00000001/art00001>
- Corrêa DD (1963) Nemerteans from Curaçao. Studies on the Fauna of Curaçao and Other Caribbean Islands 17(1): 41–56. <http://www.narcis.nl/publication/RecordID/oai%3Anaturalis.nl%3A506220>
- Crandall FB, Kajihara H, Mawatari SF, Iwata F (2001) The status of four Japanese nemertean species of Yamaoka. Hydrobiologia 456(1–3): 175–185. <https://doi.org/10.1023/A:1013054022264>
- Crandall FB, Norenburg JL (1999) Checklist of the Nemertean Fauna of the United States. Smithsonian Institution, Washington, D.C., 36 pp. <https://repository.si.edu/handle/10088/14814>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature methods 9(8): 772–772. <https://doi.org/10.1038/nmeth.2109>
- Dirzo R, Raven P (2003) Global State of Biodiversity and Loss. Annual Review of Environment and Resources 28: 137–167. <https://doi.org/10.1146/annurev.energy.28.050302.105532>
- Dueñas PR (1998) Sucesión, distribución y ecología de las comunidades macrozoobentónicas de fondos blandos de la región de Santa Marta, con énfasis en los poliquetos (Annelida). INVEMAR Informe final línea: Biodiversidad y Ecosistemas Marinos. Santa Marta, 66. [http://gis.invemar.org.co:8088/cgi-bin/wwwi32/\[in=cdo/Pel\\_libr.in\]?MFN=000096](http://gis.invemar.org.co:8088/cgi-bin/wwwi32/[in=cdo/Pel_libr.in]?MFN=000096)
- Filatov DA (2009) Processing and population genetic analysis of multigenic datasets with ProSeq3 software. Bioinformatics 25(23): 3189–3190. <https://doi.org/10.1093/bioinformatics/btp572>

- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek RC (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Biology and Biotechnology* 3: 294–299. <https://www.ncbi.nlm.nih.gov/pubmed/7881515>
- Friedrich H (1957) Beiträge zur Kenntnis der Arktischen Hoplonemertinen. *Videnskabelige Meddelelser fra Dansk naturhistorisk Forening i Kjobenhavn* 119: 129–154.
- Friedrich H (1968) *Sagaminemertes*, eine bemerkenswerte neue Gattung der Hoplonemertinen und ihre systematische Stellung. *Zoologischer Anzeiger* 180: 33–36.
- Gibson R (1995) Nemertean genera and species of the world: an annotated checklist of original names and description citations, synonyms, current taxonomic status, habitats and recorded zoogeographic distribution. *Journal of Natural History* 29(2): 271–561. <https://doi.org/10.1080/00222939500770161>
- Gibson R, Crandall FB (1989) The genus *Amphiporus* Ehrenberg (Nemertea, Enopla, Monostiliferoidea). *Zoologica Scripta* 18(4): 453–470. <https://doi.org/10.1111/j.1463-6409.1989.tb00140.x>
- Gonzalez-Cueto J, Quiroga S, Norenburg J (2014) A shore-based preliminary survey of marine ribbon worms (Nemertea) from the Caribbean coast of Colombia. *ZooKeys* 439: 83. <https://doi.org/10.3897/zookeys.439.5965>
- Griffin BB (1898) Description of some marine nemerteans of Puget Sound and Alaska. *Annals of the New York Academy of Sciences* 11(1): 193–218. <https://doi.org/10.1111/j.1749-6632.1898.tb54969.x>
- Hebert PD, Cywinska NA, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. *Proceedings Royal Society London Biology Science* 270(1512): 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Hubrecht AA (1887) Report on the Nemertea collected by H.M.S. “Challenger” during the years 1873-1876. *Challenger Zoological Reports* 19: 1–151.
- Iwata F (2001) *Nipponnemertes fernaldi*, a new species of swimming monostiliferous hoplonemertean from the San Juan Archipelago, Washington, USA. *Proceedings of the Biological Society of Washington* 114(4): 833–857.
- Iwata F (2008) On Three Monostiliferous Hoplonemerteans from the San Juan archipelago, Washington State, USA. *Publications of the Seto Marine Biological Laboratory* 40(5/6): 9–45. <https://doi.org/10.5134/72819>
- Johnston G (1837) *Miscellanea Zoologica*. II. A description of some planarian worms, *Magazine of Zoology and Botany* 1: 529–538.
- Kajihara H, Chernyshev AV, Sun SC, Sundberg P, Crandall FB (2007) Checklist of nemertean genera and species published between 1995 and 2007. *Species Diversity: an International Journal for Taxonomy, Systematics, Speciation, Biogeography, and Life History Research of Animals* 13(4): 245–274.
- Kajihara H, Nishi E, Kawabata M, Kohtsuka H, Uyeno D (2014) Records of the poorly known ribbon worm *Nipponnemertes ogumai* (Nemertea: Monostilifera) and its phylogenetic position. *Marine Biodiversity* 45(2): 175–182. <https://doi.org/10.1007/s12526-014-0252-1>
- Kirsteuer E (1965) Über das Vorkommen von Nemertinen in einem tropischen Korallenriff. 4. Hoplonemertini Monostilifera, *Zoologische Jahrbücher, Abteilungen Systematik, Ökologie und Geographie der Tiere* 92: 289–326.

- Kirsteuer E (1973) A new polystiliferous hoplonemertean, *Curranemertes natans* gen. et sp. n., from the Caribbean Sea (Nemertina, Polystilifera Reptantia). *Zoologica Scripta* 2(4): 125–140. <https://doi.org/10.1111/j.1463-6409.1974.tb00744.x>
- Kirsteuer E (1974) Description of *Poseidonemertes caribensis* sp. n., and discussion of other taxa of Hoplonemertini Monostilifera with divided longitudinal musculature in the body wall. *Zoologica Scripta* 3(4): 153–166. <https://doi.org/10.1111/j.1463-6409.1974.tb00812.x>
- Kirsteuer E (1977) Remarks on Taxonomy and Geographic Distribution of the Genus *Ototyphlonemertes* Diesing (Nemertina, Monostilifera). *Mikrofauna Meeresboden* 61: 167–181.
- Korotkevich VS (1983) O stiletakh nemertin i novom vide *Cratenemertes* (Hoplonemertini, Amphiporidae) iz Antarktiki, Antarktika Doklady Komissii 22: 137–143.
- McDermott J (1998) Observations on feeding in a South African suctorial hoplonemertean, *Nipponnemertes* sp. (Family Cratenemertidae). *Hydrobiologia* 365: 251–256. <https://doi.org/10.1023/A:1003114117397>
- Mora C, Tittensor DP, Adl S, Simpson AG, Worm B (2011) How Many Species Are There on Earth and in the Ocean?. *PLoS Biology* 9(8): e1001127. <http://dx.doi.org/10.1371/journal.pbio.1001127>
- Punnett RC (1903) On the nemerteans of Norway. *Bergens Museums Årbog* 2: 3–35.
- Riser NW (1998) New Zealand nemertines from kelp holdfasts: Hoplonemertinea 1. *Nipponnemertes sanguinea* sp. n. *New Zealand Journal of Zoology* 25(3): 287–294. <http://dx.doi.org/10.1080/03014223.1998.9518157>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Scheffers B, Joppa LN, Pimm SL, Laurance WF (2012) What we know and don't know about Earth's missing biodiversity. *Trends in Ecology and Evolution* 27(9): 501–510. <http://dx.doi.org/10.1016/j.tree.2012.05.008>
- Schwartz ML, Norenburg JL (2005) Three new species of *Micrura* (Nemertea: Heteronemertea) and a new type of heteronemertean larva from the Caribbean Sea. *Caribbean Journal of Science* 41: 528–543. <https://repository.si.edu/handle/10088/3960>
- Sundberg P, Andrade S, Bartolomaeus T, Beckers P, Döhren J, Krämer D, Gibson R, Giribet G, Herrera-Bachiller A, Junoy J, Kajihara H, Kvist S, Kånneby T, Sun S-C, Thiel M, Turbeville JM, Strand M (2016a) The future of nemertean taxonomy (phylum Nemertea) - a proposal. *Zoologica Scripta* 45(6): 579–582. <https://doi.org/10.1111/zsc.12182>
- Sundberg P, Kvist S, Strand M (2016b) Evaluating the Utility of Single-Locus DNA Barcoding for the Identification of Ribbon Worms (Phylum Nemertea). *PloS One* 11(5): e0155541. <http://dx.doi.org/10.1371/journal.pone.0155541>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10): 2731–2739. <https://doi.org/10.1093/molbev/msr121>

- Tittensor DP, Mora C, Jetz W, Lotze HK, Ricard D, Vanden Berghe E, Worm B (2010) Global patterns and predictors of marine biodiversity across taxa. *Nature* 466: 1098–1103. <https://doi.org/10.1038/nature09329>
- Trujillo C, Sosa Z, Linero K (2009) Estructura de la macroinfauna asociada a los fondos blandos del Caribe norte colombiano. *INTROPICA* 4(1): 101–112. <http://revistas.unimagdalena.edu.co/index.php/intropica/article/view/144>
- Turbeville JM (2002) Progress in nemertean biology: development and phylogeny. *Integrative and Comparative Biology* 42(3): 692–703. <https://doi.org/10.1093/icb/42.3.692>
- Uschakov P (1927) Eine neue Nemertine des Japanischen Meeres. *Zoologischer Anzeiger* 72: 289–290.
- Wheeler JFG (1934) Nemerteans from the South Atlantic and southern oceans. *Discovery Reports* 9: 215–294. [https://archive.org/details/cbarchive\\_106347\\_nemerteansfromthesouthatlantic1934](https://archive.org/details/cbarchive_106347_nemerteansfromthesouthatlantic1934)
- Wheeler JFG (1940) Some Nemerteans from South Africa and a note on *Lineus corrugatus* M'Intosh. *Journal of the Linnean Society of London, Zoology* 41(276): 20–49. <https://doi.org/10.1111/j.1096-3642.1940.tb02371.x>
- Yamaoka T (1947) *Emplectonema mitsuii*, *Paranemertes katoi*, *Amphiporus ogumai*, and *Prostoma roseocephalum*. As submitted by Shiro Okuda, in Uchida, Seinosuke *et al.*, 1947, *Illustrated Encyclopedia of the Fauna of Japan (Exclusive of Insects)*, Revised Edition (1947), Hokuryukan Co. Ltd., Tokyo. [This edition, in Japanese, was the work of 55 joint authors/submitters with Seinosuke Uchida as their representative]

## Supplementary material I

### COI partial gene alignments of all *Nipponnemertes* sequences from GenBank

Authors: Jaime Gonzalez-Cueto, Lyda R. Castro, Sigmer Quiroga

Data type: molecular data

Explanation note: Fasta format

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Link: <https://doi.org/10.3897/zookeys.693.12015.suppl1>



# Five new species of the armored scale genus *Andaspis* MacGillivray (Hemiptera, Coccoomorpha, Diaspididae) from New Caledonia

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## Abstract

New Caledonia is home to many endemic species of plants and animals. Here, we improve our grasp on that biota by describing five new species of armored scale insects in the genus *Andaspis*: *Andaspis brevicornuta* sp. n., *A. conica* sp. n., *A. nothofagi* sp. n., *A. novaecaledoniae* sp. n., and *A. ornata* sp. n. Each is known exclusively from collections on southern beeches (*Nothofagus* spp.) in New Caledonia. A key to the species of *Andaspis* of New Caledonia is provided.

## Keywords

Alpha-taxonomy, armored scale insects, Southern Hemisphere biota

## Introduction

New Caledonia is an archipelago located in the South Pacific Ocean east of Australia. It was once part of the ancient supercontinent Gondwana, but has been isolated for approximately 80 million years (Grandcolas et al. 2008). It encompasses an array of soil

types and biomes – including evergreen humid forests, sclerophyll dry forests, maquis, and savannas (Murienne 2009). Due to its isolation and geographic heterogeneity, much of New Caledonian biodiversity is endemic and New Caledonia is considered to be a biodiversity hotspot (Ladiges and Cantrill 2007, Grandcolas et al. 2008). Among the emblematic elements of the New Caledonian biota are the southern beeches (*Nothofagus* spp.) (Nothofagaceae). Southern beeches are archetypal Gondwanan plants, which might suggest biotic interchanges with other Southern Hemisphere landmasses when they were contiguous (Cook and Crisp 2005, Ladiges and Cantrill 2007). However, the ultramafic soils which cover much of the surface of New Caledonia suggest a period in which it was entirely submerged (Grandcolas et al. 2008). Hence, although southern beeches may have long been an important component of the New Caledonian terrestrial biota, the ones found there today must have dispersed over an ocean, from elsewhere in the Southern Hemisphere. Here, we improve our characterization of New Caledonian biota by describing five new species of armored scale insects associated with *Nothofagus*.

An estimated 4,000 species of insects have been recorded from New Caledonia (Lowry et al. 2004) and 118 of these are scale insects (Hemiptera: Coccomorpha) (Mille et al. 2016). Scale insects mostly feed on plant sap or the contents of plant cells. They live on a variety of host-plant species, and many are ecological and agricultural pests (Campbell et al. 2014). The New Caledonian species represent ten scale insect families: Asterolecaniidae, Coccidae, Conchaspidiidae, Dactylopiidae, Diaspididae, Eriococcidae, Monophlebidae, Ortheziidae, Pseudococcidae, and Rhizoecidae (Mille et al. 2016).

The most species-rich family of scale insects is Diaspididae, colloquially referred to as armored scale insects. We currently recognize 2,650 valid species of armored scale insects, in 418 genera (García Morales et al. 2016). Fifty species of armored scale insects and 26 genera have been collected from New Caledonia. About 20% are thought to be endemic (Mille et al. 2016). Given its long history of isolation, this may seem to be a strikingly low proportion. It can be explained in part by the fact that scale insects are extremely invasive (Miller et al. 2005). But the low proportion of endemic species could also reflect that the scale insect fauna has been little studied; much of the endemic diversity could be undocumented. In what follows, we describe five new species of New Caledonian endemic armored scales in the genus *Andaspis* MacGillivray, a genus which had not yet been recorded from New Caledonia. Before this work, *Andaspis* comprised 46 species distributed mostly throughout Eastern Asia and Australia (Northern Territory and Queensland) (Williams and Watson 1988, García Morales et al. 2016).

With the additions made here, the total number of described species in *Andaspis* is 51. We provide a key to the New Caledonian species.

## Materials and methods

The following descriptions are based on forty-six slide-mounted specimens of adult female scale insects which had been prepared from material collected by J. S. Dugdale and P. N. Johnson in 1978. All of the specimens examined were collected from two

sites in New Caledonia: Mont Mou and Rivière Bleue. These specimens are part of the scale insect collection of The Natural History Museum, London, UK (BMNH). D. J. Williams delimited the species, and F. B. Hamilton described them. They are joint authors of the five new species names introduced below.

Specimens were viewed through a phase contrast light microscope. NIS elements software was used to take photographs and measurements. All of the given measurements are maximum dimensions and are expressed as a range across specimens. The body of the adult female and a higher magnification image of the pygidium were traced and inked from the photographs. Illustrations were refined with the Adobe Creative Suite. In each of the illustrations, the dorsal body surface is displayed on the left side and the ventral body surface on the right side. Surrounding the main image are enlargements of diagnostic features. Measurements are given in millimeters (mm) and micrometers ( $\mu\text{m}$ ). The morphological terminology used in the descriptions follows that of Williams and Watson (1988) and Miller and Davidson (2005). Specimens are deposited at BMNH and NMNH (National Museum of Natural History, Beltsville, MD).

## Taxonomy

### *Andaspis* MacGillivray, 1921

*Andaspis* MacGillivray, 1921: 275; Rao and Ferris 1952: 17; Williams 1963: 13; Takagi 1970: 20; Williams and Watson 1988: 27.

**Type species.** *Mytilaspis flava* var. *hawaiiensis* Maskell, 1895, by original designation

**Generic diagnosis.** Body shape of adult female variable: oblong, fusiform, obovate, or oval. Antennae with 2 or more setae. Prominent median lobes with short inner edges, long outer margins, and conspicuous scleroses and paraphyses. A pair of small gland spines between lobes. Second lobes greatly reduced or absent and 4–6 marginal macroducts present on each side of pygidium. Anal opening in anterior portion of pygidium or near apex. Vulva either without perivulvar pores or surrounded by three or five groups of them (Takagi and Kawai 1966, Takagi 1970, Williams and Watson 1988).

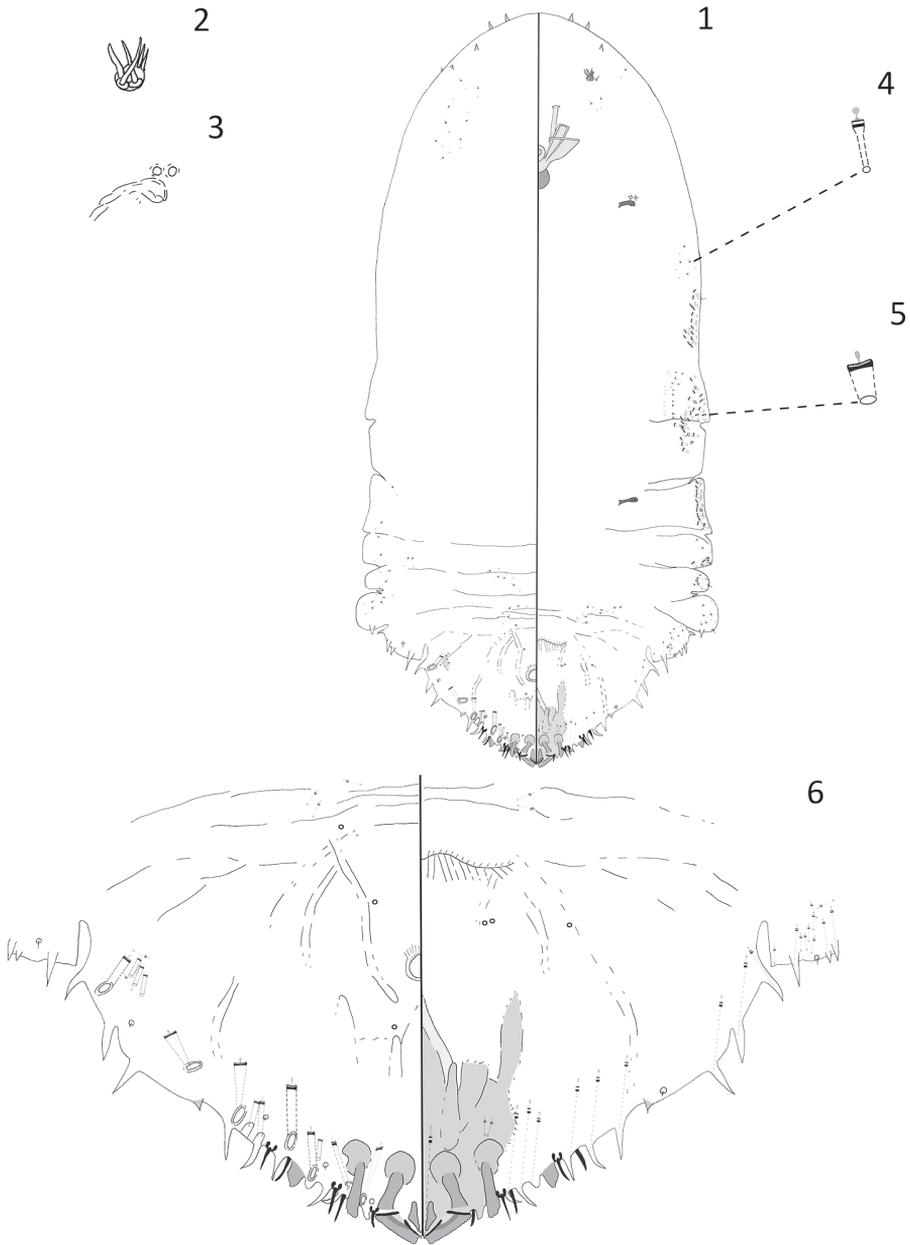
### *Andaspis brevicornuta* Hamilton & Williams, sp. n.

<http://zoobank.org/4D4A2A7C-9239-4054-9F31-18EC93C1ADED>

Figures 1–6

**Material examined.** Holotype: adult female, slide-mounted. Original label: “New Caledonia, Rivière Bleue, *Nothofagus codonandra*, J.S. Dugdale, 10.x.1978, *Andaspis*” (handwritten in black ink). Deposited at BMNH.

**Description. Adult female.** Slide-mounted adult female 1.06 mm long; widest at third abdominal segment, 0.48 mm wide. Body outline oblong, derm membranous



**Figure 1–6.** *Andaspis brevicornuta* Hamilton and Williams, sp. n., adult female; **1** whole body **2** antenna **3** anterior spiracle **4** microduct **5** macroduct **6** pygidium.

except for pygidium. A distinctive species with six spurs or minute horns present on anterior edge of head. Each antenna with five setae. Anterior spiracles each with 2 disc pores, each about 4  $\mu\text{m}$  in diameter, trilocular; posterior spiracles lacking pores. An-

terior abdominal segments well-developed with convex margins; tooth-like tubercles present on segments 2 and 4. In addition to those on pygidium, gland spines located along margins of abdominal segments 3 and 4. Many short macroducts distributed around ventral margins extending from mesothorax to abdominal segment 1.

Pygidium with well-developed median lobes, approximately triangular in shape. Two short gland spines present between median lobes. Each median lobe with paraphysis arising from outer and inner basal angles, anterior ends almost touching, also with short sclerosis arising from inner angle and a large club-shaped sclerosis arising from outer basal angle. Second lobes present; each with a large club-shaped basal sclerosis. Third lobes present, each with outer edge notched but without basal sclerosis. Additionally, a pointed tubercle located on each side of abdominal segment 6. Eight gland spines present along the margin of each side of the pygidium, each with a long microduct, about 40  $\mu\text{m}$ . Marginal setae present, each about 14  $\mu\text{m}$  long, setae on abdominal segment 7 shorter, about 10  $\mu\text{m}$  long. Macroducts on pygidium restricted to dorsal margin and submargin, with eight marginal macroducts and five smaller and narrower submarginal macroducts located on each side. Marginal macroduct openings narrowly oval, each about 8  $\mu\text{m}$  long  $\times$  3  $\mu\text{m}$  wide. Opening of each macroduct on segment 7 narrow, 5  $\mu\text{m}$  long  $\times$  3  $\mu\text{m}$  wide. Perivulvar pores absent. Identity of dark-rimmed circular structures on venter and dorsum of pygidium near vulva unknown and they could be orifices of pores or setal sockets.

**Remarks.** The adult female of this species differs from those of all other currently described *Andaspis* species by having 6 tooth-like spurs (or minute horns) present on the anterior margin of the head. This species is somewhat similar to *Andaspis halli* Rao, 1952, a species known to occur in Zimbabwe. Adult females of the two species share an elongate body shape and a distinct second lobe. However, this species differs from it by the following characters (those for *A. halli* in parentheses): two scleroses located above each median lobe (no scleroses present above each median lobe), a club-shaped sclerosis present above each second lobe (an elongate sclerosis present above each second lobe), eight marginal macroducts located on the dorsum (six marginal macroducts located on the dorsum), and lacking perivulvar pores (three groups of perivulvar pores).

**Etymology.** The specific epithet is the Latin adjective meaning short horned, referring to the projections on the head margin.

***Andaspis conica* Hamilton & Williams, sp. n.**

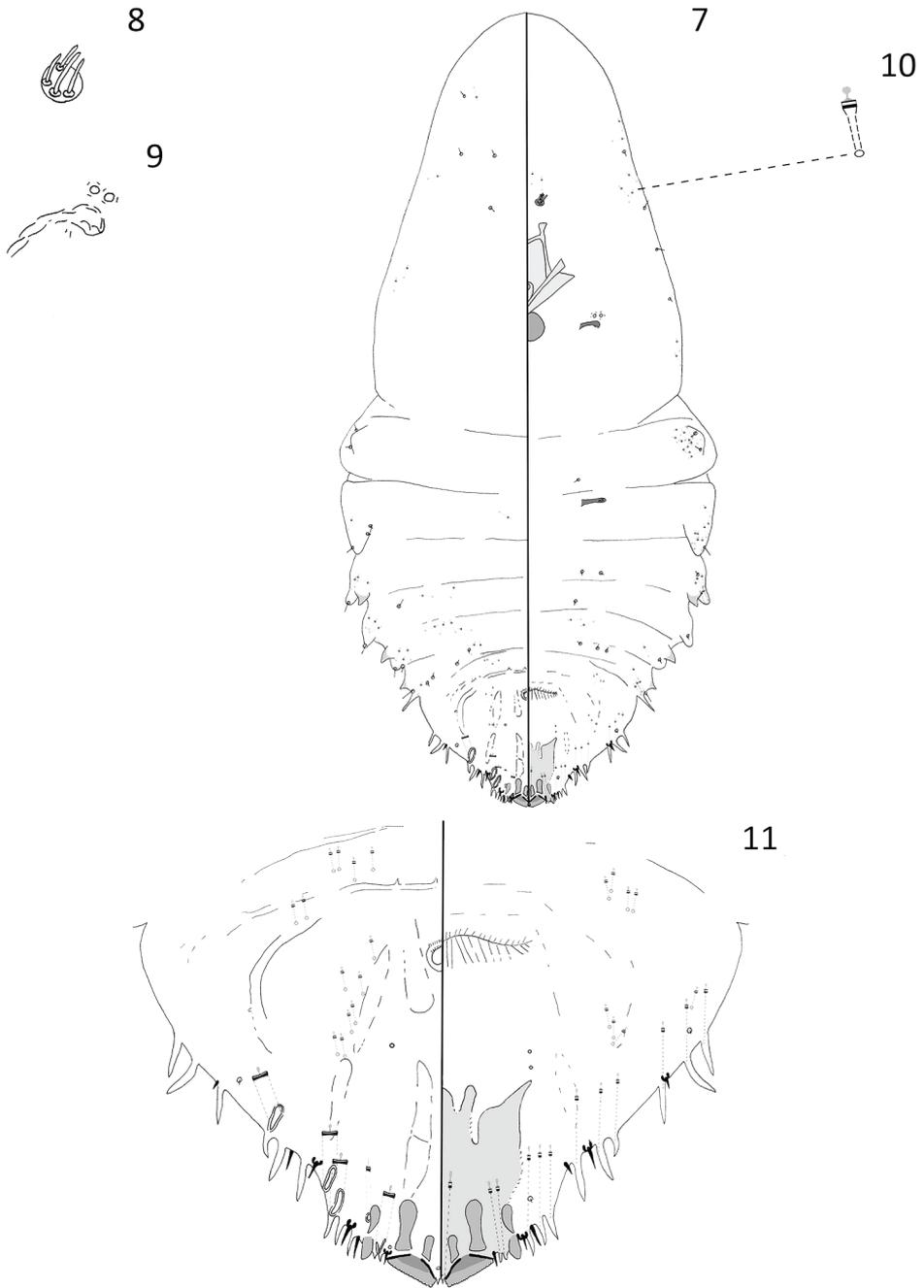
<http://zoobank.org/F31D9577-607E-4E92-9F0E-E24418422622>

Figures 7–11

**Material examined.** Holotype: adult female, slide-mounted. Original label: “New Caledonia, Rivière Bleue, *Nothofagus codonandra*, J.S. Dugdale, 10.x.1978, *Andaspis*” (handwritten in black ink). Deposited at BMNH.

Paratypes: 13 adult females. Same data as holotype. Deposited at BMNH and NMNH.

**Description. Adult female.** Slide-mounted adult female 0.88–1.52 mm long; 0.42–0.54 mm wide. Body outline fusiform, derm membranous except for pygidium.



**Figure 7–11.** *Andaspis conica* Hamilton and Williams, sp. n., adult female; **7** whole body **8** antenna **9** anterior spiracle **10** microduct **11** pygidium.

Each antenna with four setae. Anterior spiracles each with 1 or 2 disc pores, each about 3  $\mu\text{m}$  in diameter, trilocular; posterior spiracles lacking pores. Anterior abdominal segments well-developed with convex margins; tooth-like tubercles present on margins of segments 1, 3, and 4. In addition to those on pygidium, a pair of gland spines present along lateral margins of abdominal segment 4.

Pygidium with well-developed median lobes, approximately triangular in shape. Two short gland spines present between median lobes, extending almost halfway down lobes. Each median lobe with a transversal paraphysis arising from each basal angle, inner ends almost touching; a short sclerosis arising from inner basal angle, and a longer club-like sclerosis extending from lateral basal angle of each median lobe. Second lobes present; each with a short pyriform sclerosis near base. Eleven gland spines present on each lateral margin of pygidium, each gland spine with a long microduct, about 45  $\mu\text{m}$ . Marginal setae each about 12  $\mu\text{m}$  in length, setae on abdominal segment 7 shorter, each about 9  $\mu\text{m}$  long. Macroducts on pygidium restricted to margin and submargin. Four marginal macroducts and one smaller, narrower submarginal macroduct located on each side of dorsum. Macroduct openings almost vertical to margin, narrowly oval, each about 11  $\mu\text{m}$  long  $\times$  3  $\mu\text{m}$  wide, except for a very narrow submarginal macroduct located on abdominal segment 7 with an opening 9  $\mu\text{m}$  long  $\times$  2  $\mu\text{m}$  wide. Perivulvar pores absent. Identity of dark-rimmed circular structures on venter and dorsum of pygidium near vulva unknown and they could be orifices of pores or setal sockets.

**Remarks.** The adult female of this species most resembles that of *Andaspis kazimiae* Williams, 1963, a species known to occur in Pakistan. Adult females of the two species share four marginal macroducts located on the dorsum and have a second lobe. This species differs from *A. kazimiae* by the following characters (those for *A. kazimiae* in parentheses): a pair of scleroses located above each median lobe (scleroses absent above each median lobe), second lobe with pyriform sclerosis near base (second lobe without pyriform sclerosis near base), lacking perivulvar pores (three groups of perivulvar pores), and antennae with four setae (antennae with two setae).

**Etymology.** The specific epithet *conica* is the Latin feminine adjective meaning conical and refers to the conical head.

***Andaspis nothofagi* Hamilton & Williams, sp. n.**

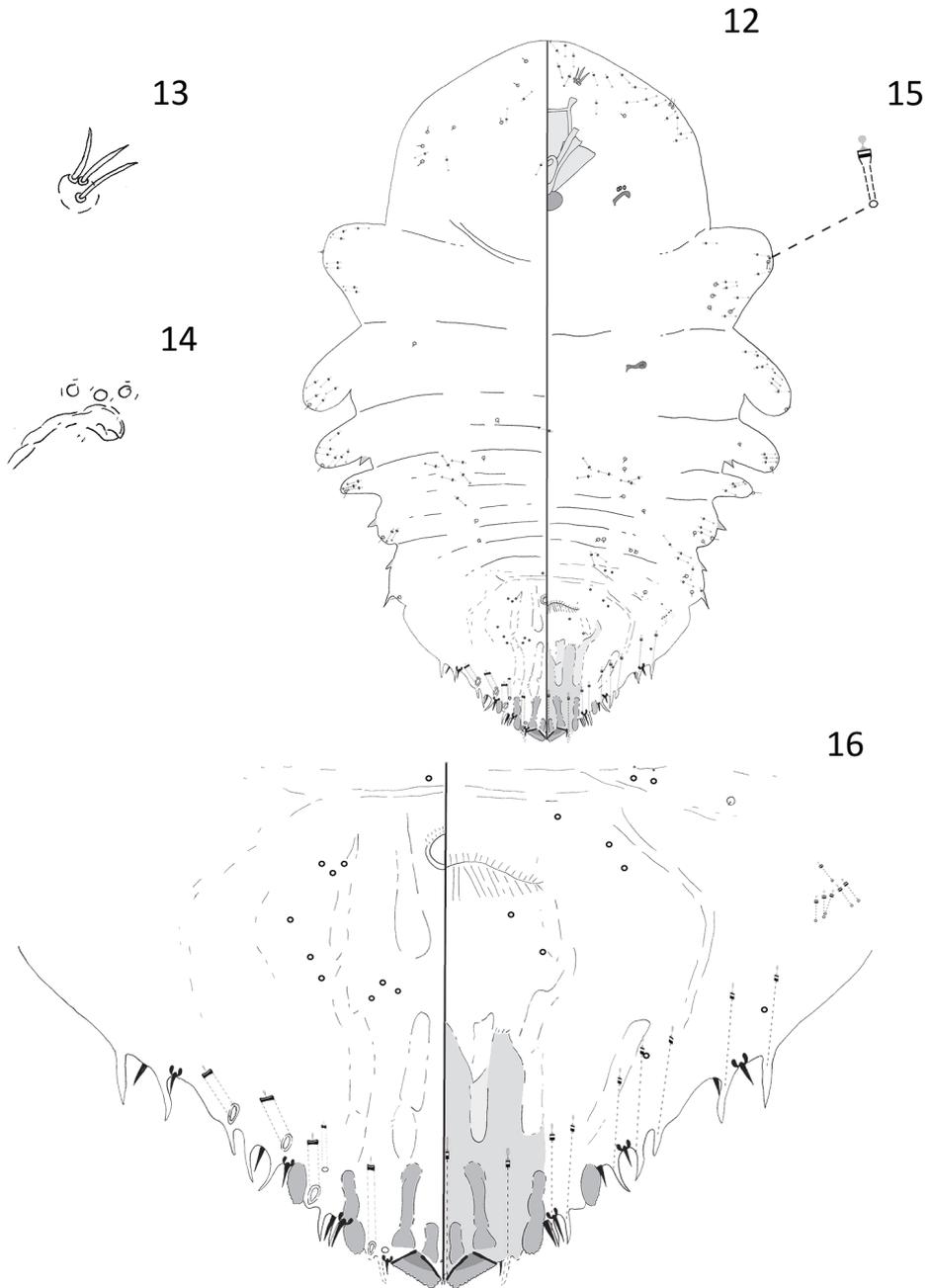
<http://zoobank.org/0073B165-8A89-4163-B2CF-14B3D2DA7629>

Figures 12–16

**Material examined.** Holotype: adult female, slide-mounted. Original label: “New Caledonia, Mt. Mou, *Nothofagus baumanii* twigs, P.N. Johnson, 2.xi.1978, *Andaspis*” (handwritten in black ink). Deposited at BMNH.

Paratypes: 5 adult females. Same data as holotype. Deposited at BMNH and NMNH.

**Description. Adult female.** Slide-mounted adult female 1.15–1.72 mm long; widest at first abdominal segment, 0.64–0.74 mm wide. Body outline oval or oblong,



**Figure 12–16.** *Andaspis nothofagi* Hamilton and Williams, sp. n., adult female; **12** whole body **13** antenna **14** anterior spiracle **15** microduct **16** pygidium.

derm membranous except for pygidium. Each antenna with three setae. Anterior spiracles each with 3–5 disc pores, each about 4  $\mu\text{m}$  in diameter, trilocular; posterior spiracles lacking pores. Anterior abdominal segments well-developed with convex margins; tooth-like tubercles present on margins of segments 1, 3, and 4. In addition to those on pygidium, a pair of gland spines along margin of abdominal segment 4 on each side. Many microducts distributed along margins of abdomen, thorax, and head.

Pygidium with well-developed median lobes, approximately triangular in shape. Two short gland spines present between lobes. Each median lobe with a paraphysis extending anterolaterally from inner angle, and another extending medially from outer angle with inner ends almost touching. A short sclerosis arising from inner base of each median lobe and a longer club-like sclerosis arising from near lateral base. Second and third lobes present; second lobes each with an hourglass-shaped sclerosis arising from base and third lobes each with a short oval sclerotic base. Seven to eight gland spines present along margin of each side of pygidium, each with long microducts, each about 60  $\mu\text{m}$ . Marginal setae each about 15  $\mu\text{m}$  in length, setae on abdominal segment 7 shorter, about 11  $\mu\text{m}$  long. Macroducts on pygidium restricted to margin and submargin. Four marginal macroducts and one smaller and narrower submarginal macroduct located on each side of dorsum. Macroduct openings narrowly oval, each about 9  $\mu\text{m}$  long  $\times$  4  $\mu\text{m}$  wide. Macroduct located on abdominal segment 7 with a much narrower opening compared to the others, about 8  $\mu\text{m}$  long  $\times$  2  $\mu\text{m}$  wide. Perivulvar pores absent. Identity of dark-rimmed circular structures on dorsum and venter of pygidium near vulva unknown and they could be orifices of pores or setal sockets.

**Remarks.** The adult female of this species is somewhat similar to that of *Andaspis laingi* Rao, 1952, a species known to occur in India. Adult females of the two species share an oval body shape and four marginal macroducts located on the dorsum. This species differs from *A. laingi* by the following characters (those for *A. laingi* in parentheses): well-developed lateral lobes (slightly developed lateral lobes), two scleroses present above each median lobe (one paraphysis above each lobe), and, in addition a short sclerosis present above each second lobe (sclerosis absent and second lobe obsolete), and a short sclerotic area present above each third lobe (sclerotic area absent and third lobe obsolete), also lacking perivulvar pores (three groups of perivulvar pores).

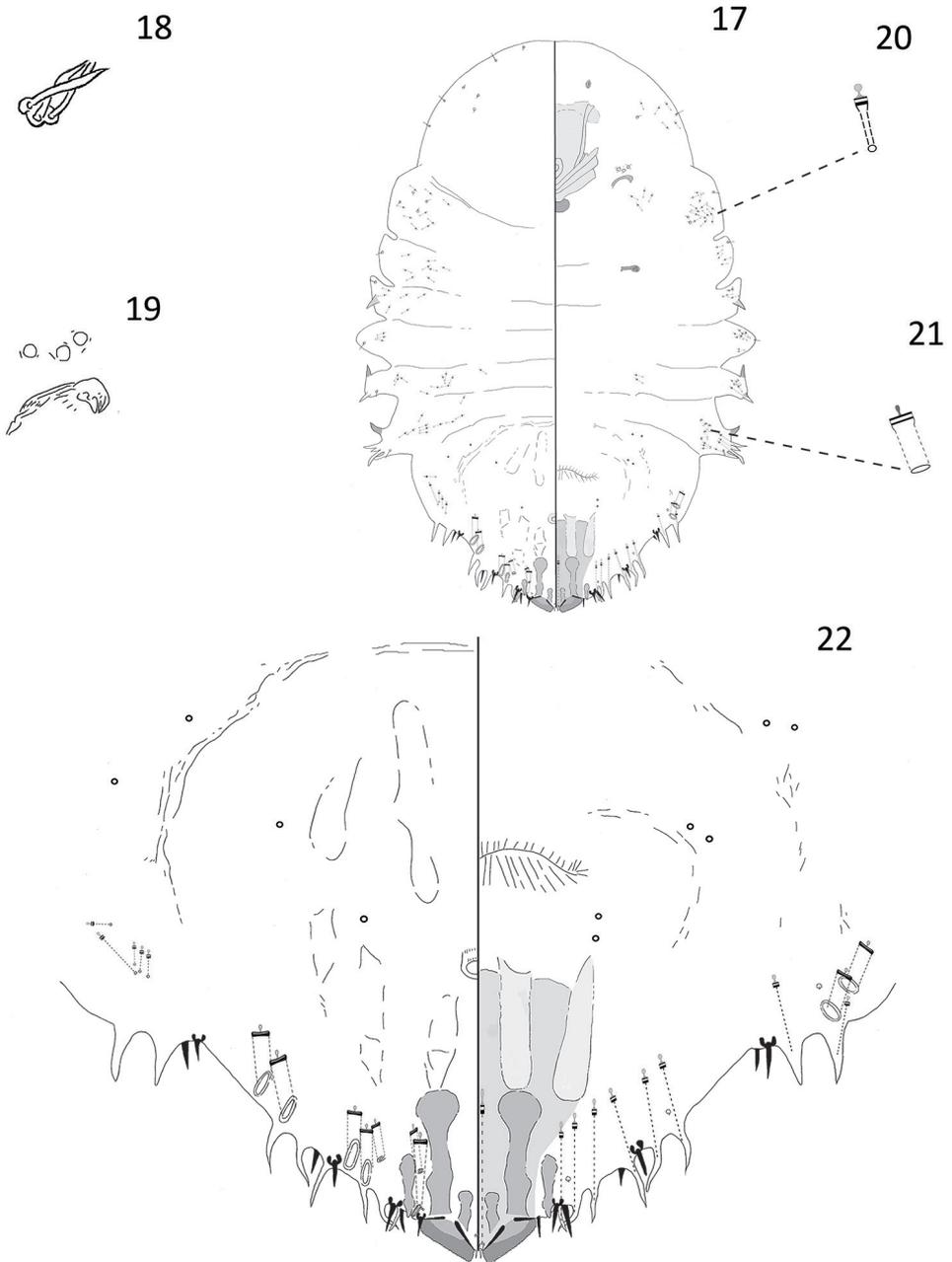
**Etymology.** The specific epithet is the Latin genitive of the host plant genus, *Nothofagus*.

***Andaspis novaecaledoniae* Hamilton & Williams, sp. n.**

<http://zoobank.org/5368CA1F-D60B-49FC-A36B-4BCB2BC8144C>

Figures 17–22

**Material examined.** Holotype: adult female, slide-mounted. Original label: “New Caledonia, Rivière Bleue, *Nothofagus codonandra*, J.S. Dugdale, 10.x.1978, *Andaspis*” (handwritten in black ink). Deposited at BMNH.



**Figure 17–22.** *Andaspis novaecaledoniae* Hamilton and Williams, sp. n., adult female; **17** whole body **18** antenna **19** anterior spiracle **20** microduct **21** macroduct **22** pygidium.

Paratypes: 21 adult females. New Caledonia: Rivière Bleue and Mt. Mou. Collected on *Nothofagus baumanii* and *N. codonandra*, J.S. Dugdale and P.N. Johnson, 10.x.1978 and 2.xi.1978. Deposited at BMNH and NMNH.

**Description. Adult female.** Slide-mounted adult female 0.84–1.46 mm long; widest at first abdominal segment, 0.52–0.84 mm. Body outline oval or oblong, derm membranous except for pygidium. Each antenna with three setae. Anterior spiracles each with 1–4 disc pores, each about 5  $\mu\text{m}$  in diameter, trilocular; posterior spiracles lacking pores. Anterior abdominal segments well-developed with convex margins; tooth-like tubercles present on segments 1, 3, and 4. In addition to those on pygidium, gland spines present along margins of abdominal segments 3 and 4. Many microducts distributed along margins and submargins of thorax and abdomen on both venter and dorsum, plus several on head.

Pygidium with well-developed median lobes, each approximately triangular in shape. Two short gland spines present between median lobes, extending almost halfway down lobes. Each median lobe with a paraphysis arising from outer angle of lobe and another arising from inner basal angle of lobe, both paraphyses often pointing medially with inner ends almost touching. A short sclerosis arising from inner basal part of lobe and a longer club-like sclerosis extending from lateral half. Second lobes present; short and pointed, each with a short sclerosis arising from base. Eight gland spines present along margin of each side of pygidium, each with a long microduct, about 90  $\mu\text{m}$  in length. Marginal setae each about 16  $\mu\text{m}$  long, setae on abdominal segment 7 shorter, about 13  $\mu\text{m}$  long. Macroducts on pygidium restricted to margin and submargin. Five marginal macroducts located on each side of dorsum and two on venter. Macroduct openings narrowly oval, almost perpendicular to margin, each about 13  $\mu\text{m}$  long  $\times$  3  $\mu\text{m}$  wide. Each macroduct located on segment 7 with much narrower opening compared to others, about 8  $\mu\text{m}$  long  $\times$  2  $\mu\text{m}$  wide. Two narrower submarginal macroducts located posterior to macroducts on dorsum on segments 6 and 7, each opening about 6  $\mu\text{m}$  long  $\times$  3  $\mu\text{m}$  wide. Perivulvar pores absent. Identity of dark-rimmed circular structures on venter and dorsum of pygidium near vulva unknown and they could be orifices of pores or setal sockets.

**Remarks.** The adult female of this species is different from those of all other species in the genus described so far, in having two marginal macroducts located on the venter. Similarly, *A. ornata* sp. n. has nine marginal macroducts located on the venter. However, this species is somewhat similar to *Andaspis tokyoensis* Takagi and Kawai, 1966, a species known to occur in Japan. Adult females of *A. novaecaledoniae* and *A. tokyoensis* share well-developed lateral lobes on the abdomen, a club-shaped sclerosis arising from each median lobe, a narrow macroduct located on abdominal segment 7, and a sclerosis located anterolateral to each median lobe. This species differs from *A. tokyoensis* by the following characters (those for *A. tokyoensis* in parentheses): two scleroses located above each median lobe (one sclerosis located above each median lobe), five marginal macroducts and two submarginal macroducts located on the dorsum (six marginal macroducts and one submarginal macroduct located on the dorsum), lacking perivulvar pores (three groups of perivulvar pores), and antennae with three setae (antennae with two setae).

**Etymology.** The specific epithet is taken from the latinized name of the country in which it occurs meaning “of New Caledonia”.

***Andaspis ornata* Hamilton & Williams, sp. n.**

<http://zoobank.org/F90EFDEA-5B2C-437E-8419-065C50C6AF65>

Figures 23–29

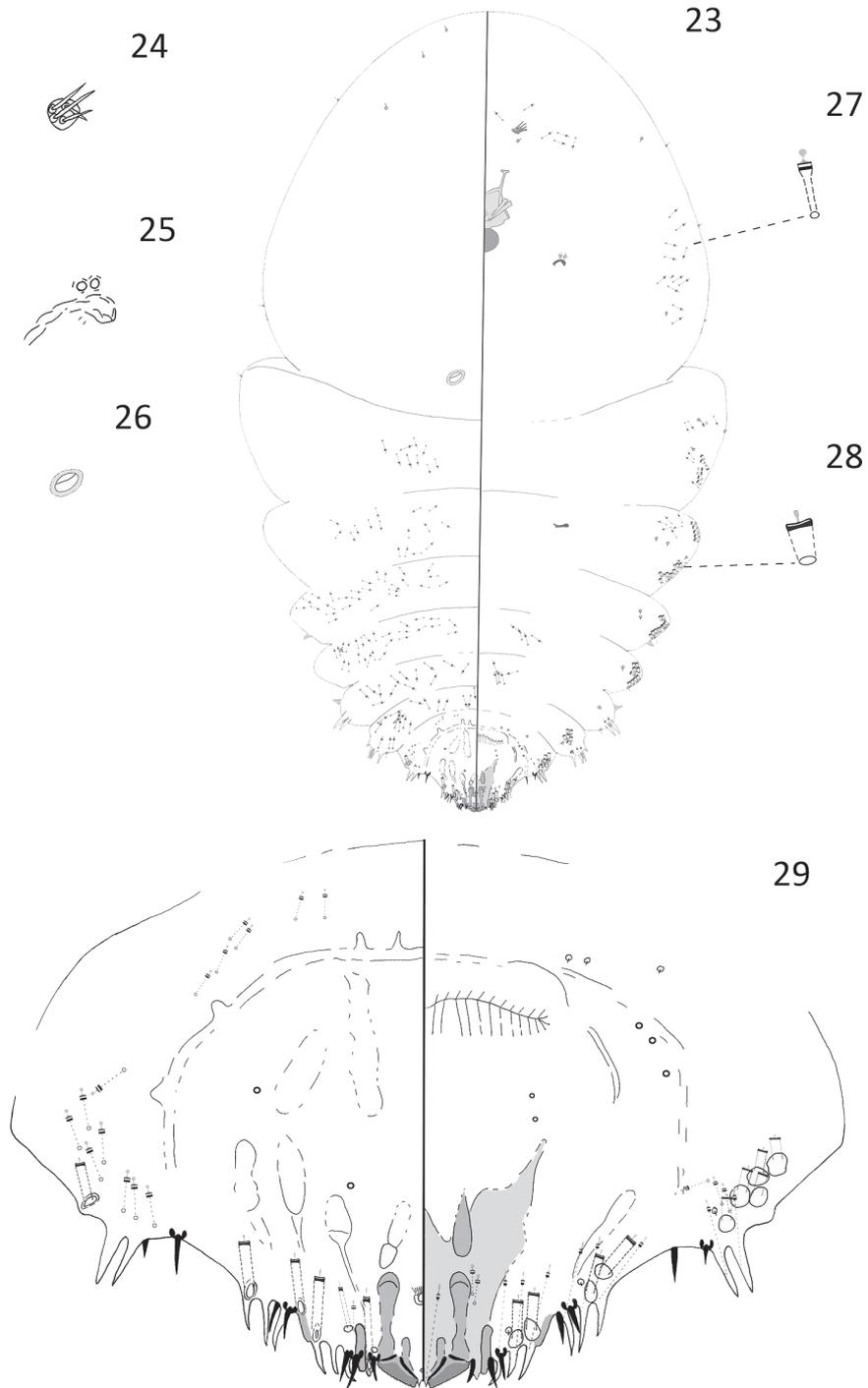
**Material examined.** Holotype: adult female, slide-mounted. Original label: “New Caledonia, Mt. Mou, *Nothofagus baumani* twigs, P.N. Johnson, 2.xi.1978, *Andaspis*” (handwritten in black ink). Deposited at BMNH.

Paratypes: 2 adult females. Same data as holotype. Deposited at BMNH and NMNH.

**Description. Adult female.** Slide-mounted adult female 1.36–2.72 mm long; widest at mesothorax, 0.90–1.67 mm. Body outline obovate, derm membranous except for pygidium. Each antenna with four setae. Anterior spiracles each with 1–2 disc pores, each about 4  $\mu\text{m}$  in diameter, indistinguishable number of loculi; posterior spiracles lacking pores. A prominent cicatrix located on each side of mesothorax on dorsal side of body. Anterior abdominal segments well-developed with convex margins; tooth-like tubercles present on margins of segments 1, 3, and 4. In addition to gland spines on pygidium, gland spines also present along the margins of abdominal segments 3 and 4. Many microducts located on the dorsum of the metathorax and abdominal segments 1, 2, 3, and 4. Short macroducts present along margins of venter.

Pygidium considerably shorter and narrower in comparison to rest of body, with well-developed median lobes that are approximately triangular in shape. Two short gland spines present between median lobes. Each median lobe with a paraphysis arising from inner and outer basal angles, ends almost touching. Each median lobe with a short medial sclerosis arising from inner basal angle and a longer club-like sclerosis extending from lateral basal angle. Second lobes present, rounded, much smaller than median lobes, each with a short basal sclerosis. Third lobes short and rounded, each with a short sclerotized area extending along margin. Eight gland spines present along the margin of each side of the pygidium, each gland spine with a long microduct, about 35  $\mu\text{m}$ . Marginal setae on pygidium each about 20  $\mu\text{m}$  in length, setae on abdominal segment 7 shorter, about 15  $\mu\text{m}$  long. Macroducts on pygidium restricted to margin and submargin. Five marginal macroducts and one smaller and narrower submarginal macroduct located on each side of the dorsum; macroduct openings on dorsum narrowly oval, each about 8  $\mu\text{m}$  long  $\times$  4  $\mu\text{m}$  wide. Nine marginal macroducts located on each side of the venter; openings of marginal macroducts on venter slightly larger and nearly circular in shape, about 9  $\mu\text{m}$  long  $\times$  7  $\mu\text{m}$  wide. Anal opening in the adult female of this species is placed close to pygidium apex. Apex of pygidium to anal opening about 53  $\mu\text{m}$ ; apex of pygidium to vulva about 213  $\mu\text{m}$ . Perivulvar pores absent. Identity of dark-rimmed circular structures on venter and dorsum of pygidium near vulva unknown and they could be orifices of pores or setal sockets.

**Remarks.** The adult females of this species are different from all other species in the genus described so far, in having nine marginal macroducts located on the venter



**Figure 23–29.** *Andaspis ornata* Hamilton and Williams, sp. n., adult female; **23** whole body **24** antenna **25** anterior spiracle **26** cicatrix **27** microduct **28** macroduct **29** pygidium.

of the pygidium. This species is somewhat similar to *Andaspis retrusa* Williams, 1963, a species known to occur in India. Adult females of the two species have microducts present in groups across the body surface and have a second lobe. This species differs from *A. retrusa* by the following characters (those for *A. retrusa* in parentheses): five marginal macroducts on the dorsum (four marginal macroducts on the dorsum), one submarginal macroduct on the dorsum (numerous dorsal ducts on the dorsum), two scleroses arising from each median lobe (no scleroses arising from each median lobe), lacking perivulvar pores (five groups of perivulvar pores), and antennae with four setae (antennae with three setae).

**Etymology.** The specific epithet *ornata* is the Latin feminine adjective meaning ornate and refers to the many marginal macroducts located on the pygidium.

**Key to adult females of *Andaspis* MacGillivray from New Caledonia**

- 1       Spurs present along anterior margin of head, body shape oblong ..... ***Andaspis brevicornuta* sp. n.**
- No spurs present along anterior margin of head; body shape fusiform, oblong, obovate, or oval..... **2**
- 2       Cicatrix present on each side of mesothorax, anal opening located between scleroses on median lobes, and nine marginal macroducts located on venter . ..... ***Andaspis ornata* sp. n.**
- Cicatrices absent on mesothorax, anal opening located near base (anterior edge) of pygidium, and two or fewer marginal macroducts located on venter ..... **3**
- 3       Five marginal macroducts located on the dorsum and two marginal macroducts located on the venter ..... ***Andaspis novaecaledoniae* sp. n.**
- Four marginal macroducts located on the dorsum and no marginal macroducts located on the venter ..... **4**
- 4       Body shape oval or oblong and with hourglass-shaped sclerosis located anterior to each second lobe ..... ***Andaspis nothofagi* sp. n.**
- Body shape fusiform and with a small pyriform sclerosis located anterior to each second lobe ..... ***Andaspis conica* sp. n.**

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## References

- Campbell AM, Lawrence AJ, Hudspath CB, Gruwell ME (2014) Molecular identification of Diaspididae and elucidation of non-native species using the genes 28s and 16s. *Insects* 5: 528–538. <https://doi.org/10.3390/insects5030528>
- Cook LG, Crisp MD (2005) Not so ancient: the extant crown group of *Nothofagus* represents a post-Gondwanan radiation. *Proceedings of the Royal Society B* 272: 2535–2544. <https://doi.org/10.1098/rspb.2005.3219>
- García Morales M, Denno BD, Miller DR, Miller GL, Ben-Dov Y, Hardy NB (2016) ScaleNet: A literature-based model of scale insect biology and systematics. Database <https://doi.org/10.1093/database/bav118>
- Grandcolas P, Murienne J, Robillard T, Desutter-Grandcolas L, Jourdan H, Guilbert E, Deharvenge L (2008) New Caledonia: a very old Darwinian island? *Philosophical Transactions of the Royal Society B* 363: 3309–3317. <https://doi.org/10.1098/rstb.2008.0122>
- Ladiges PY, Cantrill D (2007) New Caledonia-Australian connections: biogeographic patterns and geology. *Australian Systematic Botany* 20: 383–389. <https://doi.org/10.1071/SB07018>
- Lowry PP II, Munzinger J, Bouchet P, G'eraux H, Bauer A, Langrand O, Mittermeier RA (2004) New Caledonia. In: Mittermeier RA, Gil PR, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, Da Fonseca GAB (Eds) *Hotspots revisited: earth's biologically richest and most endangered terrestrial ecoregions*. CEMEX, Mexico, 192–197.
- MacGillivray AD (1921) *The Coccidae. Tables for the identification of the subfamilies and some of the more important genera and species, together with discussions*. Scarab, Urbana, Illinois, 502 pp.
- Mille C, Henderson RC, Cazeres S, Jourdan H (2016) Checklist of the scale insects (Hemiptera: Sternorrhyncha: Coccoomorpha) of New Caledonia. *Zoosystema* 38: 129–176. <https://doi.org/10.5252/z2016n2a1>
- Miller DR, Davidson JA (2005) *Armored scale insect pests of trees and shrubs*. Cornell University Press, 442 pp.
- Miller DR, Miller GL, Hodges GS, Davidson JA (2005) Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on U.S. agriculture. *Proceedings of the Entomological Society of Washington* 107: 123–158.
- Murienne J (2009) New Caledonia: biology. *Encyclopedia of islands*, 643–645.
- Rao VP, Ferris GF (1952) The Genus *Andaspis* MacGillivray (Insecta: Homoptera: Coccoidea) (Contrib. No. 77). *Microentomology* 17: 17–32.
- Takagi S, Kawai S (1966) Some Diaspididae of Japan (Homoptera: Coccoidea). *Insecta Matsumurana* 28: 93–119.
- Takagi S (1970) Diaspididae of Taiwan based on material collected in connection with the Japan-U.S. Co-operative Science Programme, 1965 (Homoptera: Coccoidea) Part II. *Insecta Matsumurana* 33: 1–142.
- Williams DJ (1963) Synoptic revisions of I. *Lindingaspis* and II. *Andaspis* with two new allied genera (Hemiptera: Coccoidea). *Bulletin of the British Museum* 15: 3–31.
- Williams DJ, Watson GW (1988) *The Scale Insects of the Tropical South Pacific Region. Pt. 1: The Armoured Scales (Diaspididae)*. CAB International, Wallingford, 290 pp.



# Next-generation morphological character discovery and evaluation: an X-ray micro-CT enhanced revision of the ant genus *Zasphinctus* Wheeler (Hymenoptera, Formicidae, Dorylinae) in the Afrotropics

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## Abstract

New technologies for imaging and analysis of morphological characters offer opportunities to enhance revisionary taxonomy and better integrate it with the rest of biology. In this study, we revise the Afrotropical fauna of the ant genus *Zasphinctus* Wheeler, and use high-resolution X-ray microtomography (micro-CT) to analyse a number of morphological characters of taxonomic and biological interest. We recognise and describe three new species: *Z. obamai* **sp. n.**, *Z. sarowiwai* **sp. n.**, and *Z. wilsoni* **sp. n.** The species delimitations are based on the morphological examination of all physical specimens in combination with 3D scans and volume reconstructions. Based on this approach, we present a new taxonomic discrimination system for the regional fauna that consists of a combination of easily observable morphological characters visible at magnifications of around 80–100 ×, less observable characters that require higher magnifications, as well as characters made visible through virtual dissections that would otherwise require destructive treatment. *Zasphinctus* are rarely collected ants and the material available to us is comparatively scarce. Consequently, we explore the use of micro-CT as a non-invasive tool for the virtual examination, manipulation, and dissection of such rare material. Furthermore, we delineate the treated species by providing a diagnostic character matrix illustrated by numerous images and supplement that with additional evidence in the form of stacked montage images, 3D PDFs and 3D rotation videos of scans of major body

parts and full body (in total we provide 16 stacked montage photographs, 116 images of 3D reconstructions, 15 3D rotation videos, and 13 3D PDFs). In addition to the comparative morphology analyses used for species delimitations, we also apply micro-CT data to examine certain traits, such as mouthparts, cuticle thickness, and thoracic and abdominal muscles in order to assess their taxonomic usefulness or gain insights into the natural history of the genus. The complete datasets comprising the raw micro-CT data, 3D PDFs, 3D rotation videos, still images of 3D models, and coloured montage photos have been made available online as cybertypes (Dryad, <http://dx.doi.org/10.5061/dryad.4s3v1>).

### Keywords

3D model, cuticle, cybertype, micro-CT, morphology, mouthparts, new species, taxonomy

### Introduction

The primary goal of taxonomic science is to organize life by developing hypotheses delimiting species and higher groups (Winston 1999; Wägele et al. 2011), but a secondary goal is to generate and curate information about species that can be useful for future taxonomic work as well as the broader fields of biology (Wheeler and Valdecasas 2007). The moment of description is particularly important, at which time an account of the species, supported and illustrated with information of some nature, is put on the record for posterity (Wheeler et al. 2004). The emergence of new technologies offers new opportunities for enhancing taxonomic descriptions and broadening their utility for other biological disciplines (La Salle et al. 2009; Schlick-Steiner et al. 2010; Faulwetter et al. 2013). Here, we continue a recent series of taxonomic works (Fischer et al. 2016; Sarnat et al. 2016; Hita Garcia et al. 2017a) exploring the use of three-dimensional (3D) data generated from X-Ray microcomputed tomography (micro-CT) for ant taxonomy, with a revision of the Afrotropical species of the genus *Zasphinctus* Wheeler. In our treatment of this group, we focus on exploring the potential for using micro-CT data for new character discovery and evaluation, enhancing the way descriptions themselves are organized and presented given the new data, and identifying anatomical characters that can be linked to the biology and ecology of the organism.

Micro-CT is a powerful imaging technology that enables the generation of high-resolution, virtual, and interactive 3D reconstructions of whole specimens or parts thereof. Such reconstructions can be virtually rotated, sectioned, measured, and dissected, thus allowing a comprehensive 3D analysis of the anatomy and morphology of the studied organisms (e.g. Faulwetter et al. 2013; Friedrich et al. 2014). Since its initial use for the study of insect cephalic morphology (Hörschemeyer et al. 2002), micro-CT has gradually gained popularity as a fundamental tool for a variety of research fields that rely on the exact examination of animal morphology. It has been primarily employed for comparative and functional morphology (e.g. Beutel et al. 2008 2010; Zimmermann et al. 2011; Wojcieszek et al. 2012; Lipke et al. 2015), but also for the study of insect fossils in amber (Dierick et al. 2007; Barden and Grimaldi 2012), forensic entomology (Richards et al. 2012), and developmental biology (Metscher 2009). Surprisingly late, micro-CT has also been applied for invertebrate taxonomy

of myriapods (Stoev et al. 2013; Akkari et al. 2015), spiders (Michalik and Ramírez 2013), earthworms (Fernandez et al. 2014), and flatworms (Carbayo et al. 2016; Carbayo and Lenihan 2016). Despite its great potential for the taxonomy of extant insects, it has so far only been used in lepidopterans (Simonsen and Kitching 2014) and ants (Fischer et al. 2016; Sarnat et al. 2016; Hita Garcia et al. 2017a, b).

Compared to traditional methods like histology, the use of micro-CT provides the means for a quick and non-invasive generation of almost artefact-free morphological raw data for visualisation in 3D (Faulwetter et al. 2013; Friedrich et al. 2014). The non-destructive nature of the technique is of crucial importance for systematic research since it permits the scanning of very rare species and/or museum material, and it can be very well applied to type material. One drawback of modern collections-based systematics is that often important reference or type material is not available or accessible for examination, thus effectively slowing taxonomic progress (Smith and Blagoderov 2012; Wheeler et al. 2012; Faulwetter et al. 2013). One recent development with the potential to improve this situation is the establishment of virtual natural history collections that provide rapid access to anatomically correct and permanent digital reconstructions of type material. Based on the idea of Godfray (2007) to create virtual types, Faulwetter et al. (2013) introduced the concept of “cybertypes” and proposed a workflow to generate such virtual collections. Shortly afterwards Stoev et al. (2013) and Akkari et al. (2015) used micro-CT scanning for the description of new myriapod species and presented the first invertebrate cybertypes. Recently, Hita Garcia et al. (2017a, 2017b) critically explored the use of micro-CT for ant taxonomy and proposed the first ant cybertypes. Another great advantage is the application of micro-CT for virtual dissections and character identification, which has proven successful for a variety of invertebrates, such as myriapods (Blanke and Wesener 2014), Neuroptera (Zimmermann et al. 2011), and dragonflies (Simonsen and Kitching 2014).

Compared to other insect groups, ant taxonomy is thoroughly founded on the morphology of the very simplified worker caste. Despite that several authors also examine reproductive castes (e.g. Boudinot 2014; Yoshimura and Fisher 2014; Probst et al. 2015), this is often not possible due to the sheer absence of reproductives in collections and unknown associations between castes. Consequently, ant taxonomy predominantly focuses on phenotypical differences between workers of different species (Ward 2010). Due to the simplified female morphology of the worker caste, the vast majority of studies use very basic characters for species delimitations, mostly setation, surface sculpture, size, and shape differences of few body parts, especially eyes, mandibles, propodeal spines and the waist segments (e.g. Fernández 2004; Sarnat 2008; Bolton 2007; Fischer et al. 2012; Branstetter 2013; Hita Garcia et al. 2014). This approach offers the advantage that taxonomic studies of ants are relatively easy to perform. However, such a simplified approach increasingly often reaches its limits and cannot provide discriminatory evidence for species delimitations. Integrative taxonomy approaches including morphometrics (e.g. Csósz and Schulz 2010; Csósz and Fisher 2015), molecular phylogenetics (e.g. Branstetter 2012; Blaimer 2012), next generation sequencing (Fischer et al. 2015; Jesovnik et al. 2017), or combinations of multiple data sources

(e.g. Schlick-Steiner et al. 2010; Csósz et al. 2014), have proven efficient to resolve the relationships within morphologically challenging ant genera. Nevertheless, in recent years, there have been very few approaches of advancing and improving traditional worker-based character sets used for species diagnostics (e.g. Bolton and Fisher 2012; Yoshimura and Fisher 2014).

The ant genus *Zasphinctus* Wheeler is a moderately small genus distributed in the Afrotropical, Indomalayan, and Australasian regions. Currently, 20 valid species are recognised (Bolton 2017), of which the vast majority occur in the Australasian region (15 spp. from Australia, one from New Caledonia, and one from New Guinea). By contrast, there is only one species known from South East Asia and two from the Afrotropical region. These ants are rarely collected and the material housed in natural history collections is somewhat limited. Perhaps due to its moderate species richness and relative rarity of collections, knowledge about the biology of *Zasphinctus* is rather incomplete. Wilson (1958) and Brown (1975) provided field notes about the biology of *Z. steinheili* (Forel) and laboratory observations of *Z. caledonicus* (Wilson). Both species turned out to be myrmecophagous feeding on larvae and adults of a variety of ant species captured during nest raids. Later, Buschinger (1989) confirmed this behaviour in *Z. steinheili* under laboratory conditions. Based on data from *Z. steinheili* and *Z. caledonicus*, colonies are found in soil and range in size from 100 to 500 workers. However, whether or not this is true for other *Zasphinctus* species, especially outside Australia, remains unknown.

A taxonomic problem commonly encountered in doryline ants is the existence of two or even three parallel taxonomic systems: a female-based one, which often splits into worker-based and queen-based, and a male-based one (e.g. Wilson 1964; Jaitrong and Yamane 2011; Borowiec 2016). Workers and reproductives are rarely collected together, and usually only one caste is available for taxonomic evaluation, which creates great difficulties for the association of workers with queen and/or males. This situation is especially problematic in, but not restricted to, the army ant genera *Aenictus* Shuckard and *Dorylus* Fabricius (Wilson 1964; Gotwald 1995). Prior to this study the taxonomy of *Zasphinctus* in the Afrotropical region was solely based on two male-based species described more than a hundred years ago from West African savannahs (Santschi 1915). Since then, no further taxonomic studies on *Zasphinctus* were published and the scarce male-based or worker-based material in collections has been tentatively assigned to one of these male-based species without evidence of any association. Recent collections in Kenya (Hita Garcia et al. 2009), Mozambique, and Uganda have yielded worker-based material without any males, thus not associable to any valid species name. Consequently, in order to use the genus for ant diversity inventories or conservation studies, it is imperative to create a taxonomic system founded on the worker caste.

In this study, we provide a taxonomic revision of the genus for the Afrotropical region on the basis of the worker caste. All three species treated herein are newly described. The taxonomic decision-making was founded on the examination of all physical specimens, as well as on 3D volume reconstructions of high-resolution micro-CT

scanning data from several specimens per species, if available. Based on that approach, our newly developed taxonomic discrimination system consists of a new character set, which is unusual in ant taxonomy. The backbone of it is still based on easily observable morphological characters visible at magnifications of around 80 to 100 ×. On the basis of micro-CT scanning data, we also present less perceivable characters that require higher magnifications, previously only achieved through scanning electron microscopy (SEM), as well as characters that are usually hidden or partly obscured and would require destructive treatment of the physical material. Through virtual dissections of 3D reconstructed specimens, we recovered several of these hidden characters. Furthermore, we present our results in a different way compared to previous ant taxonomy revisions by including numerous stacked montage images, micro-CT microphotographs, 3D PDFs, and 3D rotation videos of relevant body parts in addition to full specimens. We argue that such a wealth of illustrative power obviates the need for lengthy descriptions and a traditional identification key. Instead, we opt for a thorough genus description and brief species accounts supplemented by a detailed diagnostic species character matrix with high quality illustrations for all characters. Finally, we use micro-CT data to examine traits, such as mouthparts, cuticle thickness, thoracic and abdominal muscles, and the sting in order to gain insights into the natural history of the genus. The complete datasets comprising the micro-CT raw data, 3D PDFs, 3D rotation videos, and coloured montage photos have been made available online as cybertypes (Hita Garcia et al. 2017c).

## Material and methods

### Abbreviations of depositories

Institutional museum collection abbreviations follow Evenhuis (2017). The material on which this study is based is located and/or was examined at the following institutions:

<b>BMNH</b>	The Natural History Museum, London, U.K.
<b>CASC</b>	California Academy of Sciences, San Francisco, U.S.A.
<b>MCZC</b>	Museum of Comparative Zoology, Harvard University, Cambridge, U.S.A.
<b>NMKE</b>	National Museums of Kenya, Nairobi, Kenya
<b>ZFMK</b>	Zoological Research Museum Alexander Koenig, Bonn, Germany

### Material examined and terminology

The general terminology for ant morphology predominantly follows Keller (2011) and to a lesser extent Bolton (1990) and Borowiec (2009, 2016). For the description of mouthparts, we used the terminology of Gotwald (1969) and Keller (2011). The terminology for the description of surface sculpturing follows Harris (1979).

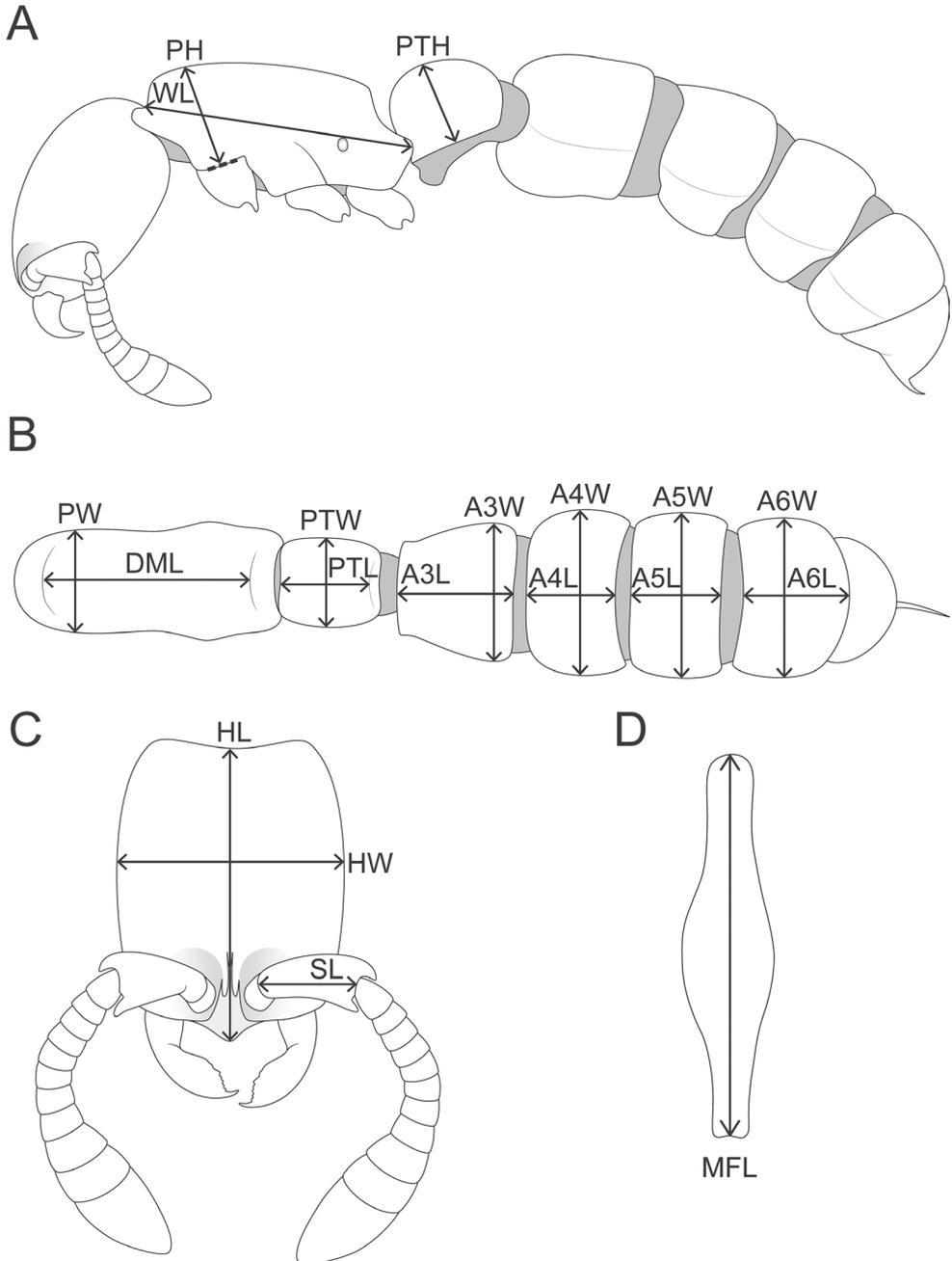
## Montage images and line drawings

All raw images were taken with a Leica DFC450 camera attached to a Leica M205C microscope and Leica Application Suite (version 4.1). The raw photo stacks were then processed to single montage images with Helicon Focus (version 6). All montage images used in this publication are available online and can be seen on AntWeb. Vector illustrations were created with Adobe Illustrator (version CS 5) by tracing specimen photographs.

## Measurements and indices

We measured 17 physical workers with a Leica M125 equipped with an orthogonal pair of micrometers under magnifications of 80 to 100 ×. Measurements and indices are presented as minimum and maximum values with arithmetic means in parentheses. In addition, measurements are expressed in mm to two decimal places. Since the workers of all three species treated herein are eyeless we omit any eye measurements and do not generate an ocular (or eye) index. We refrain from using total length since it is difficult to measure in already dry-mounted specimens that are not orientated in a straight line. The standard measurements HW and WL provide sufficient information about general body size dimensions. The following measurements and indices partly follow Bolton and Fisher (2012), Hita Garcia and Fischer (2014) and Hita Garcia et al. (2014) or are used here for the first time (Fig. 1):

- HL** Head Length: maximum distance from the midpoint of the anterior clypeal margin or from a line spanning the anterior-most points of the frontal lobes (depending on which projects farthest forward) to the midpoint of the posterior margin of head, measured in full-face view (Fig. 1C).
- HW** Head Width: the maximum width of the head capsule, measured in full-face view (Fig. 1C).
- SL** Scape Length: the maximum straight-line length of the scape, excluding the basal constriction or the neck (Fig. 1C).
- PH** Pronotal Height: the maximum height of the pronotum in profile (Fig. 1A).
- PW** Pronotal Width: the maximum width of the pronotum in dorsal view (Fig. 1B).
- DML** Dorsal Mesosoma Length: maximum length of mesosomal dorsum from antero-dorsal margin of pronotum to dorsal margin of propodeal declivity (Fig. 1B).
- WL** Weber's Length of Mesosoma: the maximum diagonal length of the mesosoma in profile, from the angle at which the pronotum meets the cervix to the posterior basal angle of the metapleuron (Fig. 1A).
- MFL** Metafemur Length: the maximum straight-line length of the metafemur, measured in dorsal view (Fig. 1D).
- PTL** Abdominal Segment II (petiole) Length: the maximum length of abdominal segment II (petiole), measured in dorsal view (Fig. 1B).



**Figure 1.** Schematic line drawings illustrating the measurements used in this study. **A** Body in profile with measuring lines for PH, PTH, and WL **B** Mesosoma and metasoma in dorsal view with measuring lines for A3L, A3W, A4L, A4W, A5L, A5W, A6L, A6W, DML, PW, PTL, and PTW **C** Head in full-face view with measuring lines for HL, HW, and SL **D** Metafemur in dorsal view with measuring line for MFL.

- PTH** Abdominal Segment II (petiole) Height: the maximum height of the petiolar tergum in profile view, including laterotergite, excluding petiolar sternum (Fig. 1A).
- PTW** Abdominal Segment II (petiole) Width: the maximum width of abdominal segment II (petiole), measured in dorsal view (Fig. 1B).
- A3L** Abdominal Segment III Length: the maximum length of abdominal segment III, measured in dorsal view (Fig. 1B).
- A3W** Abdominal Segment III Width: the maximum width of abdominal segment III, measured in dorsal view (Fig. 1B).
- A4L** Abdominal Segment IV Length: the maximum length of abdominal segment IV, measured in dorsal view (Fig. 1B).
- A4W** Abdominal Segment IV Width: the maximum width of abdominal segment IV, measured in dorsal view (Fig. 1B).
- A5L** Abdominal Segment V Length: the maximum length of abdominal segment V, measured in dorsal view (Fig. 1B).
- A5W** Abdominal Segment V Width: the maximum width of abdominal segment V, measured in dorsal view (Fig. 1B).
- A6L** Abdominal Segment VI Length: the maximum length of abdominal segment VI, measured in dorsal view (Fig. 1B).
- A6W** Abdominal Segment VI Width: the maximum width of abdominal segment VI, measured in dorsal view (Fig. 1B).
- CI** Cephalic Index:  $HW / HL \times 100$
- SI** Scape Index:  $SL / HL \times 100$
- DMI** Dorsal Mesosoma Index:  $PW / WL \times 100$
- DMI2** Dorsal Mesosoma Index 2:  $DML / WL \times 100$
- LMI** Lateral Mesosoma Index:  $PH / WL \times 100$
- MF** Metafemur Index:  $MFL / HW \times 100$
- LPI** Lateral Petiole Index:  $PTL / PTH \times 100$
- DPI** Dorsal Petiole Index:  $PTW / PTL \times 100$
- DA3I** Dorsal Abdominal Segment III Index:  $A3W / A3L \times 100$
- DA4I** Dorsal Abdominal Segment IV Index:  $A4W / A4L \times 100$
- DA5I** Dorsal Abdominal Segment V Index:  $A5W / A5L \times 100$
- DA6I** Dorsal Abdominal Segment VI Index:  $A6W / A6L \times 100$

### Micro X-ray computed tomography

Micro-CT scans were performed using a ZEISS Xradia 510 Versa 3D X-ray microscope and the ZEISS Scout and Scan Control System software (version 10.7.2936). The scanned specimens were left attached to their paper point, which was clamped to a holding stage. Scan settings were selected according to yield optimum scan quality: optical magnification of 4 ×, exposure times of 1–3 s, binning of two by two pixels, source filter “air”, voltage of 35–85 keV, power of 3–7.5 W, current of 71–88 μA, and

**Table 1.** Data summary for micro-CT scanning giving an overview of the specimens and body parts scanned for the three species and presenting specimen data, scan settings, and voxel sizes for the resulting scans (all specimens are workers and all files are in DICOM format).

Species	Body part scanned	Specimen identifier	voxel size ( $\mu\text{m}$ )	exposure time (s)	Power (W)	Voltage (kV)	Amperage ( $\mu\text{A}$ )
<i>Z. obamai</i>	full body	CASENT0764125	3.003	2	3	40	75
<i>Z. obamai</i>	head	CASENT0764127	0.945	3	6	70	85
<i>Z. obamai</i>	mesosoma	CASENT0764127	1.604	2	5	55	82
<i>Z. obamai</i>	metasoma	CASENT0764127	1.952	2	4	50	80
<i>Z. sarowiwai</i>	full body	CASENT0764650	4.606	1.8	4	50	81
<i>Z. sarowiwai</i>	mouthparts	CASENT0764652	0.945	3	6	65	84
<i>Z. sarowiwai</i>	full body	CASENT0764654	3.861	2	3	40	76
<i>Z. sarowiwai</i>	head	CASENT0764654	1.267	3	5	60	83
<i>Z. sarowiwai</i>	mesosoma	CASENT0764654	1.931	2	4	50	80
<i>Z. sarowiwai</i>	metasoma	CASENT0764654	2.834	1	4	45	78
<i>Z. wilsoni</i>	full body	MCZ-ENT-00512764	3.137	2.5	3	35	71
<i>Z. wilsoni</i>	head	MCZ-ENT-00512764	0.965	3	6	70	86
<i>Z. wilsoni</i>	mesosoma	MCZ-ENT-00512764	1.292	2.7	5	55	82
<i>Z. wilsoni</i>	metasoma	MCZ-ENT-00512764	2.312	2	4	45	78

field mode “normal”. The combination of voltage, power and exposure time was set to yield intensity levels of between 10,000 and 15,000 across the whole specimen. Scanning duration varied from 1.2 to 2.2 h, depending on exposure time. Full 360 degree rotations were done with a number of 1601 projections. The resulting scans have resolutions of  $1013 \times 1013$  pixels and voxel sizes are in range of 0.94–4.6  $\mu\text{m}$ . The original file size was 3113.577 MB for all scans. We scanned a varying number of specimens per species, depending on specimen availability and character suitability. An overview of the specimens used and scanning settings is provided in Table 1.

### Virtual reconstruction and post-processing of raw data

3D reconstructions of the resulting scans were done with XMReconstructor (version 10.7.2936) and saved in DICOM file format (default settings; USHORT 16 bit output data type). Post-processing of DICOM raw data was performed with Amira software (version 6.1.1). Virtual examinations of 3D surface models were performed by using either the “volren” or “volume rendering” functions. The desired volume renderings were generated by adjusting colour space range to a minimum so that the exterior surface of specimens remained visible at the highest available quality. The 3D models were rotated and manipulated to allow a complete virtual examination of the scanned specimens. Images of shaded surface display volume renderings were made with the “snapshot” function at the highest achievable resolution (usually at around 1500 by 893 pixels). Volumetric surface rendering rotational videos of head, mesosoma, metasoma, and full body scans were created with the “camera path” object (5–10 keyframes,

constant velocity for constant rotation speed) and “movie maker” function (parameters: MPEG format, AntiAlias2, total of 1200 frames at 60 frames per second, and resolution of 1920 × 1080 pixels).

### **Character recognition and virtual dissections**

In addition to the traditional morphological examination of the physical specimens under a light microscope with magnifications up to 100 ×, we virtually examined the full external morphology of the treated species in Amira. For this we compared more than 50 morphological characters potentially significant for dorylines (Bolton 1990; Keller 2011; Borowiec 2016), especially the genera previously grouped as Cerapachyinae, in the 3D models and made more than 350 snapshots to assess which characters have diagnostic value. A series of characters were hidden or obscured by other body parts, thus not observable by light microscopy, or only after destructive dissection. Due to the severe lack of material the latter was not an option. In order to examine such characters and explore a wider range of morphology, we used the segmentation function in Amira to deselect body parts obscuring segmented body parts. By doing so we were able to expose every desired structure. We examined the head capsule from all sides, which is usually ventrally and posteriorly obscured by antennae, legs, or anterior mesosoma, as well as ventral metasomal characters usually hidden between propodeum, legs, and different abdominal segments. Based on more than 110 character images per species, we chose to highlight 24 characters of high diagnostic significance for our newly developed species delimitation system (see Table 2 for complete list of examined characters). Furthermore, the volume reconstructions of the mouthparts and musculature were generated by using the segmentation function in Amira. Targeted structures were first visualised by adjusting density and contrast and then segmented by manually tracing their outline slide by slide.

### **Virtual measuring of cuticle thickness**

In addition to the taxonomic standard measurements of external morphology given above, we also measured the thickness of the exoskeleton cuticle of the cephalic capsule, the pronotum, and abdominal segments II (petiole) and III. Measuring was performed with Amira by using the 2D measuring tool on slices representing sagittal sections along the median axis of the chosen body parts. For each body part, we measured five times over a defined area (Fig. 2) and calculated the average thickness. Based on Peeters et al. (2017) we put the measurements in context to body size by using the following measurements and indices:

Cephalic capsule cuticle thickness (CCC): thickness of cuticle of head measured in profile a short distance posterior of torulo-posttorular complex (Fig. 2A).

**Table 2.** List of all important characters examined in this study with assessment of diagnostic potential and information on usage in this study (characters marked with \* were used for species delimitations).

Characters examined	Diagnostic assessment and usage
<b>Head characters</b>	
Shape of head in full-face view	none, no significant interspecific variation observed, not used in this study
Shape of head in profile *	high, used in this study
Shape of mandibles	none, no significant interspecific variation observed, not used in this study
Mandibular dentition	none, no significant interspecific variation observed, not used in this study
Shape of clypeus	low, no significant interspecific variation observed, not used in this study
Presence of median clypeal tooth *	high, used in this study
Cuticular apron of clypeus	none, no significant interspecific variation observed, not used in this study
Torulo-postorular complex *	high, used in this study
Antennal bulb	none, no significant interspecific variation observed, not used in this study
Antennal scapes *	high, used in this study
Antennal pedicel and funiculus	none, no significant interspecific variation observed, not used in this study
Anterior tentorial pits	none, no significant interspecific variation observed, not used in this study
Parafrenal ridges *	high, used in this study
Eyes	none, absent in the worker caste
Vertex *	high, used in this study
Occipital margin in posterodorsal view *	high, used in this study
Occiput in posterior view *	high, used in this study
Occipital margin in posteroventral view *	high, used in this study
Hypostoma *	high, used in this study
Mouthparts (maxillae, labium, labrum)	unclear, none in closed condition; described in open condition for <i>Z. sarowiwai</i> , but needs further investigation with better preserved alcohol material for $\mu$ CT scanning
Tentorium (internal)	unclear, tentatively examined in this study and appears species-specific, but needs further investigation with better preserved alcohol material for $\mu$ CT scanning
<b>Mesosoma characters</b>	
Mesosoma in profile *	high, used in this study
Endosternum (internal)	unclear, tentatively examined in this study and appears species-specific, but needs further investigation with better preserved alcohol material for $\mu$ CT scanning
Transverse mesopleural groove	moderately variable among species, not used in this study
Propleuron	none, no significant interspecific variation observed, not used in this study
Pleural endophragmal pit *	high, used in this study
Mesopleuron	moderately variable among species, not used in this study
Metapleuron	low, no significant interspecific variation observed, not used in this study
Mesosoma dorsal *	high, used in this study
Probasitarsus	low, no significant interspecific variation observed, not used in this study
Calcar of strigil	low, no significant interspecific variation observed, not used in this study
<b>Metasoma characters</b>	
Levator of petiole	unclear, not examined in this study, very difficult to virtually dissect
Petiolar tergum in profile *	high, used in this study
Laterotergites	low, no significant interspecific variation observed, not used in this study
Subpetiolar process of petiolar sternum in profile *	high, used in this study

Characters examined	Diagnostic assessment and usage
Petiolar tergum in dorsal view *	high, used in this study
Disc of petiole	none, no significant interspecific variation observed, not used in this study
Subpetiolar process in ventral view *	high, used in this study
Helcium	unclear, not examined in this study
Abdominal segment III in dorsal view *	high, used in this study
Abdominal segment III in ventral view *	high, used in this study
Posterior end of abdominal segment III in ventral view *	high, used in this study
Prora in anteroventral view *	high, used in this study
Abdominal segment IV in dorsal view	moderate, relatively variable within species, not used in this study
Abdominal segment IV in ventral view	moderate, relatively variable within species, not used in this study
Abdominal segment V in dorsal view	low, no significant interspecific variation observed, not used in this study
Abdominal segment V in ventral view	low, no significant interspecific variation observed, not used in this study
Abdominal segment VI in dorsal view *	high, used in this study
Abdominal segment VI in ventral view	high, not used in this study
Girdling constrictions abdominal segments IV, V, VI *	high, used in this study
Pygidium	low, no significant interspecific variation observed, not used in this study
Hypopygium	low, no significant interspecific variation observed, not used in this study
Spiracles abdominal segments II-VII	none, no significant interspecific variation observed, not used in this study
General surface sculpture *	high, used in this study
Cuticle thickness (internal)	unclear, examined in this study but needs further investigation with more specimens

Dorsal pronotum cuticle thickness (PRC): thickness of cuticle of pronotum measured in profile a short distance posterior of anterodorsal margin (Fig. 2B).

Dorsal abdominal segment II (petiole) cuticle thickness (ASIIC): thickness of cuticle of abdominal segment II measured in profile a short distance posterior of anterodorsal margin (Fig. 2C).

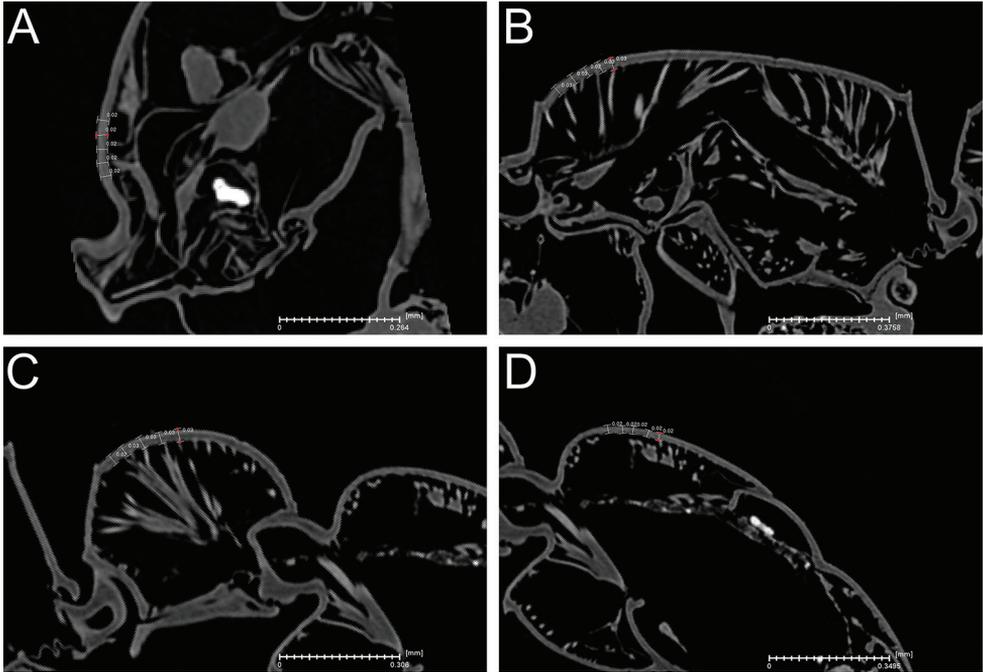
Dorsal abdominal segment III cuticle thickness (ASIIIC): thickness of cuticle of abdominal segment III measured in profile a short distance posterior of anterodorsal margin (Fig. 2D).

Cephalic capsule cuticle thickness index (CCCI):  $CCC / HW \times 1000$

Dorsal pronotum cuticle thickness index (PRCI):  $PRC / HW \times 1000$

Dorsal abdominal segment II (petiole) cuticle thickness index (ASIICI):  $ASIIC / HW \times 1000$

Dorsal abdominal segment III cuticle thickness index (ASIIICI):  $ASIIIC / HW \times 1000$



**Figure 2.** Microtomographic slides showing cuticle thickness measurements (with measuring lines in white). **A** Head in profile **B** Mesosoma in profile **C** Petiole (abdominal segment II) in profile **D** Abdominal segment III in profile.

### 3D PDFs

The first step to creating unicoloured 3D PDFs was to make 3D renderings of ant specimens in Amira using the Isosurface function (deselect compactify) for exporting surface meshes in the STL file format. These were imported into Meshlab (version 1.3.3) where the number of vertices per specimen was reduced in three steps to decrease total file size and before importing into Adobe Acrobat. First, the scan files were cleaned from isolated vertices (Filters > Cleaning and Repairing > Remove isolated pieces (wrt diameter) [set max diameter: 0.05–1%]) and the paper tips on which the ants are mounted were digitally removed as much as possible. The next step removed all internal vertices so that only the exoskeleton remained (1. Filters > Color Creation and Processing > Ambient Occlusion Per Vertex; 2. Filters > Selection > Select Faces By Vertex Quality (min = 0, max = 0.001); 3. Remove Selected Faces). In the last step, the number of total vertices was reduced to the final number of <750,000 (Filters > Remeshing, Simplification and Reconstruction > Quadratic Edge Collapse Decimation) in order to get a manageable resolution resulting in 3D PDF files of approximately 20 MB in size for supplementary files (the final step was omitted for files uploaded to Dryad). The processed STL files were annotated and exported as 3D PDFs in Adobe Acrobat Pro DC (version 2015.006.30119) using the Tetra4D Converter plug-in (version 5.1.2). When viewing the 3D PDFs with Adobe Acrobat

Reader (version 8 or higher), trusting the document by clicking on the image will activate the interactive 3D-mode and allows rotating, moving and zooming into the 3D model.

To generate the coloured 3D PDF of the mouthparts, we first segmented each mouthpart (maxillae, labium, labrum) independently and labelled each with a different colour. A surface mesh of the combined segmentation data was then generated using Generate Surface function in Amira with Unconstrained Smoothing (Smoothing Extent set to 1.5). We exported the surface data into Open Inventor Format, where it was converted to U3D format using IvTuneViewer plugged in Amira. Finally, the 3D PDF was generated by importing the U3D file to Adobe Acrobat Pro DC (version 2015.006.30119) with Tetra4D plugged in (version 5.1.2).

## Data availability

All specimens used in this study have been databased and the data is freely accessible on AntWeb (<http://www.antweb.org>). Each specimen can be traced by a unique specimen identifier attached to its pin (e.g. CASENT0764125). The Cybertype datasets provided in this study consist of the full micro-CT original volumetric datasets, 3D PDFs, 3D rotation video files, all light photography montage images, and all image plates including all important images of 3D models for each species. In addition to the cybertype datasets, we also provide high-resolution 3D videos and/or 3D PDFs of the mouthparts and musculature reconstructions, as well as the full micro-CT original volumetric dataset of the mouthpart scan. All data have been archived and are freely available from the Dryad Digital Repository (Hita Garcia et al. 2017c, <http://dx.doi.org/10.5061/dryad.4s3v1>). In addition to the cybertype data at Dryad, we also provide freely accessible 3D surface models of type material on Sketchfab (<https://sketchfab.com/arilab>).

## Results

### Taxonomy of *Zasphinctus* in the Afrotropical region

#### Notes on the genus in the region

At the beginning of our study we encountered a situation in which the only two valid species from the region were described from males from West Africa (Santschi 1915), whereas the material available to us consisted of three worker-based species not associated to any males. A DNA-based association is currently not possible since the two male-based species are only known from their respective type material, thus not available for any molecular analysis. There are additional males available from Nigeria and Uganda, but they are also not associated with any workers and their conspecificity with the other male-based species is uncertain. Since *Zasphinctus* is one of the rarest ant genera in the region, it is not likely that more specimens than currently available will be collected anytime soon, which means that the lack of male-worker associa-

tion problems will remain. Furthermore, the two male-based species were collected in relatively arid savannah areas in West Africa while two of the worker-based species are from humid equatorial rainforests. The third worker-based species is from a savannah in Mozambique, thus geographically distant from the two male-based species.

These discrepancies led us to describe the three worker-based species independently from the already known male-based species and create a comprehensive worker-based taxonomic system for the genus in the Afrotropical region. With this approach, we follow Wilson (1964) who suggested temporarily ignoring the male-based names and establishing a sound worker-based taxonomy until males are found together with workers and the different taxonomic names can be harmonised. More recent authors concur with that approach and also opine that male-based names will eventually be matched with worker-based names using molecular data (Jaitrong and Yamane 2011; Liu et al. 2015; Staab 2015).

### **Diagnosis of Afrotropical *Zasphinctus* (workers)**

The following diagnosis is based on Borowiec (2016) with modifications and additions to encompass just the Afrotropical species:

**HEAD:** Antennae with 12 segments and relatively short (SI 47–57), far from approaching posterior head margin. Apical antennal segment conspicuously enlarged, longer than two preceding segments combined. Head distinctly longer than broad (CI 78–86). Clypeus with cuticular apron. Lateroclypeal teeth absent. Parafrontal ridges present and well developed. Torulo-posttorular complex vertical. Antennal scrobes absent. Labrum with median notch or concavity. Proximal face of stipes not projecting beyond inner margin of sclerite, prementum exposed when mouthparts fully closed, even though only slightly so. Maxillary and labial palps 3-segmented (see section on mouthparts below). Mandibles elongate triangular, masticatory margin with 4 or 5 small denticles on basal half, denticles usually strongly reduced and inconspicuous. Eyes and ocelli absent. Head capsule with weakly to well differentiated vertical posterior surface above occipital foramen. Ventrolateral margins of head without lamella or ridge extending towards mandibles and beyond carina surrounding occipital foramen. Posterior head corners dorsolaterally immarginate. Carina surrounding occipital foramen ventrally present.

**MESOSOMA:** Mesosoma in profile relatively low and elongate to moderately high and stocky (LMI 34–41). In dorsal view usually slightly more than twice as long as broad (DMI2 49–58). Pronotal flange separated from collar by distinct ridge. Promesonotal connection with suture completely fused. Pronotomesopleural suture absent. Mesometapleural groove not impressed or weakly impressed. Transverse groove dividing mesopleuron absent. Pleural endophragmal pit concavity present, weakly to well developed. Mesosoma dorsolaterally immarginate. Metanotal depression or groove on mesosoma absent. Propodeal spiracle situated low on sclerite. Propodeal declivity with distinct dorsal edge or margin and rectangular in posterior view. Metapleural gland without bulla visible through cuticle. Propodeal lobes present and well developed.

LEGS: Mesotibia with single pectinate spur. Metatibia with single pectinate spur. Metabasitarsus not widening distally, circular in cross-section. Posterior flange of hind coxa not produced as raised lamella. Metatibial gland an oval patch of whitish cuticle. Metabasitarsal gland absent. Pretarsal claws of metatibia simple. Metafemur short to moderately long (MFI 75–100).

METASOMA: Abdominal segment II (petiole) sessile without peduncle and petiolar node well developed. In profile petiolar tergum between 1.0 to 1.2 times longer than high (LPI 102–123). Petiole anterodorsally marginate, dorsolaterally rounded, and laterally above spiracle weakly marginate. Laterotergites well developed and clearly demarcated. Sternum of petiole well developed with strongly anteroventrally projecting subpetiolar process, process with or without fenestra. Helcium axial and in relation to tergosternal suture placed at posttergite. Prora simple, not delimited by carina. Prora forming a U-shaped margin with median ridge. Spiracle openings of abdominal segments IV–VI circular. Abdominal segment III anterodorsally immarginate and dorsolaterally immarginate. In profile view abdominal segment III distinctly larger than succeeding segment IV, in dorsal view abdominal segment III longer than segment IV. Cinctus of abdominal segment IV not impressed. Girdling constrictions of segments IV, V, VI present and distinct, either unsculptured or cross-ribbed. Abdominal tergite IV not folding over sternite, and anterior portions of sternite and tergite equally well visible in lateral view. Pygidium large, with weakly impressed medial field.

SETATION: Most of body with numerous short to moderately long, appressed to suberect (very rarely erect) setae. Pygidium armed with modified, thick, and often peg-like setae. Hypopygium armed with modified setae.

COLOURATION: All known species predominantly dark brown to black with often lighter appendages.

### **Synoptic list of Afrotropical *Zasphinctus***

*Zasphinctus chariensis* Santschi, 1915 \* [Chad]

*Zasphinctus sarowiwai* Hita Garcia sp. n. [Cameroon, Democratic Republic of Congo, Ghana, Ivory Coast, Uganda]

*Zasphinctus obamai* Hita Garcia sp. n. [Kenya]

*Zasphinctus rufiventris* Santschi, 1915 \* [Benin, Mali]

*Zasphinctus wilsoni* Hita Garcia sp. n. [Mozambique]

\* Only known from males and not treated in this study.

### **Diagnostic treatment**

Based on a thorough examination of external morphology and character evaluation, we provide the following character matrix (Table 3) that contains 24 morphological characters of high diagnostic value.

**Table 3.** Character matrix showing all diagnostic characters used for worker-based species delimitation system of Afrotropical *Zasphinctus*.

Species	<i>Z. obamaei</i>	<i>Z. sarovituwai</i>	<i>Z. wilsoni</i>
<b>Head in profile</b>	appearing longer and thinner (Fig. 4A)	appearing shorter and thicker (Fig. 4B)	appearing longer and thinner (Fig. 4C)
<b>Clypeal area</b>	without conspicuous median tooth (Fig. 4D)	with conspicuous median tooth (Fig. 4E)	without conspicuous median tooth (Fig. 4F)
<b>Parafrenal ridges</b>	dorsal outline irregularly convex and conspicuously thickened (Fig. 4G)	dorsal outline regularly convex and not conspicuously thickened (Fig. 4H)	dorsal outline mostly regularly convex and conspicuously thickened (Fig. 4I)
<b>Torulo-posttorular complex in dorsal view</b>	comparatively thicker and shorter (Fig. 4G)	comparatively thinner and longer (Fig. 4H)	comparatively thicker and shorter (Fig. 4I)
<b>Antennal scapes</b>	scape thicker: 2.2 to 2.4 times longer than broad at apex (SI2 215–242) (Fig. 4J)	scape moderately thick: 2.4 to 2.6 times longer than broad at apex (SI2 238–261) (Fig. 4K)	scape thinner: 2.7 times longer than broad at apex (SI2 267) (Fig. 4L)
<b>Vertex</b>	vertexal margin and posterior face of head strongly developed (Fig. 4M)	vertexal margin and posterior face of head weakly developed (Fig. 4N)	vertexal margin and posterior face of head strongly developed (Fig. 4O)
<b>Occipital margin in postero-dorsal view</b>	outline sharp and irregularly defined (Fig. 4M)	outline sharp and very regularly defined (Fig. 4N)	outline weakly and irregularly defined (Fig. 4O)
<b>Occiput in posterior view</b>	posterior and ventral margins similarly broad; ventral margin medially protruding (Fig. 4P)	more ellipsoid; posterior and ventral margins similarly broad; ventral margin not medially protruding (Fig. 4Q)	posterior clearly broader than ventral margin; ventral margin weakly medially protruding (Fig. 4R)
<b>Occipital margin in postero-ventral view</b>	outline sharp and irregularly defined (Fig. 5A)	outline sharp and very regularly defined (Fig. 5B)	outline moderately sharp and irregularly defined (Fig. 5C)
<b>Hypostoma</b>	less diverging with relatively thin and mostly rounded lateral arms (Fig. 5D)	strongly diverging with very thick and strongly rounded lateral arms (Fig. 5E)	strongly diverging with moderately thick and strongly angulate lateral arms (Fig. 5F)
<b>Mesosoma in profile</b>	relatively lower and elongate (LMI 34–36) (Fig. 5G)	moderately higher and compact (LMI 40–41) (Fig. 5H)	relatively lower and elongate (LMI 37) (Fig. 5I)
<b>Pleural endophragmal pit</b>	weakly developed and shallow but visible (Fig. 5G)	strongly developed and deep (Fig. 5H)	very weakly developed and inconspicuous (Fig. 5I)
<b>Mesosoma dorsal</b>	appearing thinner (DMI 38–40; DMI2 49–53) (Fig. 5J)	appearing thicker (DMI 41–44; DMI2 53–58) (Fig. 5K)	appearing intermediate (DMI 40; DMI2 53) (Fig. 5L)
<b>Petiolar tergum in profile</b>	relatively lower: 1.2 times longer than high (LPI 117–123) (Fig. 5M)	relatively higher: 1.0 to 1.1 times longer than high (LPI 102–112) (Fig. 5N)	relatively higher: 1.1 times longer than high (LPI 112) (Fig. 5O)
<b>Subpetiolar process of petiolar sternum in profile</b>	with thickened anterior and ventral margins and well developed concavity with differentiated fenestra (Fig. 5M)	with thickened anterior and ventral margins and well developed concavity with differentiated fenestra (Fig. 5N)	with thickened anterior and ventral margins and weak concavity without differentiated fenestra (Fig. 5O)

Species	<i>Z. obamaei</i>	<i>Z. saroviniwai</i>	<i>Z. wilsoni</i>
<b>Petiole tergum in dorsal view</b>	relatively thinner: around 1.2 times longer than broad (DPI 82–85) (Fig. 5P)	relatively thicker: around 1.0 to 1.1 times broader than long (DPI 101–111) (Fig. 5Q)	relatively thinner: around 1.1 times longer than broad (DPI 93) (Fig. 5R)
<b>Subpetiolar process in ventral view</b>	forklike, ventral margin very thick and short (Fig. 6A)	forklike, ventral margin moderately thick and short (Fig. 6B)	forklike, ventral margin thin and long (Fig. 6C)
<b>Abdominal segment III in dorsal view</b>	appearing more trapezoidal with anterior margin more angulate (Fig. 6D)	appearing more rounded with anterior margin usually more rounded (Fig. 6E)	appearing more trapezoidal with anterior margin more angulate (Fig. 6F)
<b>Abdominal segment III in ventral view</b>	comparatively thinner, longer, and only gently narrowing towards prora (Fig. 6G)	comparatively broad, short and strongly narrowing towards prora (Fig. 6H)	comparatively broad, short and moderately narrowing towards prora (Fig. 6I)
<b>Posterior end of abdominal segment III in ventral view</b>	with thick, deep, sharply and irregularly outlined transverse groove (Fig. 6G)	with thinner, deep, sharply and relatively regularly outlined transverse groove (Fig. 6H)	transverse groove absent, instead with irregular grooves and rugosity (Fig. 6I)
<b>Prora in anteroventral view</b>	well-developed with thick, irregularly shaped and rounded lateroventral margins (Fig. 6J)	well-developed with sharply and very regularly shaped lateroventral margins (Fig. 6K)	very weak to almost absent lateroventral margins (Fig. 6L)
<b>Abdominal segment VI in dorsal view</b>	distinctly longer: 1.7 times broader than long (DA6I 163–173) (Fig. 6M)	distinctly shorter: around 1.9 to 2 times broader than long (DA6I 186–197) (Fig. 6N)	distinctly longer: 1.6 times broader than long (DA6I 159) (Fig. 6O)
<b>Girdling constrictions abdominal segments IV, V, VI</b>	unsculptured (Fig. 6P)	cross-ribbed, much weaker on IV than V & VI (Fig. 6Q)	unsculptured (Fig. 6R)
<b>General surface sculpture</b>	mostly smooth and shining with abundant, relatively deep piliferous punctures, except for reticulate-punctate anteromedian area of cephalic dorsum, anterior pronotum, mesopleuron, lateral propodeum, most of lateral petiole, and hypopygium	almost completely smooth and very shining with scattered, relatively deep piliferous punctures; sometimes with punctate sculpture on metapleuron	cephalic dorsum mostly reticulate-rugose, mesosoma and petiole laterally mostly reticulate-punctate, hypopygium reticulate-rugose, remainder of body predominantly smooth and shining with abundant piliferous punctures

**Identification key to Afrotropical *Zasphinctus* species (workers)**

- 1 With head in full-face view median clypeal area with conspicuous tooth (Fig. 4E, H) and torulo-posttorular complex comparatively long (Fig. 4H); in posterodorsal view vertexal margin and posterior face of head weakly developed (Fig. 4N) ..... *Z. sarowiwai*
- With head in full-face view median clypeal area without any tooth (Fig. 4D, 4F, G, I) and torulo-posttorular complex comparatively short (Fig. 4G, I); in posterodorsal view vertexal margin and posterior face of head strongly developed (Fig. 4M, O) ..... 2
- 2 With head in full-face view parafrontal ridges with irregularly shaped dorsal outline (Fig. 4G); petiolar tergum in profile relatively lower, ca. 1.2 times longer than high (LPI 117–123) (Fig. 5M) ..... *Z. obamai*
- With head in full-face view parafrontal ridges with regularly shaped dorsal outline (Fig. 4I); petiolar tergum in profile relatively higher, ca. 1.1 times longer than high (LPI 112) (Fig. 5O) ..... *Z. wilsoni*

***Zasphinctus obamai* Hita Garcia, sp. n.**

<http://zoobank.org/2B973F61-641C-436D-89AC-5955B519563A>

Figs 3, 4A, D, G, J, M, P, 5A, D, G, J, M, P, 6A, D, G, J, M, P, 7, 8, 13A, 16, Video 1

**Type material. Holotype**, pinned worker, KENYA, Western Province, Kakamega Forest, Buyangu, 0.35222, 34.8647, 1640 m, secondary rainforest, leaf litter, collection code FHG00001, VII.-VIII.2004 (*F. Hita Garcia*) (NMKE: CASENT0764125). **Paratypes**, seven pinned workers: two with same data as holotype (BMNH: CASENT0764126; MCZC: CASENT0764127); one from KENYA, Western Province, Kakamega Forest, Isecheno, equatorial rainforest, sifted litter and soil under *Morus mesozygia*, 0.34, 34.85, 1550 m, 6.XI.2002 (*W. Okeka*) (LACM: CASENT0178218; ZFMK: CASENT0764648); two from KENYA, Western Province, Kakamega Forest, Kisere Forest Fragment, 0.38505, 34.89378, 1650 m, rainforest, ex leaf litter, Transect 11, collection code FHG00036, 16.VII.2007 (*F. Hita Garcia*) (NMKE: CASENT0764128; NMKE: CASENT0764129); and one from KENYA, Western Province, Kakamega Forest, Bunyala Forest Fragment, 0.37889, 34.69917, 1448 m, Winkler leaf litter extraction, collection code ANTC39476, VIII.2008 (*G. Fischer*) (ZFMK: CASENT0764647).

**Cybertypes**, the cybertype dataset consists of all volumetric raw data in DICOM format, 3D PDFs and 3D rotation videos of scans of head, mesosoma, metasoma, and the full body of the physical holotype (NMKE: CASENT0764125) and/or one paratype (MCZC: CASENT0764127) in addition to montage photos illustrating head in full-face view, profile and dorsal views of the body of both specimens. The data is deposited at Dryad and can be freely accessed as virtual representation of both types (Hita Garcia et al. 2017c, <http://dx.doi.org/10.5061/dryad.4s3v1>). In addition to the cyber-

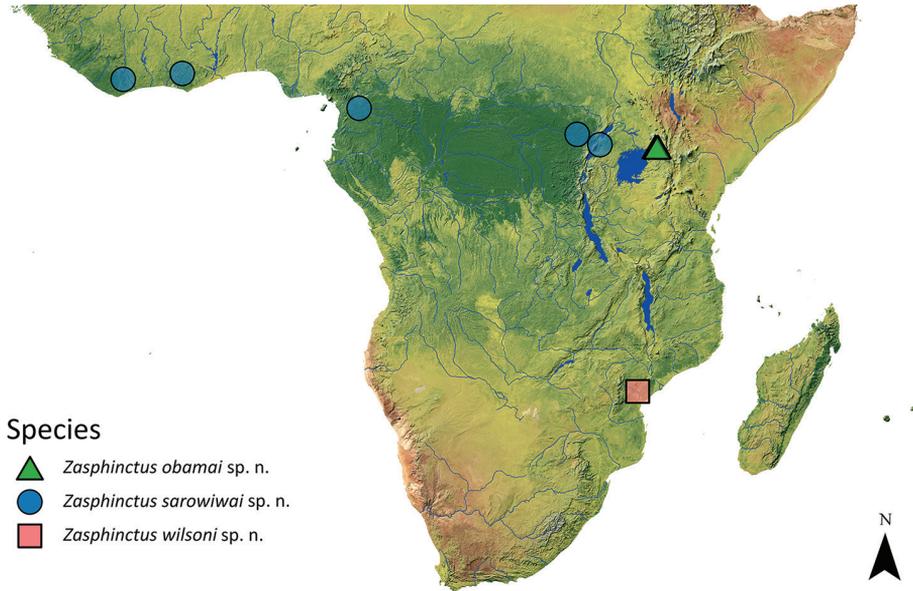
**Table 4.** Morphometric data of the three species treated in this study.

	<i>Z. obamai</i> (N=6)			<i>Z. sarowiwai</i> (N=11)			<i>Z. wilsoni</i> (N=1)
	Min	Max	Mean	Min	Max	Mean	
HL	0.55	0.59	0.56	0.78	0.90	0.86	0.60
HW	0.44	0.47	0.45	0.64	0.77	0.73	0.49
SL	0.26	0.31	0.28	0.41	0.50	0.48	0.32
SW	0.12	0.14	0.13	0.17	0.21	0.19	0.12
PH	0.26	0.29	0.27	0.44	0.52	0.49	0.32
PW	0.28	0.33	0.30	0.47	0.55	0.52	0.35
DML	0.53	0.65	0.59	0.85	0.99	0.95	0.66
WL	0.73	0.81	0.77	1.08	1.30	1.22	0.87
MFL	0.33	0.37	0.35	0.58	0.69	0.64	0.49
PTL	0.27	0.29	0.28	0.40	0.47	0.44	0.29
PTH	0.22	0.24	0.23	0.39	0.45	0.42	0.26
PTW	0.23	0.26	0.24	0.41	0.50	0.47	0.27
A3L	0.33	0.39	0.36	0.50	0.59	0.55	0.48
A3W	0.38	0.43	0.41	0.56	0.67	0.63	0.43
A4L	0.26	0.29	0.28	0.41	0.56	0.50	0.31
A4W	0.46	0.52	0.49	0.71	0.83	0.79	0.54
A5L	0.25	0.29	0.27	0.40	0.49	0.45	0.32
A5W	0.47	0.52	0.49	0.71	0.85	0.80	0.55
A6L	0.26	0.30	0.28	0.36	0.41	0.39	0.32
A6W	0.45	0.49	0.47	0.67	0.78	0.73	0.51
CI	78	80	80	82	86	84	82
SI	47	53	50	53	57	55	53
SI2	215	242	228	238	261	247	267
DMI	38	40	39	41	44	42	40
DMI2	49	53	51	53	58	55	53
LMI	34	36	36	40	41	40	37
MFI	75	79	77	88	91	89	100
LPI	117	123	120	102	112	105	112
DPI	82	93	88	101	111	105	93
DA3I	108	115	112	112	117	114	90
DA4I	170	181	176	145	173	159	174
DA5I	174	188	180	167	181	177	172
DA6I	163	173	169	186	197	189	159

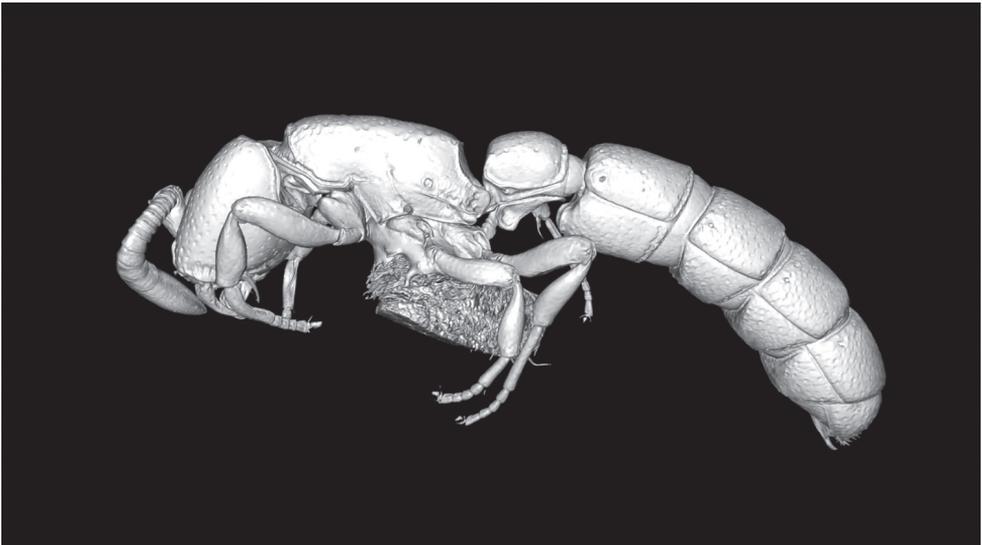
type data at Dryad, we also provide a freely accessible 3D surface model of the holotype at Sketchfab (<https://sketchfab.com/models/dfe15a58514c4be89cdef7f9713091c>).

**Differential worker diagnosis.** See Table 3.

**Worker measurements and indices.** See Table 4.

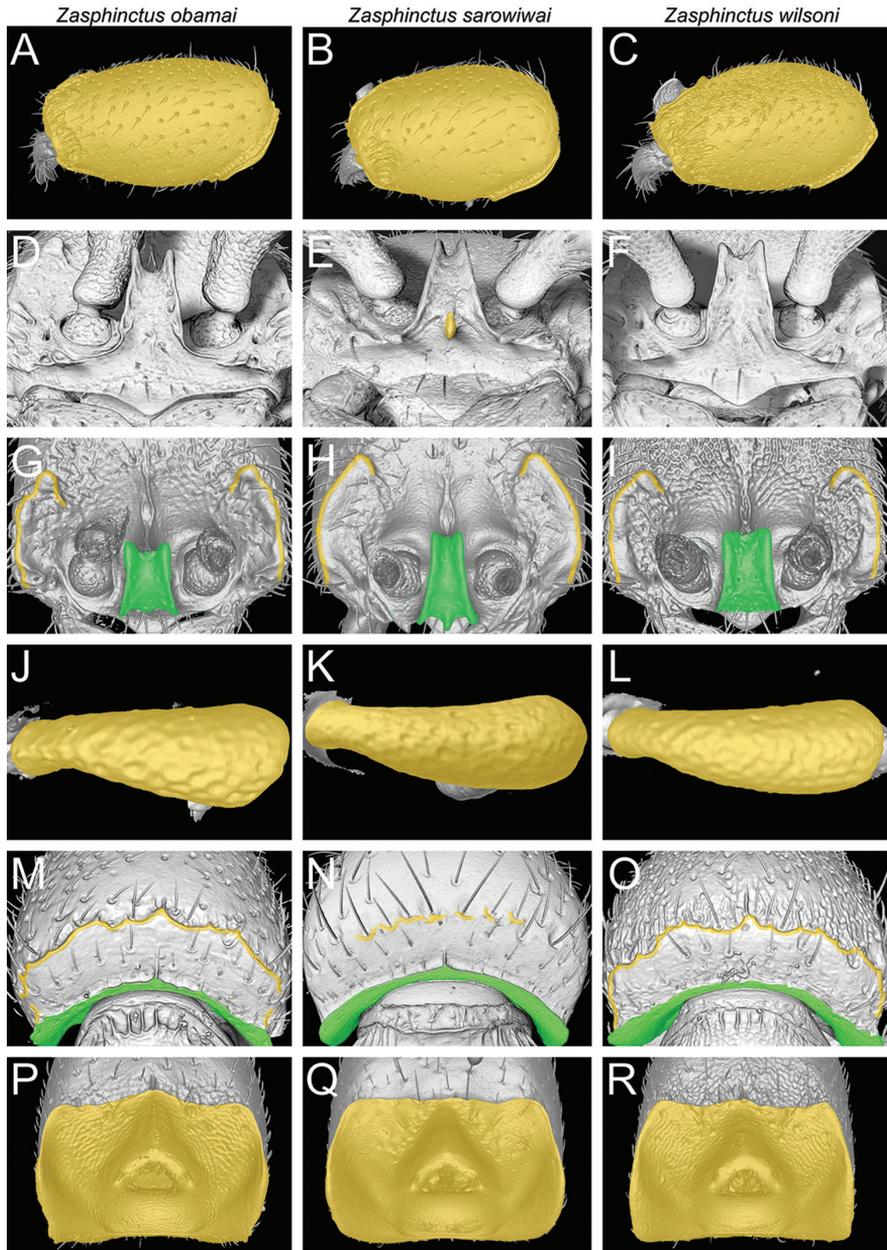


**Figure 3.** Map of sub-Saharan Africa showing the known distribution of the *Z. obamai* sp. n., *Z. sarowiwai* sp. n., and *Z. wilsoni* sp. n.

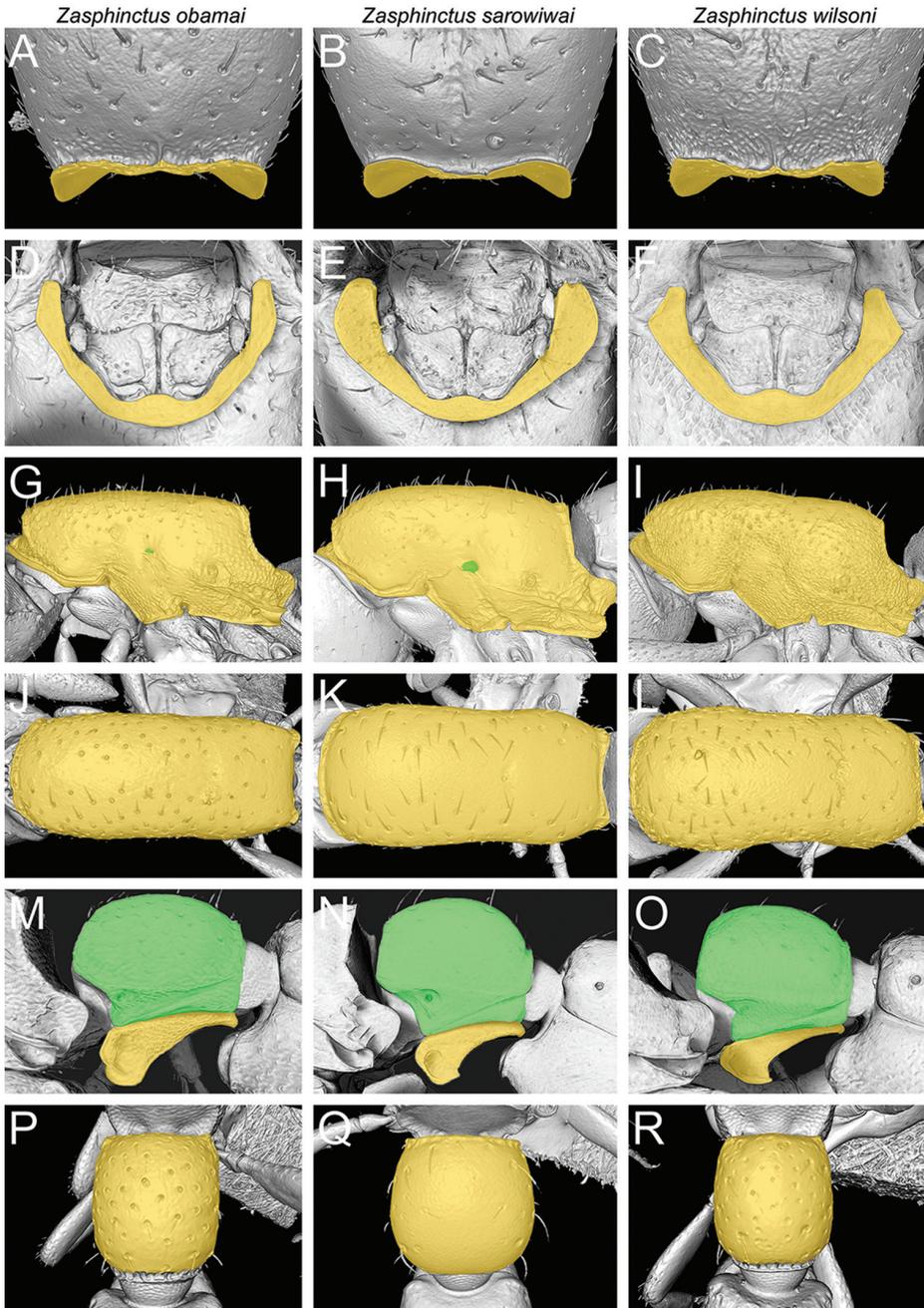


**Video 1.** 3D rotation video of *Zaspinctus obamai* sp. n. holotype worker (CASENT0764125) based on shaded volumetric surface rendering of full body.

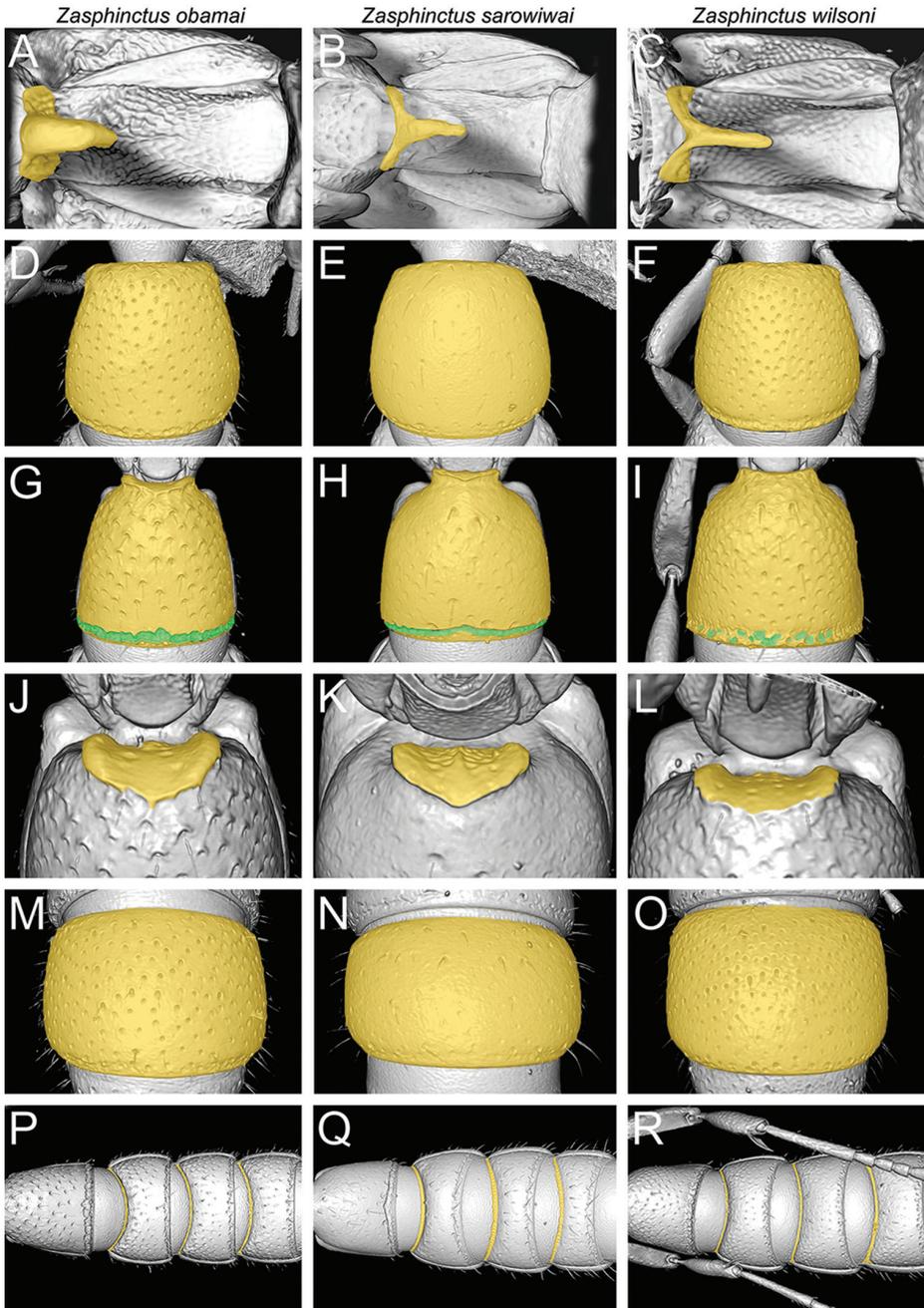
**Etymology.** This species is named in honour of Barack Hussein Obama, the 44th President of the United States of America. We want to acknowledge his important efforts undertaken for the conservation of fragile natural habitats around the globe. Also, the type locality of *Z. obamai* is geographically close to the hometown of Obama's paternal family in Western Kenya.



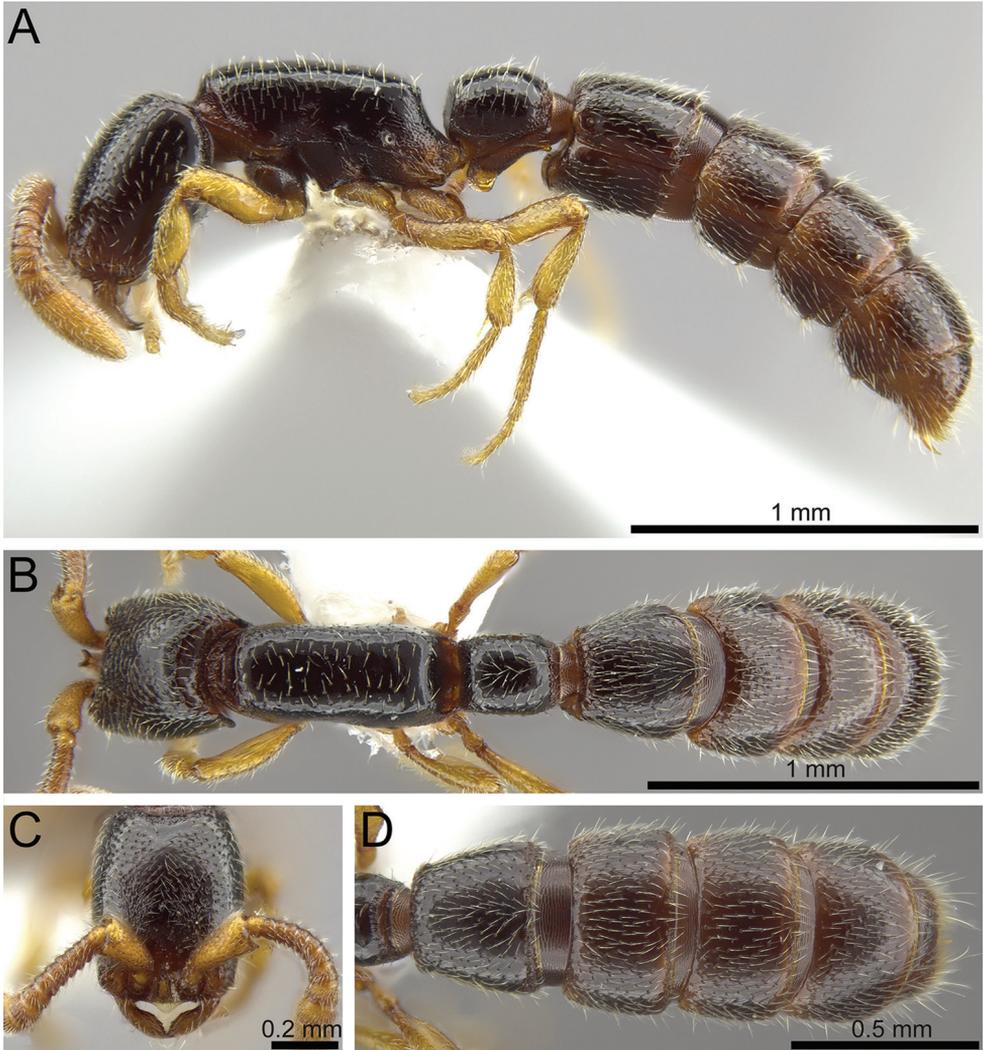
**Figure 4.** Illustrated diagnostic character matrix based on micro-CT images used for species delimitations (*Z. obamai* = left column, *Z. sarowiwai* = middle column, *Z. wilsoni* = right column). **A, B, C** Cephalic capsule in profile (virtually dissected) **D, E, F** Clypeus and torulo-posttorular complex in anterior view **G, H, I** Anterior head (antennae virtually removed) showing parafrontal ridges (orange) and torulo-posttorular complex (green) **J, K, L** Antennal scape in dorsal view (virtually dissected) **M, N, O** Head in posterodorsal view showing vertexal margin (orange), posterior face, and occipital margin (green) **P, Q, R** Head in posterior view showing occiput and occipital foramen (virtually dissected) (ventral head facing upwards).



**Figure 5.** Illustrated diagnostic character matrix based on micro-CT images used for species delimitations (*Z. obamai* = left column, *Z. sarowiwai* = middle column, *Z. wilsoni* = right column). **A, B, C** Posterior head in ventral view showing ventral occipital margin (virtually dissected) **D, E, F** Head in ventral view showing mouthparts and hypostoma (virtually dissected). **G, H, I** Mesosoma in profile (orange) with pleural endophragmal pit (green) **J, K, L** Mesosoma in dorsal view **M, N, O** Petiole in profile showing petiolar tergum (green) and petiolar sternum (orange) with subpetiolar process **P, Q, R** Petiole in dorsal view.

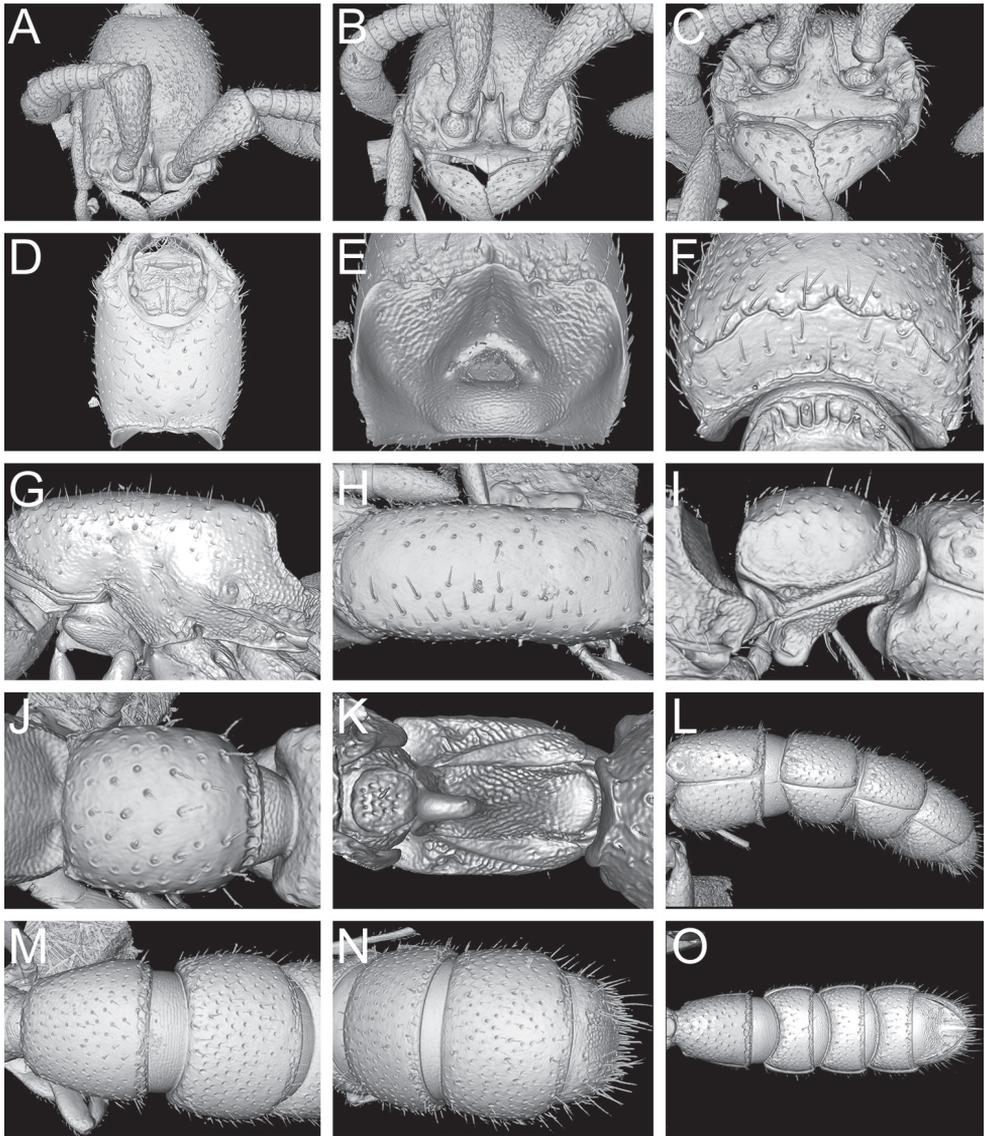


**Figure 6.** Illustrated diagnostic character matrix based on micro-CT images used for species delimitations (*Z. obamai* = left column, *Z. sarowiwai* = middle column, *Z. wilsoni* = right column). **A, B, C** Subpetiolar process of petiolar sternum in ventral view (virtually dissected) **D, E, F** Abdominal segment III in dorsal view **G, H, I** Abdominal segment III (orange) in ventral view with posterior end (green) **J, K, L** Abdominal segment III in anteroventral view showing prora (virtually dissected) **M, N, O** Abdominal segment VI in dorsal view **P, Q, R** Abdominal segments III, IV, V, and VI in ventral view showing girdling constrictions.



**Figure 7.** *Zaspinctus obamai* sp. n. holotype worker (CASENT0764125). **A** Body in profile **B** Body in dorsal view **C** Head in full-face view **D** Abdominal segments III–VII in dorsal view.

**Distribution and biology.** *Zaspinctus obamai* is only known from the type locality, the Kakamega Forest in Western Kenya, which is a tropical equatorial rainforest. Despite a thorough ant inventory (Hita Garcia et al. 2009), *Z. obamai* was only sampled four times making it one of the rarest ant species of the Kakamega Forest. It was only found in the leaf litter layer of primary or near-primary forest habitats. Considering the rarity of this species in the type locality it is possible that it might also be encountered in other rainforest localities westwards of Kakamega, but eluded collections in the past. However, presently, *Z. obamai* appears to be endemic to this one forest.



**Figure 8.** Shaded surface display volume renderings of 3D models of *Zaspinctus obamai* sp. n. paratype worker (CASENT0764127). **A** Head in full-face dorsal view **B** Head in anterodorsal view **C** Anterior cephalic dorsum and mandibles in anterodorsal view **D** Head in ventral view **E** Occiput in posterior view (ventral head facing upwards) **F** Head in posterodorsal view **G** Mesosoma in profile **H** Mesosoma in dorsal view **I** Abdominal segment II (petiole) in profile **J** Abdominal segment II (petiole) in dorsal view **K** Abdominal segment II (petiole) in ventral view **L** Abdominal segments III–VII in profile **M** Abdominal segments III and IV in dorsal view **N** Abdominal segments V–VII in dorsal view **O** Abdominal segments III–VII in ventral view.

**Diagnostic comments.** *Zasphinctus obamai* appears to be morphologically closer to *Z. wilsoni* than to *Z. sarowiwai*. Among other important differences, *Z. obamai* and *Z. wilsoni* are significantly smaller, lack a median clypeal tooth, and have a clearly defined vertexal margin compared to *Z. sarowiwai*. *Zasphinctus obamai* and *Z. wilsoni* can be easily separated by the characters provided above in Table 3. On the basis of the type series, there is no observable intraspecific variation.

***Zasphinctus sarowiwai* Hita Garcia sp. n.**

<http://zoobank.org/DB20AFDC-3644-44A5-AA74-9B53249B5C0D>

Figs 3, 4B, E, H, K, N, Q, 5B, E, H, K, N, Q, 6B, E, H, K, N, Q, 9, 10, 13B, 14, 15, Videos 2, 4, 5

**Type material. Holotype**, pinned worker, CAMEROON, Centre Province, Mbal-mayo, 3.4597, 11.4714, ca. 600 m, rainforest, XI.1993 (*N. Stork*) (BMNH: CASENT0764654). **Paratypes**, three pinned workers with same data as holotype (BMNH: CASENT0764646; CASENT0764649; CASENT0764650).

**Cybertypes**, the cybertype dataset consists of all volumetric raw data in DICOM format, 3D PDFs and 3D rotation videos of scans of head, mesosoma, metasoma, and the full body of the physical holotype (BMNH: CASENT0764654) and/or one paratype (BMNH: CASENT0764650) in addition to montage photos illustrating head in full-face view, profile and dorsal views of the body of both specimens. The data is deposited at Dryad and can be freely accessed as virtual representation of both types (Hita Garcia et al. 2017c, <http://dx.doi.org/10.5061/dryad.4s3v1>). In addition to the cybertype data at Dryad, we also provide a freely accessible 3D surface model of the holotype at Sketchfab (<https://sketchfab.com/models/3e5a54cb8ea94028a49f0722bd5eefe8>).

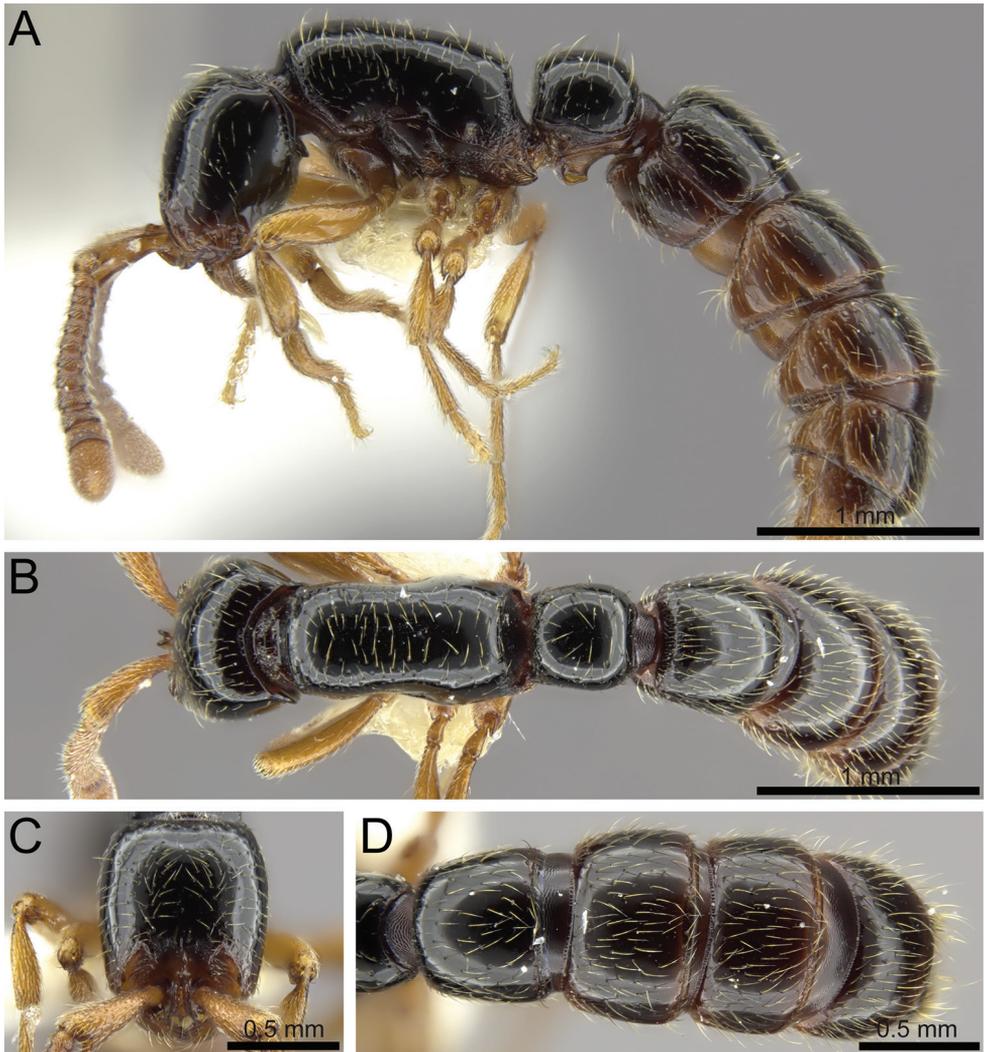
**Non-type material.** DEMOCRATIC REPUBLIC OF CONGO: Epulu, 1.38333, 28.58333, 750 m, rainforest, 1.XI.1995 (*S.D. Torti*); GHANA: Wiawso, 6.2158, -2.485, ca. 160 m, 25.IV.1969 (*D. Leston*); IVORY COAST: Tai Forest, 5.75, -7.12, ca. 250 m, rainforest, 18.–20.V.1977 (*T. Diomande*); UGANDA: Western, Kabarole, Kibale National Park, Kanyawara Biological Station, 0.56437, 30.36059, 1510–1520 m, rainforest, 6.–16.VIII.2012 (different independent collectors: *F.A. Esteves, F. Hita Garcia & P.G. Hawkes*).

**Differential worker diagnosis.** See Table 3.

**Worker measurements and indices (N=11).** See Table 4.

**Etymology.** The name of the new species is a patronym in honour of the famous Nigerian writer, environmentalist, and human rights activist Kenule Beeson “Ken” Saro-Wiwa. By naming a species from threatened rainforest habitats after him, we want to acknowledge his environmental legacy and draw attention to the often-problematic conservation situation in most Afrotropical rainforests.

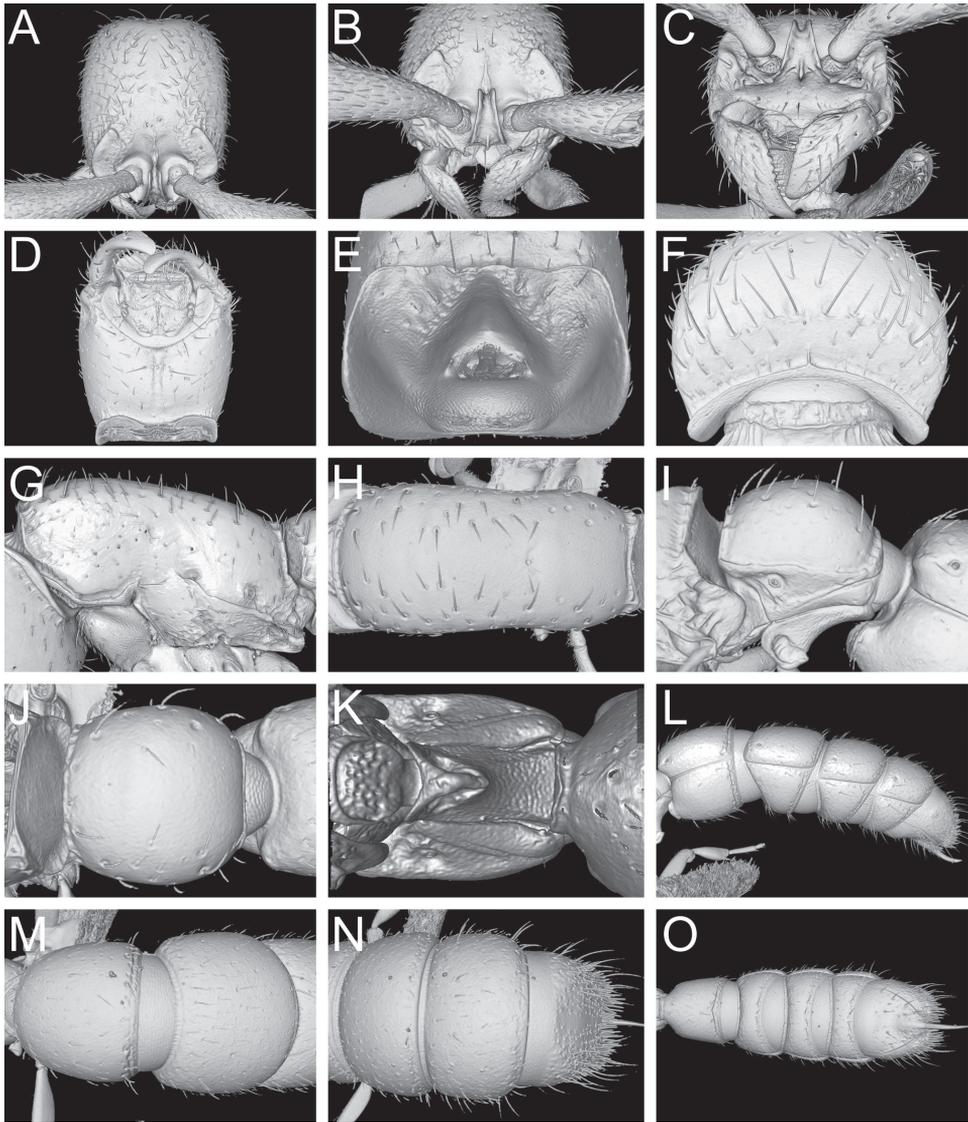
**Distribution and biology.** The new species has a comparatively wide distribution ranging from Ivory Coast to Uganda, even though it is not known from all countries in-between. However, this is likely based on a sampling artefact considering the rarity of *Zasphinctus* in general and the poor sampling in most African countries. Therefore,



**Figure 9.** *Zaspinctus sarowiwai* sp. n. paratype worker (CASENT0764650). **A** Body in profile **B** Body in dorsal view **C** Head in full-face view **D** Abdominal segments III–VI in dorsal view.

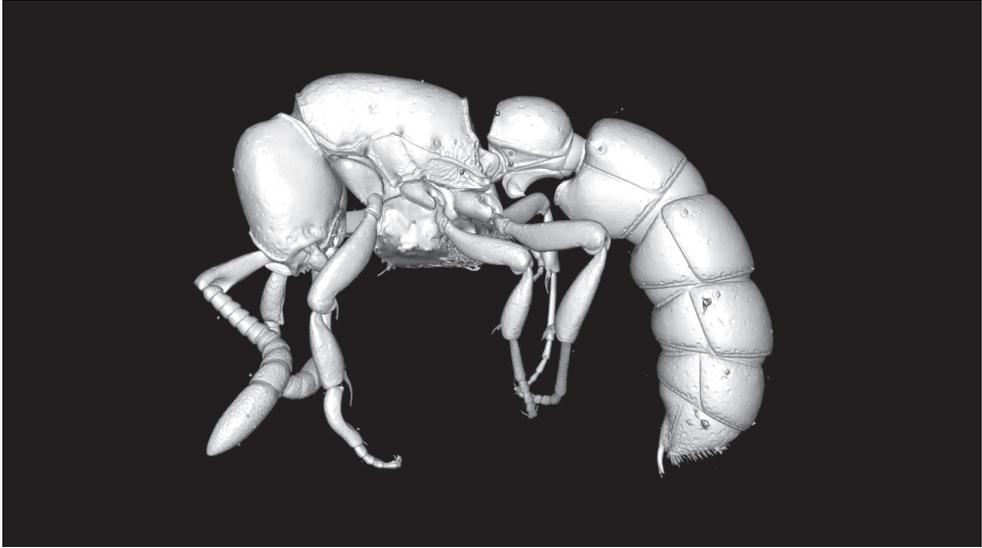
we expect future collections in all countries in-between. All samples are from rainforest habitats at elevations from 250 to 1510 m. Based on the available collection data, the species lives in soil and leaf litter.

**Diagnostic comments.** *Zaspinctus sarowiwai* differs in most diagnostic characters from the other two Afrotropical species. Most obviously, it can be separated from the other species by its much larger body size, the prominent median clypeal tooth, and the almost complete lack of surface sculpture. Despite its wide distribution range, there is very little observable variation. Most notably, the colour appears to be generally darker in the specimens from Uganda and Cameroon, which are uniformly very dark



**Figure 10.** Shaded surface display volume renderings of 3D models of *Zasphectus sarowiwai* sp. n. holotype worker (CASENT0764654). **A** Head in full-face dorsal view **B** Head in anterodorsal view **C** Anterior cephalic dorsum and mandibles in anterodorsal view **D** Head in ventral view **E** Occiput in posterior view (ventral head facing upwards) **F** Head in posterodorsal view **G** Mesosoma in profile **H** Mesosoma in dorsal view **I** Abdominal segment II (petiole) in profile **J** Abdominal segment II (petiole) in dorsal view **K** Abdominal segment II (petiole) in ventral view **L** Abdominal segments III–VII in profile **M** Abdominal segments III and IV in dorsal view **N** Abdominal segments V–VII in dorsal view **O** Abdominal segments III–VII in ventral view.

brown to black, while the specimens from West Africa tend to have a much lighter abdomen and often relatively bright legs. Furthermore, we observed some variation in the material from Uganda. In some specimens, the subpetiolar process of the petiolar



**Video 2.** 3D rotation video of *Zaspinctus sarowiwai* sp. n. paratype worker (CASENT0764650) based on shaded volumetric surface rendering of full body.

sternum had a slightly weaker, but still distinct, fenestra compared to the material from other localities, and the ventral margin of the process had a posteroventral tooth-like projection. In addition, the anterodorsal margin of abdominal segment III was slightly more angulate in a few specimens while in several other specimens the metapleuron had some weak punctate sculpture. Overall, we consider this variation as geographic and very well within the intraspecific range of such a widespread species.

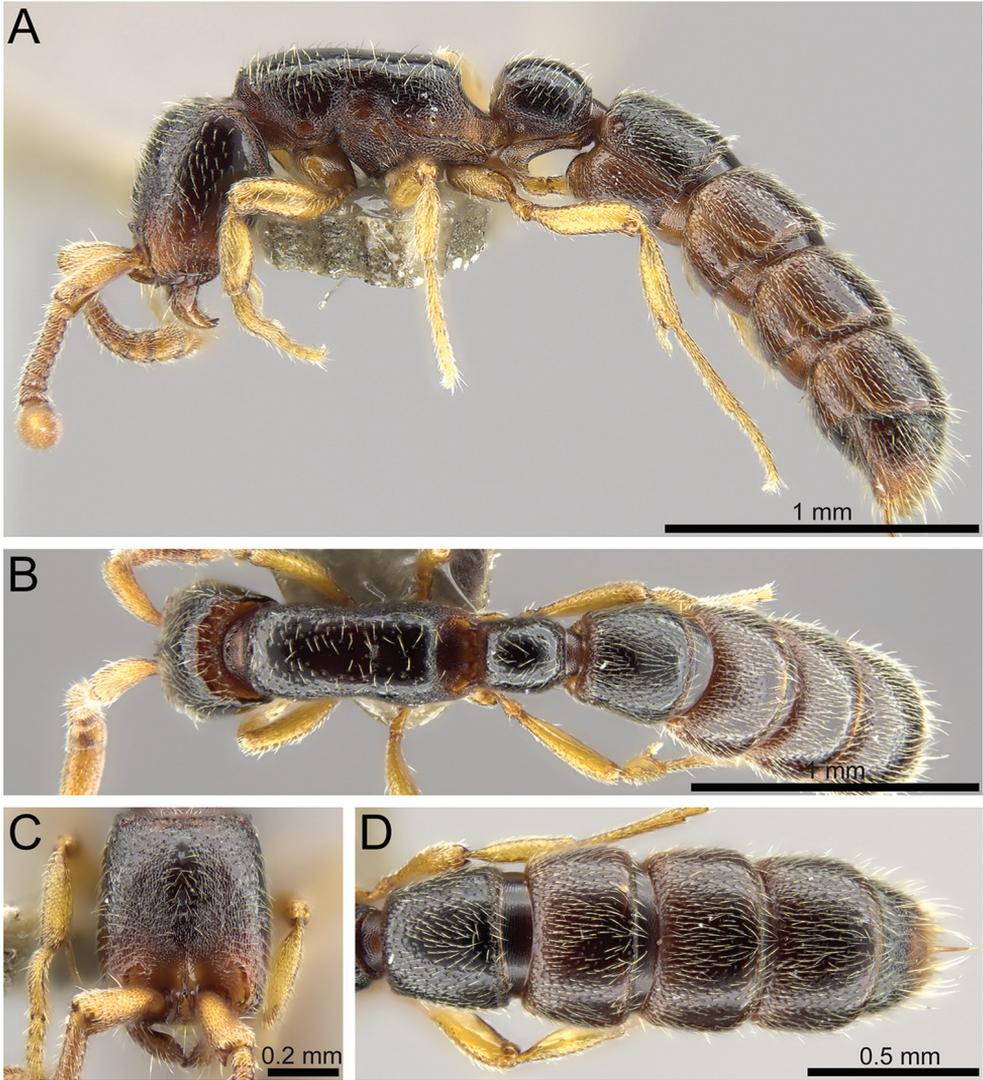
***Zaspinctus wilsoni* Hita Garcia sp. n.**

<http://zoobank.org/355B3D80-3029-4C8A-B48C-939C11914552>

Figs 3, 4C, F, I, L, O, R, 5C, F, I, L, O, R, 6C, F, I, L, O, R, 11, 12, 13C, Video 3

**Type material. Holotype**, pinned worker, Mozambique, Sofala, Gorongosa National Park, 2 km S Chitengo, -18.99472, 34.35769, 1 m, secondary forest, leaf litter, collection code ANTC37418, 30.V.2012 (*G.D. Alpert*) (MCZC: MCZ-ENT-00512764).

**Cybertype**, the cybertype dataset consists of the volumetric raw data in DICOM format, as well as 3D PDFs and 3D rotation videos of scans of the head, mesosoma, metasoma, and the full body of the physical holotype (MCZC: MCZ-ENT-00512764) in addition to montage photos illustrating head in full-face view, profile and dorsal views of the body. The data is deposited at Dryad and can be freely accessed as virtual representation of the holotype (Hita Garcia et al. 2017c, <http://dx.doi.org/10.5061/dryad.4s3v1>). In addition to the cybertype data at Dryad, we also provide a freely ac-



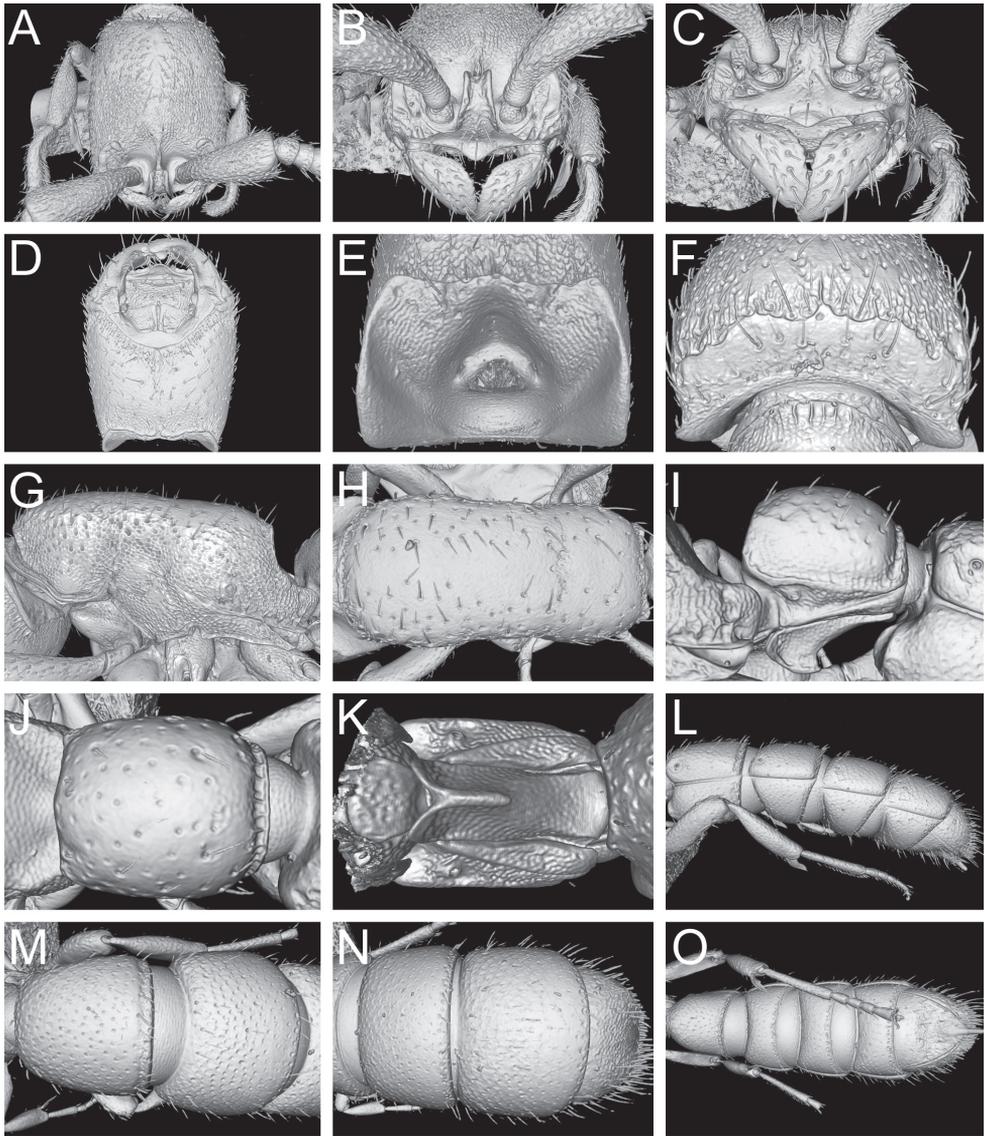
**Figure 11.** *Zaspinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764). **A** Body in profile **B** Body in dorsal view **C** Head in full-face view **D** Abdominal segments III–VII in dorsal view.

cessible 3D surface model of the holotype at Sketchfab (<https://sketchfab.com/models/36bab7ecaa8d45b18013ea679b7ca54a>).

**Differential worker diagnosis.** See Table 3.

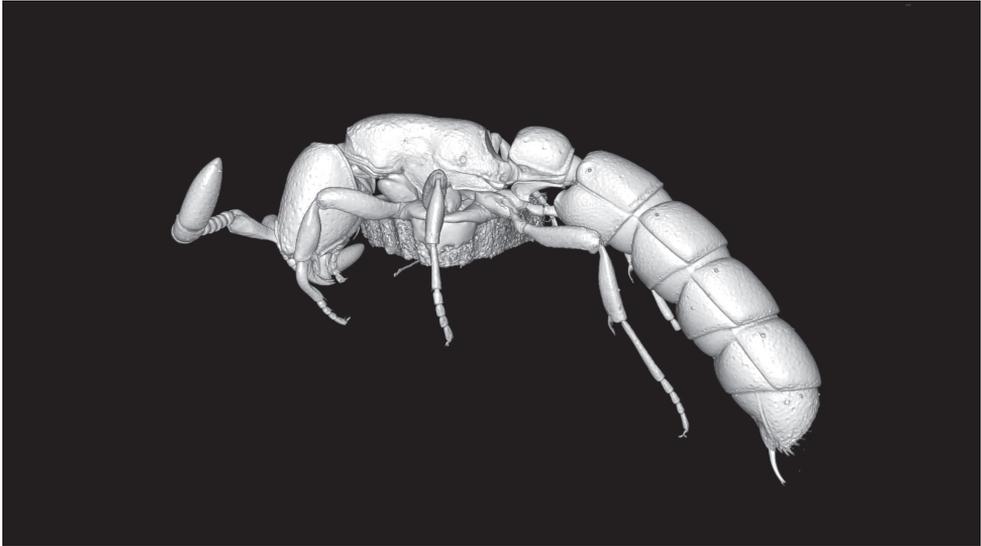
**Worker measurements and indices (N=1).** See Table 4.

**Etymology.** This new species is dedicated to the renowned scientist, author, and conservationist Edward O. Wilson from Harvard University in honour of his more than six decades of accomplishments to the fields of myrmecology, sociobiology, biodiversity, and conservation.



**Figure 12.** Shaded surface display volume renderings of 3D models of *Zaspinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764). **A** Head in full-face dorsal view **B** Head in anterodorsal view **C** Anterior cephalic dorsum and mandibles in anterodorsal view **D** Head in ventral view. **E** Occiput in posterior view (ventral head facing upwards) **F** Head in posterodorsal view **G** Mesosoma in profile **H** Mesosoma in dorsal view **I** Abdominal segment II (petiole) in profile **J** Abdominal segment II (petiole) in dorsal view **K** Abdominal segment II (petiole) in ventral view **L** Abdominal segments III–VII in profile **M** Abdominal segments III and IV in dorsal view **N** Abdominal segments V–VII in dorsal view **O** Abdominal segments III–VII in ventral view.

**Distribution and biology.** Currently, *Z. wilsoni* is only known from its type locality, the Gorongosa National Park where it was collected in the leaf litter of a secondary dry forest. Considering how generally undersampled south-eastern Africa is, it is likely that *Z. wilsoni* might be encountered in other woodland localities in Mozambique, Tanzania, or Zimbabwe.



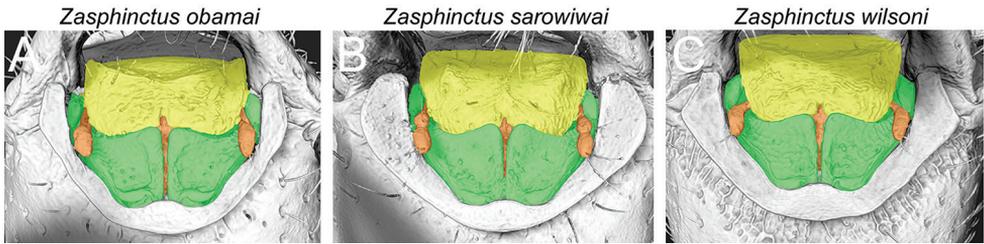
**Video 3.** 3D rotation video of *Zaspinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764) based on shaded volumetric surface rendering of full body.

**Diagnostic comments.** *Zaspinctus wilsoni* is morphologically closer to *Z. obamai* than to *Z. sarowiwai*. It shares the smaller body size, the lack of median clypeal tooth, and a clearly defined vertexal margin with *Z. obamai*, separating both from *Z. sarowiwai*. However, the conspicuous surface sculpture on the cephalic dorsum and the sides of mesosoma and petiole clearly distinguishes *Z. wilsoni* from the other two species. Since *Z. wilsoni* is only known from the holotype there is no available information about intraspecific variation.

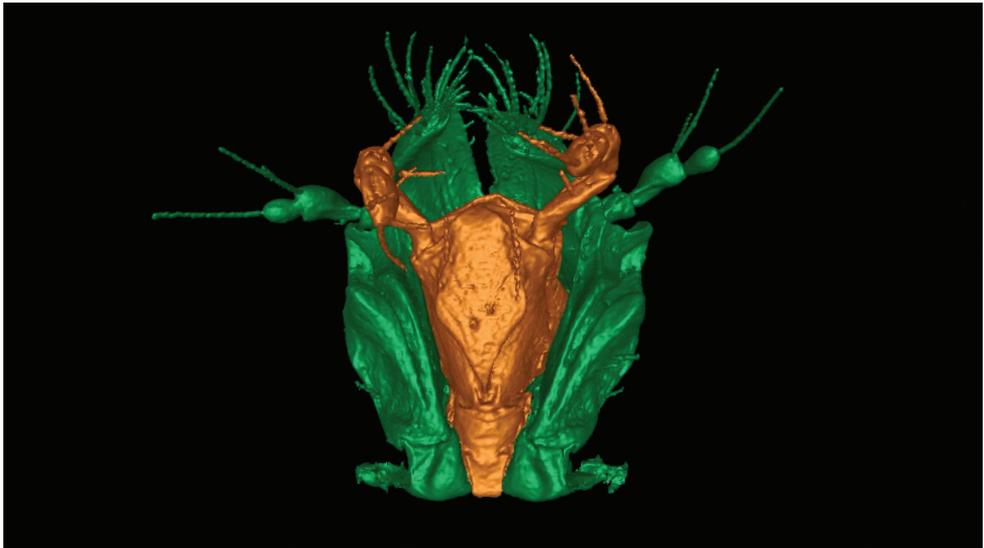
### 3D mouthparts morphology (excluding mandibles)

The small number and the preservation conditions of the specimens available for this study provided some limitations for the examination of mouthparts. It was not possible to dissect *in vivo* or micro-CT scan the open mouthparts of *Z. obamai*, nor of *Z. wilsoni*. Fortunately, the mouthparts of one pinned specimen of *Z. sarowiwai* were open and mostly exposed, thus available for superficial examination under the light microscope and for micro-CT scanning. Consequently, we were unable to test mouthpart morphology in detail for species delimitation. However, based on the limited information observable in closed condition, there appears to be no significant difference between the three species (Fig. 13). In the following we briefly describe the open mouthparts of *Z. sarowiwai* based on a 3D reconstruction of segmented micro-CT data (Fig. 14 and Video 4):

*Labrum*: distal margin conspicuously cleft medially; median area from anterior cleft to proximal articulation very thin, dividing labrum into two lobes; each lobe bulging medially; lateroventrally with two conspicuous hook-like labral arms projecting parallel to remainder of labrum; row of ten to twelve setae (1 very long pair plus four/five shorter



**Figure 13.** Shaded surface display volume renderings of 3D models of mouthparts (excluding mandibles) in closed configuration (green=maxillae; yellow=labrum; orange=labium). **A** *Zaspinctus obamai* sp. n. (CASENT0764127) **B** *Zaspinctus sarowiwai* sp. n. (CASENT0764654) **C** *Zaspinctus wilsoni* sp. n. (MCZ-ENT-00512764).

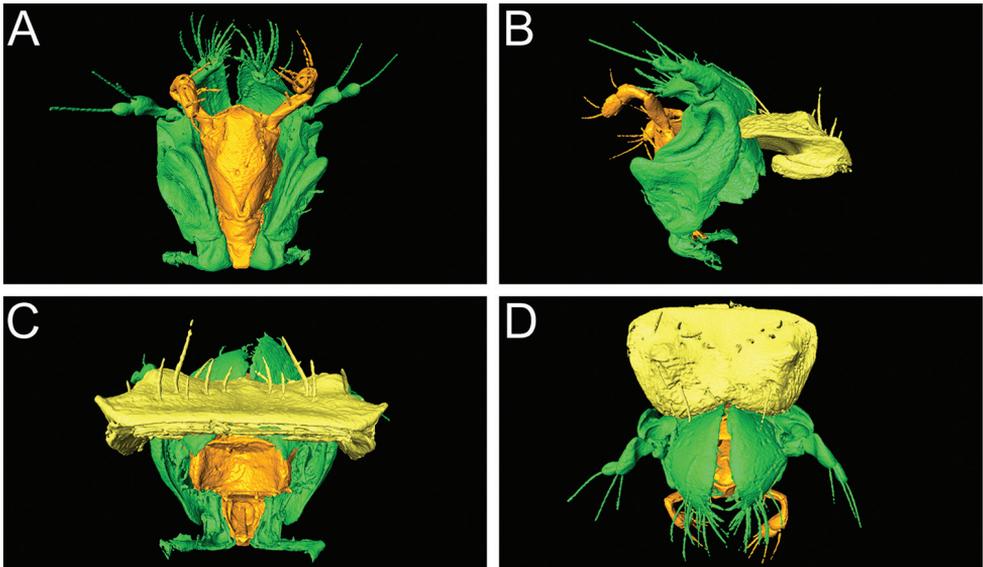


**Video 4.** 3D rotation video of segmented surface reconstructions of the mouthparts of *Zaspinctus sarowiwai* sp. n. (CASENT0764652) in open configuration (green= maxillae; yellow=labrum; orange=labium).

pairs) on basal third of exterior face; row of four to six setae (1 very long pair plus one/two shorter pairs) on exterior face close to distal margin; labral tubercles absent.

*Maxillae:* maxillary palp three-segmented with second segment being greatly enlarged, third segment with very long seta, second with two long setae; deep and conspicuous diagonal, transverse stipital groove present dividing stipes into proximal external face and distal external face; articulation of labrum with maxillae of labro-stipital type via lateral extension/shoulder; proximal faces projecting beyond inner margin of stipites, thus almost completely concealing prementum; galea with well-developed galeal crown and maxillary brush, galeal comb apparently absent; lacinial comb not observable.

*Labium:* labial palp three-segmented with first segment being greatly enlarged, first and second segment with one long seta, third segment with three long setae; premental shield with several moderately long setae; shape of glossa not observable (structure collapsed); subglossal brush present and conspicuous with numerous long and thick setae; paraglossae absent.



**Figure 14.** Volumetric 3D model of segmented surface reconstructions of the mouthparts of *Zaspheinctus sarowiwai* sp. n. (CASENT0764652) in open configuration (green=maxillae; yellow=labrum; orange=labium). **A** Frontal view **B** Lateral view **C** Posterior view **D** Dorsal view.

### Cuticle thickness

The results of our cuticle thickness data are provided in Table 5. The measurements show absolute values of 13–22  $\mu\text{m}$  in *Z. obamai*, 20–30  $\mu\text{m}$  in *Z. sarowiwai*, and 14–21  $\mu\text{m}$  in *Z. wilsoni*, and by putting these in relation to body size the thickness indices range between 31–44. On the basis of cuticle thickness data throughout nine subfamilies of ants (Peeters et al. 2017), the cuticle thickness values of our three *Zaspheinctus* species are at the upper range meaning that these species possess among the thickest cuticles measured so far.

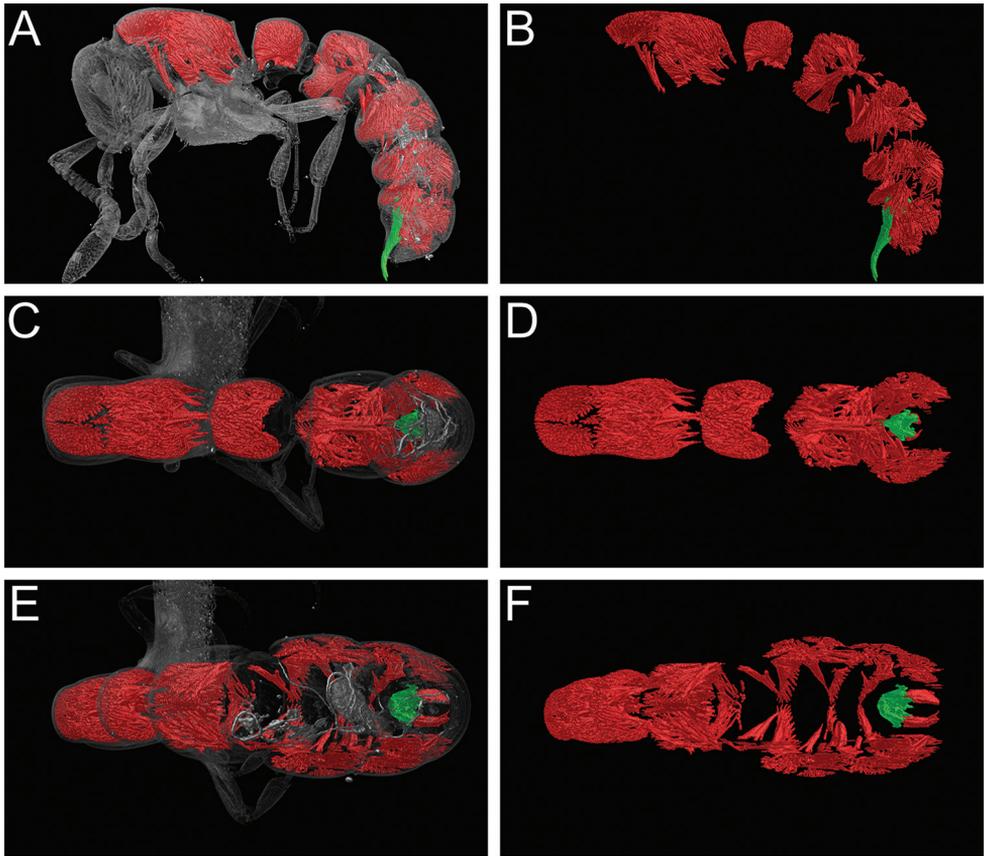
### Thoracic and abdominal musculature

Based on our virtually reconstructed and segmented data, we can show that the mesosoma and metasoma both contain high degrees of musculature (Fig. 15; Video 5). The propodeum is tightly packed with dorsal and ventral muscles moving the abdominal segment II (petiole) and stabilizing the weight of the abdominal segments III to VII. Due to the massive size of the latter, the volume of the propodeal muscles attaching to the anterior petiole is high and comparable to that of the neck muscles in the pronotum. The petiole and the following segments also have a high muscle density, which is prominently visible in lateral (Fig. 15A, 15B), dorsal (Fig. 15C, 15D), and posterodorsal views (Fig. 15E, 15F) in the segmented 3D models of the metasoma. While the muscles in abdominal segment II – and to a lesser extent in segment III – evenly fill almost the entire segment, those in segments IV to VII are mostly limited to

**Table 5.** Morphometric data generated from 3D measuring cuticle thickness. For each species the five raw measurements with corresponding calculations into indices are given, as well as mean values and standard deviations (SD).

Species	<i>Z. obamai</i>		<i>Z. sarowiwai</i>		<i>Z. wilsoni</i>	
	in mm	CCCI	in mm	CCCI	in mm	CCCI
CCC 1	0.019	41	0.022	30	0.014	29
CCC 2	0.018	41	0.023	32	0.014	29
CCC 3	0.019	41	0.022	29	0.016	32
CCC 4	0.021	47	0.024	33	0.017	34
CCC 5	0.022	49	0.023	31	0.015	30
MEAN	0.019	<b>44</b>	0.023	<b>31</b>	0.015	<b>31</b>
SD	0.001	3	0.001	1	0.001	2
	in mm	PRCI	in mm	PRCI	in mm	PRCI
PRC 1	0.017	37	0.027	36	0.018	38
PRC 2	0.017	38	0.027	36	0.020	40
PRC 3	0.016	36	0.025	34	0.019	39
PRC 4	0.016	35	0.029	39	0.020	41
PRC 5	0.015	34	0.030	40	0.021	42
MEAN	0.016	<b>36</b>	0.027	<b>37</b>	0.020	<b>40</b>
SD	0.001	1	0.002	2	0.001	1
	in mm	ASIICI	in mm	ASIICI	in mm	ASIICI
ASIIC 1	0.013	29	0.025	33	0.017	34
ASIIC 2	0.014	31	0.026	35	0.019	39
ASIIC 3	0.016	35	0.027	37	0.020	41
ASIIC 4	0.013	29	0.026	35	0.020	42
ASIIC 5	0.014	31	0.030	40	0.020	40
MEAN	0.014	<b>31</b>	0.027	<b>36</b>	0.019	<b>39.2</b>
SD	0.001	2	0.002	2	0.001	3
	in mm	ASIIICI	in mm	ASIIICI	in mm	ASIIICI
ASIIIC 1	0.013	29	0.022	30	0.018	37
ASIIIC 2	0.015	33	0.029	39	0.018	37
ASIIIC 3	0.013	29	0.021	29	0.017	34
ASIIIC 4	0.016	36	0.022	30	0.017	35
ASIIIC 5	0.013	30	0.020	28	0.019	38
MEAN	0.014	<b>31</b>	0.021	<b>31</b>	0.018	<b>36</b>
SD	0.001	3	0.003	4	0.001	1

positions along the lateral and ventral walls. Finally, attached to the sting apparatus are two separate muscle sets, the protractors and retractors of the sting shaft. The former set is responsible for extending the sting from the tip of the abdomen during attack or defence and the latter for retracting it back to its resting position within the abdomen (visible in dorsal view in Fig. 15F).

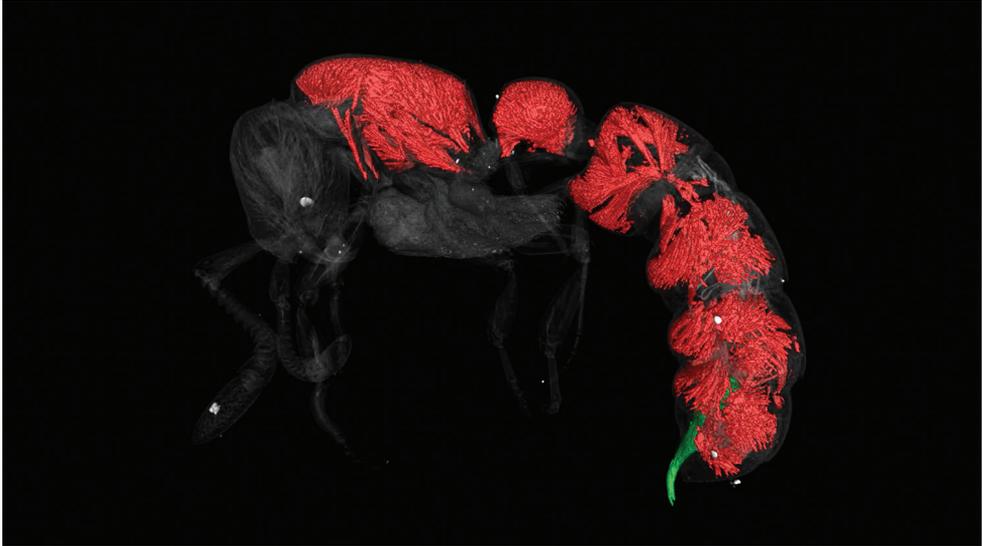


**Figure 15.** Still images of 3D model of full body of *Zasphinctus sarowiwai* sp. n. holotype worker (CASENT0764654). False-colour volume rendering of segmented mesosoma and metasoma musculature (red) and sting apparatus (green) superimposed on semi-transparent surface model (**A, C, E**) or stand-alone (**B, D, F**). **A, B** Body in profile view **C, D** Body in dorsal view **E, F** Body in posterodorsal view.

## Discussion

### Virtual recovery of morphology

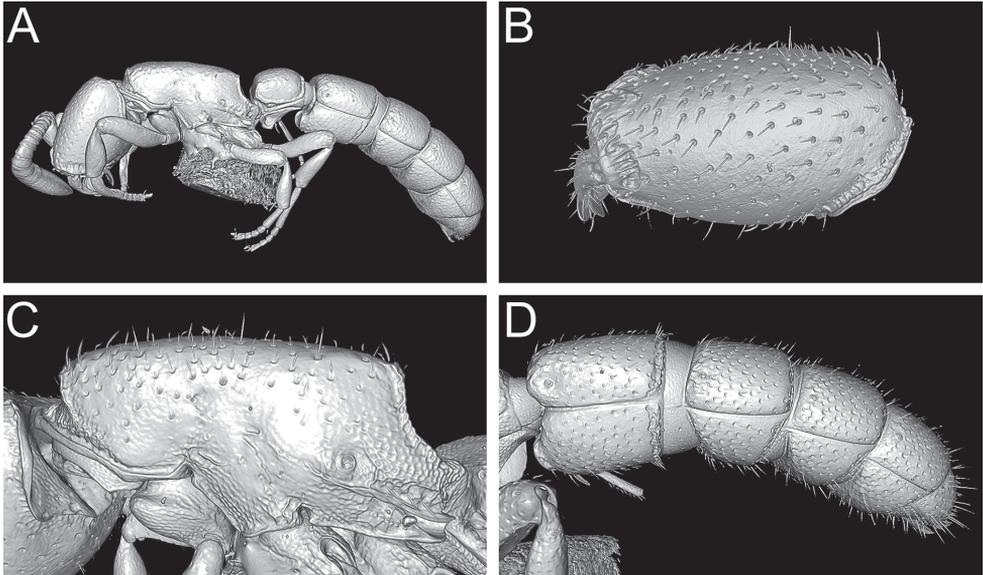
Almost all previous studies that used micro-CT for invertebrate taxonomy encountered problems with the achievable voxel resolution in relation to body size resulting in a poor recovery of certain, very fine or small structures, such as setae, ommatidia, and microsculpture (Faulwetter et al. 2013, 2014; Fernández et al. 2014; Carbayo et al. 2016; Fischer et al. 2016). In the case of ant taxonomy this was intensively discussed by Hita Garcia et al. (2017a) who achieved voxel sizes of around 5  $\mu\text{m}$  for full body scans of the two treated species. Based on these results and in order to improve the voxel resolution and present better resolved 3D reconstructions, we scanned the head, the mesosoma, and the metasoma separately in addition to a full body scan for each species. As a consequence, we attained smaller voxel sizes for the



**Video 5.** 3D rotation video of full body of *Zaspinctus sarowiwai* sp. n. holotype worker (CASENT0764654). False-colour volume rendering of segmented mesosoma and metasoma musculature (red) and sting apparatus (green) superimposed on semitransparent surface model.

3D models of the single body parts (0.95–2.83  $\mu\text{m}$  versus 3.00–4.61  $\mu\text{m}$ ) resulting in a much higher resolution, and significantly reduced or eliminated the problems encountered by Hita Garcia et al. (2017a) (Fig. 16), except for ommatidia that are absent in *Zaspinctus* workers. While setae were poorly recovered by Hita Garcia et al. (2017a), they are very well visible in our 3D models of single body parts presented in this study. However, due to the higher voxel size, our full body scans of the *Zaspinctus* species have a weaker resolution of setae. Furthermore, compared to the physical specimens, surface sculpture was recovered with high morphological accuracy in the 3D models of single body parts, whereas it was only poorly noticeable in the full body scans. Surprisingly, fine surface sculpture on some body parts was even more observable in the 3D models than in the physical material, due to a limited magnification of our light microscope to 100  $\times$ .

The visualised reconstruction of the mouthparts provides a comparatively adequate and detailed 3D model of the maxillae, labium, and labrum, but also presents some important limitations. The general morphology of maxillae, labium and labrum are well recovered, and they are very similar to the mouthparts of *Z. steinheili* that were described by Gotwald (1969). The vast majority of setae are well visible, as is the surface sculpture of most structures. More importantly though, for the first time it is possible to examine these structures in their natural position in 3D, as well as their configuration with respect to each other. This is a significant advantage compared to traditional histological dissections that always remove all parts from the head and then separate each structure for separate examination. Our 3D volume reconstruction



**Figure 16.** Comparison of full body scan versus single body part scans based on *Zasphectus obamai* sp. n. holotype (CASENT0764125) and paratype worker (CASENT0764127). **A** Full body scan (CASENT0764125) **B** Scan of head (CASENT0764125) **C** Scan of mesosoma (CASENT0764127) **D** Scan of metasoma showing abdominal segments III to VII in profile (CASENT0764127).

allows detailed examinations from all possible angles and the segmented mouthparts can be observed independently or in combination with each other.

Nevertheless, there are some problems with our 3D reconstructed model. The most problematic structure is the glossa. As already pointed out by Gotwald (1969), due to its highly membranous nature it is deformed in most dead specimens independently of preservation agent. In our specimen, the glossa was already collapsed to a crater-like appearance prior to micro-CT scanning, thus not available for any shape examination.

Another important limitation is that not all structures could be satisfactorily outlined during the segmentation process. This was especially difficult for the delineation of some components, such as the cardo, the lacinial comb, the regions where labium and maxillae meet, and generally everywhere where membranous and chitinous tissues are in contact. These problems are caused by scanning a dry mounted specimen, in which most internal structures have undergone desiccation, shrinkage, and deformation. In such specimens, the dissimilarities in density and contrast between different tissues or components are minimal to zero, thus causing significant problems for the proper recognition and subsequent outlining of borders between structures. In general, our 3D reconstructed model provides fewer details compared to histological dissections. However, these problems might be solvable in future studies if specimens are preserved and prepared in a way more suitable for micro-CT scanning and virtual reconstruction. Based on unpublished data, the use of freshly killed material or specimens in alcohol combined with the use of potassium hydroxide (KOH) and iodine

staining provides much better resolution of internal structures than the use of dry material. This allows a much more sophisticated recovery of mouthparts morphology.

The application of micro-CT scanning to obtain information about cuticle thickness is novel. Based on our data however, we refrain from using it for taxonomic diagnostics at the moment. There are some differences in the cuticle thickness among the three species, most notably the very thick head of *Z. obamai* (CCCI 44 vs. CCCI 31 in *Z. sarowiwai* and *Z. wilsoni*). It also appears that the head of *Z. obamai* is thicker than the pronotum and abdominal segments II and III, whereas the heads of the other two species are thinner than or as thin as most other body parts (see Table 5). However, these results could be based on measuring the cuticle at a wrong angle resulting in a distorted result. Even though we have tried to find the best possible sagittal section slides for virtual measuring, it cannot be ruled out that we did not measure the thickest part of the cuticle. For future use, we recommend measuring more slides and more body parts in order to achieve a more complete picture of cuticle thickness. Even though we do not use it for taxonomic diagnostics at the moment, we believe there is potential for such use (unpublished data). However, this should be investigated with more taxa and more specimens first. Nevertheless, our results permit to place the treated species in an evolutionary context and make interpretations about natural history. Currently, the use of micro-CT data for virtual cuticle measuring has rather limited applicability due to the sparse availability of scanning resources in the myrmecological community. Nevertheless, we believe such data provides important information that might be valuable for future systematic and evolutionary studies.

## Cybertypes

As pointed out in previous studies, a crucial advantage of using 3D models based on micro-CT data is its potential application as cybertypes (Faulwetter et al. 2013, 2014; Akkari et al. 2015; Hita Garcia et al. 2017a, 2017b). The concept of a cybertype is to present a detailed and as complete as possible virtual reconstruction of a physical type specimen that is freely accessible. Hita Garcia et al. (2017a) critically assessed the usefulness of cybertypes for ant taxonomy, and due to the limitations in voxel resolution in their scan data suggested to use a combination of micro-CT data (raw data, 3D PDF, and 3D rotation video) and montage photos (three standard view: head in full-face view, full body in profile and dorsal view) as a minimal cybertype for ants. Although we have achieved much higher quality 3D reconstructions compared to previous ant studies using micro-CT and strongly reduced the limitations discussed by Hita Garcia et al. (2017a), we still believe that a minimum ant cybertype should include optical montage photographs. The main reason for this is the lack of natural colour in the micro-CT, which can only be shown with visible light photography. However, in this study we improve the previous ant cybertypes by providing micro-CT scans of single body parts and scan data for the holotype and one paratype, if available, thus increasing the usefulness of the cybertype datasets.

## Virtual character evaluation and presentation

One aim of this study was to evaluate new taxonomic characters for species level taxonomy on the basis of traditional morphological analysis and virtual examination of 3D reconstructions. Unfortunately, only dry mounted material was available for this study. As pointed out in Hita Garcia et al. (2017a), most internal anatomical structures of such specimens have undergone significant desiccation, shrinking and deformation. Consequently, micro-CT data from dry specimens provides much less useful information for comparative examination. Based on our initial investigation, however, some internal sclerotized structures, such as the tentorium, several apodemes, and the endosternum appear to have significant potential for comparative morphology among species. However, due to the poor recovery of these structures in our raw data, we could not examine these in detail and focused our character evaluation on external morphology, with the exception of cuticle thickness. For future taxonomic studies using micro-CT, we propose to examine internal characters in more detail by using material preserved in ethanol.

Initially, our intention was to omit a species identification key, which may appear counterintuitive and substandard. However, there are several problems with dichotomous identification keys that lead us to take a different approach in this study. Identification keys for well-studied regions, such as Japan and Central Europe, generally work well and are very stable since new species are rarely encountered and nomenclatorial benchmarking is rare. This is certainly not the case for most tropical and subtropical regions because our knowledge of the local and regional diversity is fragmentary to non-existent. One major limitation of keys for such regions is that they usually only work for the known species at the time of publication. Later discoveries of new species render keys less useful and often less reliable for identification purposes. To avoid this, it is necessary to update older keys in additional publications after new species are discovered, as done by Hita Garcia and Fischer (2014) to update Hita Garcia et al. (2010). However, this is comparatively work-intensive and most authors of revisionary taxonomy studies are hesitant to revisit previously “finished” groups. This situation is especially problematic in hyperdiverse genera, for which there is no obvious solution except for continuously revising the taxa until most or “all” species are known and described. However, for genera with small or moderate species richness there might be alternatives to traditional identification keys with a few characters per key couplet.

In the case of *Zasphinctus* it is very likely that future collecting in the Afrotropical region will reveal additional species, even though not too many. This assessment is based on the apparent rarity of these ants and the fact that the region is largely under-sampled. Accordingly, any identification key that covers only the three species treated here is doomed to obsolescence with the discovery of additional species, especially if only a few characters are listed per key couplet. Instead of simply providing a short key, we decided to present an illustrated matrix with numerous characters, in which we only present the ones that have proven to be diagnostic. Future users of our identification system can check multiple character illustrations and compare them with

their specimens at hand. Nevertheless, despite our concerns with a short key with few diagnostic characters, we understand that some users would still prefer to use a short dichotomous key and we still provide one in this study.

Furthermore, the characters chosen are suited for diverse audiences with different interests and resources. For users with limited microscopy resources or little taxonomic training, we have included many easy-to-examine characters that are visible at lower magnifications, such as the shapes of head, mesosoma, or abdominal segment II in profile (e.g. Figs 4A–F, J–L, 5G–R). Based on that, we have added numerous further characters that are not completely necessary for simple identification purposes of faunistic or ecological studies, but target a more taxonomically oriented audience with better microscopy resources and deeper knowledge of ant morphology. We present many characters that require detailed examinations, such as structures on the posterior or ventral head (Figs 4P–R, 5A–F) and the ventral metasoma (Fig. 6A–C, G–L, P–R). These are intended to provide important comparative data for future systematic studies, and will very likely improve delimitations of any additional species.

As outlined above, compared to the taxonomy of most insect groups, the character sets used in the field of ant worker taxonomy are very often rather limited and rely heavily on setation, sculpture, body size, and colour. These are often problematic since they can be highly variable within species and prone to geographic variability, such as shown for the Neotropical *Tatuidris* Brown & Kempf (Donoso 2012), Malagasy *Tetramorium* Mayr (Hita Garcia and Fisher 2011) and Malagasy *Creमतogaster* Lund (Blaimer 2012). Against the background of a resilient taxonomic impediment with continuously declining taxonomic resources and funding (e.g. Wheeler et al. 2004; Ebach et al. 2011), it is imperative to deliver taxonomic works that provide high quality species delimitations at a more accelerated speed that offer a stable taxonomic foundation upon which future discoveries can be based. We believe that the application of 21<sup>st</sup> century taxonomic tools, such as molecular phylogenetics/phylogenomics and 3D next-generation morphology techniques, can strongly improve ant taxonomy.

For the revision of Afrotropical *Zasphectus*, we have evaluated every single character that could be of diagnostic importance based on the literature record (Bolton 1990; Keller 2011; Borowiec 2016) through a combination of examination of physical material under the light microscope and virtually reconstructed 3D models of micro-CT scans. The latter is of crucial importance since it provides numerous advantages. The most important turned out to be the use of micro-CT data for virtual character examinations and dissections. As noted above, the available material was too scarce to perform physical dissections or dangerous manipulations of specimens. Fortunately, we were able to examine the virtual specimens from all imaginable angles by rotating the 3D models. In addition, in order to examine characters that were hidden behind other body parts, we virtually removed any obstructing structure. By doing this we were able to observe and reveal characters that are challenging to see in most dry-mounted specimens, thus rarely used for ant taxonomy. In particular, we found that the ventral and posterior head possesses a series of useful diagnostic characters, such as the hypostoma (Fig. 5D–F), the vertex (Fig. 4M–O), the occiput (Fig. 4P–R), and

several margins around these structures (Fig. 4M–O, 5A–C). The same applies for the ventral metasoma since we found that abdominal segments II and III in ventral view provided some valuable characters, such as the subpetiolar process (Fig. 5M–O, 6A–C) or the prora (Fig. 6J–L). Physical examination of most of these would require damaging the specimens by removing legs, moving the head, separating abdominal segments, or in order to examine the occiput, detaching the head from the pronotum. By using micro-CT based 3D models, we were able to accomplish this with valuable type material without any damage.

Furthermore, the use of virtually reconstructed 3D models permits a quick and effective use of time and resources. Dissections and manipulations of physical specimens are usually very time-consuming, especially if histology and SEM are involved. By contrast, the application of micro-CT scanning enables highly accelerated examinations of morphology compared to these methods (Faulwetter et al. 2013; Friedrich et al. 2014). The initial data generation with a powerful scanner and subsequent 3D reconstructions can be done quickly with minimal effort, if the necessary scanning and visualisation resources are available (see limitations below). The virtual examination of characters is easy and very straightforward. The 3D models can be manipulated in many ways to observe the targeted morphological structures and once a character is in focus one can generate high-quality images within seconds. It is also easy to make images of structures from different angles in order to find the best one for presentation purposes. In our study, we generated several hundred character snapshots within a few days, thus allowing us to choose the most suitable ones for the illustrations used in this study. Generating so many images by using montage photography, SEM, or histology would require much more time and significantly slow down the speed of the publication process.

As already discussed in previous studies employing micro-CT data (Faulwetter et al. 2014; Carbayo et al. 2016; Hita Garcia et al. 2017a), the most important weakness is the limited access to scanning resources since most universities or museums do not have their own micro-CT scanners. Currently, the acquisition of scanners and access to external scanning facilities require substantial economic resources, and this situation will remain unchanged for some time. Some natural history museums and universities already have or are in the process of establishing scanning resources, but presently only a small minority of the taxonomic community has access to the technology. In addition, generating, post-processing, and handling the usually rather large data requires time and technical skills. Nonetheless, as with all new technologies, it is likely that technological and computational developments will reduce the costs of scanning, increase the availability for the taxonomic community, and simplify data management (Faulwetter et al. 2014; Hita Garcia et al. 2017a).

### **Functional morphology and lifestyle**

As outlined above, there is no knowledge of the natural history of Afrotropical *Zasphinctus*, except that they might live in leaf litter since most specimens were collected

in litter samples. Against the background that they are dorylines and that their Australian congeners are predators of other ants, it is likely that the species treated in this study pursue a similar lifestyle. The examination of the micro-CT data generated during this study allows some inference about the lifestyle of the studied species.

As mentioned above, all three Afrotropical *Zasphinctus* species possess a very thick cuticle. Peeters et al. (2017) found the species with the thickest cuticle in relation to body size to be predominantly large ponerine genera, such as *Diacamma* Mayr, *Odonotoponera* Mayr, *Leptogenys* Roger, and *Ectomomyrmex* Mayr. However, the thickest cuticle was observed in the species *Ooceraea biroi* (Forel), which is a doryline in relative close phylogenetic proximity to *Zasphinctus*. Based on that result, it is not that surprising that African *Zasphinctus* display such thick cuticle. Notwithstanding that Peeters et al. (2017) found that the best predictors for thick cuticle were body size (larger ants have thicker cuticle) and phylogeny (poneroid ants have thicker cuticle), the thick cuticle of African *Zasphinctus* is likely related to a predatory lifestyle. Based on observations of other *Zasphinctus* species mentioned above, it is highly probable that African *Zasphinctus* are top predators that feed predominantly on other ants, which is also the case in the clonal raider ant *Ooceraea biroi*, which feeds primarily on ant brood. Furthermore, Peeters et al. (2017) concluded that cuticle thickness was also negatively correlated with larger colony size in more phylogenetically derived ant lineages (formicoid clade). Despite a severe lack of observation and natural history data, it appears that African *Zasphinctus* live in small colonies, which is well in accordance with the findings of Peeters et al. (2017).

In *Zasphinctus*, the relative amount of muscles responsible for moving the abdomen seems to be largely increased compared to other ants from the formicoid clade, e.g. *Pheidole* Westwood & *Terataner* Emery, studied in previous publications (Sarnat et al. 2016; Hita Garcia et al. 2017a). It may be tempting to think that the genus *Zasphinctus* – as compared to the majority of ant lineages which have (evolved) greatly reduced abdominal musculature – has retained a more ‘primitive’ and wasp-like internal morphology in its abdomen. Yet, its species have a morphology that makes them rather special among ants: their relatively long abdomen is serially constricted between individually rotating presclerital plates. This apparent adaptation may have gained *Zasphinctus* additional functionality during predation and defence by increasing overall flexibility of use for its well-developed sting apparatus (Fig. 15) in the apical abdominal segment. It seems that other doryline genera such as for example *Eusphinctus* Emery, *Sphinctomyrmex* Mayr, and to a lesser degree possibly some *Cylindromyrmex* Mayr, and *Leptanilloides* Mann have evolved very similar features independently from *Zasphinctus*. It may be interesting to investigate the evolution of these specialised morphologies with respect to the different army ant lifestyles. Since studies on internal ant morphology are generally rare, we have little opportunity to compare our present results with those of others. Hashimoto (1996) for example found that, in some ant subfamilies, the muscles in abdominal segment II and III (petiole and postpetiole) show ‘positional and functional modifications’. Although he also gives an anatomically based discussion on the functional morphology of these modifications, there is no information given

on related behavioural or ecological functions nor on musculature modifications in the posterior abdominal segments. With our main focus on the taxonomy of the three newly described species we refrain from further speculations and more detailed analyses in this publication and defer to future internal functional ant morphology studies using micro-CT scanning technology.

## Conclusions

Our study highlights the potential of in-depth comparative morphology analyses for taxonomy founded on a combined investigation of physical specimens under light microscopy and virtual 3D models generated from micro-CT data. Our approach reveals a wealth of morphological characters with high diagnostic potential that we use to successfully delimit species within Afrotropical *Zasphinctus*. Even though the worker caste of ants is highly simplified and the presence of cryptic species in many ant genera is increasingly recognised (e.g. Schlick-Steiner et al. 2006; Seifert 2009), we believe that in many cases the whole range of comparative morphology for alpha taxonomy has not been fully explored yet (Keller 2011). Virtual and interactive examination of morphology and anatomy in 3D can fill the gap and improve our understanding of functionality and homology of characters and provide the means for the discovery of new diagnostic characters (Zimmermann et al. 2011; Blanke and Wesener 2014).

Furthermore, considering the lack of material and apparent rarity of Afrotropical *Zasphinctus*, our study also emphasises the strength of micro-CT scanning as a tool for the non-destructive virtual examination of valuable and scarce type material. Based on our results, micro-CT scanning opens up promising possibilities for the integration of very rare type (and non-type) material into systematic studies, as demonstrated here with the singleton holotype of *Z. wilsoni*.

In general, even though it often appears as if the modern era of molecular systematics has dwarfed the importance of morphology-based systematics, we strongly concur with previous authors that by embracing and employing new technologies, such as micro-CT scanning, the study of morphology can still have a significant impact and remain a strong field in systematic and evolutionary biology (Giribet 2010; Keller 2011; Friedrich et al. 2014). Perhaps the most interesting aspect of using micro-CT for ant taxonomy, however, is the potential to bridge different fields of research. Our study and previous ones for spiny *Pheidole* (Sarnat et al. 2016; Sarnat et al. 2017) show that by examining morphology and anatomy in detail insights about potential behavioural adaptations can be gained. By including new and internal morphological characters in our taxonomic studies we can draw conclusions about and make a connection with functional morphology and ecology. In more holistic approaches combined with statistical analyses and controlled for phylogenetic relationships we can study the evolution of morphological adaptations and learn about the mechanisms that make ants so successful in their respective environments.

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## References

- Akkari N, Enghoff H, Metscher BD (2015) A new dimension in documenting new species: high-detail imaging for myriapod taxonomy and first 3D cybertype of a new millipede species (Diplopoda, Julida, Julidae). PLoS ONE 10(8): e0135243. <https://doi.org/10.1371/journal.pone.0135243>
- Barden P, Grimaldi D (2012) Rediscovery of the bizarre Cretaceous ant *Haidomyrmex* Dlussky (Hymenoptera: Formicidae), with two new species. American Museum Novitates 3755: 1–16. <https://doi.org/10.1206/3755.2>
- Beutel RG, Ge S-Q, Hörschemeyer T (2008) On the head morphology of *Tetraphalerus*, the phylogeny of Archostemata and the basal branching events in Coleoptera. Cladistics 24: 270–298. <https://doi.org/10.1111/j.1096-0031.2007.00186.x>
- Beutel RG, Friedrich F, Aspöck U (2010) The larval head of Nevrothidae and the phylogeny of Neuroptera (Insecta). Zoological Journal of the Linnean Society 158: 533–562. <https://doi.org/10.1111/j.1096-3642.2009.00560.x>
- Blaimer BB (2012) Untangling complex morphological variation: taxonomic revision of the subgenus *Crematogaster* (*Oxygyne*) in Madagascar, with insight into the evolution and biogeography of this enigmatic ant clade (Hymenoptera: Formicidae). Systematic Entomology 37: 240–260. <https://doi.org/10.1111/j.1365-3113.2011.00609.x>
- Blanke A, Wesener T (2014) Revival of forgotten characters and modern imaging techniques help to produce a robust phylogeny of the Diplopoda (Arthropoda, Myriapoda). Arthropod Structure & Development 43: 63–75. <https://doi.org/10.1016/j.asd.2013.10.003>
- Bolton B (1990) Abdominal characters and status of the cerapachyine ants (Hymenoptera, Formicidae). Journal of Natural History 24: 53–68. <https://doi.org/10.1080/00222939000770051>
- Bolton B (2007) Taxonomy of the dolichoderine ant genus *Technomyrmex* Mayr (Hymenoptera: Formicidae) based on the worker caste. Contributions of the American Entomological Institute 35: 1–149.

- Bolton B, Fisher BL (2012) Taxonomy of the cerapachyine ant genera *Simopone* Forel, *Vicinopone* gen. n. and *Tanipone* gen. n. (Hymenoptera: Formicidae). *Zootaxa* 3283: 1–101.
- Bolton B (2017) An online catalog of the ants of the world. <http://antcat.org> [accessed 7 March 2017]
- Borowiec ML (2009) New ant species related to *Cerapachys sexspinus* and discussion of the status of *Yunodorylus* (Hymenoptera: Formicidae). *Zootaxa* 2069: 43–58
- Borowiec ML (2016) Generic revision of the ant subfamily Dorylinae (Hymenoptera, Formicidae). *Zookeys* 608: 1–280. <https://doi.org/10.3897/zookeys.608.9427>
- Boudinot B (2014) Rediscovery of *Tricytarus* Donisthorpe, 1947, a new genus-level synonym of *Meranoplus* F. Smith, 1853 (Hymenoptera: Formicidae: Myrmicinae). *Myrmecological News* 20: 95–100.
- Branstetter MG (2012) Origin and diversification of the cryptic ant genus *Stenamamma* Westwood (Hymenoptera: Formicidae), inferred from multilocus molecular data, biogeography and natural history. *Systematic Entomology* 37: 478–496. <https://doi.org/10.1111/j.1365-3113.2012.00624.x>
- Branstetter MG (2013) Revision of the Middle American clade of the ant genus *Stenamamma* Westwood (Hymenoptera, Formicidae, Myrmicinae). *Zookeys* 295: 1–277. <https://doi.org/10.3897/zookeys.295.4905>
- Brown WL (1975) Contributions toward a reclassification of the Formicidae. V. Ponerinae, tribes Platythyreini, Cerapachyini, Cyldromyrmecini, Acanthostichini, and Aenictogitini. *Search Agriculture Entomology* (Ithaca) 5: 1–115
- Buschinger A, Peeters C, Crozier RH (1989) Life-pattern studies on an Australian Sphinctomyrmex (Formicidae: Ponerinae; Cerapachyini): functional polygyny, brood periodicity and raiding behavior. *Psyche* 96: 287–300. <https://doi.org/10.1155/1989/13614>
- Carbayo F, Francoy TM, Giribet G (2016) Non-destructive imaging to describe a new species of *Obama* land planarian (Platyhelminthes, Tricladida). *Zoologica Scripta* 45: 566–578. <https://doi.org/10.1111/zsc.12175>
- Carbayo F, Lenihan JW (2016) Micro-computed tomography scan and virtual histological slide data for the land planarian *Obama otavioi* (Platyhelminthes). *GigaScience* 5: 1–6. <https://doi.org/10.1186/s13742-016-0119-4>
- Csösz S, Schulz A (2010) A taxonomic review of the Palaearctic *Tetramorium ferox* species-complex (Hymenoptera, Formicidae). *Zootaxa* 2401: 1–29.
- Csösz S, Wagner HC, Bozsó M, Seifert B, Arthofer W, Schlick-Steiner BC, Steiner FM, Péntes Z (2014) *Tetramorium indocile* Santschi, 1927 stat. rev. is the proposed scientific name for *Tetramorium* sp. C sensu Schlick-Steiner et al. (2006) based on combined molecular and morphological evidence (Hymenoptera: Formicidae). *Zoologischer Anzeiger* 253: 469–481. <https://doi.org/10.1016/j.jcz.2014.06.002>
- Csösz S, Fisher BL (2015) Diagnostic survey of Malagasy *Nesomyrmex* species-groups and revision of *hafahafa* group species via morphology based cluster delimitation protocol. *Zookeys* 526: 19–59. <https://doi.org/10.3897/zookeys.526.6037>
- Dierick M, Cnudde V, Masschaele B, Vlassenbroeck J, Van Hoorebeke L, Jacobs P (2007) Micro-CT of fossils preserved in amber. *Nuclear Instruments and Methods in Physics Research A* 580: 641–643. <https://doi.org/10.1016/j.nima.2007.05.030>

- Donoso DA (2012) Additions to the taxonomy of the armadillo ants (Hymenoptera, Formicidae, *Tatuidris*). *Zootaxa* 3503: 61–81.
- Ebach MC, Valdecasas AG, Wheeler QD (2011) Impediments to taxonomy and users of taxonomy: accessibility and impact evaluation. *Cladistics* 27: 550–557. <https://doi.org/10.1111/j.1096-0031.2011.00348.x>
- Evenhuis NL (2017) The insect and spider collections of the world website. <http://hbs.bishop-museum.org/codens> [accessed 12 March 2017]
- Faulwetter S, Dailianias T, Vasileiadou A, Arvanitidis C (2013) Contrast enhancing techniques for the application of micro-CT in marine biodiversity studies. *Microscopy and Analysis*. John Wiley & Sons, EU, S4–S7.
- Fernández F (2004) The American species of the myrmicine ant genus *Carebara* Westwood (Hymenoptera: Formicidae). *Caldasia* 26: 191–238.
- Fernández R, Kvist S, Lenihan J, Giribet G, Ziegler A (2014) Sine systemate chaos? A versatile tool for earthworm taxonomy: Non-destructive imaging of freshly fixed and museum specimens using micro-computed tomography. *PLoS ONE* 9: e96617. <https://doi.org/10.1371/journal.pone.0096617>
- Fischer G, Hita Garcia F, Peters MK (2012) Taxonomy of the ant genus *Pheidole* Westwood (Hymenoptera: Formicidae) in the Afrotropical zoogeographic region: definition of species groups and systematic revision of the *Pheidole pulchella* group. *Zootaxa* 3232: 1–43
- Fischer G, Hita Garcia F, Azorsa F, Mikheyev AS, Economo EP (2015) Two new phragmotic ant species from Africa: morphology and next-generation sequencing solve a caste association problem in the genus *Carebara* Westwood. *Zookeys* 525: 77–105. <https://doi.org/10.3897/zookeys.525.6057>
- Fischer G, Sarnat E, Economo EP (2016) Revision and microtomography of the *Pheidole knowlesi* group, an endemic ant radiation in Fiji (Hymenoptera, Formicidae, Myrmicinae). *PLoS ONE* 11: e0158544. <https://doi.org/10.1371/journal.pone.0158544>
- Friedrich F, Matsumura Y, Pohl H, Bai M, Hörschemeyer T, Beutel RG (2014) Insect morphology in the age of phylogenomics: innovative techniques and its future role in systematics. *Entomological Science* 17: 1–24. <https://doi.org/10.1111/ens.12053>
- Giribet G (2010) A new dimension in combining data? The use of morphology and phylogenomic data in metazoan systematics. *Acta Zoologica* 91: 11–19. <https://doi.org/10.1111/j.1463-6395.2009.00420.x>
- Godfray HCJ (2007) Linnaeus in the information age. *Nature* 446: 259–260. <https://doi.org/10.1038/446259a>
- Gotwald WH (1969) Comparative morphological studies of the ants, with particular reference to the mouthparts (Hymenoptera: Formicidae). *Memoirs of the Cornell University Agricultural Experiment Station* 408: 1–150
- Gotwald WH (1995) *Army ants: the biology of social predation*. Cornell University Press, Ithaca, New York, 302 pp.
- Harris RA (1979) A glossary of surface sculpturing. *California Department of Food and Agriculture, Bureau of Entomology* 28: 1–31.
- Hashimoto Y (1996) Skeletomuscular modifications associated with the formation of an additional petiole on the anterior abdominal segments in aculeate Hymenoptera. *Japanese Journal of Entomology* 64: 340–356

- Hita Garcia F, Fischer G, Peters MK, Snelling RR, Wägele JW (2009) A preliminary checklist of the ants (Hymenoptera: Formicidae) of Kakamega Forest (Kenya). *Journal of East African Natural History* 98: 147–165. <https://doi.org/10.2982/028.098.0201>
- Hita Garcia F, Fischer G, Peters MK (2010) Taxonomy of the *Tetramorium weitzckeri* species group (Hymenoptera: Formicidae) in the Afrotropical zoogeographical region. *Zootaxa* 2704: 1–90.
- Hita Garcia F, Fisher BL (2011) The ant genus *Tetramorium* Mayr (Hymenoptera: Formicidae) in the Malagasy region – introduction, definition of species groups, and revision of the *T. bicarinatum*, *T. obesum*, *T. sericeiventris* and *T. tosii* species groups. *Zootaxa* 3039: 1–72
- Hita Garcia F, Fischer G (2014) Additions to the taxonomy of the Afrotropical *Tetramorium weitzckeri* species complex (Hymenoptera, Formicidae, Myrmicinae), with the description of a new species from Kenya. *European Journal of Taxonomy* 90: 1–16. <https://doi.org/10.5852/ejt.2014.90>
- Hita Garcia F, Hawkes PG, Alpert GD (2014) Taxonomy of the ant genus *Proceratium* Roger (Hymenoptera, Formicidae) in the Afrotropical region with a revision of the *P. arnoldi* clade and description of four new species. *Zookeys* 447: 47–86. <https://doi.org/10.3897/zookeys.447.7766>
- Hita Garcia F, Fischer G, Liu C, Audisio TL, Alpert GD, Fisher BL, Economo EP (2017a) X-Ray microtomography for ant taxonomy: An exploration and case study with two new *Terataner* (Hymenoptera, Formicidae, Myrmicinae) species from Madagascar. *PLoS ONE* 12: e0172641. <https://doi.org/10.1371/journal.pone.0172641>
- Hita Garcia F, Fischer G, Liu C, Audisio TL, Alpert GD, Fisher BL, Economo EP (2017b) Data from: X-ray microtomography for ant taxonomy: an exploration and case study with two new *Terataner* (Hymenoptera, Formicidae, Myrmicinae) species from Madagascar. Dryad Digital Repository. <https://doi.org/10.5061/dryad.sk6s0>
- Hita Garcia F, Fischer G, Liu C, Audisio TL, Economo EP (2017c) Data from: Next-generation morphological character discovery and evaluation: an X-ray micro-CT enhanced revision of the ant genus *Zasphinctus* Wheeler (Hymenoptera, Formicidae, Dorylinae) in the Afrotropics. Dryad Digital Repository. <https://doi.org/10.5061/dryad.4s3v1>
- Hörschemeyer T, Beutel RG, Pasop F (2002) Head structures of *Priacma serrata* Leconte (Coleoptera, Archostemata) inferred from X-ray tomography. *Journal of Morphology* 252: 298–314. <https://doi.org/10.1002/jmor.1107>
- Jaitrong W, Yamane S (2011) Synopsis of *Aenictus* species groups and revision of the *A. currax* and *A. laeviceps* groups in the eastern Oriental, Indo-Australian, and Australasian regions (Hymenoptera: Formicidae: Aenictinae). *Zootaxa* 3128: 1–46.
- Jesovnik A, Sosa-Calvo J, Lloyd JW, Branstetter MG, Fernández F, Schultz TR (2017) Phylogenomic species delimitation and host-symbiont coevolution in the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera: Formicidae): ultraconserved elements (UCEs) resolve a recent radiation. *Systematic Entomology* early view. <https://doi.org/10.1111/syen.12228>
- Keller RA (2011) A phylogenetic analysis of ant morphology (Hymenoptera, Formicidae) with special reference to the poneromorph subfamilies. *Bulletin of the American Museum of Natural History* 355: 1–90. <https://doi.org/10.1206/355.1>
- La Salle J, Wheeler QD, Jackway P, Winterton S, Hobern D, Lovell D (2009) Accelerating taxonomic discovery through automated character extraction. *Zootaxa* 2217: 43–55.

- Lipke E, Ramírez M, Michalik P (2014) Ultrastructure of spermatozoa of Orsolobidae (Haplonyxae, Araneae) with implications on the evolution of sperm transfer forms in Dysderoidea. *Journal of Morphology* 275: 1238–1257. <https://doi.org/10.1002/jmor.20298>
- Liu C, Hita Garcia F, Peng Y-Q, Economo EP (2015) *Aenictus yangi* sp. n. – a new species of the *A. ceylonicus* species group (Hymenoptera, Formicidae, Dorylinae) from Yunnan, China. *Journal of Hymenoptera Research* 42: 33–45. <https://doi.org/10.3897/JHR.42.8859>
- Metscher BD (2009) MicroCT for developmental biology: a versatile tool for high-contrast 3D imaging at histological resolutions. *Developmental Dynamics* 238: 632–640. <https://doi.org/10.1002/dvdy.21857>
- Michalik P, Ramírez MJ (2013) First description of the male of *Thaيدا chepu* Platnick, 1987 (Araneae, Austrochilidae) with micro-computed tomography of the palpal organ. *Zookeys* 352: 117–125. <https://doi.org/10.3897/zookeys.352.6021>
- Peeters C, Molet M, Lin C-C, Billen J (2017) Evolution of cheaper workers in ants: comparative study of exoskeleton thickness. *Biological Journal of the Linnean Society* 121: 556–563. <https://doi.org/10.1093/biolinnean/blx011>
- Probst RS, Guénard B, Boudinot BE (2015) Toward understanding the predatory ant genus *Myopias* (Formicidae: Ponerinae), including a key to global species, male-based generic diagnosis, and new species description. *Sociobiology* 62: 192–212. <https://doi.org/10.13102/sociobiology.v62i2.192-212>
- Richards CS, Simonsen TJ, Abel RL, Hall MJR, Schwyn DA, Wicklein M (2012) Virtual forensic entomology: Improving estimates of minimum post-mortem interval with 3D micro-computed tomography. *Forensic Science International* 220: 251–264. <https://doi.org/10.1016/j.forsciint.2012.03.012>
- Santschi F (1915) Nouvelles fourmis d’Afrique. *Annales de la Société Entomologique de France* 84: 244–282.
- Sarnat EM (2008) A taxonomic revision of the *Pheidole roosevelti*-group (Hymenoptera: Formicidae) in Fiji. *Zootaxa* 1767: 1–36
- Sarnat E, Fischer G, Economo EP (2016) Inordinate spinescence: taxonomic revision and microtomography of the *Pheidole cervicornis* species group (Hymenoptera, Formicidae). *PLoS ONE* 11: e0156709. <https://doi.org/10.1371/journal.pone.0156709>
- Sarnat EM, Friedman NR, Fischer G, Lecroq B, Economo EP (2017) Rise of the spiny ants: diversification, ecology, and function of extreme traits in the hyperdiverse genus *Pheidole* (Formicidae: Hymenoptera). *Biological Journal of the Linnean Society* blx081. <https://doi.org/10.1093/biolinnean/blx081>
- Schlick-Steiner BC, Steiner FM, Moder K, Seifert B, Sanetra M, Dyreson E, Stauffer C, Christian E (2006) A multidisciplinary approach reveals cryptic diversity in Western Palearctic *Tetramorium* ants (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution* 40: 259–273. <https://doi.org/10.1016/j.ympev.2006.03.005>
- Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH (2010) Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421–438. <https://doi.org/10.1146/annurev-ento-112408-085432>
- Seifert B (2009) Cryptic species in ants (Hymenoptera: Formicidae) revisited: we need a change in the alpha-taxonomic approach. *Myrmecological News* 12: 149–166.

- Simonsen TJ, Kitching IJ (2014) Virtual dissections through micro-CT scanning: a method for non-destructive genitalia 'dissections' of valuable Lepidoptera material. *Systematic Entomology* 39: 606–618. <https://doi.org/10.1111/syen.12067>
- Smith VS, Blagoderov V (2012) Bringing collections out of the dark. *Zookeys* 209: 1–6. <https://doi.org/10.3897/zookeys.209.3699>
- Staab M (2015) *Aenictus hoelldobleri* sp. n., a new species of the *Aenictus ceylonicus* group (Hymenoptera, Formicidae) from China, with a key to the Chinese members of the group. *Zookeys* 516: 137–155. <https://doi.org/10.3897/zookeys.516.9927>
- Stoev P, Komerički A, Akkari N, Liu S, Zhou X, Weigand AM, Hostens J, Hunter CI, Edmunds SC, Porco D, Zapparoli M, Georgiev T, Mietchen D, Roberts D, Faulwetter S, Smith V, Penev L (2013) *Eupolybothrus cavernicolus* Komerički & Stoev sp. n. (Chilopoda: Lithobiomorpha: Lithobiidae): the first eukaryotic species description combining transcriptomic, DNA barcoding and micro-CT imaging data. *Biodiversity Data Journal* 1: e1013. <https://doi.org/10.3897/BDJ.1.e1013>
- Wägele H, Klussmann-Kolb A, Kuhlmann M, Haszprunar G, Lindberg D, Koch A, Wägele JW (2011) The taxonomist - an endangered race. A practical proposal for its survival. *Frontiers in Zoology* 8: 25. <https://doi.org/10.1186/1742-9994-8-25>
- Ward PS (2010) Taxonomy, phylogenetics and evolution. In: Lach L, Parr CL, Abbott KL (Eds) *Ant ecology*. Oxford University Press, Oxford, 3–17.
- Wheeler QD, Raven PH, Wilson EO (2004) Taxonomy: Impediment or Expedient? *Science* 303: 285. <https://doi.org/10.1126/science.303.5656.285>
- Wheeler QD, Valdecasas AG (2007) Taxonomy: myths and misconceptions. *Anales del Jardín Botánico de Madrid* 64: 237–241.
- Wheeler QD, Bourgoïn T, Coddington JA, Gostony T, Hamilton A, Larimer R, Polaszek A, Schauff M, Solis MA (2012) Nomenclatural benchmarking: the roles of digital typification and telemicroscopy. *Zookeys* 209: 193–202. <https://doi.org/10.3897/zookeys.209.3486>
- Wheeler WM (1918) The Australian ants of the ponerine tribe Cerapachyini. *Proceedings of the American Academy of Arts and Sciences* 53: 215–265. <https://doi.org/10.2307/25129989>
- Wilson EO (1958) Observations on the behavior of the cerapachyine ants. *Insectes Sociaux* 5: 129–140. <https://doi.org/10.1007/BF02222432>
- Wilson EO (1964) The true army ants of the Indo-Australian area (Hymenoptera: Formicidae: Dorylinae). *Pacific Insects* 6: 427–483.
- Winston JE (1999) *Describing species*. Columbia University Press, New York, 512 pp.
- Wojcieszek JM, Austin P, Harvey MS, Simmons LW (2012) Micro-CT scanning provides insight into the functional morphology of millipede genitalia. *Journal of Zoology* 287: 91–95. <https://doi.org/10.1111/j.1469-7998.2011.00892.x>
- Yoshimura M, Fisher BL (2014) A revision of the ant genus *Mystrium* in the Malagasy region with description of six new species and remarks on *Amblyopone* and *Stigmatomma* (Hymenoptera, Formicidae, Amblyoponinae). *Zookeys* 394: 1–99. <https://doi.org/10.3897/zookeys.394.6446>
- Zimmermann D, Randolf S, Metscher BD, Aspöck U (2011) The function and phylogenetic implications of the tentorium in adult Neuroptera (Insecta). *Arthropod Structure & Development* 40: 571–582. <https://doi.org/10.1016/j.asd.2011.06.003>

## Supplementary material 1

### 3D PDF 1

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus obamai* sp. n. holotype worker (CASENT0764125).  
3D PDF of volumetric surface rendering model of full body.

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl1>

## Supplementary material 2

### 3D PDF 2

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus obamai* sp. n. paratype worker (CASENT0764127).  
3D PDF of volumetric surface rendering model of head (most of antennae virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl2>

## Supplementary material 3

### 3D PDF 3

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus obamai* sp. n. paratype worker (CASENT0764127).  
3D PDF of volumetric surface rendering model of mesosoma (head and metasoma mostly virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl3>

## Supplementary material 4

### 3D PDF 4

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zaspinctus obamai* sp. n. paratype worker (CASENT0764127). 3D PDF of volumetric surface rendering model of metasoma (virtually separated from mesosoma and most of legs virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl4>

## Supplementary material 5

### 3D PDF 5

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zaspinctus sarowiwai* sp. n. holotype worker (CASENT0764654). 3D PDF of volumetric surface rendering model of full body.

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl5>

## Supplementary material 6

### 3D PDF 6

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus sarowiwai* sp. n. holotype worker (CASENT0764654).  
3D PDF of volumetric surface rendering model of head (most of antennae virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl6>

## Supplementary material 7

### 3D PDF 7

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus sarowiwai* sp. n. holotype worker (CASENT0764654).  
3D PDF of volumetric surface rendering model of mesosoma (head and metasoma mostly virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl7>

## Supplementary material 8

### 3D PDF 8

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus sarowiwai* sp. n. holotype worker (CASENT0764654).  
3D PDF of volumetric surface rendering model of metasoma (virtually separated from mesosoma and most of legs virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl8>

## Supplementary material 9

### 3D PDF 9

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764).  
3D PDF of volumetric surface rendering model of full body.

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl9>

## Supplementary material 10

### 3D PDF 10

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764).  
3D PDF of volumetric surface rendering model of head (most of antennae virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl10>

## Supplementary material 11

### 3D PDF 11

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764). 3D PDF of volumetric surface rendering model of mesosoma (head and metasoma mostly virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl11>

## Supplementary material 12

### 3D PDF 12

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764). 3D PDF of volumetric surface rendering model of metasoma (virtually separated from mesosoma and most of legs virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl12>

## Supplementary material 13

### 3D PDF 13

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Adobe PDF file

Explanation note: *Zasphinctus sarowiwai* sp. n. worker (CASENT0764652). 3D PDF of volumetric surface rendering of segmented mouthparts excluding mandibles (green= maxillae; yellow=labrum; orang=labium).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl13>

## Supplementary material 14

### Video 1

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zasphinctus obamai* sp. n. paratype worker (CASENT0764127). 3D rotation video of volumetric surface rendering of head (most of antennae virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl14>

## Supplementary material 15

### Video 2

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zasphinctus obamai* sp. n. paratype worker (CASENT0764127). 3D rotation video of volumetric surface rendering of mesosoma (head and metasoma mostly virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl15>

## Supplementary material 16

### Video 3

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zasphinctus obamai* sp. n. paratype worker (CASENT0764127). 3D rotation video of volumetric surface rendering of metasoma (virtually separated from mesosoma and most of legs virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl16>

## Supplementary material 17

### Video 4

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zasphinctus sarowiwai* sp. n. holotype worker (CASENT0764654). 3D rotation video of volumetric surface rendering of head (antennae virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl17>

## Supplementary material 18

### Video 5

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zasphinctus sarowiwai* sp. n. holotype worker (CASENT0764654). 3D rotation video of volumetric surface rendering of mesosoma (head and metasoma mostly virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl18>

## Supplementary material 19

### Video 6

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zasphinctus sarowiwai* sp. n. holotype worker (CASENT0764654).  
3D rotation video of volumetric surface rendering of metasoma (virtually separated from mesosoma and most of legs virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl19>

## Supplementary material 20

### Video 7

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zasphinctus sarowiwai* sp. n. paratype worker (CASENT0764650).  
3D rotation video of volumetric surface rendering of full body.

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl20>

## Supplementary material 21

### Video 8

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zaspinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764). 3D rotation video of volumetric surface rendering of head (antennae virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl21>

## Supplementary material 22

### Video 9

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zaspinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764). 3D rotation video of volumetric surface rendering of mesosoma (head and metasoma mostly virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl22>

## Supplementary material 23

### Video 10

Authors: Francisco Hita Garcia, Georg Fischer, Cong Liu, Tracy L. Audisio, Evan P. Economo

Data type: Video File (.mov)

Explanation note: *Zaspinctus wilsoni* sp. n. holotype worker (MCZ-ENT-00512764).

3D rotation video of volumetric surface rendering of metasoma (virtually separated from mesosoma and most of legs virtually removed).

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Link: <https://doi.org/10.3897/zookeys.693.13012.suppl23>



# *Lithobius (Chinobius) yuchernovi*, a new lithobiid species from northeastern Siberia and the Kamchatka Peninsula, Russia (Chilopoda, Lithobiomorpha)

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<http://zoobank.org/E9E8771F-BB86-4E24-9753-ECE80C9FAC9D>

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## Abstract

*Lithobius yuchernovi* sp. n. is described, based on type material from the Ola Plateau, Magadan Region, Russia. The new species is widely distributed in northeastern Siberia, ranging from the Magadan Region, until the eastern Chukot Autonomous Region and the Kamchatka Peninsula in the southeast, whence non-type material is documented. This species belongs to the subgenus *Chinobius* Matic, 1973 based on the structure of the female gonopodal claw (more than two denticles on the internal ridge). It differs from all Palearctic species of the genus *Lithobius* by the following apomorphy: distoventral tubercle supporting a cluster of long and curved setae situated on the last male tibia. In addition, it shows dorsal and ventral sulci on the last legs. New replacement names are introduced: *Lithobius zachuii* nom. n. for *Lithobius (Chinobius) orientalis* (Matic, 1973) and *Lithobius carli* nom. n. for *Lithobius (Alokobius) orientalis* Attems, 1953.

## Keywords

*Lithobius*, new species, nomina nova, Russian Far East, Siberia, taxonomy

## Introduction

Northeastern Siberia, Russia, is a large territory lying east of the Lena and Aldan rivers and extending until the Bering Strait and the shores of the Bering Sea, between the Arctic and Pacific oceans. The Kamchatka Peninsula borders northeastern Siberia from the south and belongs to the Kamchatka-Kurilian geographical province (Davydova et al. 1960).

The lithobiomorph fauna of both regions is still poorly studied. To date, northeastern Siberia and the Kamchatka Peninsula are known to support only four species of one genus (Zalesskaja 1978, Eason 1996). A new species of lithobiid centipede currently has been recorded from the Ola Plateau highlands, Magadan Region. Additional material from that region, as well as the Chukot Autonomous Region and the Kamchatka Peninsula, mostly deposited in the Zoological Museum of the Lomonosov Moscow State University, Russia, shows this new species to actually be widespread in the area.

The present paper provides a description of the new species, with short comments on the taxonomic problems encountered in the subgenus *Chinobius* Matic, 1973 it belongs to. Additionally, new replacement names are introduced for junior homonyms.

## Materials and methods

Fifty-four specimens of both sexes of the new species treated below were collected from the Ola Plateau highlands, Magadan Region, by O.L. Makarova and A.B. Babenko. The type locality of the new species (Ola Plateau) is situated 130 km inland from the Sea of Okhotsk coast, northwest of the city of Magadan. It occupies an area of about 100 km<sup>2</sup> (Yurtsev and Khokhryakov 1975) and consists of flat-topped mountains (photographs and detailed descriptions of typical habitats see in Makarova et al. 2014) mainly formed by Tertiary basalts, with a well-developed river network. The altitudes of the plateau average 1100–1600 m (hereafter, all altitudes are given above sea-level), with some peaks reaching 2000 m. The tundra belt in the study area (the upper reaches of the Ola River, 60°39'N, 151°16'E) begins from 900–1200 m, depending on slope exposition.

Additional non-type material (139 specimens) was collected from several localities in the Magadan Region, the Chukot Autonomous Region, and the Kamchatka Peninsula.

Material is currently deposited in the collections of the Zoological Museum of the Lomonosov Moscow State University, Moscow, Russia (ZMUM), the Perm State University, Perm, Russia (PSU), and the Manchester Museum of the University of Manchester, UK (MMUM).

The terminology of the external anatomy follows Bonato et al. (2010).

Measurements. The total body length was measured from the fore margin of the cephalic plate to the posterior end of the postpedal tergite. Leg length was measured excluding the length of the claw. Lengths are given as the minimum and maximum values. All measurements are given in millimeters (mm).

Plectrotaxy. Legs spinulation data are given in a separate table for holotype only. The number of coxal pores on legs 12–15 is presented in a formula where a sequence of Arabic numerals means the number of pores on these legs, respectively.

SEM micrographs were prepared at the PSU using a Hitachi TM3000 scanning electron microscope with a back-scatter electron detector. The drawings were executed by G. Sh. Farzalieva using a Meiji EMZ-5 stereo microscope and a RA-5 drawing tube.

**The following abbreviations are used in the text and table:**

<b>V</b>	ventral	<b>F</b>	femur
<b>D</b>	dorsal	<b>Ti</b>	tibia
<b>T, TT</b>	tergite, tergites	<b>Ts1</b>	tarsus 1
<b>S</b>	sternite	<b>Ts2</b>	tarsus 2
<b>C</b>	coxa	<b>a</b>	anterior
<b>t</b>	trochanter	<b>m</b>	median
<b>P</b>	prefemur	<b>p</b>	posterior

## Results

### Taxonomy

***Lithobius (Chinobius) yuchernovi* sp. n.**

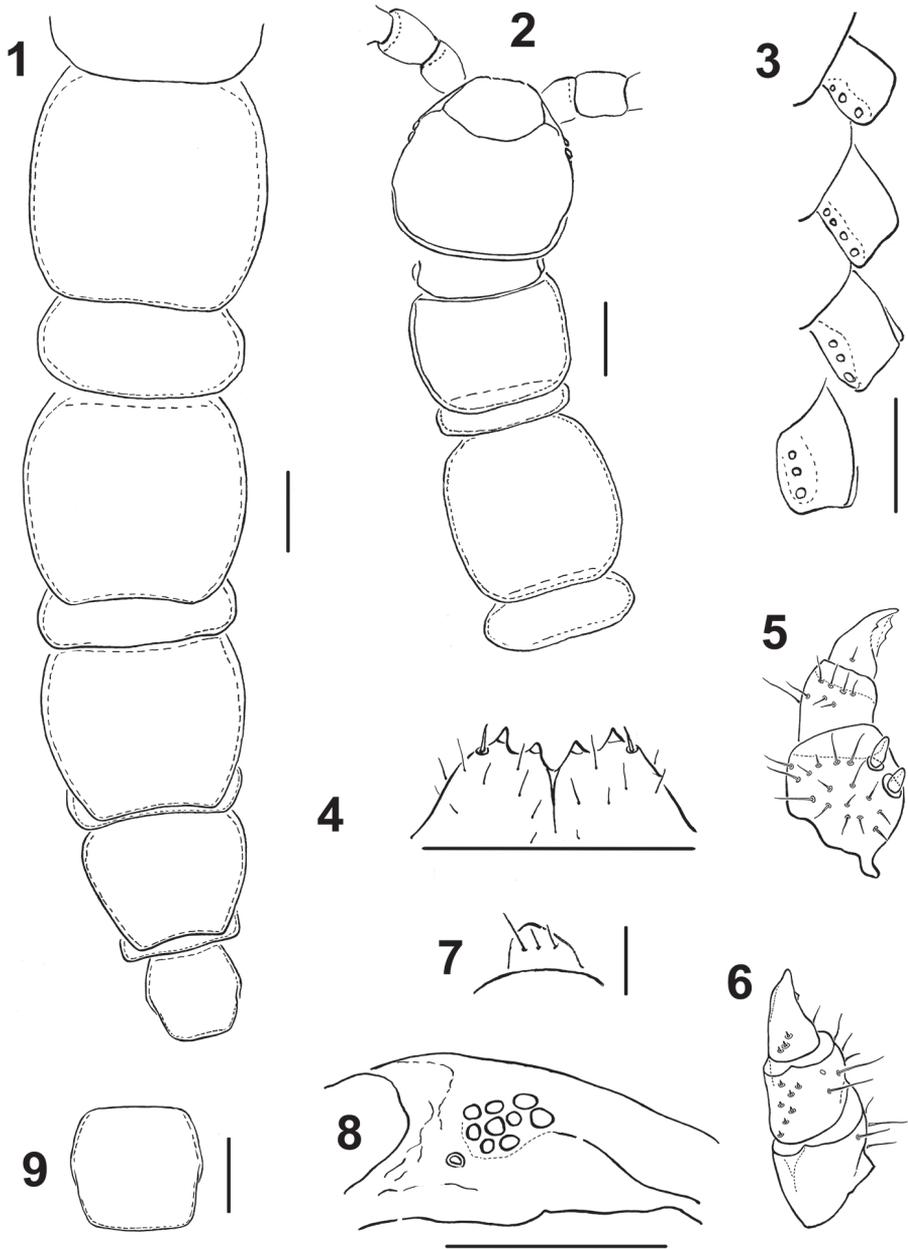
<http://zoobank.org/70178FA1-C117-4FFA-B915-C47783BA339B>

Figs 1–28

**Type material.** Holotype ♂ (ZMUM): Russia, Magadan Region, Kolyma Uplands, Ola Plateau highlands, 60°39'N, 151°16'E, nival community with *Cassiope tetragona*, 1275 m, 9.08.2011, leg. O.L. Makarova and A.B. Babenko.

**Paratypes:** 3 ♂♂, 4 ♀♀ (ZMUM), 2 ♂♂, 1 ♀ (MMUM, No. G7593), same data as holotype; 5 ♂♂, 4 ♀♀ (PSU), same locality, grass meadow, forb meadow with abundant legumes, nival community with *Cassiope tetragona*, dryas-forb tundra and ridge-top dryas-moss tundra, ca 1225–1470 m, 8–9.08.2011, leg. O.L. Makarova and A.B. Babenko; 5 ♂♂, 5 ♀♀ (ZMUM), 3 ♂♂, 12 ♀♀ (PSU), same locality, along bed of a nameless stream, at timber line, snowbed at ca 1150 m, 10.08.2011, leg. O.L. Makarova and A.B. Babenko; 3 ♂♂, 6 ♀♀ (PSU), same locality, forest belt of Ola Valley, willow bog, ca 820 m, 10.08.2011, leg. O.L. Makarova.

**Non-type material:** Chukot Autonomous Region: 6 ♂♂, 5 ♀♀, 1 subadult ♀ (ZMUM), environs of Anadyr Town, Maria Hill, under stones, 14–17.07.1971, leg. A.L. Tikhomirova and V.A. Turchaninova; 1 ♂, 7 ♀♀ (ZMUM), upper reaches of Bolshaya Osinovaya River, 66°52'N, 175°13'E, 30.07.1992, leg. D.I. Berman; 1



**Figures 1–9.** *Lithobius (Chinobius) yuchernovi* sp. n., paratypes. **1** male 8–16 tergites, dorsal view **2** male front body part, dorsal view **3** female coxal pores of legs 12–15, ventral view **4** male forcipular coxosternite, ventral view **5–6** female gonopod, ventral and dorsal view, respectively **7** male gonopod, ventral view **8** female ocelli and Tömösváry's organ, lateral view **9** female intermediate tergite, dorsal view. Scale bars: 0.5 mm (**1–6, 8, 9**), 0.1 mm (**7**).

♂ (ZMUM), near Markovo, 64°41'N, 170°26'E, spring alluvium of Anadyr River, 9.07.1971, leg. A.L. Tikhomirova; 8 ♂♂, 1 subadult ♂, 1 ♀♀ (ZMUM), Anadyr River, estuary of Balaganchik River, 64°54'N, 168°36'E, no date, leg. P.S. Tomkovich; 3 ♂♂, 16 ♀♀, 11 epimorph juveniles (PSU), Chaun Bay, Mt. Neitlin, 69°19'N, 171°27'E, pitfall traps, 5–25.08.1992, leg. D.I. Berman. Kamchatka Peninsula: 1 ♂ (ZMUM), near Elizovo, 53°10'N, 158°28'E, *Betula* forest with Poaceae, 11.08.1987, leg. A.V. Tanasevitch; 1 ♂, 1 ♀ (ZMUM), Kronotsky Nature Reserve, near Valley of Geysers, 54°31'N, 159°48'E, mountain tundra with mosses and lichens, 1200 m, 2–3.09.1978, leg. A.V. Tanasevitch; 1 ♂, 3 ♀♀ (ZMUM), same locality, multiherbaceous *Betula* forest on slope, 1.09.1978, leg. A.V. Tanasevitch; 2 ♀♀ (ZMUM), southern border of Kronotsky Nature Reserve, Zhupanovo, *Abies* forest, 30.08.1987, leg. A.V. Tanasevitch. Magadan Region: 19 ♂♂, 49 ♀♀, Tenkinsky District, near Sibik-Tyellakh, “Aborigin” Field Station, 61°54'N, 149°18'E, Mt. Medvezhya, no date, leg. D.I. Berman; 1 ♂, 1 ♀ (ZMUM), Annachag Mts, Jack London Lake, 62°04'N, 149°31'E, ca 800 m, litter, 5–6.08.1985, leg. Lyubimova.

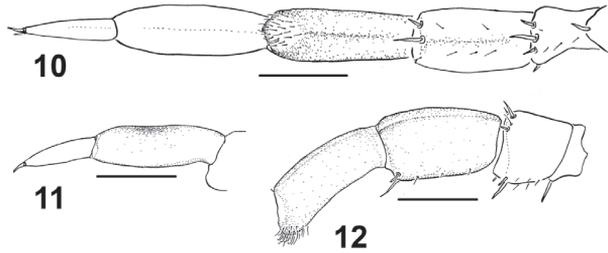
**Name.** The new species honours Academician **Yury Ivanovich Chernov** (1934–2012), the outstanding researcher of the Arctic (Reznikova 2012).

**Diagnosis.** A species of the genus *Lithobius* Leach, 1814, subgenus *Chinobius* Matic, 1973, normally with 20+20 elongate antennal articles; 9–11 ocelli, arranged in three rows; Tömösváry’s organ similar in size to the nearest ocellus; 2+2 teeth and setiform prodonts at dental margin of coxosternite; tergites without processes at posterior angles; tarsi 2-segmented, articulation being well-defined on all legs; legs 14–15 with DCa, leg 15 with an accessory apical claw; female gonopods with 2+2 spurs, gonopodal claw with 2–3 poorly-expressed denticles on internal ridge and with a single well-defined denticle on external ridge; last pair of legs swollen in male, ventrodistally with a group of curved setae on a round tubercle on tibiae, as well as shallow dorsal and ventral sulci on femora and tibiae.

**Distribution (Figure 29).** Northeastern Siberia and the Kamchatka Peninsula.

**Description.** Holotype ♂. Body 12.9 mm long; colour in alcohol yellow-brownish, with a distinct, darker, axial stripe on forcipular T–T 10, thereafter axial stripe poorly-expressed. Tergites: almost smooth, without visible setae, T 15 distinct; posterior angles rounded from forcipular T to T 9; posterior margin of TT 10, 12 and 14 slightly sinuate; TT 9, 11, 13 and 15 without triangular projections, but TT 13 and 15 with posterior angles slightly drawn back (Fig. 1); posterior margin of intermediate T straight, breadth/length ratio 1.04 (length 0.68 mm, breadth 0.70 mm); T 10 broadened, breadth/length ratio 1.16 (length 1.23 mm, breadth 1.43 mm). Sternites: sparsely setose, breadth/length ratio of S 10, 1.23 (length 0.88 mm, breadth 1.08 mm); breadth/length ratio of S 15, 0.65 (length 0.55 mm; genital sternite more densely setose, as in Fig. 18).

Cephalic plate: breadth/length ratio 1.04 (length 1.20 mm, breadth 1.23 mm); much broader than forcipular T (breadth 0.90 mm) (Fig. 2). Antennae: ca 5.25 mm long, reaching the middle of T 6, composed of 20+19 elongate articles (Fig. 20). Ocelli: 11 on each side, dark, arranged in three broken rows; posterior ocellus slightly larger



**Figures 10–12.** *Lithobius (Chinobius) yuchernovi* sp. n., male paratype. **10** leg 15, ventral view **11** tarsus 15, lateral view **12** femur, femur and tibia of leg 15, lateral view. Scale bars: 0.5 mm.

than posterosuperior ocellus and other seriated ocelli. Tömösváry's organ as large as nearest ocellus, rounded (Figs 8, 26). Forcipular coxosternite: dental margin slightly concave, with 2+2 acute teeth and setiform prodonts, median diastema V-shaped; shoulders of coxosternite strongly sloping, as in Figs 4 and 23.

Tarsal articulation of all legs distinct (Figs 13–15). Legs 14 slightly incrassate, without visible modifications (Fig. 13). P, F, Ti and Ts1 of legs 15 incrassate, last three with modifications: F with a clearly expressed dorsal sulcus and a poorly-developed ventral one; Ti with a poorly-expressed dorsal sulcus and a well-developed ventral sulcus, the latter reaching the distoventral tubercle supporting a cluster of curved and long setae (distal setae more strongly curved, unciform, proximal ones almost straight) (Figs 12, 15); Ts1 slightly flattened, dorsally with an implicit impression (Fig. 11) bearing a few erect setae. Length of legs 15: P = 0.78, F = 0.83, Ti = 0.83, Ts1 = 0.83, Ts2 = 0.48. Legs 13–15 with DCa. Accessory claw on leg 15 large, well-developed. Plectrotaxy as in Table 1. Coxal pores: present on legs 12–15, rounded, separated from one another by distances 2–2.5 times greater than their own diameter; inner pores smaller than neighbouring ones; formula 3, 4(5), 4(5), 4. Gonopod 1-segmented, with three setae (Figs 7, 18).

Paratype ♂♂. Length 10.2–12.2 mm. All characters as in holotype, but ocelli 8–10, usually 9 (Figs 8, 26). Antennae normally with 20+20 segments, but 3 specimens with asymmetric numbers of antennal articles (19+20, 20+19 and 18+20); 6 specimens with broken antenna (20+?). Posterior and posterosuperior ocelli slightly different in size from seriate ocelli. All specimens with a well-visible distoventral tubercle, the latter carrying a group of straight or curved setae. Expression of other sulci on legs 15 variable: in some males, dorsal sulci on F and Ti poorly visible or absent at all, while in others, ventral sulci not developed. In some specimens, dorsal impression on Ts1 without erect setae (Fig. 11). Plectrotaxy as in holotype, but in some specimens Vtm can start with legs 13, while VTia from legs 3 to 14. Coxal pores as in holotype, their number varying from 3 to 5. Gonopods as in holotype, with 2–3 setae (Figs 7, 18).

Paratype ♀♀. Length 12.1–16.0 mm. Antennae usually with 20+20 segments, but in two specimens 20+19 and 20+18, respectively; 3 specimens with broken antenna (20+?). Intermediate tergite broadened, breadth/length ratio 1.19 (length

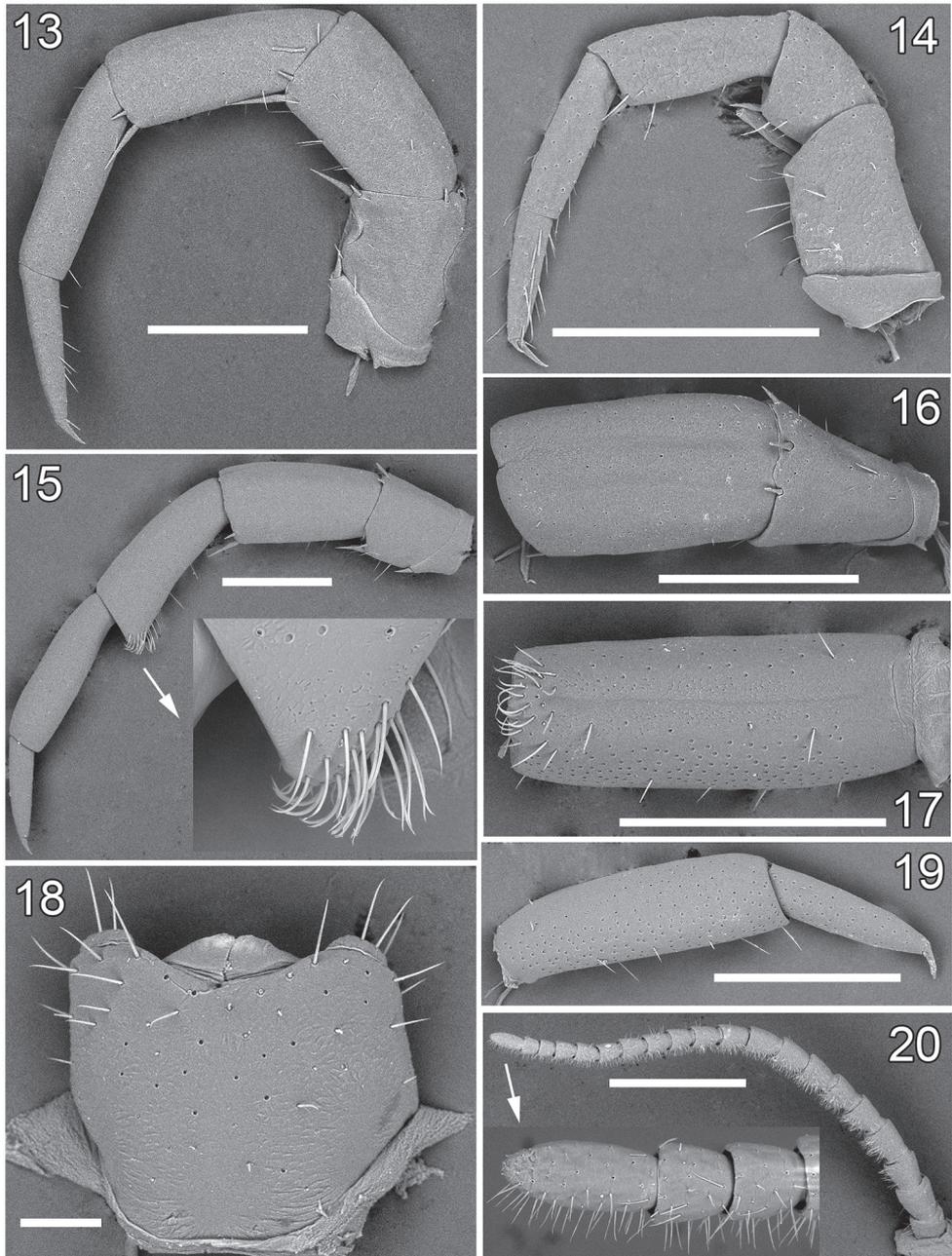
**Table 1.** Plectrotaxy of *L. yuchernovi* sp. n. Holotype. Brackets show the presence of an asymmetric spine in one of leg pairs.

Leg	Ventral				Dorsal			
	t	P	F	Ti	C	P	F	Ti
1	–	–	am	m	–	p	a	a
2	–	–	am	m	–	p	a p	a
3	–	–	am	m	–	p	a p	a (p)
4	–	–	am	m	–	p	a p	a p
5	–	–	am	m	–	(a) p	a p	a p
6	–	–	am	(a)m	–	a p	a p	a p
7	–	(m)	am	am	–	a p	a p	a p
8	–	m	am	am	–	a p	a p	a p
9–11	–	mp	amp	am	–	a p	a p	a p
12	–	mp	amp	am	–	amp	a p	a p
13	–	mp	amp	am	a	amp	p	p
14	m	amp	am	m	a	amp	p	–
15	m	amp	am	–	a	amp	–	–

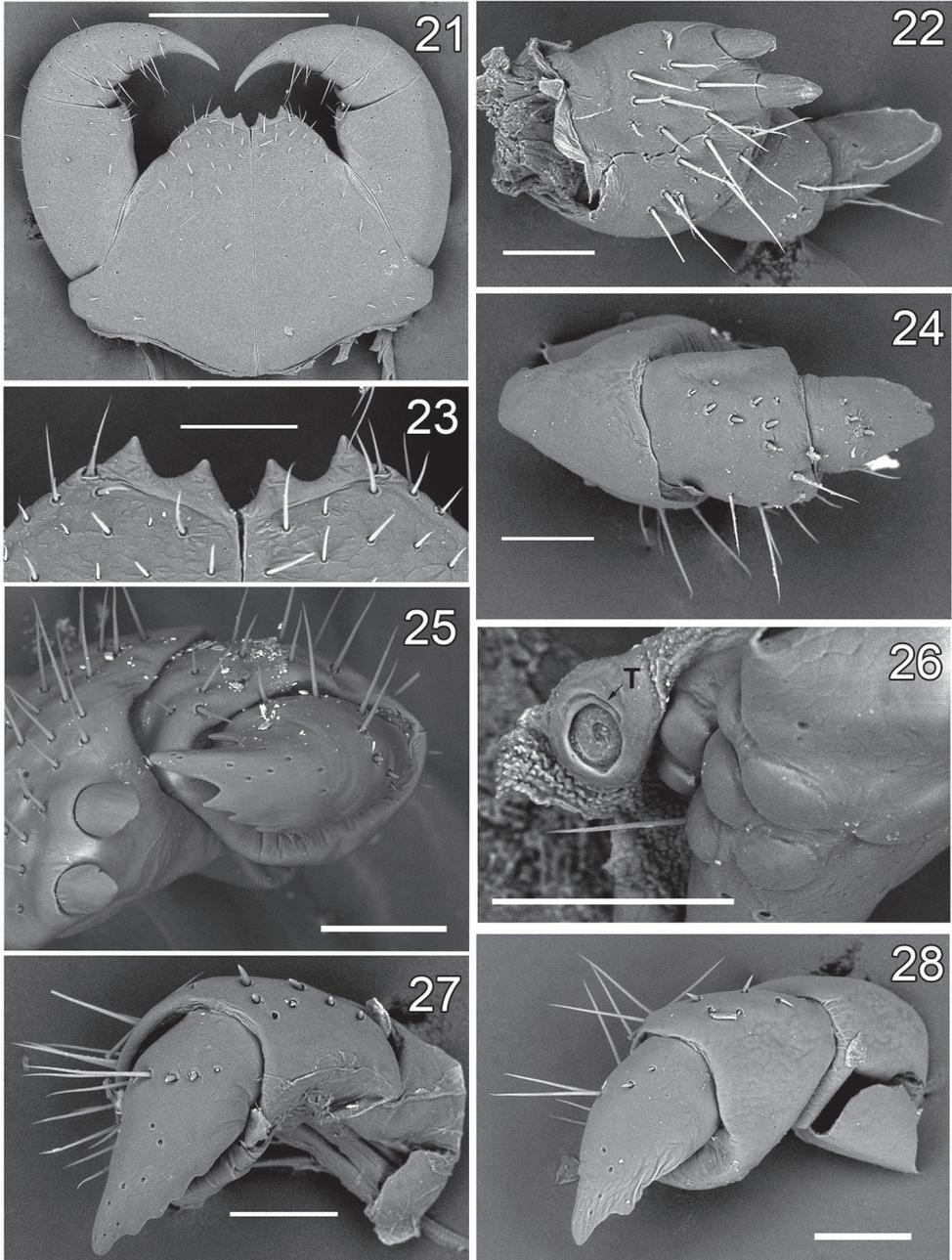
0.80 mm, breadth 0.95 mm) (Fig. 9). Legs 12–15 with 4–6 coxal pores, formula 4(5),5(4),5(4),5(6). Gonopods without setae on internal face, with 2+2 short spurs separated from one another by distances not exceeding the diameter of the widest part of a spur (Figs 5, 22). First segment of gonopod without spines, second with 6–8, third with 2–3 dorsal short spines in two uneven rows (Figs 6, 24). In some specimens, the spines very short. Apical claw of gonopod with one well-defined lateral denticle located in the middle of external ridge, as well as with 2–4 blunt denticles on internal ridge. In some specimens, the denticles almost abraded. In most specimens, the lateral denticles on internal ridge of claw blunt, as in Figs 27 and 28. Other characters as in holotype.

**Variation.** Although males from different localities in northeastern Siberia show relatively stable morphological features, females from various places demonstrate certain variability in the structure of the gonopods. Thus, in females from Chukotka, the lateral denticles on the gonopodal claw are better expressed (Fig. 25) than in the type material from the Magadan Region (Figs 5, 22). The Kamchatka specimens differ from all others to the greatest extent: both sexes are with faint, but still visible tarsal articulations on the first eleven pairs of legs (in one large male, the tarsal articulations were truly distinct); males with the distoventral tubercle on Ti 15 that supports fewer setae and less strongly expressed dorsal and ventral sulci. In females, the lateral denticle on the external ridge of the gonopodal claw is very weakly expressed, up to absent in some females. In addition, the internal ridge of the claw with one well-expressed and one almost abraded denticle, the latter looks like an uneven edge at the claw base (Fig. 28).

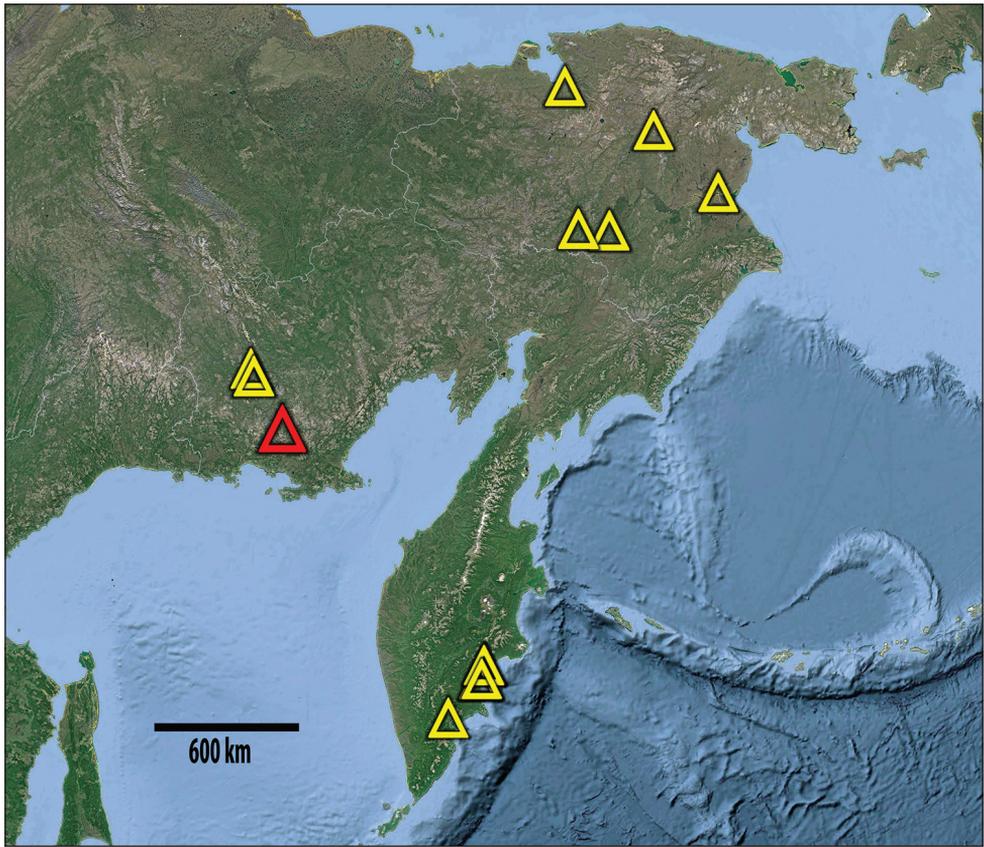
**Habitats.** Almost all specimens were collected in montane environments (800 to 1470 m), with only a few obtained from a plain area at ca 60 m near the town of Anadyr. In the mountains, the new species dwells in mountain *Betula* forest, alpine meadow, montane tundra with *Dryas* dwarf bush and *Cassiope tetragona* nival habitats.



**Figures 13–20.** *Lithobius (Chinobius) yuchernovi* sp. n., male paratype. **13** leg 14, lateral view **14** leg 1, lateral view **15** leg 15, lateral view **16** prefemur and femur of leg 15, dorsal view **17** tibia 15, ventral view **18** genital sternite with gonopods, ventral view **19** tarsus 15, lateral view **20** antenna. Scale bars: 0.1 mm (**18**), 0.5 mm (**13, 14, 16, 17, 19**), 1 mm (**15, 20**).



**Figures 21–28.** *Lithobius (Chinobius) yuchernovi* sp. n., female paratype (21–24, 26) and non-type females (25, 27–28). **21, 23** forcipule and dental margin of forcipular coxosternite, ventral view **22** gonopod, ventral view **24** gonopod, dorsal view **25** gonopod, ventrolateral view (Chukotka) **26** ocelli and Tömösváry's organ, lateral view **27** gonopod, dorsolateral view (Magadan Region) **28** same (Kamchatka Peninsula). Scale bars: 0.5 mm (21), 0.1 mm (22–28).



**Figure 29.** Distribution of *Lithobius (Chinobius) yuchernovi* sp. n. The type locality is shown in red.

## Remarks

As a nomenclatural remark, *Lithobius zachiui* nom. n., is proposed herewith as a new replacement name for *Lithobius (Chinobius) orientalis* (Matic, 1973). The name *Lithobius (Chinobius) orientalis* (Matic, 1973) must be considered as permanently invalid (ICZN 1999: Art. 57.2) since it is a junior primary homonym of *Lithobius orientalis* Sselivanoff, 1880, even though the two were originally described in different genera (ICZN 1999: Art. 57.2). Since there are no junior synonyms, a new name, *Lithobius zachiui* nom. n., is advanced here to replace the preoccupied name *Lithobius orientalis* (Matic, 1973). The new name is dedicated to Zachiui Matic (1924–1994). Besides *Lithobius matici* Prunescu, 1966, which is a synonym of *Lithobius punctulatus* (C.L. Koch, 1847), this is the second species to honour the outstanding Romanian myriapodologist who described 193 taxa of centipedes (Bonato et al. 2016, Zapparoli and Minelli 1995).

Another nomenclatural remark, *Lithobius carli* nom. n., is proposed herewith as a new replacement name for *Lithobius (Alokobius) orientalis* Attems, 1953. This name must be considered as permanently invalid (ICZN 1999: Art. 57.2) since it is a junior

primary homonym of *Lithobius orientalis* Sseliwanoff, 1880 too (ICZN 1999: Art. 57.2). Since there are no junior synonyms, a new name, *Lithobius carli* nom. n., is advanced here to replace the preoccupied name *Lithobius (Alokobius) orientalis* Attems, 1953. The new name is dedicated to Carl August Graf Attems-Petzenstein (1868–1952) to honour this prolific Austrian myriapodologist who described almost 400 taxa of centipedes (Strouhal 1960, Bonato et al. 2016).

Males of *L. yuchernovi* sp. n. differ from those of all known Palaearctic species of the genus *Lithobius* by the presence of a distoventral tubercle on Ti 15. Based on the structure of the gonopod, females are close to species of the subgenus *Chinobius*, from which they differ by following morphological details of the body. Thus, our new species is distinguished from *L. (Ch.) orientalis* Sseliwanoff, 1880 by the 20-segmented antennae (vs. 19-segmented); from *L. (Ch.) zachiui* nom. n. by the non-swollen gonopodal segment 2 and the presence of 6–8 dorsal short spines on it (vs. segment 2 swollen and spineless); from *L. (Ch.) pectinatus* Takakuwa, 1939 by the presence of a well-defined denticle on the external ridge of the claw and thick spurs (vs. without such a denticle and with very thin spurs).

Based on the structure of the female gonopod and other main characters (body length, number of antennomeres and ocelli, 2+2 coxosternal teeth, tergites without posterolateral triangular projections), *L. yuchernovi* sp. n. also resembles *L. otasanus* Takakuwa, 1941, a species described from the southern Sakhalin Island (Takakuwa 1941) and omitted by Zalesskaja (1978) in her book on the Lithobiomorpha fauna of the former Soviet Union. However, both differ clearly in the latter species showing neither DCa nor accessory claws on the last legs. Both these taxa differ also in the plectrotaxy patterns of legs 1, 13–15.

In addition, females of the new species are close to the northern Pacific species *Lithobius (Ezembius) stejnegeri* (Bollman, 1893) which inhabits Alaska, the Pribilof and Commander Islands, as well as some Aleutian Islands. However, *L. yuchernovi* sp. n. differs by the presence of 2 or more lateral denticles on the internal ridge of the gonopodal claw (vs. a tripartite or simple gonopodal claw in young and adult females, respectively (Zalesskaja 1978)).

Another patronym in lithobiid taxonomy that honours Academician Y.I. Chernov is worth mention. This is *Lithobius (Monotarsobius) chernovi* (Zalesskaja, 1976), described from the Taymyr Peninsula in the Far North of Russia. The valid name for *L. (M.) chernovi* is *Lithobius (Monotarsobius) alticus* (Loksa, 1965) (Zalesskaja 1978: 174). Although both species, viz. *L. (M.) alticus* and *L. (Chinobius) yuchernovi* sp. n., belong to different subgenera, they share an important taxonomic character, i.e. the presence of a distal tubercle supporting a cluster of long and curved setae on Ti 15. Nevertheless, they differ significantly in the position of this tubercle: distoventral in *L. yuchernovi* sp. n., vs. distodorsal in *L. alticus*.

## Discussion

Originally, Verhoeff (1934) proposed *Chinobius* as a subgenus of *Lithobius* to receive two new species from central China: *L. svenhedini* Verhoeff, 1934 and *L. hummelii* Verhoeff, 1934, the former based on a male, the latter based on a damaged female.

Since neither have been chosen to serve as the type species, *Chinobius* had languished as an invalid name until Matic (1973) typified it through the selection of *L. svenhedini* as the generotype. He also elevated *Chinobius* to full generic status.

The taxonomic problems existing within *Chinobius* were discussed by Eason (1996), who again downgraded *Chinobius* to the status of a subgenus of *Lithobius*, albeit improperly ascribing the authorship of *Chinobius* to Verhoeff (Jeekel 2005: 14). At present, the main feature characterizing *Chinobius* lies in the development of two or more denticles on the internal ridge of the female gonopodal claw. However, as noted above, the females of *L. (Ch.) yuchernovi* sp. n. show significant variations in this trait, both intra- and interpopulational. Specimens with well-defined 2–3 lateral denticles at the internal margin of the gonopod, as well as with almost abraded or completely absent denticles are observed within and between various populations. This severely undermines the acceptance of *Chinobius* as a generic-level taxon.

On the other hand, females of *L. (Ch.) yuchernovi* sp. n. from the Kamchatka Peninsula are extremely similar to those of *L. (E.) stejnegeri* Bollman, 1893. When redescribing *L. (E.) stejnegeri* from Kamchatka and the Iturup Island, Kuriles, Eason (1996) recorded several specimens of both sexes which had been collected from some localities in Kamchatka and support what we identify as *L. (Ch.) yuchernovi* sp. n. Although the males of these two species are clearly distinguished, morphological differences between the females are quite subtle: (1) 8–9 ocelli in the new species vs. 9–18 in *L. stejnegeri*, i.e. 12–18 ocelli after Bollman (1893), but 9–15 ocelli following Eason (1996); (2) gonopodal segment 1 dorsally with a single, short, setiform spine in *L. yuchernovi* sp. n. vs. devoid of a spine in *L. stejnegeri*; (3) gonopodal segment 2 dorsally with 5–6 short and setiform spines in *L. yuchernovi* sp. n., vs. only 3 short and setiform spines in *L. stejnegeri*.

As a result, Kamchatka appears to harbour both these species which are best distinguished based on male specimens, whereas females are difficult to separate. Whether these species occur not only sympatrically, as is the case concerning the Kamchatka Peninsula, but also syntopically remains open to question.

Males of the new species are characterised by a feature unique among the Palaearctic Lithobiinae Verhoeff, 1907, i.e. a distoventral rounded tubercle supporting a group of setae on Ti 15. In addition, they show more or less strongly expressed dorsal and ventral sulci on ultimate legs. Similar distoventral modifications of male Ti 15 are observed in the North American genus *Nothembius* Chamberlin, 1916. *L. yuchernovi* sp. n. seems to be particularly close to *N. aberrans* Chamberlin, 1916 (see Chamberlin 1916, pl. 9, fig. 5), based on this feature, but differs by the number of antennomeres: 20 in *L. yuchernovi* sp. n., vs. usually 22 in *N. aberrans*; 2+2 teeth at the dental margin of the forcipular coxosternite in *L. yuchernovi* sp. n., vs. 3+3 in *N. aberrans*; the presence of sulci on the dorsal and ventral sides of Ti 15 in *L. yuchernovi* sp. n., vs. their absence in *N. aberrans*; and the claw of the female gonopod equipped with more than two lateral denticles in *L. yuchernovi* sp. n. (Figs 5, 22, 25, 27), vs. simple in *N. aberrans* (cf. Chamberlin 1916).

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## References

- Bollman CH (1893) The Myriapoda of North America. Bulletin of the United States National Museum 46: 1–210.
- Bonato L, Chagas Junior A, Edgecombe GD, Lewis JGE, Minelli A, Pereira LA, Shelley RM, Stoev P, Zapparoli M (2016) ChiloBase 2.0 – A World Catalogue of Centipedes (Chilopoda). <http://chilobase.biologia.unipd.it>.
- Bonato L, Edgecombe GD, Lewis JGE, Minelli A, Pereira LA, Shelley RM, Zapparoli M (2010) A common terminology for the external anatomy of centipedes (Chilopoda). ZooKeys 69: 17–51. <https://doi.org/10.3897/zookeys.69.737>
- Bonato L, Edgecombe GD, Zapparoli M (2011) Chilopoda – Taxonomic overview. In: Minelli A (Ed.) Treatise on Zoology – Anatomy, Taxonomy, Biology. The Myriapoda, Volume 1. Brill, Leiden–Boston, 363–443.
- Chamberlin RV (1916) The lithobiid genera *Oabius*, *Kiberbius*, *Paobius*, *Arebius*, *Nothembius* and *Tigobius*. Bulletin of the Museum of Comparative Zoology, Harvard College 57: 115–201.
- Davydova MI, Kamensky AI, Neklyukova NP, Tushinsky GK (1960) [Physiography of the USSR]. Prosveshcheniye Publ., Moscow, 680 pp. [in Russian].
- Eason EH (1996) Lithobiomorpha from Sakhalin Island, the Kamchatka Peninsula and the Kurile Islands (Chilopoda). Arthropoda Selecta 5(3/4): 117–123.
- ICZN [International Code of Zoological Nomenclature] (1999) International Code of Zoological Nomenclature. Fourth edition. The International Trust for Zoological Nomenclature, London, 306 pp.
- Jeekel CAW (2005) Myriapod memoranda. Supplement 1. Nomenclator generum et familiarum Chilopodorum: a list of the genus and family-group names in the class Chilopoda from the 10<sup>th</sup> edition of Linnaeus, 1758, to the end of 1957. Ulvenhout, 1–130.
- Makarova OL, Makarov KV, Berman DI (2014) Ground beetles (Coleoptera, Carabidae) of the Ola Plateau highlands, Kolyma Uplands. Entomological Review 94(1): 49–57. <https://doi.org/10.1134/S0013873814010060>
- Matic Z (1973) Revision du genre *Chinobius* Verhoeff avec des descriptions de trois espèces nouvelles (Chilopoda Lithobiidae). Annales Zoologici 30: 33–47.
- Reznikova ZhI (2012) Memory of Yurii Ivanovich Chernov (1934–2012). Eurasian Entomological Journal 11(3): 291–296.

- Strouhal H (1960) Hofrat Dr. Carl Graf Attems zum Gedenken. Annalen des Naturhistorischen Museums in Wien 64: 1–38.
- Takakuwa Y (1941) Weitere japanische *Lithobius*-Arten und zwei neue Diplopoden. Transactions of the Sapporo Natural History Society 42(1): 1–9.
- Verhoeff KW (1934) Schwedisch-chinesische wissenschaftliche Expedition nach den nordwestlichen Provinzen Chinas, unter Leitung von Dr. Sven Hedin und Prof. Sü Ping-chang. Myriapoda. Arkiv för Zoologi 26a(10): 1–41.
- Yurtsev BA, Khokhryakov AP (1975) [Analysis of the flora of the Ola Plateau as related to the history of the plant cover of the Kolyma Uplands]. Byulleten' Moskovskogo obshchestva ispytateley prirody, Otdel. biologicheskii 80(2): 120–133. [in Russian]
- Zalesskaja NT (1978) [Identification book of the lithobiomorph centipedes of the USSR]. Nauka Publ., Moscow, 212 pp. [in Russian]
- Zapparoli M, Minelli A (1995) Zachiu Matic (1924–1994). Fragmenta Entomologica 26: 419–427.

# Faunistic and bibliographical inventory of moth flies from Ukraine (Diptera, Psychodidae)

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## Abstract

All important published records for 11 moth fly species known so far from Ukraine are reviewed (Phlebotominae 10 species, Psychodinae 1 species). Occurrences of two problematic taxa, *Phlebotomus (Adlerius) brevis* Theodor & Mesghali, 1964 and *P. (Larrousius) major major* Annandale, 1910, and some synonymies are discussed. *Threticus negrobovi* Vaillant, 1972 must be deleted for Ukraine (misstatement). First records of 34 species of Psychodinae (tribes Mormiini, Paramormiini, Psychodini, Pericomaini) and one of Sycoracinae from Ukraine are also listed.

## Keywords

Diptera, faunistics, first records, Psychodidae, Phlebotominae, Sycoracinae, Psychodinae, Ukraine

## Introduction

Moth flies (Psychodidae) are represented only by 11 species previously recorded in Ukraine (mainly Perfil'ev 1966; Lewis 1982; Artemiev and Neronov 1984; Vaillant 1972; Wagner 1990, 2013). Most of these species are members of subfamily Phlebotominae: *Phlebotomus (Phlebotomus) papatasi* (Scopoli, 1786),

*P. (Adlerius) balcanicus* Theodor, 1958, *P. (A.) brevis* Theodor & Mesghali, 1964 (doubtful evidence for Ukraine), *P. (A.) longiductus* Parrot, 1928, *P. (Larroussius) neglectus* Tonnoir, 1921, *P. (L.) major major* Annandale, 1910 (doubtful evidence for Ukraine), *P. (L.) perfiliewi perfiliewi* Parrot 1930, *P. (Paraphlebotomus) alexandri* Sinton, 1928, *P. (P.) similis* Perfil'ev, 1963 and *Sergentomyia minuta minuta* (Rondani, 1843); only one species from subfamily Psychodinae has been listed: *Threticus negrobovi* Vaillant, 1972. This is probably due to erroneous distribution data. In the current paper, the fauna of moth flies in Ukraine is enriched with 35 newly recorded species. The number of known species of Psychodidae from Ukraine is elevated to 43.

## Materials and methods

The material presented here comes from two different sampling campaigns (see Table 1). The first part of the material was taken during field work as an implementation of the project “Ephemeroptera, Plecoptera, Diptera biodiversity trip along the Uzh river, Ukraine“ (2015 and 2016). The material was collected in the upper part of the Uzh River basin, in the eastern Ukraine (Transcarpathia) and preserved in 75% ethanol. All samples were collected by sweep netting from vegetation along streams (Uzh River and its tributaries) and lakes by Jozef Oboňa, Peter Manko and Ľuboš Hrivniak. Captured moth flies were mounted on slides in Canada balsam in laboratory – 51 slides. The same collecting method was used by Pavel Chvojka during sampling trip to eastern Carpathians (1996) – 19 slides. All material, determined by the first author, is deposited in the National Museum, Natural History Museum, Department of Entomology, Prague, Czech Republic (NMPC). Slides are numbered by Inv. No. = Inventory Slide Number of the family Psychodidae (Tkoč et al. 2014). Nomenclature utilised is according to Perfil'ev (1966), Lewis (1982) and Artemiev and Neronov (1984) for Phlebotominae; for Psychodinae and Sycoracinae the nomenclature is modified from Vaillant (1972) and Wagner (1990, 2013) using the classifications of Oboňa and Ježek (2014) and Kroča and Ježek (2015).

## The following abbreviations are used in the paper:

<b>C</b>	P. Chvojka leg.,	<b>NMPC</b>	collections of the National Museum Prague, Czech Rep.,
<b>H</b>	Ľ. Hrivniak leg.,	<b>M</b>	male,
<b>J</b>	J. Ježek det.,	<b>F</b>	female.
<b>Ma</b>	P. Manko leg.,		
<b>O</b>	J. Oboňa leg.,		

**Table 1.** List of collecting sites.

Site name	Latitude/N	Longitude/E
Hropynets' Stream	48°11'54.0"	24°16'42.0"
Kamenychky Stream, below Novoselytsya	48°48'36.9"	22°28'33.4"
Kevele Stream, left tributary of Chorna Tysa River	48°11'11.0"	24°18'37.0"
Lubnya River, below Lubnya	49°00'36.9"	22°43'27.8"
Lyuta River, above Chornoholova	48°51'43.1"	22°36'21.4"
Paporotnyi Stream, in Stuzhytsya	49°01'40.0"	22°35'17.1"
Preluchnyi Stream, bridge above the water reservoir	48°46'22.0"	22°52'32.6"
Pylypets' River	48°40'12.0"	23°20'17.0"
Shypot River, below hydroelectric power plant	48°42'58.2"	22°49'20.4"
Shypotyky Stream, tributary of the Shypot River near hydroelectric power plant	48°44'23.7"	22°50'18.4"
Strychavka Stream, below Strychava	48°56'43.6"	22°28'17.0"
Sukchyi Stream, above Kam'yanytsya	48°42'46.2"	22°25'24.9"
Synevir–Polyans'ke Lake	48°37'00.0"	23°40'54.0"
Tereblya River, below Kolochava	48°25'22.0"	23°41'36.0"
Tikhyi Stream, above Stuzhytsya	49°02'09.2"	22°34'43.5"
tributary of the Uzh River, near Zahorb	49°00'41.9"	22°38'39.1"
Turiya River above Simer	48°43'41.5"	22°32'41.9"
Uzh River, at the last building in Stuzhytsya	48°40'49.9"	22°24'09.6"
Uzh River, Nevyts'ke near bridge	48°40'49.9"	22°24'09.6"
Uzh River, above Uzhgorod	48°36'37.5"	22°13'49.6"

## Results

### Faunistic records

#### PSYCHODIDAE

#### PHLEBOTOMINAE

#### *Phlebotomus* Rondani, 1840 (sensu Sabrosky, 1999)

#### *Phlebotomus* s. str.

#### 1. *Phlebotomus* (*Phlebotomus*) *papatasi* (Scopoli, 1786)

**Published records.** Perfil'ev (1966): 252, 254; Lewis (1982): 138, 140; Artemiev and Neronov (1984): 39, 40; Wagner (1990, 2013): 13.

**Distribution.** Afghanistan, Albania, Algeria, Azerbaijan, Bulgaria, Crete, Crimea, Croatia, Cyprus, Egypt, Ethiopia, France (southern), Georgia, Greece, Hungary, India, Iran, Iraq, Israel, Italy, Jordan, Kazakhstan (southern), Kuwait, Libya, Malta, Moldavia, Montenegro, Morocco, Oman, Pakistan, Portugal, Romania, Sardinia, Saudi Arabia, Serbia, Spain, Sudan, Syria, Tunisia, Turkey, Ukraine (southern), Yemen (Perfil'ev 1966; Lewis 1982; Artemiev and Neronov 1984; Wagner 1990, 2013).

***Adlerius* Nitzulescu, 1931****2. *Phlebotomus (Adlerius) balcanicus* Theodor, 1958**

**Published records.** Perfil'ev (1966) as *Phlebotomus chinensis balcanicus* Theodor, 1958: 316, 317; Lewis (1982): 165; Artemiev and Neronov (1984): 114; Wagner (1990, 2013): 17.

**Distribution.** Azerbaijan, Bulgaria, Caucasus, Crimea, Greece, Iran (north-western), Romania, Turkey, Ukraine, former Yugoslavia (Perfil'ev 1966; Lewis 1982; Artemiev and Neronov 1984; Wagner 1990, 2013).

***Phlebotomus (Adlerius) brevis* Theodor & Mesghali, 1964**

(doubtful evidence for Ukraine, see quite different views below)

Syn. *Phlebotomus chinensis ismailicus* Perfil'ev, 1966

**Published records.** Perfil'ev (1966) as *Phlebotomus chinensis ismailicus* Perfil'ev, 1966: 314; Lewis (1982): 165; Artemiev and Neronov (1984): 115; Wagner (1990, 2013): 17.

**Comments on distribution.** Artemiev and Neronov (1984): only Iran, Southern Caucasus, Turkey; Perfil'ev's statement Izmail is erroneous (published as *ismailicus*). See the full synonymy in Lewis (1982). Wagner (1990, 2013) lists records from the republic of Moldova and Ukraine; however, these records are **not verified so far**.

**3. *Phlebotomus (Adlerius) longiductus* Parrot, 1928**

Syn. *Phlebotomus (Adlerius) tauriae* Perfil'ev, 1966: 312, 314, Crimea

**Published records.** Perfil'ev (1966): 310, 312; Lewis (1982): 167; Artemiev and Neronov (1984): 121; Wagner (1990, 2013): 18.

**Comments on distribution.** Romania Perfil'ev (1966): as *Phlebotomus chinensis longiductus* Parrot, 1928 – occurrence only in Central Asia. Lewis (1982) and Artemiev and Neronov (1984): Afghanistan (northern and central), Crimea, Kazakhstan, Romania, Ukraine (southern); Caucasus (northern) and Central Asia. Wagner (1990, 2013) lists Afghanistan, Kazakhstan, Moldova, Romania and the Ukraine.

***Larrousius* Nitzulescu, 1931****4. *Phlebotomus (Larrousius) neglectus* Tonnoir, 1921**

Syn. *Phlebotomus (Larrousius) major krimensis* Perfil'ev, 1966

**Published records.** Perfil'ev (1966): 282, 285; Lewis (1982): 156, 157 (as *major krimensis*), 157 (as *major neglectus*); Artemiev and Neronov (1984): 93, 95 (as *neglectus*); Wagner (1990, 2013): 16, (as *major krimensis*), 16 (as *major neglectus*).

**Comments on distribution.** Perfil'ev (1966): southern coast of Crimea; Lewis (1982): *krimensis* – Crimea, *neglectus* – Albania, Dalmatia, Italy; Artemiev and Neronov (1984): *neglectus* – Italy, Balkan Peninsula, Crimea, Turkey, Caucasus (southern), former Palestine, ?Iran (north-western). Wagner (1990, 2013) lists the species (as *major krimensis*) from Crimea, Ukraine, and as *major neglectus* from Albania, Austria, Greece (Crete), Italy (Sicily), Romania, and former Yugoslavia.

### ***Phlebotomus (Larrousius) major major* Annandale, 1910**

(doubtful evidence for Ukraine, see quite different views below)

**Published records.** Perfil'ev (1966): 280; Lewis (1982): 157; Artemiev and Neronov (1984): 92, 93; Wagner (1990, 2013): 16.

**Comments on distribution.** Perfil'ev (1966): India only. Lewis (1982): India, Nepal, Pakistan. Artemiev and Neronov (1984): Afghanistan, India (southern slopes of the Himalayas), Nepal, Pakistan (northern). Wagner (1990) lists Afghanistan, India, Iran, and the Oriental Region. Wagner (2013) lists the species from Albania, Austria, Crete, Romania, Sicily, Ukraine, and former Yugoslavia; however, these records have **not been verified**.

### **5. *Phlebotomus (Larrousius) perfiliewi perfiliewi* Parrot, 1930**

**Published records.** Perfil'ev (1966): 289, 291; Lewis (1982): 160; Artemiev and Neronov (1984): 98; Wagner (1990, 2013): 16.

**Distribution.** Albania, Algeria, Bosnia and Herzegovina, Crete, Crimea, Croatia, Cyprus, France (south-eastern), Georgia, Greece, Hungary, Italy, Libya, Macedonia, Malta, Moldova, Montenegro, Morocco, Portugal, Romania, Sardinia, Serbia, Sicily, Spain, Tunisia, Turkey, and Ukraine (Perfil'ev 1966; Lewis 1982; Artemiev and Neronov 1984; Wagner 1990, 2013).

### ***Paraphlebotomus* Theodor, 1948**

### **6. *Phlebotomus (Paraphlebotomus) alexandri* Sinton, 1928**

**Published records.** Perfil'ev (1966): 264, 267; Lewis (1982): 143; Artemiev and Neronov (1984): 45; Wagner (1990, 2013): 14.

**Distribution.** Afghanistan, Algeria, Armenia, Azerbaijan, China (western), Crimea, Cyprus, Djibouti, Ethiopia, Georgia, Greece, India, Iran, Iraq, Israel, Ka-

zakhstan (southern), Moldova, Mongolia, Morocco, Pakistan, Romania, Saudi Arabia, Spain, Sudan, Tunisia, Turkey, Ukraine, United Arab Emirates, Yemen; northern Sahara, Caucasus (southern), Near and Middle East, Central and Eastern Asia; Afrotropical and Oriental Regions (Perfil'ev 1966; Lewis 1982; Artemiev and Neronov 1984; Wagner 1990, 2013).

### 7. *Phlebotomus (Paraphlebotomus) similis* Perfil'ev, 1963

Sensu Artemiev and Neronov (1984)

Syn: *Phlebotomus (Paraphlebotomus) sergenti similis* Perfil'ev, 1963

**Published records.** Perfil'ev (1966): 276; Lewis (1982): 148 – as *sergenti similis*; Artemiev and Neronov (1984): 58, 59 – as *similis*.

**Comments on distribution.** Perfil'ev (1966): southern coast of Crimea and southern Ukraine, Russia, northern Caucasus. Lewis (1982): same, Azerbaijan, Uzbekistan. Artemiev and Neronov (1984): Crimea, southern Ukraine, ?northern Caucasus, Albania, Romania, and former Yugoslavia.

### *Sergentomyia* França & Parrot, 1920

#### 8. *Sergentomyia minuta minuta* (Rondani, 1843)

**Published records.** Perfil'ev (1966): 323, 327; Wagner (1990): 22.

**Distribution.** Crimea, France, Greece, Italy, Malta, Sardinia, Sicily, Spain, former Yugoslavia (Perfil'ev 1966; Wagner 1990).

## PSYCHODINAE

### MORMIINI

#### MORMIINA

#### *Oomormia* Ježek, 1984

#### 9. *Oomormia andrenipes* (Strobl, 1910)

**Material examined.** Rachiv, tributary of Chorna Tysa (Kvasy), 7.vi.1996, 1M, C leg., slide Inv. No. 12216, NMPC.

**Distribution.** A quite rare species known from Austria, Bosnia, Czech Republic, Great Britain, Slovakia, and Slovenia (Ježek and Omelková 2012; Wagner 2013). **New species for Ukraine.**

PARAMORMIINI

PARAMORMIINA

*Jungiella* Vaillant, 1972

*Jungiella* s. str.

10. *Jungiella (Jungiella) hygrophila* Ježek, 1987

**Material examined.** Lyuta River, above Chornoholova, 27.v.2016, 1M, O Ma H leg., slide Inv. No. 22612, NMPC.

**Distribution.** Probably a central European species, uncommon, known only from the Czech Republic, Poland, and Slovakia (Ježek 1987; Ježek and Omelková 2012; Wagner 2013; Oboňa and Ježek 2014). **New species for Ukraine.**

11. *Jungiella (Jungiella) valachica* (Vaillant, 1963)

**Material examined.** Lyuta River, above Chornoholova, 27.v.2016, 1M, O Ma H leg., slide Inv. No. 22610, NMPC.

**Distribution.** A quite sporadic species, known from Austria, Bosnia and Herzegovina, Croatia, Czech Republic, Poland, Serbia, Slovakia, and Switzerland (Krek 1999; Ježek and Omelková 2012; Kvifte et al. 2013; Wagner 2013; Oboňa and Ježek 2014). **New species for Ukraine.**

*Psychogella* Ježek, 1983

12. *Jungiella (Psychogella) bohemica* Ježek, 1979

**Material examined.** Uzh River, Nevyts'ke near bridge, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22502, NMPC.

**Distribution.** A rare species, known only from Czech Republic, Greece, and Germany (Ježek 1979; Ježek and Goutner 1995; Wagner 2013). **New species for Ukraine.**

*Parajungiella* Vaillant, 1972

13. *Parajungiella longicornis* (Tonnoir, 1919)

**Material examined.** Lyuta River, above Chornoholova, 27.v.2016, 1M, O Ma H leg., slide Inv. No. 22611, NMPC.

**Distribution.** Generally, a very common European and west Siberian species (Ježek 1992). In Europe, known from Austria, Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece,

Hungary, Ireland, Norway, Poland, Russia, Slovakia, Sweden, and the Netherlands (Ježek 1992; Ježek and Goutner 1995; Krek 1999; Kvitte et al. 2011, 2013). **New species for Ukraine.**

## TRICHOPSYCHODINA

*Philosepedon* Eaton, 1904

*Philothreticus* Krek, 1999

### 14. *Philosepedon (Philothreticus) soljani* Krek, 1971

**Material examined.** Hropynets' Stream, 7.vi.1996, 1M, C leg., slide Inv. No. 12217, NMPC.

**Distribution.** A very rare species, so far known only from Bosnia and Herzegovina, Czech Republic, Finland, and Slovenia (Krek 1999; Salmela et al. 2014; Kroča and Ježek 2015). **New species for Ukraine.**

*Threticus* Eaton, 1904

*Threticus negrobovi* Vaillant, 1972

**Published records.** Vaillant (1972): 104 (Taberda in Russian Caucasus, not Ukraine, compare with Wagner 1990, 2013)

**Distribution.** Very rare mountain species, so far known from Czech Republic, Slovenia, Slovakia, Russia, Ukraine (**error**), Abkhazia and Georgia (Ježek 2004; Kroča and Ježek 2015).

**Note.** *Threticus negrobovi* Vaillant, 1972 was mentioned in the Palaearctic catalogue of moth flies from South European territory (Ukraine) based on a record from Teberda village (Wagner 1990; Taberda 2000 m a.s.l. sensu Vaillant 1972). This village is, however, in the Russian part of the Caucasus and the listing from the Ukraine must be regarded as an error.

## PSYCHODINI

*Chodopsycha* Ježek, 1984

### 15. *Chodopsycha lobata* (Tonnoir, 1940)

**Material examined.** Preluchnyi Stream, bridge above the water reservoir, 10.viii.2015, 1F, O Ma H leg., slide Inv. No. 22513, NMPC.

**Distribution.** A very common European (Transcaucasian) species, present in lowlands as well as in hills and mountains; associated with fungi. In Europe, known from

Belgium, Bulgaria, France, Germany, Great Britain, Hungary, Ireland, Italy, Norway, and Slovenia (Kvifte et al. 2011; Ježek and Omelková 2012; Ježek et al. 2013). **New species for Ukraine.**

*Logima* Eaton, 1904

16. *Logima satchelli* (Quate, 1955)

**Material examined.** Preluchnyi Stream, bridge above the water reservoir, 10.viii.2015, 1F, O Ma H leg., slide Inv. No. 22515; Shypoty Stream, tributary of the Shypot River near hydroelectric power plant, 11.viii.2015, 1F, O Ma H leg., slide Inv. No. 22500, all NMPC.

**Distribution.** A common Holarctic species. In Europe, known from e.g. Austria, Czech Republic, Ireland, Italy, Norway, Russia, Slovakia, Slovenia, Sweden, the Netherlands, and former Yugoslavia; Canada, U.S.A. (Ježek and Goutner 1995; Svensson 2009; Kvifte et al. 2011). **New species for Ukraine.**

*Psycha* Ježek, 1984

17. *Psycha grisescens* (Tonnoir, 1922)

**Material examined.** Synevir – Polyans'ke Lake, 3.vi.1996, 1F, C leg., slide Inv. No. 122241; Hropynets' Stream, 7.vi.1996, 1F, C leg., slide Inv. No. 12221, all NMPC.

**Distribution.** A European and North African species. Recorded from Algeria, Austria, Belgium, Bosnia and Herzegovina, Czech Republic, Denmark, the Faroe Islands, France, Finland, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Mallorca, Morocco, the Netherlands, Norway, Slovakia, Slovenia, Sweden, Tunisia, and Turkey (Andersen 1999; Ježek 2004; Ježek and Yažci 2005; Salmela et al. 2014; Kvifte et al. 2016). **New species for Ukraine.**

*Psychodocha* Ježek, 1984

18. *Psychodocha cinerea* (Banks, 1894)

**Material examined.** Preluchnyi Stream, bridge above the water reservoir, 10.viii.2015, 1F, O Ma H leg., slide Inv. No. 225145, NMPC.

**Distribution.** A very common cosmopolitan species. Known from Austria, Azores, Belgium, Bosnia and Herzegovina, Bulgaria, Canary Islands, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy (incl. Sardinia), Madeira, the Netherlands, Norway, Poland, Romania, Russia, Serbia,

Slovakia, Slovenia, Spain, Sweden, Switzerland, and Turkey; Abkhazia, Afghanistan, Africa mer., Algeria, Argentina, Australia, Azores, Brazil, Canada, Chile, Iran, Israel, Juan Fernández Islands, New Zealand, Puerto Rico Islands, Tunisia, U.S.A. (Krek 1985; Ježek and Goutner 1995; Ježek and Yağci 2005; Kvifte et al. 2011; Wagner 1990, 2013; Salmela et al. 2014). **New species for Ukraine.**

### 19. *Psychodocha gemina* (Eaton, 1904)

**Material examined.** Preluchnyi Stream, bridge above the water reservoir, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22512; Shypot River, below the hydroelectric power plant, 10.viii.2015, 1F, O Ma H leg., slide Inv. No. 22517; Lyuta River, above Chornoholova, 11.viii.2015, 1F, O Ma H leg., slide Inv. No. 22492; Uzh River, at the last building in Stuzhytsya, 12.viii.2015, 1F, O Ma H leg., slide Inv. No. 22507, all NMPC.

**Distribution.** A common European species. Recorded from Austria, Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Great Britain, Denmark, France, Finland, Germany, Greece, Hungary, Ireland, Norway, Romania, Serbia, Slovakia, Slovenia, Spain, Switzerland, and the Netherlands (Ježek and Goutner 1995; Krek 1999; Ježek 2002; Kvifte et al. 2011, 2013; Salmela et al. 2014). **New species for Ukraine.**

### *Psychodula* Ježek, 1984

#### 20. *Psychodula minuta* (Banks, 1894)

**Material examined.** Uzh River, at the last building in Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22510, NMPC.

**Distribution.** A generally common Holarctic species. Recorded from Abkhazia, Austria, Belgium, Britain, Bulgaria, Cyprus, Czech Republic, Denmark, France, Finland, Germany, Greece, Hungary, Ireland, Italy (incl. Sardinia), Madeira, the Netherlands, Norway, Romania, Slovakia, Slovenia, Spain (incl. Balearic Islands), Sweden, and Switzerland; Canada, Syria, U.S.A. (Ježek 1990; Wagner 2013; Salmela et al. 2014). **New species for Ukraine.**

### *Psychomora* Ježek, 1984

#### 21. *Psychomora mycophila* (Vaillant, 1988)

(syn. *vanharai* Ježek, 1995)

**Material examined.** Shypoty Stream, tributary of the Shypot River near hydroelectric power plant, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22497, NMPC.

**Distribution.** A rare species associated with fungi, so far known only from Czech Republic, France, Slovakia, Slovenia, and Switzerland (Ježek and Omelková 2012). **New species for Ukraine.**

## 22. *Psychomora trinodulosa* (Tonnoir, 1922)

**Material examined.** Shypotyck Stream, tributary of Shypot River near hydroelectric power plant, 11.viii.2015, 1F, O Ma H leg., slide Inv. No. 22499, NMPC.

**Distribution.** A very common Holarctic species. Known from Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greek mainland, Hungary, Ireland, Italy, the Netherlands, Norway, Poland, Romania, Russia, Sardinia, Slovakia, Slovenia, Spanish mainland, and Sweden; Algeria, U.S.A. (Ježek 1990; Wagner 2013; Salmela et al. 2014). **New species for Ukraine.**

## *Tinearia Schellenberg, 1803*

### 23. *Tinearia alternata* (Say, 1824)

**Material examined.** Uzh River, Nevyts'ke near bridge, 10.viii.2015, 1F, O Ma H leg., slide Inv. No. 22501, NMPC.

**Distribution.** A cosmopolitan species, generally very common. Known from Austria, Balearic Is., Belgium, Bulgaria, Canary Is., Crete, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greek mainland, Hungary, Ireland, Italy, Madeira, Norway, Poland, Romania, Sardinia, Slovenia, Spanish mainland, Sweden, Switzerland, the Netherlands, and Norway; Afghanistan, whole Africa, Australia, Bangladesh, Borneo, Canal Zone, Cape Verde Islands, Philippines, Hawaii, India, Jamaica, Japan, Macquarie Islands, Malaysia, Micronesia, Mongolia, New Zealand, North and South America, North Korea, Puerto Rico, Ryukyu Islands, Samoa, Socotra Island, Taiwan, Trinidad (Ježek 1981; Ježek and van Harten 1996; Kvifte et al. 2011; Ježek and Tkoč 2012; Wagner 2013). **New species for Ukraine.**

## PERICOMAINI

### *Berdeniella* Vaillant, 1976

#### 24. *Berdeniella kocii* Ježek, 2006

**Material examined.** Shypot River, below hydroelectric power plant, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22519; Shypotyck Stream, tributary of the Shypot River near hydroelectric power plant, 11.viii.2015, 1M, O Ma H leg., slide Inv. No.

22498; Tikhyi Stream, above Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22523; tributary of the Uzh River, near Zahorb, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22524; Paporotnyi Stream, in Stuzhytsya, 12.vii.2015, 1M, O Ma H leg., slide Inv. No. 22495 – all slides deposited in NMPC.

**Distribution.** A rare mountain species, known only from Czech Republic and Slovakia (Ježek 2006, 2009; Ježek and Omelková 2012). **New species for Ukraine.**

## 25. *Berdeniella matthesi* (Jung, 1954)

**Material examined.** Synevir – Polyans'ke Lake, 3.vi.1996, 1M, C leg., slide Inv. No. 12223, NMPC.

**Distribution.** A locally common species in Europe, the knowledge on its distribution is, however, quite poor: so far, it has been collected in Austria, Czech Republic, Germany, Italy, and Slovakia (Ježek and Omelková 2012; Kroča and Ježek 2015). **New species for Ukraine.**

## 26. *Berdeniella stavniensis* (Krek, 1969)

**Material examined.** Synevir – Polyans'ke Lake, 3.vi.1996, 1M, C leg., slide Inv. No. 12222; Hropynets' Stream, 7.vi.1996, 1M, C leg., slide Inv. No. 12219, all NMPC.

**Distribution.** European species, apparently not quite common, known from Austria, Bosnia and Herzegovina, Czech Republic, France, Germany, Serbia, and Slovakia (Krek 1999; Ježek 2003). **New species for Ukraine.**

## 27. *Berdeniella vimmeri* Ježek, 1997

**Material examined.** Hropynets' Stream, 7.vi.1996, 1M, C leg., slide Inv. No. 12233, NMPC.

**Distribution.** A central European species (knowledge of distribution is poor so far); known from Czech Republic and Slovakia (Ježek 1997, 2009; Ježek and Omelková 2012). **New species for Ukraine.**

## *Clytocerus* Eaton, 1904

## *Boreoclytocerus* Duckhouse, 1978

## 28. *Clytocerus (Boreoclytocerus) longicorniculatus* Krek, 1987

**Material examined.** Sukchyi Stream, above Kam'yanytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22521, NMPC.

**Distribution.** Probably not common European species. Known only from Bosnia and Herzegovina, Czech Republic, and Slovakia (Krek 1999; Ježek 2009; Ježek et al. 2013). **New species for Ukraine.**

**29. *Clytocerus (Boreoclytocerus) ocellaris* (Meigen, 1804)**

**Material examined.** Lyuta River, above Chornoholova, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22494, NMPC.

**Distribution.** A very common central and western European species. Known from Austria, Belgium, Great Britain, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Lithuania, the Netherlands, Norway, Poland, Sardinia, Slovenia, and Switzerland (Wagner 2013; Ježek et al. 2014). **New species for Ukraine.**

***Pericoma* Walker, 1856**

***Pachypericoma* Vaillant, 1978**

**30. *Pericoma (Pachypericoma) blandula* Eaton, 1893**

**Material examined.** Tereblya River, below Kolochava, 3.vi.1996, 1M, C leg., slide Inv. No. 12230; Hropynets' Stream, 7.vi.1996, C leg., slide Inv. No. 12234; Uzh river, above Uzhgorod, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22520; Tikhyi Stream, above Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22484; tributary of the Uzh River, near Zahorb, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22525; Paprotnyi Stream, in Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22496; Sukhyi Stream, above Kam'yanytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22522, all slides deposited in NMPC.

**Distribution.** Species widespread in Europe and recorded also in Transcaucasia, Tunisia, and Morocco. In Europe, known from Austria, Belgium, Bosnia and Herzegovina, Croatia, Great Britain, Bulgaria, Czech Republic, Denmark, European Turkey, Finland, France, Germany, Greek mainland, Hungary, Ireland, Italy, Macedonia, Montenegro, the Netherlands, Norway, Poland, Romania, Russia, Sardinia, Serbia, Slovakia, Slovenia, Spanish mainland, Sweden, and Switzerland (Ježek 2004; Kvitte et al. 2011, 2013). **New species for Ukraine.**

**31. *Pericoma (Pachypericoma) fallax* Eaton, 1893**

**Material examined.** Tereblya River, below Kolochava, 3.vi.1996, 1M, C leg., slide Inv. No. 12229; Shypot River, below hydroelectric power plant, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22518; Kamenychky Stream, below Novoselytsya, 11.viii.2015,

1M, O Ma H leg., slide Inv. No. 22485; Tikhyi Stream, above Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22483, all slides deposited in NMPC.

**Distribution.** A European and western Siberian species. In Europe, known from Belgium, Bosnia and Herzegovina, Czech Republic, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Macedonia, Montenegro, the Netherlands, Slovenia, and Spain (Ježek 1992, 2004; Wagner 2013). **New species for Ukraine.**

### 32. *Pericoma (Pachypericoma) nielseni* Kvifte, 2010

Syn: *Pericoma formosa* Nielsen, 1964

**Material examined.** Tereblya River, below Kolochava, 3.vi.1996, 1M, C leg., slide Inv. No. 12232; Uzh River, Nevyts'ke near bridge, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22504, all in NMPC.

**Distribution:** Not common European species, known from Czech Republic, Denmark, Finland, France, Norway, and Slovakia (Ježek 2009; Kvifte 2010; Kvifte et al. 2011). **New species for Ukraine.**

*Pericoma s. str.*

### 33. *Pericoma (Pericoma) exquisita* Eaton, 1893

**Material examined.** Uzh River, Nevyts'ke near bridge, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22503; Lyuta River, above Chornoholova, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22493; Uzh River, at the last building in Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22509, all in NMPC.

**Distribution.** Species widespread in Europe, North Africa, and Transcaucasia (Armenia). In Europe, known from Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Czech Republic, Crete, Croatia, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Macedonia, Montenegro, Poland, Serbia, Slovakia, Slovenia, and Spain (Ježek 2004, 2009; Kvifte et al. 2013; Wagner 2013; Oboňa and Ježek 2014). **New species for Ukraine.**

*Pneumia* Enderlein, 1935

### 34. *Pneumia crispi* (Freeman, 1953)

**Material examined.** Lubnya River, below Lubnya, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22505, NMPC.

**Distribution.** A rare European species. So far recorded from Bosnia and Herzegovina, Czech Republic, France, Germany, Great Britain, Greece, Hungary, Macedonia, Romania, and Slovakia (Ježek and Omelková 2012). **New species for Ukraine.**

**35. *Pneumia gracilis gracilis* (Eaton, 1893)**

**Material examined.** Strychavka Stream, below Strychava, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22516; Tikhyi Stream, above Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22482, all in NMPC.

**Distribution.** A species published from several countries, not too frequent in Europe: Albania, Belgium, Bosnia and Herzegovina, Czech Republic, France, Germany, Great Britain, Greece, Italy, Serbia, Slovakia, and Slovenia; Transcaucasia: Abkhazia (Ježek 2002, 2004; Ježek et al. 2012). **New species for Ukraine.**

**36. *Pneumia mutua* (Eaton, 1893)**

**Material examined.** Synevir – Polyans'ke Lake, 3.vi.1996, 1M, C leg., slide Inv. No. 12227; Hropynets' Stream, 7.vi.1996, 1M, C leg., slide Inv. No. 12235, all in NMPC.

**Distribution.** A common species distributed largely in western and central Europe, and Scandinavia; known from Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, the Netherlands, Norway, Poland, Slovakia, and Slovenia (Kvifte et al. 2011, 2013; Oboňa and Ježek 2014). **New species for Ukraine.**

**37. *Pneumia nubila* (Meigen, 1818)**

**Material examined.** Lyuta River, above Chornoholova, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22489; 27.v.2016, 1M, O Ma H leg., slide Inv. No. 22609, all in NMPC.

**Distribution.** A very common species, recorded from throughout Europe and the Canary Islands. Known from Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Luxembourg, Macedonia, Montenegro, Poland, Romania, Sardinia, Serbia, Slovakia, Slovenia, Spain, Switzerland, and the Netherlands (Ježek and Goutner 1995; Krek 1999; Ježek 2002; Kvifte et al. 2013). **New species for Ukraine.**

**38. *Pneumia stammeri* (Jung, 1956)**

**Material examined.** Pylypets' River, 2.vi.1996, C leg., 1M, slide Inv. No. 12228; Synevir – Polyans'ke Lake, 3.vi.1996, C leg., 1M, slide Inv. No. 12226, all in NMPC.

**Distribution.** A quite rare European species known from Austria, Belgium, Bosnia, Czech Republic, Denmark, France, Finland, Germany, Great Britain, Hungary, Norway, Slovakia, and Sweden (Ježek 2003; Kvitte et al. 2011; Salmela et al. 2014). **New species for Ukraine.**

### 39. *Pneumia trivialis* (Eaton, 1893)

**Material examined.** Lyuta River, above Chornoholova, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22491; Tikhyi Stream, above Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22481; tributary of the Uzh River, near Zahorb, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22488, all slides deposited in NMPC.

**Distribution.** A very common European species. Known from Austria, Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, the Netherlands, Norway, Poland, Serbia, Slovakia, Slovenia, Spain, Sweden, and Switzerland (Krek 1999; Ježek 2002; Kvitte et al. 2011, 2013). **New species for Ukraine.**

### 40. *Pneumia tjentistensis* (Krek, 1969)

**Material examined.** Hropynets' Stream, 7.vi.1996, 1M, C leg., slide Inv. No. 12218, NMPC.

**Distribution.** So far known only from Bosnia and Herzegovina, and Czech Republic (Krek 1999; Ježek and Omelková 2012). **New species for Ukraine.**

## *Ulomyia* Walker, 1856

### 41. *Ulomyia cognata* (Eaton, 1893)

**Material examined.** Synevir – Polyans'ke Lake, 3.vi.1996, 1M, C leg., slide Inv. No. 12225; Hropynets' Stream, 7.vi.1996, 1M, C leg., slide Inv. No. 12220; Lubnya River, below Lubnya, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22506; tributary of the Uzh River, near Zahorb, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22487; Uzh River, at the last building in Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22508, all slides deposited in NMPC.

**Distribution.** A common European species known from Austria, Czech Republic, Finland, France, Germany, Great Britain, Italy, Lithuania, Poland, Slovakia, and Slovenia (Ježek and Omelková 2012; Salmela et al. 2014). **New species for Ukraine.**

#### 42. *Ulomyia fuliginosa* (Meigen, 1804)

**Material examined.** Preluchnyi Stream, bridge above the water reservoir, 10.viii.2015, 1M, O Ma H leg., slide Inv. No. 22511; Lyuta River, above Chornoholova, 11.viii.2015, 1M, O Ma H leg., slide Inv. No. 22490; Tikhyi Stream, above Stuzhytsya, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22480; tributary of the Uzh River, near Zahorb; same, 12.viii.2015, 1M, O Ma H leg., slide Inv. No. 22486, all slides deposited in NMPC.

**Distribution.** A very common and often frequently recorded European species, known from Austria, Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Kosovo, Macedonia, the Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, and Switzerland (Krek 1999; Ježek 2002; Kvitte et al. 2011, 2013; Salmela et al. 2014).

**New species for Ukraine.**

### SYCORACINAE

#### *Sycorax* Haliday in Curtis, 1839

#### 43. *Sycorax silacea* Haliday in Curtis, 1839

**Material examined.** Turiya River above Simer, 27.v.2016, 1M, O Ma H leg., slide Inv. No. 22613, NMPC.

**Distribution.** European species, locally common; known from Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Norway, Poland, Romania, Slovakia Sweden, and Switzerland (Ježek 1996; Krek 1999; Kvitte et al. 2013; Wagner 2013; Salmela et al. 2014). **New species for Ukraine.**

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## References

- Andersen T (1999) Moth flies (Diptera, Psychodidae) from the Faroes. *Norwegian Journal of Entomology* 46: 66.
- Artemiev MM, Neronov VM (1984) Distribution and ecology of sandflies of the Old World (genus *Phlebotomus*). The USSR committee for the UNESCO programme on man and the biosphere (MAB). Institute of evolutionary morphology and animal ecology USSR Academy of Sciences, Moscow, 207 pp. [in Russian]
- Ježek J (1979) *Jungiella bohémica* sp. n. from Czechoslovakia (Diptera, Psychodidae). *Acta entomologica bohemoslovaca* 76: 341–344.
- Ježek J (1981) Taxonomy of tribes Mormiini End., Paramormiini End. and Psychodini End. (Diptera, Psychodidae) of Czechoslovakia. Dissertation Thesis, Department of Entomology, Faculty of Natural Sciences, Charles University, Praha, Vol. I, 453 pp., Vol. II, 9 pp. [40 tables of figures; In Czech, completely unpubl. manuscript]
- Ježek J (1987) *Jungiella hygrophila* sp. n. (Diptera, Psychodidae, Paramormiini) with redescrptions of Czechoslovak species of *Jungiella* s. str. *Acta Entomologica Musei Nationalis Pragae* 42: 207–223.
- Ježek J (1990) Redescrptions of nine common Palaearctic and Holarctic species of Psychodini End. (Diptera: Psychodidae). *Acta Entomologica Musei Nationalis Pragae* 43: 33–83.
- Ježek J (1992) Siberian moth flies (Diptera, Psychodidae) – new faunistic records. *Časopis Narodního Muzea, Řada přírodovědná* 160: 63–64.
- Ježek J (1996) New faunistic records of moth flies (Diptera, Psychodidae) from the Czech Republic. *Časopis Narodního Muzea, Řada přírodovědná* 165: 113–120.
- Ježek J (1997) Moth flies (Diptera, Psychodidae) inhabiting Prague city and adjacent localities with descriptions of five new species. *Acta Entomologica Musei Nationalis Pragae* 44 (1995): 125–165.
- Ježek J (2002) The first account of Slovenian moth flies (Psychodidae, Diptera). *Časopis Národního Muzea, Řada přírodovědná* 171: 89–116.
- Ježek J (2003) New faunistic data and check list of non phlebotomine moth flies (Diptera, Psychodidae) from the Czech and Slovak Republics. *Časopis Narodního Muzea, Řada přírodovědná* 172: 121–132.
- Ježek J (2004) New faunistic data of non-phlebotomine moth flies (Diptera: Psychodidae) from the Palearctic Region. *Folia Facultatis Scientiarum Naturalium Universitatis Masarykianae Brunensis, Biologia* 109: 141–151.
- Ježek J (2006) Psychodidae (Diptera) of the Jeseníky Protected Landscape Area and its environs with descriptions of two new *Berdeniella* species from the Czech Republic. *Acta Entomologica Musei Nationalis Pragae* 46: 151–192.
- Ježek J (2009) Further new faunistic records of moth flies (Insecta, Diptera, Psychodidae) from the Czech Republic and Slovakia. *Folia faunistica Slovaca* 14(15): 101–105.
- Ježek J, Goutner V (1995) Psychodidae (Diptera) of Greece. *Acta Musei Nationalis Pragae, Series B, Historia Naturalis* 50 (1994) (1–4): 107–124.
- Ježek J, van Harten A (1996) Psychodidae (Diptera) of the Cape Verde Islands, captured with a Johnson-Taylor suction trap. *Boletim do Museu Municipal do Funchal* 48(266): 63–86.

- Ježek J, Kubík Š, Barták M (2013) Psychodidae (Diptera) of Vráž nr. Písek (Czech Republic), 189–199. In: Kubík Š, Barták M (Eds) Workshop on biodiversity, Jevany. Česká zemědělská univerzita v Praze, 436 pp.
- Ježek J, Lukáš J, Kvitte GM, Oboňa J (2012) New faunistic records of non-biting moth flies (Diptera: Psychodidae) from the Czech Republic and Slovakia. *Klapalekiana* 48: 121–126.
- Ježek J, Omelková M (2012) Moth flies (Diptera: Psychodidae) of the Bílé Karpaty Protected Landscape Area and Biosphere Reserve (Czech Republic). In: Malenovský I, Kment P, Konvička O (Eds) Species inventories of selected insect groups in the Bílé Karpaty Protected Landscape Area and Biosphere Reserve (Czech Republic). *Acta Musei Moraviae, Scientiae biologicae*, Brno 96(2): 763–802.
- Ježek J, Omelková M, Heřman P (2014) Moth flies (Psychodidae, Diptera) of the Český kras/ Bohemian Karst and neighbouring localities (Czech Republic). *Bohemia centralis*, Praha 32: 321–344.
- Ježek J, Tkoč M (2012) A new species of the genus *Gondwanoscurus*, and two new records of non-biting moth flies (Diptera: Psychodidae: Psychodinae) from Socotra Island. *Acta Entomologica Musei Nationalis Pragae* 52 (supplementum 2): 545–557.
- Ježek J, Yağci Ş (2005) Common non-biting moth flies (Insecta, Diptera, Psychodidae) new to the fauna of Turkey. *Acta parasitologica Turcica* 29: 188–192.
- Krek S (1985) Einige neue Psychodinae – Arten (Psychodidae, Diptera) aus Serbien. *Proceedings on the Fauna of SR Serbia, Belgrade* 3: 183–191.
- Krek S (1999) Psychodidae (Diptera Insecta) Balkanskog Poluotoka. *Federacija Bosne i Hercegovine, Ministarstvo Obrazovanja, Nauke, Kulture i Sporta, Studentska Štamparija Univerziteta Sarajevo, Sarajevo*, 417 pp.
- Kročá J, Ježek J (2015) Moth flies (Diptera: Psychodidae) of the Moravskoslezské Beskydy Mts and the Podbeskydská pahorkatina Upland, Czech Republic. *Acta Musei Silesiae, Scientiae Naturales* 64: 27–50.
- Kvitte GM (2010) *Pericoma nielseni* nom. nov., a replacement name for *Pericoma formosa* Nielsen, 1964, preoccupied by *Pericoma formosa* Meunier, 1905 (Diptera: Psychodidae). *Zootaxa* 2726: 68.
- Kvitte GM, Håland Ø, Andersen T (2011) A revised check-list of Norwegian moth flies (Diptera, Psychodidae). *Norwegian Journal of Entomology* 58: 180–188.
- Kvitte GM, Ivković M, Klarić A (2013) New records of moth flies (Diptera: Psychodidae) from Croatia, with the description of *Berdeniella keroveci* sp.nov. *Zootaxa* 3737(1): 57–67. <https://doi.org/10.11646/zootaxa.3737.1.4>
- Kvitte GM, Stokkan M, Wagner R (2016) Review of the Psychodinae from Mallorca, Spain, with description of *Pericoma unipennata*, sp. n. (Diptera, Psychodidae). *ZooKeys* 577: 149–160. <https://doi.org/10.3897/zookeys.577.7679>
- Lewis DJ (1982) A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bulletin of the British Museum (Natural History). Entomology* 45(2): 121–209.
- Oboňa J, Ježek J (2014) Prodrómus of moth flies (Diptera: Psychodidae) from Slovakia. *Acta Musei Silesiae Scientiae Naturales* 63: 193–251. <https://doi.org/10.2478/cszma-2014-0020>
- Perfil'ev PP (1966) Phlebotomidae (Diptera). *Fauna SSSR* 3(2): 1–383. [in Russian]

- Sabrosky CW (1999) Family-group names in Diptera. An annotated catalog. In: Thompson FC (Ed.) Family-group names in Diptera and bibliography. *Myia* 10: 1–360.
- Salmela J, Paasivirta L, Kvifte GM (2014) Checklist of the families Chaoboridae, Dixidae, Thaumaleidae, Psychodidae and Ptychopteridae (Diptera) of Finland. *ZooKeys* 441: 37–46. <https://doi.org/10.3897/zookeys.441.7532>
- Svensson BW (2009) Fjärilsmyggfauna i ett hagmarksområde och en ladugård i östra Blekinges skogsland. Med en översikt av familjen Psychodidae:s morfologi, systematik och utforskande, samt särskilt de svenska *Psychoda* s. l. – arternas biologi. *Entomol Tidskrift* 130: 185–206.
- Tkoč M, Pecharová M, Ježek J (2014) Catalogue of the type specimens of Diptera deposited in the Department of Entomology, National Museum, Prague, Czech Republic. Moth flies (Psychodidae). *Acta Entomologica Musei Nationalis Pragae* 54: 789–837.
- Vaillant F (1971–1983) Psychodidae–Psychodinae (not finished). In: Lindner E (Ed.) Die Fliegen der palaearktischen Region. Stuttgart, 287, (1971), 1–48; 291, (1972), 49–78; 292, (1972), 79–108; 305, (1974), 109–142; 310, (1975), 143–182; 313, (1977), 183–206; 317, (1978), 207–238; 320, (1979), 239–270; 326, (1981), 271–310; 328, (1983), 311–357.
- Wagner R (1990) Family Psychodidae. In: Soós A (Ed.) Catalogue of Palaearctic Diptera, Vol. 2, Psychodidae – Chironomidae. Akadémiai Kiadó, Budapest, 11–65.
- Wagner R (2013) Fauna Europaea: Psychodidae. In: Jong H de (Ed.) Fauna Europaea: Diptera Nematocera. Fauna Europaea version 2.4. <http://www.faunaeur.org> [accessed 2.2.2016]

# A commentary on the practice of using the so-called typeless species

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## Abstract

The fears expressed by Santos et al. (2016) that description of typeless species (new species described based on field photographs) can be fatal for the practice of taxonomy which will succumb to an uncontrollable stream of “species of questionable delimitation” are, in our opinion, exaggerated. The Code already protects taxonomic practice from subjectivity quite well by limiting opportunities for descriptions of new species based on field photos by rigid requirements, and only skilled taxonomists with extensive knowledge of a group are capable of fulfilling them. If a taxonomist has omitted to compare the new typeless species with the known species externally similar to it, the latter cannot be diagnosed and its name in that case becomes *nomen nudum*. Typeless species can coincide with species described earlier, but can represent a new species differing in internal features. To describe typeless species without infringement of Article 13.1 a taxonomist should compare this species to all related and similar species described earlier.

## Keywords

ICZN, *nomen nudum*, nomenclatural type, nomenclature, typeless and standard species

## Introduction

In 2015, Marshall and Evenhuis published a description of a new African species *Marleyimyia xylocopae* (Diptera, Bombyliidae), which was based on a photograph taken in the field. The following year another article was published with a description of a new species *Nothybus absens* (Diptera, Nothybidae) based on two photographs taken in China (Lonsdale and Marshall 2016). This species also does not have a preserved holotype. To designate a species described without a reference to type specimens deposited in the collection, we will use the term ‘typeless species’ (Santos et al. 2016).

Marshall and Evenhuis (2015, p. 119) have clearly specified the two conditions that should be followed when describing a species on the basis of photographs alone, without a preserved specimen as a holotype: “even in the absence of a collected type specimen, current technologies such as high-resolution photography can often provide enough information for a proper description resulting in a readily recognizable and unequivocally distinct newly named species”. Therefore, a photograph should (1) provide enough information (2) to differentiate unequivocally new species from other already described species. However, a photograph can turn out to be insufficient when internal structures and other morphological features not shown in the photograph are used for species discrimination (Cianferoni and Bartolozzi 2016, p. 129). In such cases it simply cannot be used for the description of species.

It is important to emphasize one more detail. Aside from its valuable function as the bearer of a taxon name (Article 61.1: “Each nominal taxon in the family, genus or species groups has actually or potentially a name-bearing type”), the type also plays an equally important role of being the carrier of objective data about a species. This function allows one to reevaluate the adequacy and accuracy of initial description of a species by a repeated study of its holotype. Consequently, the need for this function can be partly dismissed if a series of high quality photographs are used since they convey the information about a species more accurately in comparison to verbal and written descriptions.

According to the second condition, a taxonomist should discriminate the “new insect species described and named solely on the basis of field photographs of the type specimen” from all the previously described known species (Marshall and Evenhuis 2015, p. 119); in this case the type of the new species is a specimen illustrated in the photograph (article 73.1.4). Surprisingly, the Code specifically demands a fulfillment of this condition for description of species by photograph, and this will be shown later. As for the claimed impossibility of strict discrimination of a species in case of finding a sibling species in the future (Cianferoni and Bartolozzi 2016), this problem is not new to systematics. As Marshall and Evenhuis (2016, p. 88) have emphasized, “every time we examine a previously named species in the course of a revision there is a possibility that it will turn out to be two or more species, and that the additional species will be undescribed. A normal part of that process is to figure out which of the multiple species corresponds with the original name”. In case of sibling species, we cannot unequivocally establish which of two species corresponds with the original name. This case is also not a serious issue for a taxonomist. For example, a putatively new insect

species has been discovered, which, when relying solely on the description, does not differ from another species described earlier, but nothing but a pin has remained from a type specimen of the latter. In this case the entomologist must formally connect one of the two species with an earlier described species at his own discretion.

At the same time we cannot completely agree with another statement of the authors (Marshall and Evenhuis 2015, p. 120), that “We expect that such descriptions [of species without preserved type specimens] that do not render new species unequivocally recognizable will be rejected, just as they should be if they were based on dead type specimens”. The first part of the statement is supported by the Code, as we will show later. As to the second part of sentence, the Code does not impose any restrictions on the traditional practice of the description of species. In any case, in the differential diagnosis does not demand full discrimination of a new species from all the previously described species.

Among entomologists, the reaction to the practice of describing new species based on a photograph only, without the specimens deposited in a collection, was both positive (Pape 2016) and negative (Santos et al. 2016).

### **Critical comments on the practice of description of typeless species**

The publication of Marshall and Evenhuis (2015) has been critically analyzed by a collective of authors (Santos et al. 2016, p. 513), who have concluded that “the idea of associating names with beautiful species of questionable delimitation based only on photographs may be highly damaging to the practice of taxonomy” that “adjustments and corrections to the ICZN (Anonymous 1999; further referred to as the Code, or ICZN) especially to Article 73.1.4, are necessary and urgent. A modification to the ICZN would prevent the creation of other species names based solely on illustrations or photographs without real and proper type specimens”. Amorim et al. (2016, p. 125) have also expressed disagreement with the practice of description of species without having a preserved holotype: “The spirit of the International Code of Zoological Nomenclature is willfully violated by a description based only on a photograph”. In this work authors analyzed the answers given by Marshall and Evenhuis (2015) to critical remarks addressed to them. In their critical analysis Amorim et al. (2016, p. 123) moved from the two following positions: “1. A specimen, as a standard of its species, has infinitely more fidelity than images thereof...”, and “2. Subsequent examination of an image is unlikely to find any new characters beyond what the pixels that were originally captured have already revealed”.

We completely agree with these statements. However, at the same time these fix the particular nomenclatural status of typeless species (the term offered by Santos et al. 2016), which is connected with serious restrictions in a choice of traits to be described. These restrictions have a double nature. Firstly, these species can be described by a finite set of mainly external characteristics. This means that from the taxonomic standpoint the information provided by a photograph can be insufficient for promot-

ing a hypothesis about a new species. If, for example, some species in a group differ in the structure of genitalia, then a photograph will be insufficient to make a judgement about the species shown in it. This is the simplest case. Secondly, there are restrictions imposed by the Code. Upon instigating the description of a species by a photograph, a taxonomist should understand whether he infringes any articles of the Code. In other words, he should solve a following question: what requirements are made by the Code for the practice of the description of species by photographs? This question will be central in our analysis.

Löbl et al. (2016, p. 84) have expressed misgivings concerning the practice of description of species by a photo believing that “Taxonomy might be threatened because of the increasing power and availability of digital photography, improving one’s chances of quickly capturing images of animals, without carefully studying the animal themselves”. This conclusion has something in common with the opinion of Cianferoni and Bartolozzi (2016, p. 129): “These new practices could make it much easier to describe new species, and this in turn would contribute to the increasing involvement of unsuitably qualified people. A number of inexperienced naturalists or even nature photographers with no biological training could begin to submit descriptions to journals, based only on photographs”.

Entomologists were not the first who ventured to describe new species without type specimens deposited in a collection before scientific community. Earlier three works with such descriptions of new species of primates have been published: the mangabey *Lophocebus kipunji* (Jones et al. 2005), the lemur *Avahi cleesei* (Thalmann and Geissmann 2005); and the capuchin *Cebus queirozi* (Mendes Pontes et al. 2006). These works also were a subject of active discussion. Its results have been summarized by Dubois and Nemésio (2007): “Whereas some authors (Timm et al. 2005, Landry 2005, Oliveira and Langguth 2006) stated that the *nomina* of these taxa [the new species of primates mentioned above and some others], if published after 1999, are nomenclaturally unavailable, others (Wakeham-Dawson et al. 2002, Polaszek et al. 2005) defended the opposite idea”.

Timm et al. (2005, p. 2163) wrote “The photographs are not valid substitutes for a type specimen. The function of a type specimen in nomenclature is to provide an objective basis for the application of a species-group name”. While agreeing with these thoughts, it is nonetheless necessary to note that this objective basis becomes actual only in the presence of material for comparison. However, what if this material is absent? Or if a species is very rare? If someone had the luck to photograph it, we cannot wait for a next happy opportunity to see it. Here is a very symptomatic example that testifies to the rarity of some dipterous species. The flat-footed fly *Agathomyia auran-tiaca* (Bezzi, 1893) (Diptera, Platypezidae) was described on a sole male from Monte Baldo (Northern Italy), caught 19. IX.1891 on a leaf of coltsfoot *Tussilago farfara* L., 1753. While this record of this species is still the only one for Europe (Chandler 2001, p. 135), our colleague Michal Tkoč (Natural History Museum, Praha) kindly informed us that he found one specimen of this species caught at the beginning of the

last century and stored in MNHN (Paris). The only small series of males and females of this species had been collected in the vicinities of the town of Zeya (the Amur area). Male *A. aurantiaca* possess a unique coloring, and can be easily differentiated from all the other species based on a photograph.

Of course, it is possible to take a different point of view and claim that there is a shortcoming in the Code, that the mentioned authors had found loopholes in the Code, and that the problem will be resolved if these loopholes are accounted for. We are, however, inclined to take a compromising position. Being the practical document regulating the work of systematists, in a number of cases the Code is forced to go for conciliatory proposals that consider the interests of different sides. The history of description of the highland mangabey *Lophocebus kipunji* (Jones et al. 2005) from southern Tanzania based on two photos shows this well (Polaszek et al. 2005). We completely agree with Polaszek et al. (2005, p. 2166) who wrote “The allowance under the Code for designation of living specimens as holotypes needs to be more widely recognized, given contemporary concerns for the conservation of threatened species. There is no doubt that many newly described taxa will be threatened (*L. kipunji* will be designated as “critically endangered” in the IUCN Red List). Dead animal specimens should not be understood to be essential to the process of establishing new taxa. In such cases, supplementation with evidence such as sonograms and oscillograms of species-specific vocalizations, and molecular information (now readily derived from noninvasive samples, e.g., hair, urine, and feces) may contribute to validation.”

Some conceptual ambiguity is certainly present in the Code. On the one hand, type specimens should be deposited in a collection, yet on the other hand this norm is not always obligatory. However, there is an understanding that it is caused by serious reasons, first of all related to the responsibility of the world community that has set for itself the task of preserving the fauna of our planet: “Due to the declining abundance of many species, access to complete anatomical specimens is becoming a vanishing luxury. (...) For many animal taxa, the lethal collection of such voucher specimens would now also be considered unethical” (Dalebout et al. 2004, p. 459). At the same time there is a danger to taxonomy in that these special cases can become the rule, and in hands of unskilled taxonomists will lead to nomenclatural chaos. That is exactly what the Code should prevent.

### **Typeless species and Article 13.1**

Now, the basic question stated in works of Santos et al. (2016) and by other zoologists touches upon possible damage for taxonomy from the practice of describing of new species based on photographs alone. In our opinion, the expressed apprehensions that many zoologists, and not only these, will instantly go for the opportunity to describe species based on field photographs, and as the result taxonomy will become flooded with typeless species, are a little exaggerated.

In our opinion, Article 13.1 of the Code imposes serious restrictions on the practice of describing of new species based on photographs. It establishes the following requirements with regard to the availability of names for new taxa:

- 13.1. Requirements. To be available, every new name published after 1930 must satisfy the provisions of Article 11 and must
- 13.1.1. be accompanied by a description or definition that states in words characters that are purported to differentiate the taxon...

Accordingly, in some insect groups new species of insects clearly cannot be described with only photographs. Species that are distinguished by structure of genitalia are an obvious example. One may object that, after all, Article 13.1.1 does not mandate that we use genitalia for differentiation of species. For further clarity we provide the following hypothetical example.

Let us consider two species of flies, *A* and *B*, belonging to some genus. Both have been described on the basis of external characters. Let us assume further, that one more species *C* has been found and this species differs from species *B* externally, and from species *A* only in a structure of genitalia. Article 13.1.1 of the Code does not limit taxonomists in the number of characters deemed to be necessary and sufficient for strict differentiation of a new species. That means that if a taxonomist had missed one or several known species in his differential diagnosis for a new species, the Code does not see any nomenclatural infringements. However, if among these missed species there was a species (for example species *A*) which does not differ from a new species *C* in the characteristics used, the consequences will be different for the practice of description of a species based on photograph, vs. the traditional practice of description of a species with a fixed specimen as a holotype. In the second case we simply repeatedly examine holotypes of new (*C*) and known (*A*) species, analyzing all accessible characteristics, and including new ones that were not used earlier for the differentiation of the species. In any case we will receive a certain result: either the species do not differ (in this case the name of a new species becomes a synonym of the known one), or their independent status is confirmed.

In a similar situation, in the case of a species without extant specimens as a holotype we will not receive an unequivocal result. If a new typeless species *C*, about the characteristics of which we judge only by photographs, does not differ from the species *A*, it does not confirm the identity of these species. They may be distinguished by other characters that are invisible in a photograph; for example, by details of genitalia. However, it also does not mean that these species are distinguished, even if the holotype of a new species *C*, i.e., that single specimen portrayed on a photograph, really differs from a known species *A* in the structure of genitalia. It is impossible to solve this issue by having only a photograph of the holotype *C*. Hence it follows that a taxonomist describing a new species by a photograph did not differentiate it, and now it is impossible to correct his mistake. As the result of nonprofessional actions of the taxonomist, the species described by him on the grounds of a photograph cannot be differentiated

in principle, i.e. it cannot be conclusively attributed neither to a new species *C*, nor to the known species *A*. By virtue of this the name of a new species, since it is impossible to diagnose this species, is, under the Code, a ‘*nomen nudum*’.

According to the Code Glossary, ‘*nomen nudum*’ is “a Latin term referring to a name that, if published before 1931, fails to conform to Article 12; or, if published after 1930, fails to conform to Article 13”. In our example of typeless species *C*, that is exactly what takes place: the zoologist did not differentiate species *C*, and thereby has not executed the requirement of Article 13.1.

So, when describing a new typeless species a specialist should differentiate it from all the species described earlier. If the taxonomist in the differential diagnosis misses the comparison to a known species with which a typeless species is similar externally, the latter cannot be diagnosed on any account, and therefore its name falls under the definition of *nomen nudum*. Once again, we need to emphasize that *nomen nudum* here would be the result of nonprofessional actions of the taxonomist, who should have formally approached the task of comparison of typeless species to the closely related species described earlier.

Thus, conditions of the description of a typeless species are more rigid, and can be granted only by a professionally established specialist with good knowledge of the studied group. Let us remember that for the traditional procedure of a species description with deposition of fixed reference specimens in public collections the Code does not demand a comparison of a new species to all the species described earlier and belonging to the same genus or closely related genera. What are the causes of these serious differences in the practice of new species description?

The particularity of taxonomic names introduced based on photographs is that they have a substantial double interpretation connected in the example given above, solved by explaining formulae: “species *C*” and “typeless species *C*”, where *C* designates the photographed specimen; for example, the holotype of *Marleyimyia xylocopae*. The first name designates taxon *C* as it is, in its all completeness and with all attributes inherent to it. That is to say that the name *Marleyimyia xylocopae* is considered without any connotation. The second name designates taxon *C* connotatively, giving its narrow definition by the means of a finite set of characters that can be recognized in a photograph. According to the accepted assumption, taxon *C* does not differ from taxon *A* in these characteristics. Hence it follows that the name “typeless species *C*” (in other words connotation of the name *Marleyimyia xylocopae*) can simultaneously designate both the taxon *C* and the taxon *A*. Once again, we shall emphasize that it will become possible only in a case when a taxonomist describing a typeless species had omitted to compare it to a similar species described earlier. This hypothetical opportunity has no relation to the real species *Marleyimyia xylocopae* (described by Marshall and Evenhuis 2015), as authors followed the requirements of Article 13.1. There is no such species indistinguishable from *Marleyimyia xylocopae* as the species *A* described above. The name *Marleyimyia xylocopae* is lawful from the point of view of the Code.

How, according to Article 13.1, is the problem of differentiation of taxa solved in cases of standard species based on extant specimen as holotype? Let us assume that

a taxonomist describing a new species *D* had not compared it to the known species *E*, which is similar to first one in external attributes. It is easy to see that the mistake of this taxonomist can always be corrected, and consequently it does not make the taxonomic position of species *D* uncertain, as it can be in cases of typeless species. Even though the species *D* and *E* do not differ in external characteristics, they can be distinguished by other attributes; for example, their genitalia or the characters of their genome. A taxonomist can repeatedly examine the holotypes of these species to solve the problem of taxonomic status for the species *D*. The latter can either appear identical to the species *E*, or will represent a separate species. The name “species *D*” does not bear any limiting connotation; therefore, taxon *D* is diagnosable in our example.

Due to the constantly extending character database of systematics, the inclusion of new categories of features in its arsenal, and additionally due to the continuous activity of taxonomists describing new species, the Code fundamentally cannot formalize Article 13.1.1. Therefore, it cannot establish the number of characteristics or species that would be necessary and sufficient for differentiation of a new species from related or similar ones. Consequently, the application of Article 13.1.1 is completely left at the discretion of taxonomists (see Recommendation 13A), and it will depend only on his readiness to seriously approach the compiling of a differential diagnosis, as well as on his desire to analyze all the currently available information on closely related species.

The seeming impression that nomenclature is not connected to taxonomy comes from this general impossibility of formalizing the procedure of description of new species. Actually, it is incorrect, there is a connection; however, it is mediated in many cases. At the same time, the analysis of names of typeless species shows that this connection can be clearly expressed.

## **Taxonomy and nomenclature**

Dubois and Nemésio (2007) have expressed the opinion that “the definition of taxa is not a matter of nomenclature but a matter of taxonomy, and the Code does not deal with taxonomy but with nomenclature”. It is possible to agree with the first part of the sentence. As for the second part of that sentence, the statement by Dubois and Nemésio (2007) does not, in our opinion, take into account the full extent of the primary principle of the Code: “The Code refrains from infringing upon taxonomic judgement, which must not be made subject to regulation or restraint.” We claim that the Code is a practical guide for the description of new taxa. The connection between nomenclature and taxonomy is created through this practice.

Nomenclature is often defined as the practice of designation of taxa. This definition considers taxonomy and nomenclature to be two different and disconnected aspects of the work of systematists. It is assumed that a systematist had discovered a new taxon, i.e., he found that it differs from all other taxa known at that time, and consequently provided a name for the new taxon. It is difficult to agree with such an interpretation. Here we follow the opinion of I. Ya. Pavlinov, who wrote in his manual of taxonomic

nomenclature (2015, p. 11): “Despite the traditional separation of two “foundations” of taxonomy recognized by Linnaeus, viz. classification (disposition) and naming, the nomenclatorial codes actually regulate both. The reason is quite simple and obvious: as there cannot be taxa appearing in scientific classifications and texts without being properly nominated, the “empty” *nomina* that are not properly assigned to particular taxa do not have any biological meaning.”

The Code of Nomenclature was written by taxonomists for taxonomists studying various groups of organisms, aiming to order and by that to facilitate, as much as possible, their activity with regard to the descriptions of taxa. The procedure for descriptions of new taxa is regulated in a number of places within the Code, and in particular the terms of availability of a taxonomic name are stipulated in Article 13.1. All things considered, the nomenclature in systematics is a system of rules created for formal descriptions and designations of new taxa. Formal requirements for the procedure of describing new taxa are aimed at avoiding the mistakes of naming, i.e., homonyms, synonyms, and *nomina nuda* (Principles 3, 5; Glossary).

As it has already been stated, Article 13.1.1 cannot be formalized with relation to the practice of description of standard species. With all this, the Code brings attention that he might act negligently within the requirements of this article, and the result of his oversight could be a synonymy between the name of a new species newly established and a species described earlier. In reference to typeless species described by photographed individuals, the Code implies severe constraints concerning the procedure of comparison of new species to related or similar species. In case of noncompliance with these conditions *nomina nuda* (see above) as well as synonyms can be created.

To date there are almost no examples of a *nomen nudum* created in consequence of the description of a typeless species. One of such example was the description of *Nembrotha yonowae* Goethel & Debelius, 1992, based on photo; later Nathalie Yonow (1994) made mention that this species is *nomen nudum*; subsequently, Pola et al. (2008) picked up on it and reinstated the name *Nembrotha yonowae*, while describing more species in the complex. Hence, the status of this species is still under consideration. An example of synonymy is the name *Strix omanensis* recently established for a new species of desert owl from northern Oman (Robb et al. 2013). Authors based the description of *Strix omanensis* on photos; as Marshall and Evenhuis (2016, p. 88) have emphasized “Robb et al. recognized that there were two species under the name *Strix butleri* (Hume), and that one of them must therefore be new. Unfortunately, they misidentified the new species as the one corresponding to the original name even though there was a type specimen corresponding to that 1878 name, and this led them to coin a new name for *S. butleri*”.

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## References

- Amorim DS, Santos CMD, Krell F-T, Dubois A, Nihei SS, Oliveira OMP, Pont A, Song H, Verdade VK, Fachin DA, Klassa B, Lamas CJE, Oliveira SS, Carvalho CJB De, Mello-Patiu CA, Hajdu E, Couri MS, Silva VC, Capellari RS, Falaschi RL, Feitosa RM, Prendini L, Pombal JPP, Fernandez F, Rocha RM, Lattke JE, Caramaschi U, Duarte M, Marques AC, Reis RE, Kurina O, Takiya DM, Tavares M, Fernandes DS, Franco FL, Cuezco F, Paulson D, Guenard B, Schlick-Steiner BC, Arthofer W, Steiner FM, Fisher BL, Johnson RA, Delsinne TD, Donoso DA, Mulieri PR, Patitucci LD, Carpenter JM, Herman L, Grimaldi D (2016) Timeless standards for species delimitation. *Zootaxa* 4137(1): 121–128. <https://doi.org/10.11646/zootaxa.4137.1.9>
- Dalebout ML, Baker CS, Mead JG, Cockcroft VG, Yamada TK (2004) A comprehensive and validated molecular taxonomy of beaked whales, family Ziphiidae. *Journal of Heredity* 95: 459–473. <https://doi.org/10.1093/jhered/esh054>
- Dubois A, Nemésio A (2007) Does nomenclatural availability of nomina of new species or subspecies require the deposition of vouchers in collections? *Zootaxa* 1409: 1–22.
- Chandler PJ (2001) The flat-footed flies (Diptera: Opetiidae, Platypezidae) of Europe. *Fauna Entomologica Scandinavica* (36). E.J. Brill, Leiden-Boston-Köln, 276 pp.
- Cianferoni F, Bartolozzi L (2016) Warning: potential problems for taxonomy on the horizon? *Zootaxa* 4139(1): 128–130. <https://doi.org/10.11646/zootaxa.4139.1.8>
- Goethel H, Debelius H (1992) Nacktschnecken der Maldiven, mit der Beschreibung einer neuen Art. *DATZ (Die aquarien- und terrarienzeitschrift)* 1992 (8): 512–519.
- ICZN (1999) International Code of Zoological Nomenclature (4<sup>th</sup> ed). International Trust for Zoological Nomenclature, London, 306 pp.
- Jones T, Ehardt CL, Butynski TM, Davenport TRB, Mpunga NE, Machaga SJ, De Luca DW (2005) The highland mangabey *Lophocebus kipunji*: a new species of African monkey. *Science* 308: 1161–1164. <https://doi.org/10.1126/science.1109191>
- Landry SO (2005) What constitutes a proper description? *Science* 309: 2164.
- Lonsdale O, Marshall SA (2016) Revision of the family Nothybidae (Diptera: Schizophora). *Zootaxa* 4098 (1): 001–042. <https://doi.org/10.11646/zootaxa.4098.1.1>
- Löbl I, Cibois A, Landry B (2016) Describing new species in the absence of sampled specimens: a taxonomist's own-goal. *Bulletin of Zoological Nomenclature* 73: 83–86. <https://doi.org/10.21805/bzn.v73i1.a2>
- Marshall SA, Evenhuis NL (2015) New species without dead bodies: a case for photo-based descriptions, illustrated by a striking new species of *Marleyimyia* Hesse (Diptera, Bombyliidae) from South Africa. *Zookeys* 525: 117–127. <https://doi.org/10.3897/zookeys.525.6143>

- Marshall SA, Evenhuis NL (2016) Proxy types, taxonomic discretion, and taxonomic progress: a response to Löbl et al. *Bulletin of Zoological Nomenclature* 73: 87–92. <https://doi.org/10.21805/bzn.v73i1.a1>
- Mendes Pontes AR, Malta A, Asfora PH (2006) A new species of capuchin monkey, genus *Cebus* Erxleben (Cebidae, Primates): found at the very brink of extinction in the Pernambuco Endemism Centre. *Zootaxa* 1200: 1–12.
- Oliveira MM de, Langguth A (2006) Rediscovery of Marcgrave's Capuchin Monkey and designation of a neotype for *Simia flavia* Schreber, 1774 (Primates, Cebidae). *Boletim do Museu Nacional, Rio de Janeiro, (n.s.)* 523: 1–16.
- Pape Th (2016) Species can be named from photos. *Nature* 537: 307. <https://doi.org/10.1038/537307b>
- Pavlinov IYa (2013). *Nomenclature in the systematics. History, theory, practice.* KMK Sci Press, Moscow, 439 pp. [In Russian]
- Pola M, Cervera JL, Gosliner TM (2008) Revision of the Indo-Pacific genus *Nembrotha* (Nudibranchia: Dorididae: Polyceridae), with a description of two new species. *Scientia Marina* 72(1): 145–183. <https://doi.org/10.3989/scimar.2008.72n1145>
- Polaszek A, Grubb P, Groves C, Ehardt CL, Butynski TM (2005) What constitutes a proper description? Response. *Science* 309: 2164–2166
- Robb MS, van den Berg AB, Constantine M (2013) A new species of *Strix* owl from Oman. *Dutch Birding* 35: 275–310.
- Santos CMD, Amorim DS, Klassa B, Fachin DA, Nihei SS, Carvalho CJB, Falaschi RL, Mello-Patiu CA, Couri MS, Oliveira SS, Silva VC, Ribeiro GC, Capellari RS, Lamas CJE. (2016) On typeless species and the perils of fast taxonomy. *Systematic Entomology* 41(3): 511–515. <https://doi.org/10.1111/syen.12180>
- Thalmann U, Geissmann T (2005) New species of woolly lemur *Avahi* (Primates: Lemniformes) in Bernaraha (central western Madagascar). *American Journal of Primatology* 67: 371–376. <https://doi.org/10.1002/ajp.20191>
- Timm RM, Ramey RR II, The Nomenclature Committee of the American Society of Mammalogists (2005) What constitutes a proper description? *Science* 309: 2163. <https://doi.org/10.1126/science.309.5744.2163c>
- Wakeham-Dawson A, Morris S, Tubbs P, Dalebout ML, Baker CS (2002) Type specimens: dead or alive? *Bulletin of Zoological Nomenclature* 59(4): 282–284.
- Yonow N (1994) Opisthobranchs from the Maldivian Islands, including descriptions of seven new species (Mollusca: Gastropoda). *Revue française d'aquariologie herpétologie*, 20(4): 97–130.



# Four new species of the genus *Delia* Robineau-Desvoidy in the Yunnan Province of China (Diptera, Anthomyiidae)

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<http://zoobank.org/5A07733F-6A4E-4CAB-AA76-3EBB370F4B60>

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## Abstract

Four new species of the genus *Delia* from Yunnan Province are described: *Delia dentiaedeagus* **sp. n.**, *Delia longiabdmina* **sp. n.**, *Delia nigerihalteres* **sp. n.**, and *Delia tuberisurstyla* **sp. n.** A catalogue of all *Delia* species recorded from Yunnan Province has also been included.

## Keywords

Anthomyiidae, *Delia*, Diptera, new species, Yunnan

## Introduction

*Delia* is one of the largest genera of Anthomyiidae (Diptera), established by Robineau-Desvoidy (1830). The type species *Delia floricola* Robineau-Desvoidy, 1830 was designated by Coquillett (1910). It can be recognized by the following combination of characteristics: eyes usually bare; frons usually narrow in male and broad in female; with pairs of inter frontal setae; legs black or yellow; fore tibia with 0–1 *ad*, 1–2 medial *p* or 1–2 *pv* and 1 apical *pv*; mid tibia with 1 *pd*; hind femur without *pv* row or apical *pv*; surstylus longer than cercus, not bifurcate apically; aedeagus slender, apical

part with paraphallus in most species (Ackland 1967 and 2008, Griffiths 1991, Dely-Draskovits 1993, Fan et al. 1988, Fan and Zheng 1993, Hennig 1974, Suwa 1977, Wei et al. 1996, Xue and Zhang 1996, Xue and Du 2008, and 2009).

There are 103 species distributed in China, among which 12 are located in Yunnan Province. Yunnan is one of the most diverse places in China: its complex and varied terrains and landforms as well as the different climates have created a natural paradise for biodiversity. After several collecting periods, four new species were found. This paper includes a complete report on these new species and a full catalogue of all species distributed in Yunnan Province.

## Materials and methods

All specimens were collected from Yunnan Province of China. Type specimens are deposited in the Diptera collection of the Institute of Entomology, Shenyang Normal University (IESNU).

### Abbreviations used in the descriptions:

<i>a</i>	anterior setae;	<i>pd</i>	posterodorsal setae;
<i>acr</i>	acrostichal setae;	<i>post acr</i>	postsutural acrostichal setae;
<i>ad</i>	anterodorsal setae;	<i>post dc</i>	postsutural dorsocentral setae;
<i>av</i>	anteroventral setae;	<i>pra</i>	prealar setae;
<i>d</i>	dorsal setae;	<i>prst acr</i>	presutural acrostichal setae;
<i>dc</i>	dorsocentral setae;	<i>prst dc</i>	presutural dorsocentral setae;
<i>ial</i>	intra-alar setae;	<i>pv</i>	posteroventral setae; and
<i>p</i>	posterior setae;	$R_{4+5}$	branch of radius.

## Taxonomy

### *Delia dentiaedeagus* sp. n.

<http://zoobank.org/8F7E8B49-7023-4191-856C-32CBAF7EE7C6>

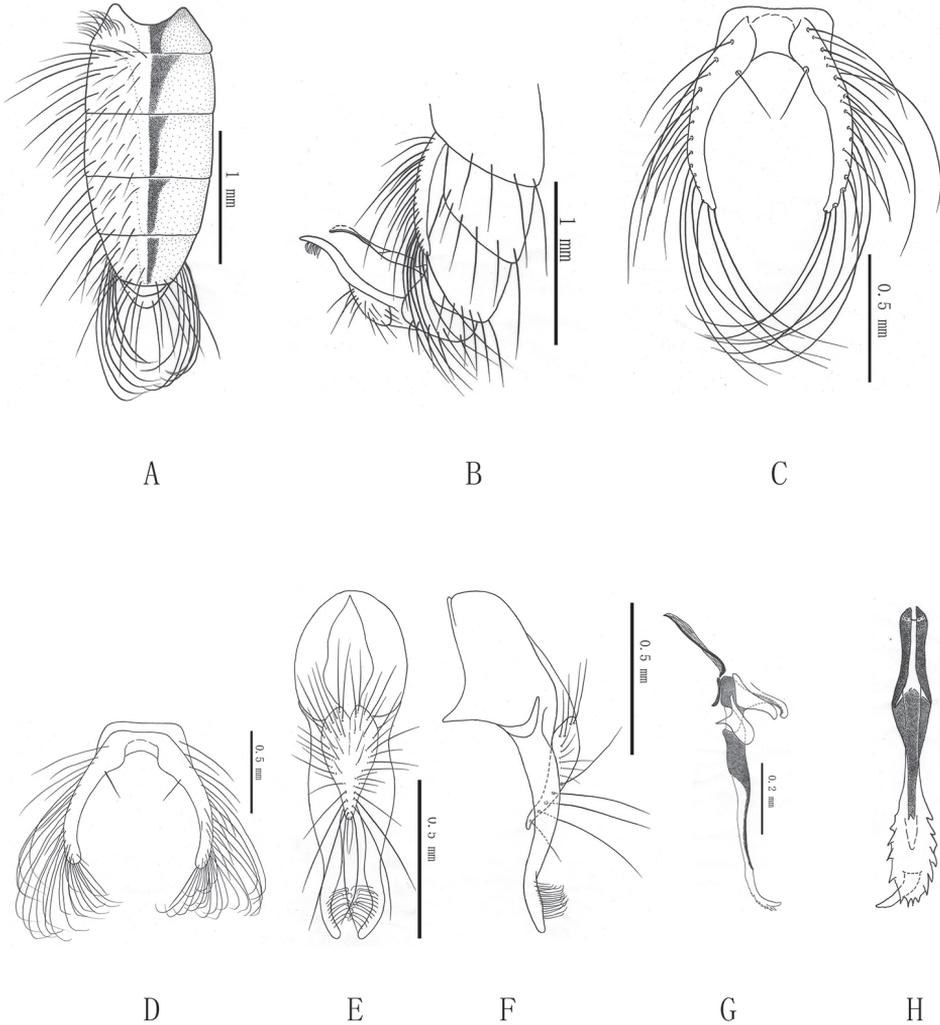
Figure 1

**Type material.** *Holotype*. China, Yunnan Province, Baimang Snowberg, 4000 m, 29 May 2007, Dandan Wang Co., ♂ (IESNU). *Paratype*. China, Yunnan Province, Baimang Snowberg, 4000 m, 31 May 2007, Lu Zhang Co., 2♂♂ (IESNU).

**Diagnosis.** Arista short ciliated, longest hairs shorter than its basal diameter; legs black; mid tarsomere 1 with long *ad*; processes of 5th sternite slender, with long and dense setae, without spine or protrusion.

**Description. Holotype Male.** Body length 4.5–5.0 mm.

*Head.* Eye bare; frontal vitta dark brown without interfrontal setule; fronto-orbital plate adjacent; frons as wide as anterior ocellus; without orbital setae; fronto-orbital



**Figure 1.** *Delia dentiaedeagus* sp. n. **A** male, abdomen in dorsal view **B** male, distal half of abdomen in profile **C** male, 5th sternite in ventral view, in holotype **D** male, 5th sternite in ventral view, in paratype **E** male, terminalia in posterior view **F** male, terminalia in profile **G** male, aedeagus in profile **H** male, distal part of aedeagus in anterior view.

plate and parafacial with grey dust; 5–6 pairs of frontal setae, situated on lower half of frons; parafacial subequal to the width of antenna; antenna black, postpedicel approx. 2.2–2.5 times longer than broad; arista short ciliated, longest hairs shorter than its basal diameter; lower facial margin not projecting, situated behind frontal angle in profile; genal height approx. 1/5 eye height; anterior margin of gena with one row of upcurved subvibrissal setulae; prementum black, without dust, approx. 3.5 times longer than broad; palpus black, slightly shorter than prementum.

*Thorax.* Black in ground colour, with grey to brownish grey dust; scutum with three black vittae, the middle one extended to scutellar suture; *prst acr* two rows, only one pair of *post acr* developed which are placed in front of scutellum, *dc* 2+3, *ial* 0+2; notopleural depression bare apart from two strong setae; *pra* as long as posterior notopleural; scutellum bare on disc centrally and basally; katepisternals 1+2.

*Wing.* Slightly transparent. Vein fuscous and basicosta black; Costa setulose near its base on ventral surface only; costal spine distinct; radial node bare; squamae brownish yellow, lower squama not projecting, approx. 2/3 length of upper; haltere yellowish.

*Legs.* Entirely black; fore tibia with one medial *p*; mid femur without distinct *av* row, a row of seta-like *pv* in basal half, only two setae in basal developed; mid tibia without *av* or *ad*, two *pd* and two *pv*; mid tarsomere 1 with distinct *ad*; hind femur with 2–3 *av* and 2–3 *pv* in distal part; hind tibia with four *av*, three *ad*, three *pd*, and 5–6 *pv* in middle part, without apical *pv*; hind tarsi longer than tibiae, all claws and pulvilli normal, slightly shorter than tarsomere 5.

*Abdomen.* Black, rhombic in dorsal view, with grey to brownish dusting; all tergites with black vittae in middle part; dorsal setae sparse, lateral setae long; 6<sup>th</sup> tergite bare; 1<sup>st</sup> sternite with setae.

**Female.** Unknown.

**Remarks.** This new species is similar to *Delia felsicanalis* Fan & Wu in Fan et al., 1984, but differs from it in the following features: male frontal vitta dark brown; fronto-orbital plate adjacent; parafacial subequal to the width of antenna; prementum 3.5 times longer than broad; basicosta black; haltere yellowish; hind tibia with four *av*, three *ad* and 5–6 *pv* in middle part.

**Etymology.** The species name is derived from the Latin words “*dent*” meaning tooth and “*aedeagus*” meaning aedeagus, referring to its apical aedeagus with tooth.

**Distribution.** China, Yunnan Province (Baimang Snowberg).

### *Delia longiabdomina* sp. n.

<http://zoobank.org/F72356C7-A54B-4721-B802-A08723D0114B>

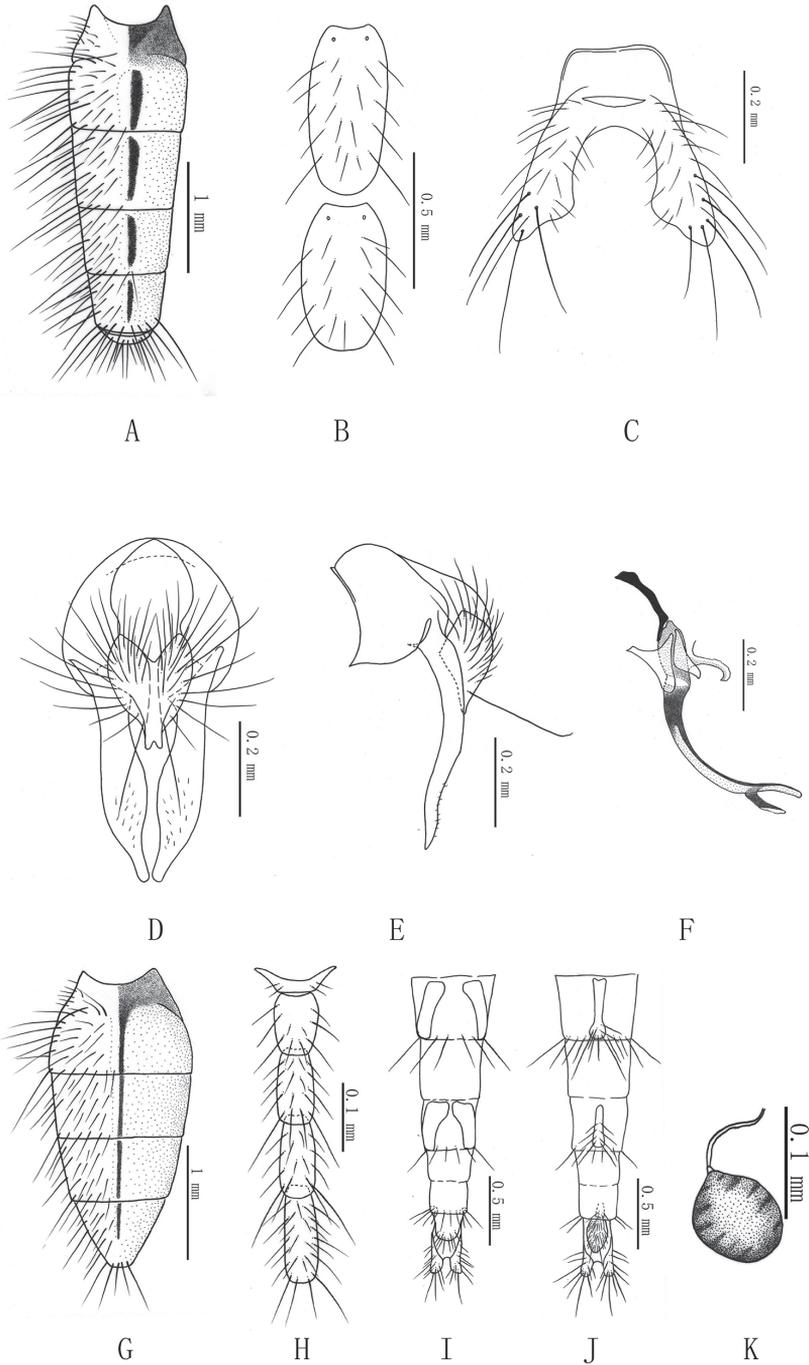
Figure 2

**Type material. Holotype.** China, Yunnan Province, Yulong Snowberg, 4506 m, 29 June 2006, Baifeng Wang Co., ♂ (IESNU). *Paratypes.* China, same data as holotype, 1 ♀ and 4♂♂ (IESNU); China, Yunnan Province, Yulong Snowberg, 4506–4571 m, 29 June 2006, You Wang Co., 5♂♂ (IESNU); China, Yunnan Province, Yulong Snowberg, Big ropeway, 4571 m, 29 June 2006, Mingfu Wang Co., 3♂♂ (IESNU).

**Diagnosis.** Arista pubescence, longest hairs shorter than its basal diameter; lower facial margin slightly projecting, situated before frontal angle in profile; legs black; hind tibia with one row of *pv*; inner side of 5th sternite processes with a protrusion.

**Description. Holotype Male.** Body length 6.5 mm.

*Head.* Eye sparsely with short ciliated; frontal vitta black, with black dust; frontal vitta with a pair of interfrontal setule; frons as wide as anterior ocellus; without or-



**Figure 2.** *Delia longiabdomina* sp. n. **A** male, abdomen in dorsal view **B** male, 3rd and 4th sternites in ventral view **C** male, 5th sternite in ventral view **D** male, terminalia in posterior view **E** male, terminalia in profile **F** male, aedeagus in profile **G** female, abdomen in dorsal view **H** female, sternites 1 to 5 **I** female, ovipositor in dorsal view **J** female, ovipositor in ventral view **K** female, spermatheca.

bital setae; fronto-orbital plate with dark grey dust; 6–7 pairs of frontal setae, situated on lower half of frons; parafacial 1.5 times wider than postpedicel; antenna black, postpedicel approx. 1.5–1.8 times longer than broad; arista pubescence, longest hairs shorter than its basal diameter; lower facial margin slightly projecting, situated before frontal angle in profile; gena sparsely with dark grey dust, genal height approx. 1/5 eye height; anterior margin of gena with 1–2 rows of upcurved subvibrissal setulae; para-occipital and postgenal hairs black; proboscis slender, prementum with grey dust, 6.0 times longer than broad; palpus black, subequal to prementum.

**Thorax.** Black in ground colour with dark green lustre and dark grey dust; scutum with three indistinct black vittae, the middle one absent; two rows of hair-like *prst acr* (1 or 2 pairs stronger), only one pair of *post acr* developed, these situated in front of scutellum, *dc* 2+3, *ial* 0+2, with one pair of outer posthumeral setae; notopleural depression bare apart from two strong setae; *pra* subequal to posterior notopleural; scutellum bare on disc centrally and basally; katepisternals 1+2(3).

**Wing.** Base fuscous, basicosta black; costa setulose only basally on ventral surface; costal spine subequal to crossvein r-m; radial node bare, squamae yellowish or white, outer marginal hairs long; lower squama short, approx. 1/2 length of upper; halter brownish yellow.

**Legs.** Entirely black; fore tibia with one medial *p*; mid femur with seta-like *a* row in basal half, *pv* rows complete, becoming shorter apically; mid tibia with two *p*, one long *pd* and 1–2 short *pv*; mid tarsomere 1 with one row of long *pd*, longer than its diameter; hind femur with complete row of *av*, becoming longer apically, with two apical *pv*; hind tibia with 8–10 *av*, 5–6 *ad*, one row of *pd* (4 developed), and one row of *pv*, similar as *Delia platura* (Meigen, 1826); all tarsi shorter than tibiae, claws and pulvilli large.

**Abdomen.** Black, long flat-shaped in dorsal view, with grey dust; all tergites with broad black vittae in middle part, the front margin slightly dark, edge unclear; lateral and post marginal setae long; 6th tergite bare; 1st sternite densely with long setae; 3<sup>rd</sup> and 4<sup>th</sup> sternites without dense long setae.

**Female.** Body length 5.0–5.5 mm; frons approx. 2/5 width of head; frontal vitta 2.5 times as wide as fronto-orbital plate; three pairs of orbital setae and three pairs of frontal setae; genal height 1/3 eye height; *dc* 2(3)+3, without outer posthumeral setae; katepisternum 1+1(2); fore tibia with 1–2 *ad*; hind tibia with 4–5 *av*, 5 *ad*, 7–8 *pv*; the other characters as same as male.

**Remarks.** This new species is similar to *Delia subnigribasis* Fan & Wang in Fan et al., 1981, but differs from it in the following features: male frontal setae 6–7 pairs; *pra* subequal to posterior notopleural seta; inner side of 5th sternite processes broad apically; cercus broad apically, surstylus broad and straight in dorsal view, straight in lateral view, becoming slender apically.

**Etymology.** The species name is derived from the Latin words “long” meaning long, referring to its very long abdomen.

**Distribution.** China, Yunnan Province (Yulong Snowberg).

***Delia nigerihalteres* sp. n.**

<http://zoobank.org/DF2FD042-384E-4D80-9588-F7232528DA76>

Figure 3

**Type material. Holotype.** China, Yunnan Province, Baimang Snowberg, 4100 m, 30 May 2007, Dandan Wang Co., ♂ (IESNU). **Paratype.** China, same data as holotype, 3♂♂.

**Diagnosis.** Frons 1.5 times wider than anterior ocellus; without orbital setae; 6–7 pairs of frontal setae; arista ciliated, longest hair longer to 1.5 times basal diameter; anterior margin of gena with 3–4 rows of upcurved subvibrissal setulae; parafacial 1.2 times wider than antenna; prementum approx. 2.5–3.0 times longer than broad; costa setulose only basally on ventral surface; legs black; mid tarsomere 2 without protuberance; all tergites with broad trapeziform spots in centre, posterior marginal setae and lateral setae strong; inner side of 5<sup>th</sup> sternite processes without protrusion.

**Description. Holotype male.** Body length 3.0–3.5 mm.

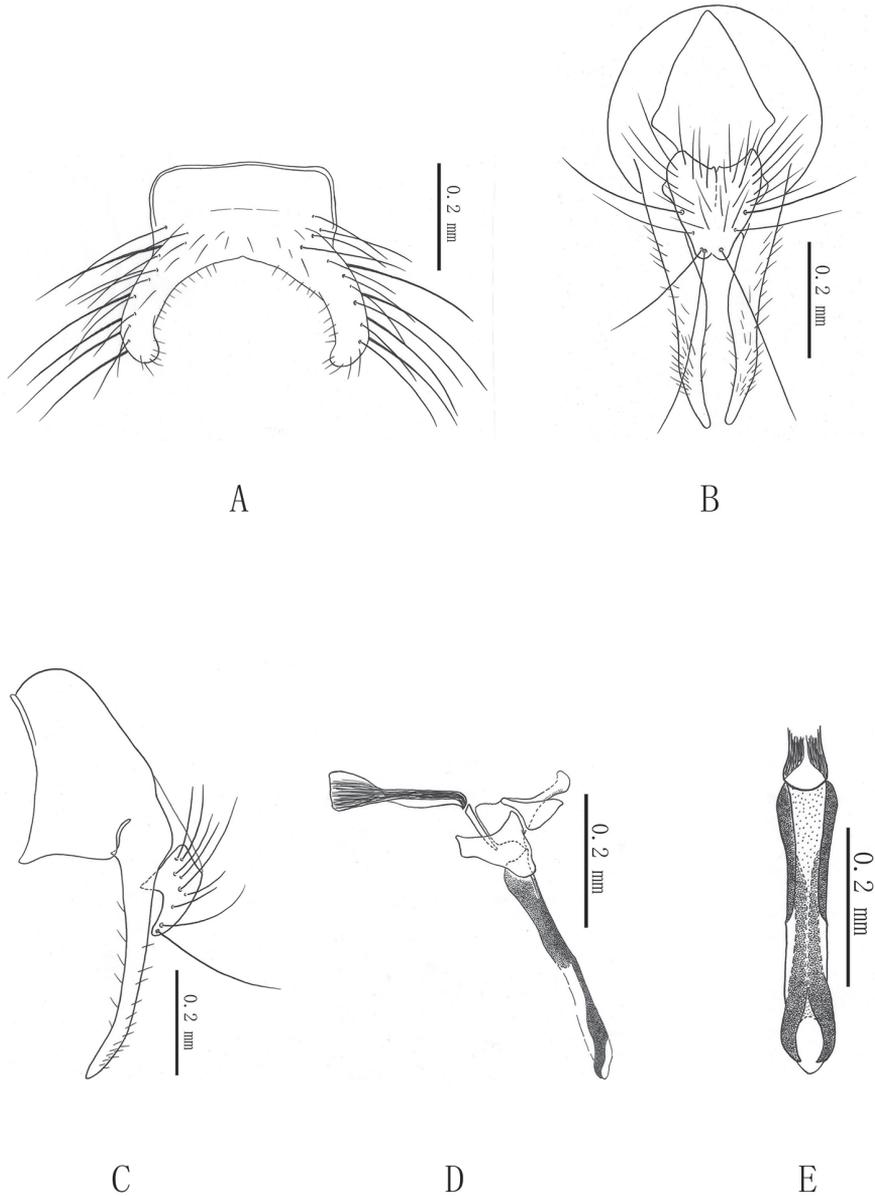
**Head.** Eye bare; frontal vitta black, 1.5 times as wide as fronto-orbital plate; frons 1.5 times wider than anterior ocellus; frontal vitta with a pair of interfrontal setule; without orbital setae; 6–7 pairs of frontal setae, situated on lower 3/5 of frons; fronto-orbital plate and parafacial with dark grey dust; parafacial 1.2 times wider than antenna; antenna black, postpedicel approx. 1.5–1.8 times longer than broad; arista ciliated, longest hair longer to 1.5 times basal diameter; lower facial margin slightly projecting, vibrissal angle situated before frontal angle in profile; genal height approx. 1/5 eye height; anterior margin of gena with 3–4 rows of upcurved subvibrissal setulae; prementum shiny, approx. 2.5–3.0 times longer than broad; palpus black, slightly shorter than prementum.

**Thorax.** Black in ground colour with brownish grey dust; scutum with three black vittae; two rows of hair-like *prst acr* (the second pair stronger), only one pair of *post acr* developed, these situated in front of scutellum, *dc* 2+3, *ial* 0+2; notopleural depression bare apart from two strong setae; *pra* subequal to the posterior notopleural; scutellum bare on disc centrally and basally; katepisternals 1+2.

**Wing.** Base and veins fuscous, basicosta black; costa setulose only basally on ventral surface; costal spine absent; radial node bare, squamae brown; lower squama approx. 1/2 length of upper; halter black.

**Legs.** Entirely black; fore tibia with one medial *p*; mid femur with a complete row of *av*, becoming shorter apically, a complete row of *pv*, becoming longer medially; mid tibia with 2–3 *pd* and two *pv*; mid tarsomere 1 with row of long *pd*, subequal to the length of its diameter; hind femur with complete row of *av* and *pv*, becoming longer medially; hind tibia with five *av*, 5–6 *ad*, 2–3 *pd*, and 8–9 *pv*; all tarsi shorter than tibiae, claws slightly longer than pulvilli, pulvilli approx. 1/2 length of tarsomere 5.

**Abdomen.** Black, flat cone-shaped in dorsal view; all tergites with broad trapeziform spots in centre, posterior marginal setae and lateral setae strong; 6<sup>th</sup> tergite bare; 1<sup>st</sup> sternite with fine hairs.



**Figure 3.** *Delia nigeribalteres* sp. n. **A** male, 5<sup>th</sup> sternite in ventral view; **B**, male, terminalia in posterior view; **C**, male, terminalia in profile; **D**, male, aedeagus in profile; **E**, male, distal part of aedeagus in anterior view.

**Female.** Unknown.

**Remarks.** This new species is similar to *Delia quadrilateralis* Fan & Zhong in Fan et al., 1981, but differs from it in the following features: male frontal vitta 1.5 times as wide as fronto-orbital plate; frons 1.5 times wider than anterior ocellus; basicosta

black; costal spine absent; squamae brown; halter black; mid tarsomere 1 with row of long *pd*, subequal to the length of its diameter.

**Etymology.** The specific name is from the Latin word “*niger*”, black, “*halter*”, halter, referring to its black halter.

**Distribution.** China, Yunnan Province (Baimang Snowberg).

***Delia tuberisurstyla* sp. n.**

<http://zoobank.org/CD4AC08A-4682-4CA8-BD74-B96336BB2783>

Figure 4

**Type material. Holotype.** China, Yunnan Province, Yulong Snowberg, Big ropeway, 4571 m, 29 June 2006, Ming-Fu Wang Co., ♂ (IESNU). **Paratype.** China, same data as holotype, 1 ♂.

**Diagnosis.** Arista pubescence, longest hairs shorter than its basal diameter; lower facial margin slightly projecting, with anterior margin of frons in same vertical plane; legs black; hind tibia with two rows of *pv*; inner side of 5<sup>th</sup> sternite processes with a protrusion.

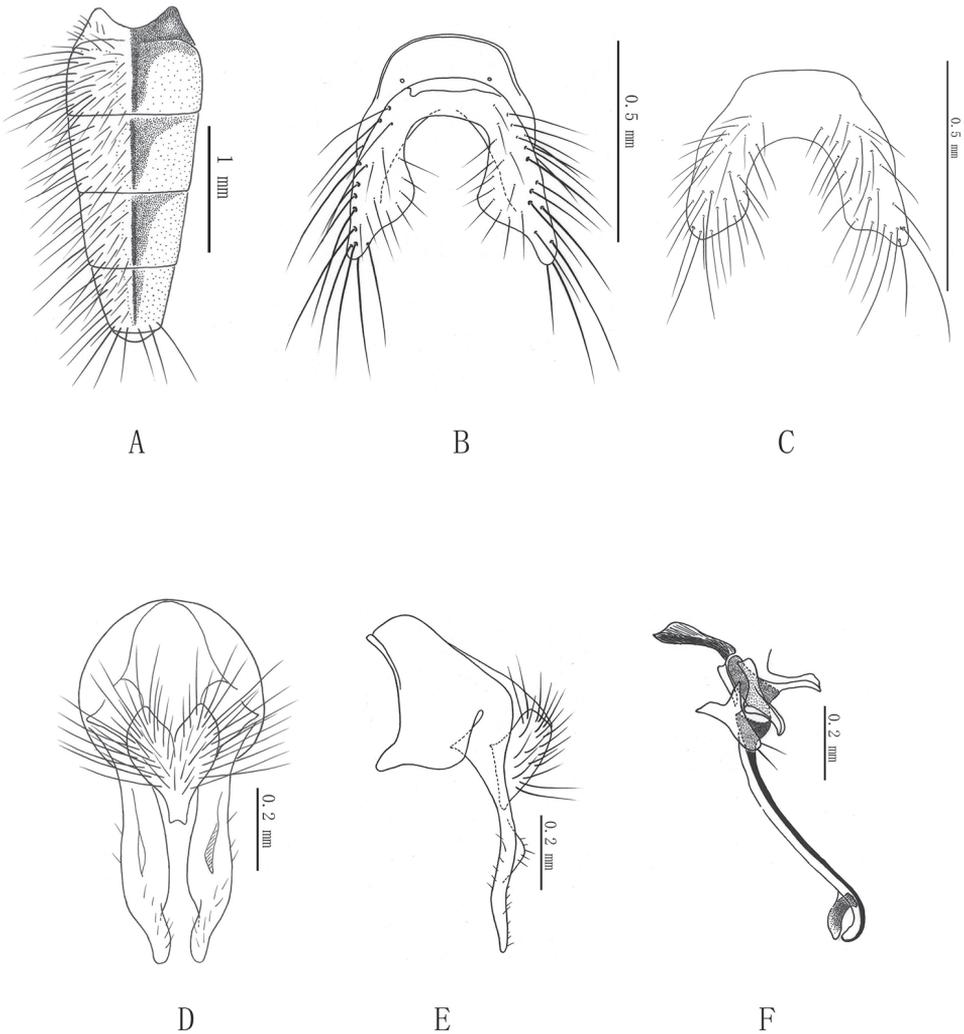
**Description. Holotype male.** Body length 5.5–6.0 mm.

**Head.** Eye with sparse and short ciliae; frontal vitta black, with black dust; frontal vitta with a pair of interfrontal setule; frons as wide as anterior ocellus; without orbital setae; fronto-orbital plate, parafacial and gena with dark grey dust; 7–8 pairs of frontal setae, situated on lower half of frons; parafacial 1.4 times wider than postpedicel; antenna black, postpedicel 1.5 times longer than broad; arista pubescence, longest hairs shorter than its basal diameter; lower facial margin slightly projecting, with anterior margin of frons in same vertical plane; genal height approx. 2/7 eye height; anterior margin of gena with two rows of upcurved subvibrissal setulae; para-occipital and post-genal hairs black; proboscis slender, prementum with grey dust, approx. 6.0–7.0 times longer than broad; palpus black, subequal to prementum.

**Thorax.** Black in ground colour with dark grey dust; scutum with three indistinct black vittae; two rows of hair-like *prst acr* (1 or 2 pairs stronger), only one pair of *post acr* developed, these situated in front of scutellum, *dc* 2+3, *ial* 0+2; notopleural depression bare apart from two strong setae; *pra* 1.2 times longer than posterior notopleural seta; scutellum bare on disc centrally and basally; katapisternals 1+2.

**Wing.** Base fuscous, basicosta black; costa setulose only basally on ventral surface; costal spine absent; radial node bare, squamae yellowish; lower squama short, approx. 1/3 length of upper; halter brown-yellow.

**Legs.** Entirely black; fore tibia with 1(0) medial *p*; mid femur with seta-like *a* row in basal half, *pv* rows complete; mid tibia with one *pd* and two *pv*; mid tarsomere 1 with one row of long *ad*, more than 1.5 times longer than its diameter; hind femur with complete row of *av*, becoming longer apically; hind tibia with 9–10 *av*, 5–6 *ad*, one row of *pd* (3–4 developed), and two rows of *pv*, slightly pectinated; all tarsi shorter than tibiae, claws and pulvilli large, slightly shorter than tarsomere 5.



**Figure 4.** *Delia tuberisurstyla* sp. n. **A** male, abdomen in dorsal view; **B** male, 5<sup>th</sup> sternite in ventral view, in holotype; **C** male, 5<sup>th</sup> sternite in ventral view, in paratype **D** male, terminalia in posterior view **E** male, terminalia in profile **F** male, aedeagus in profile.

**Abdomen.** Black, long cone-shaped in dorsal view, with grey or brownish grey dust; all tergites with hair-like setae in middle part, setae becoming longer towards lateral margin, mid black vitta expand in middle part, near rhombic; 6<sup>th</sup> tergite bare; 1<sup>st</sup> sternite dense with long fringes.

**Female.** Unknown.

**Remarks.** This new species is similar to *Delia subnigribasis* Fan & Wang in Fan et al., 1981, but differs from it in the following features: male frontal setae 7–8 pairs; *pra*

1.2 times longer than posterior notopleural seta; basicosta black; mid tarsomere 1 with one row of long *ad*, more than 1.5 times longer than its diameter.

**Etymology.** The species name is derived from the Latin words “*tuber*”, tuber, referring to the middle part of surstylus with a sheet-shaped tuber in dorsal view.

**Distribution.** China, Yunnan Province (Yulong Snowberg).

### **Distribution of the known species from Yunnan Province**

#### ***Delia absidata* Xue & Du, 2008: 113–122**

**Distribution.** China: Yunnan Province, Shangri-la, Bitahai (type loc.).

#### ***Delia antiqua* (Meigen, 1826): 166**

**Distribution.** Worldwide distribution (type loc. Germany): China (Heilongjiang, Jilin, Liaoning, Inner Mongolia, Gansu, Qinghai, Hebei, Beijing, Shanxi, Shandong, Shanghai, Sichuan, Yunnan).

#### ***Delia aurosialata* Fan, 1993 in Fan & Zheng: 1128**

**Distribution.** China: Yunnan Province, Yunlong, Mt. Zhiben (type loc.).

#### ***Delia bracata* (Rondani, 1866): 183**

**Distribution.** China (Yunnan, Tibet); India; Iran; Israel; Lebanon; Spain; France; Greece; Hungary; Italy (type loc.); Poland.

#### ***Delia echinata* (Séguy, 1923): 360**

**Distribution.** China (Sichuan, Yunnan, Tibet); Japan; North Korea ; India; Israel; Russia; Austria; Czech Republic; Slovakia; Germany; France (type loc.); England; Greece; Italy; Iceland; Roumania; Sweden; Finland; Yugoslavia.

#### ***Delia floralis* (Fallén, 1824): 71**

**Distribution.** China (Heilongjiang, Liaoning, Inner Mongolia, Qinghai, Xinjiang, Hebei, Shanxi, Yunnan); Japan; North Korea; Russia; Czech Republic; Slovakia; Germany; France; England; Denmark; Spain; Hungary; Norway; Sweden (type loc.); Finland.

#### ***Delia linearis* (Stein, 1898): 219**

**Distribution.** China (Heilongjiang, Jilin, Xinjiang, Shanxi, Yunnan); Japan; Czech Republic; Germany (type loc.); France; England; Poland; Sweden; Finland; Yugoslavia; Estonia; Latvia; Lithuania; Russia; White Russia; White Russia; Nearctic region.

#### ***Delia longithecata* Suwa, 1974: 160**

**Distribution.** China (Heilongjiang, Liaoning, Henan, Shanxi, Shaanxi, Sichuan, Guizhou, Yunnan); Japan (type loc.); North Korea; Russia.

***Delia partivitra* Fan, 1993 in Fan & Zheng: 1131**

**Distribution.** China: Yunnan Province, Lijiang, Mt. Yulong (type loc.).

***Delia platura* (Meigen, 1826): 171**

**Distribution.** Worldwide distribution (type loc. Germany).

***Delia sclerostylata* Fan, 1993 in Fan & Zheng: 1134**

**Distribution.** China: Yunnan Province, Lushui, Yaojiaping (type loc.).

***Delia subinterflua* Xue & Du, 2008: 113–122**

**Distribution.** China: Yunnan Province, Mt. Yulong, Big ropeway (type loc.); Sichuan, Mt. Balang.

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## References

- Ackland DM (1967) Diptera from Nepal (Anthomyiidae). Trustees of the British Museum (Natural History), 107–139.
- Ackland DM (2008) Revision of Afrotropical *Delia* Robineau-Desvoidy, 1830 (Diptera: Anthomyiidae), with descriptions of six new species. African Invertebrates 49(1): 1–75. <https://doi.org/10.5733/afin.049.0101>
- Coquillett DW (1910) The type-species of the North American genera of Diptera. Proceedings U.S. National Museum 37: 499–647. <https://doi.org/10.5479/si.00963801.37-1719.499>
- Dely-Draskovits Á (1993) Family Anthomyiidae. In: Soós Á, Papp L (Eds) Catalogue of Palearctic Diptera. Akadémiai Kiadó, Budapest 13: 37–51.
- Fallén (1824) Monographia Muscidum Sveciae. Part VII. Lundae, 65–72.
- Fan ZD, Chen ZZ, Ma ZY, Ge FX (1981) On some new species of Anthomyiidae from China (Diptera). Contributions from Shanghai Institute of Entomology 2: 223. [In Chinese with English summary]
- Fan ZD, Chen ZZ, Ma ZY (1984) New species of the Family Anthomyiidae, Diptera, from Qinghai Province, China, I. Contributions from Shanghai Institute of Entomology 4: 239–253. [In Chinese with English summary]
- Fan ZD, Sun CH, Chen ZZ, Ma SY, Wu L, Ge FX, Xue WQ, Cui CY, Wang CS, Ma CY, Wang CJ, Jin ZY, Jiang TJ, Qian JQ, Li R (1988) Diptera: Anthomyiidae. Economic Insect Fauna of China 37. Science Press, Beijing, China, 144–189. [In Chinese with English summary]

- Fan ZD, Zheng SS (1993) Anthomyiidae. In: Chen SX (Ed.) Insects of the Hengduan Mountains Region 2. Science Press, Beijing, 1127–1165. [In Chinese with English summary]
- Griffiths GCD (1991) Anthomyiidae [part]. In: Lindner E (Ed.) Flies of the Nearctic Region 8(2) 7–10. E. Schweizerbart, Stuttgart, 953–1632.
- Hennig W (1974) Anthomyiidae. In: Lindner E (Ed.) Die Fliegen der Palaearktischen Region 63a. Schweizerbart, Stuttgart, 687–920.
- Meigen JW (1826) Systematische Beschreibung der bekannten europäischen zweiflügeligen Insecten *Delia quadrilateralis* 5. Hamm.: Schultz, 412 pp. [pls 42–54]
- Robineau-Desvoidy JB (1830) Essai sur les Myodaires. Mémoires présentés par divers savans à l'Académie Royale des Sciences de l'Institut de France 2: 482.
- Rondani C (1866) Anthomyiinae italicae collectae distinctae et in ordinum dispositae. Atti della Società Italiana di Scienze Naturali, Milano 9: 68–217.
- Séguy E (1923) Description d'Anthomyiaires nouveaux (Diptères). Annales de la Société Entomologique de France 91: 360–368.
- Stein P (1898) Nordamerikanische Anthomyiden, Beitrag zur Dipterenfauna der Vereinigten Staaten. Berliner Entomologische Zeitschrift 42(3, 4): 161–288.
- Suwa M (1974) Anthomyiidae of Japan (Diptera). Insecta Matsumurana, new series 4: 142–166.
- Suwa M (1977) Supplementary notes on the family Anthomyiidae of Japan, I (Diptera). Insecta Matsumurana new series 10: 1–16.
- Wei LM, Xue WQ, Cui CY (1996) The family Anthomyiidae of China (Diptera). In: Xue WQ, Chao CM (Eds) Flies of China. Volume 1. Liaoning Science and Technology Press, Shenyang, 706–724. [In Chinese with English summary]
- Xue WQ, Du J (2008) Two new species of *Delia*, with a key to the males of the world species of the *interflua* group (Diptera: Anthomyiidae). Entomological News 119(2): 113–122. [https://doi.org/10.3157/0013-872X\(2008\)119\[113:TNSODW\]2.0.CO;2](https://doi.org/10.3157/0013-872X(2008)119[113:TNSODW]2.0.CO;2)
- Xue WQ, Du J (2009) A study of the world species of the *Delia alaba* subsection, with descriptions of two new species (Diptera: Anthomyiidae). Entomologica Americana 115(2): 154–159.
- Xue WQ, Zhang XZ (1996) Anthomyiidae. In: Wu SG, Feng ZJ (Eds) The Biology and Human Physiology in the Hoh Xil Region. Science Press, Beijing, 167–191. [In Chinese with English summary]

