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Three new species of *Cratera* Carbayo et al., 2013 from *Araucaria* forests with a key to species of the genus (Platyhelminthes, Continenticola)

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Abstract

Areas of *Araucaria* moist forest have been considered to constitute hotspots of land flatworm diversity, harbouring a high number of undescribed species. Herein we describe three new species of land flatworms of *Cratera* Carbayo et al., 2013 occurring in such type of forest in south Brazil. The three species are differentiated from their congeners mainly by their colour pattern, anatomy of the pharynx and prostatic vesicle, and details of the penis papilla and male atrium. An identification key to species of the genus in the Neotropical region is provided.

Keywords

Atlantic Forest, Geoplaninae, land flatworms, Neotropical region, taxonomy, Tricladida

Introduction

The subfamily Geoplaninae, which has a Neotropical distribution, shows high diversity in Brazilian tropical forests (Winsor et al. 1998, Sluys 1999, Álvarez-Prezas et al. 2014). Among the phytophysiognomies which constitute the Brazilian Atlantic Forest, the mixed ombrophilous forest (*Araucaria* moist forest) has been considered to constitute hotspots of land flatworm diversity, harbouring many yet undescribed species (Leal-Zanchet and Baptista 2009, Leal-Zanchet et al. 2011). Most flatworm species described from the *Araucaria* moist forest occur in areas from its southern portion (Lemos and Leal-Zanchet 2008, Amaral et al. 2012, Leal-Zanchet et al. 2012, Lemos et al. 2014, Rossi et al. 2014, 2015).

The subfamily Geoplaninae is currently composed of 24 genera (Sluys et al. 2009, Carbayo et al. 2013), six of them recently proposed based on a combination of morphological and molecular analyses to encompass some of the species that belonged to the genus *Geoplana* Stimpson, 1857. Among them, the genus *Cratera* Carbayo, Álvarez-Presas, Olivares, Marques, Froehlich and Riutort, 2013 was proposed for five species occurring in areas of the Brazilian Atlantic Forest. Recently, another five species were described (Rossi et al. 2014, 2015; Carbayo and Almeida 2015; Negrete and Brusa 2016). Herein three new species are described, occurring in areas covered by *Araucaria* moist forest in south Brazil and a taxonomic key provided for species of *Cratera*.

Materials and methods

Land planarians were collected in two protected areas located in the Iguassu River Drainage Basin, namely the Três Barras National Forest (26°09.27'–26°16.9'S; 50°16.0'–50°21.22'W), in Três Barras, state of Santa Catarina, and a private reserve named Araucaria Natural Heritage Private Reserve (26°20.35'–26°26.13'S; 51°19.49'–51°25.29'W), in General Carneiro, state of Paraná, both in south Brazil. They were collected during the day by direct sampling in leaf litter, under and inside fallen logs and under stones or during the night, when they are more active, by visual search.

Colour pattern and body shape and dimensions of live specimens were recorded. Specimens were then killed with boiling water and fixed in neutral formalin 10% and subsequently maintained in 70% ethyl alcohol. Methods described by Rossi et al. (2015) were used for histological processing of material and analysis of external and internal characters. The material was sectioned at intervals of 6 µm and stained with Masson's trichrome method or haematoxylin and eosin (Romeis 1989).

Type-material is deposited in the Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, state of Rio Grande do Sul, Brazil (**MZU**), and the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, state of São Paulo, Brazil (**MZUSP**).

Abbreviations used in the figures

cg	cyanophil glands
cmc	common muscle coat
cov	common glandular ovovitelline duct
db	dorsal band
de	dorsal epidermis
df	dorsal flecks
di	dorsal insertion

dm	dorsal cutaneous musculature
dsm	dorsal subcutaneous mesenchymatic musculature
e	eyes
eg	erythrophil glands
ej	ejaculatory duct
es	oesophagus
fa	female atrium
fc	female canal
gm	glandular margin
go	gonopore
h	halos
i	intestine
im	internal musculature
lu	pharyngeal lumen
m	mouth
ma	male atrium
mas	marginal stripe
mes	median stripe
n	nerve cord
o	ovary
om	outer musculature
ov	ovovitelline ducts
p	penis papilla
pp	pharyngeal pouch
ps	paramarginal stripe
pv	prostatic vesicle
rg	rhabditogen glands
sg	shell glands
sbm	sub-intestinal transverse mesenchymatic musculature
sd	sperm ducts
sp	sensory pit
spm	supra-intestinal transverse mesenchymatic musculature
sv	spermiducal vesicle
t	testes
v	vitelline follicles
ve	ventral epidermis
vi	ventral insertion
vm	ventral cutaneous musculature
xg	xanthophil glands

Taxonomy

Family Geoplanidae Stimpson, 1857
Subfamily Geoplaninae Stimpson, 1857
Cratera Carbayo et al., 2013

Cratera cryptolineata sp. n.

http://zoobank.org/0A70BDC4-AA06-46E6-A8E3-3F31686E6513

Material examined. Holotype: MZUSP PL.1690: *leg.* I. Rossi, 3 June 2015, Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 12 slides; anterior region at the level of the ovaries: sagittal sections on 25 slides; pre-pharyngeal region: transverse sections on 16 slides; pharynx: sagittal sections on 30 slides; copulatory apparatus: sagittal sections on 25 slides.

Other specimens: all specimens sampled in the same locality as the holotype. MZU PL.00217: *leg.* J. A. L. Braccini, 29 July 2015 – anterior tip: transverse sections on 15 slides; anterior region at the level of the ovaries: sagittal sections on 16 slides; pre-pharyngeal region: transverse sections on eight slides; pharynx and copulatory apparatus: sagittal sections on 19 slides. MZU PL.00218: *leg.* J. A. L. Braccini, 27 July 2015 – pre-pharyngeal region: transverse sections on 14 slides; pharynx and copulatory

Figure 1. *Cratera cryptolineata* sp. n., holotype, habitus, dorsal view.

apparatus: sagittal sections on 20 slides. MZU PL.00219: *leg*. J. A. L. Braccini, 2 June 2015 – copulatory apparatus: horizontal sections on 10 slides.

Diagnosis. Species of *Cratera* with dark-brown dorsal colour, thin median stripe and greyish margins; eyes dorsal with clear halos; pharynx cylindrical; prostatic vesicle almost horizontal; penis papilla conical and symmetrical occupying distal portion of female atrium.

Description. External features. *Body* elongate with parallel margins and dorsal surface slightly convex; anterior tip rounded and posterior tip pointed (Fig. 1). When creeping, maximum length 52mm. After fixation, maximum length 40mm (Table 1). Mouth and gonopore located at posterior fourth of body in average (Table 1).

Live specimens with *dorsal surface* homogeneous dark-brown (Fig. 1). Under stereomicroscope, greyish ground colour visible on anterior tip, on body margins, as well as on thin median stripe occurring along body except for cephalic region. Ventral surface light brown. After fixation, dorsal pigmentation becomes light brown with darker body margins, constituting marginal stripes; thin median stripe remains greyish (Figs 2, 4–5). Ventral surface becomes light grey.

Eyes monolobate, initially uniserial, surround anterior tip (Figs 3–4). After first millimetre of body, eyes become larger and spread onto dorsal surface, occupying maximum width of about one-third of body width on either side of body. Eyes remain dorsal and relatively numerous towards posterior tip (Fig. 3). Inconspicuous clear halos may occur around dorsal eyes (Figs 4–5). Diameter of pigment cups 20–30 µm.

Sensory organs, epidermis and body musculature. *Sensory pits* (Figs 6–7), as simple invaginations (30–40 µm deep), contour anterior tip and occur ventromarginally in irregular, single row in anterior 1/6th of body. Creeping sole occupies the whole body width in pre-pharyngeal region (Fig. 11).

Three types of glands discharge through whole epidermis of pre-pharyngeal region: rhabditogen glands with xanthophil secretion (ventrally with smaller rhabdites)

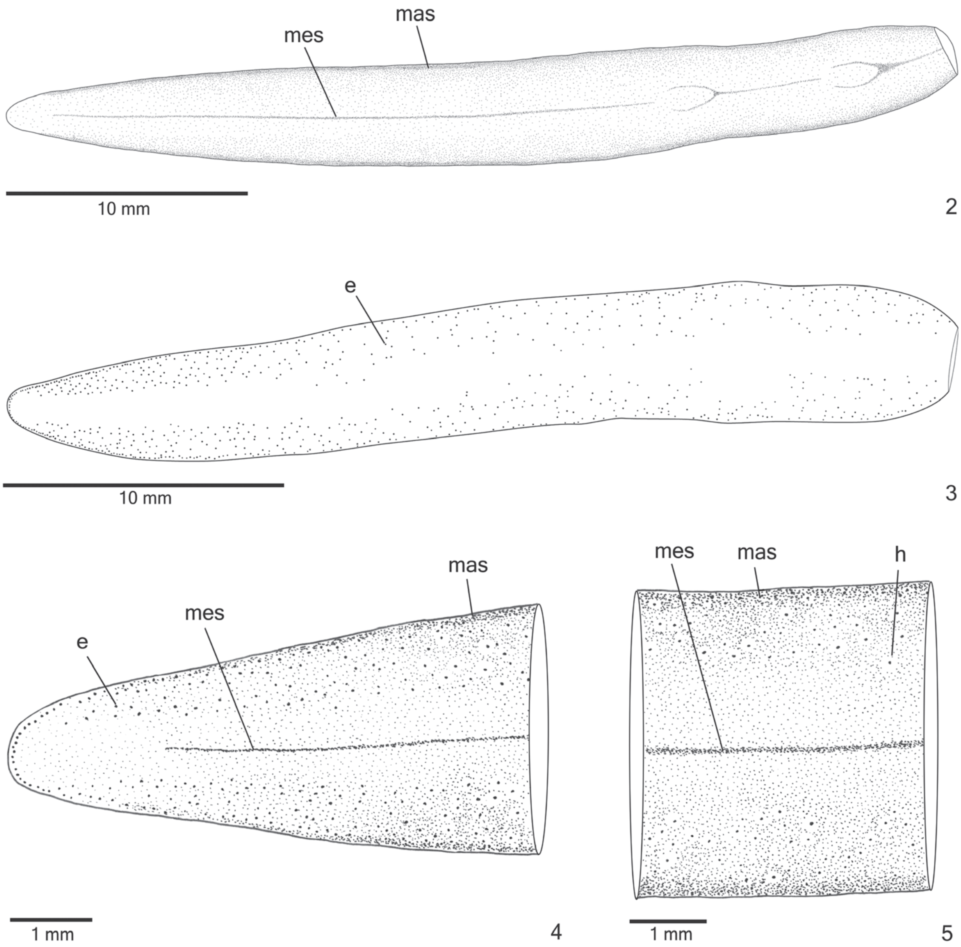
Table 1. Measurements, in mm, of specimens of *Cratera cryptolineata* sp. n. Abbreviations: – not measured; * after fixation; DG distance of gonopore from anterior end; DM distance of mouth from anterior end; DMG distance between mouth and gonopore; DPVP distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

Measurement	Holotype MZUSP PL.1690	Specimen MZU PL.00217	Specimen MZU PL.00218	Specimen MZU PL.00219
Maximum length in extension	50	43	52	45
Maximum width in extension	3	2	2	3
Length at rest	25	10	30	20
Width at rest	6	5	4	7
Length*	40	35	33	34
Width*	4	3	3.5	3.5
DM*	29 (72%)	27 (77%)	25 (76%)	27 (79%)
DG*	37 (92%)	32.5 (93%)	30 (91%)	31 (91%)
DMG*	8	5.5	5	4
DPVP*	4	2.6	2.7	–
Ovaries	9 (22%)	8 (23%)	–	–
Anteriormost testes	9.5 (24%)	10 (29%)	–	–
Posteriormost testes	25.5 (64%)	23.5 (67%)	22 (67%)	–
Length of prostatic vesicle	0.4	0.3	0.3	0.35
Length of penis papilla	0.8	0.6	0.6	0.35
Length of male atrium	0.7	0.5	0.5	–
Length of female atrium	0.5	0.3	0.3	–

and cyanophil glands with amorphous secretion, besides scarce xanthophil glands with fine granular secretion (Figs 10–11). Glandular margin (Figs 8–9) visible after first millimetre of body. At least four types of glands constitute glandular margin: numerous erythrophil glands with fine granules and xanthophil glands with coarse granules; scarcer cyanophil glands with fine granules and rhabditogen glands with xanthophil rhabdites. Glands discharging through anterior tip of body similar to those of pre-pharyngeal region (Figs 6–7).

Cutaneous musculature with usual three layers (circular, oblique and longitudinal layers); longitudinal layer with thick bundles (Figs 8–11, Table 2), becoming progressively lower towards body margins. Thickness of cutaneous musculature between two and five times that of epidermis (Table 2). Ventral musculature with similar thickness or slightly thicker than dorsal musculature at sagittal plane in pre-pharyngeal region (Table 2). In relation to body height, cutaneous musculature thinner in pre-pharyngeal region than in cephalic region (Table 2); thickness gradually diminishes towards anterior tip (Fig. 6).

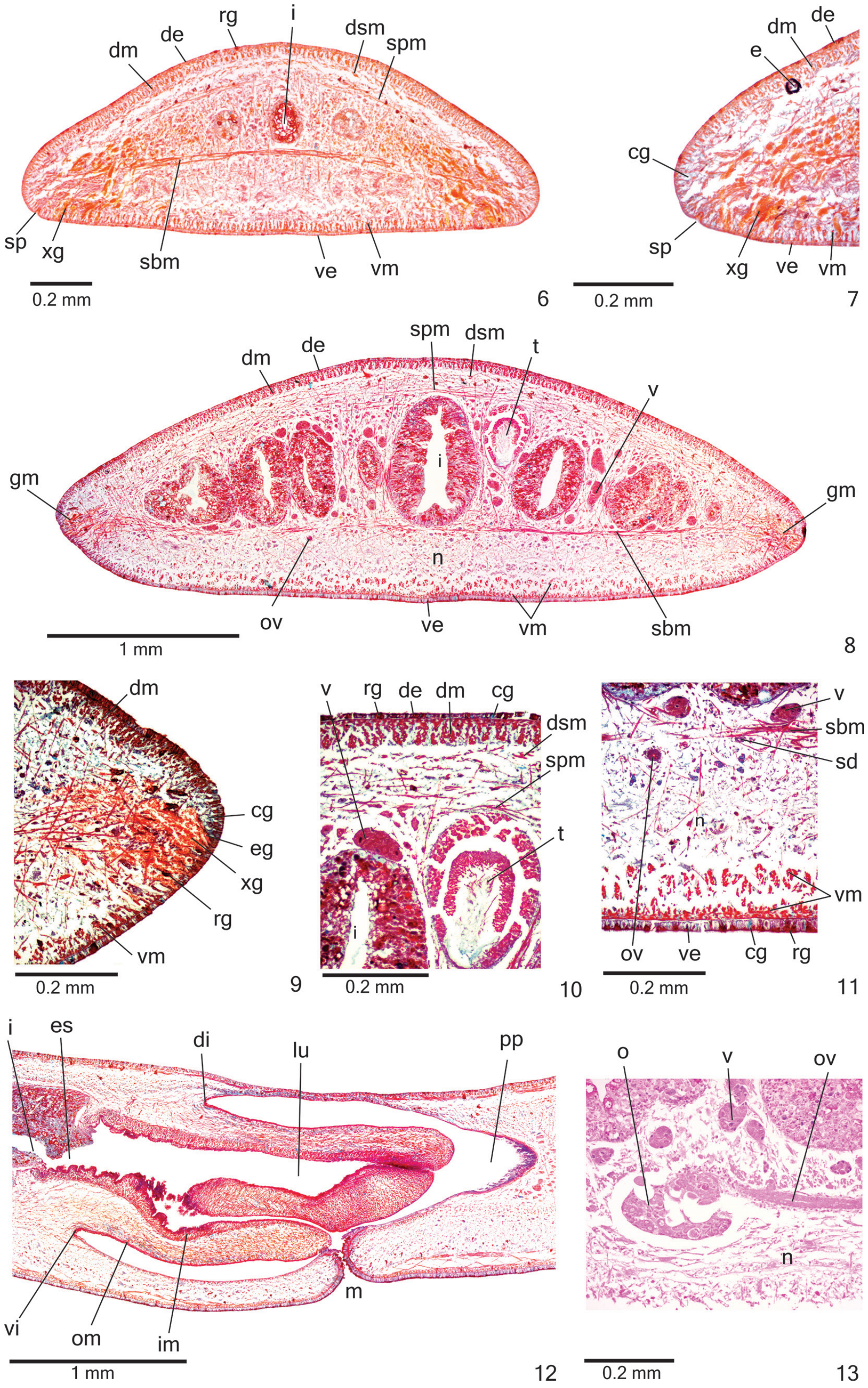
Mesenchymal musculature (Figs 6, 8, 10–11) well developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to cutaneous musculature, with decussate fibres (3–9 fibres thick), (2) supra-intestinal transverse (5–10 fibres thick) and (3) sub-intestinal transverse (6–15 fibres thick). Mesenchymal musculature less developed in anterior region (Fig. 6) than in pre-pharyngeal region.



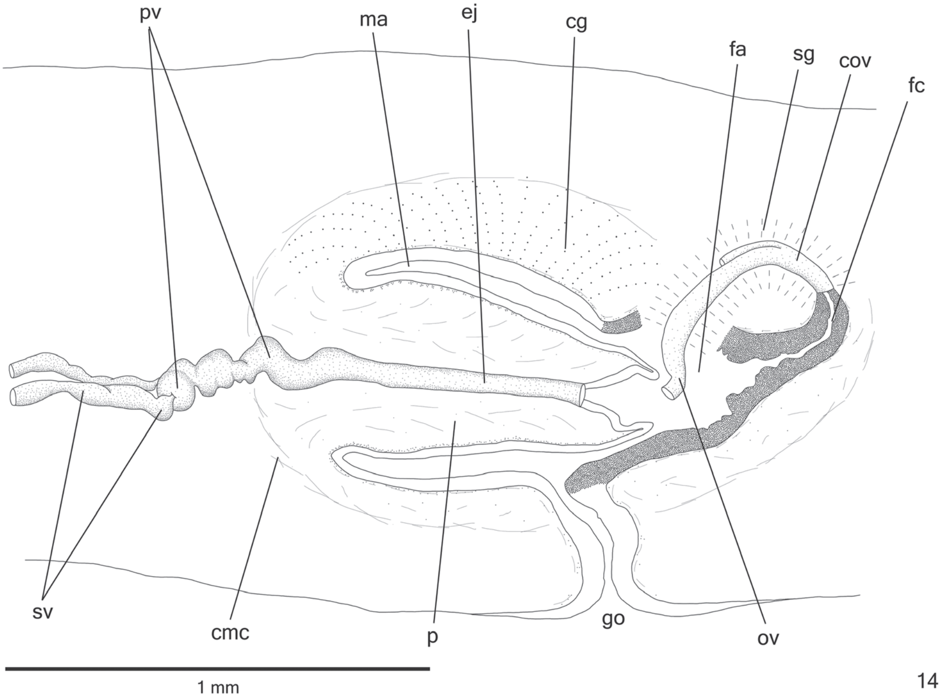
Figures 2–5. *Cratera cryptolineata* sp. n., dorsal view, **2** holotype, pattern of pigmentation **3** specimen MZU PL.00217, eye pattern **4–5** holotype, anterior extremity (**4**) and median third of body (**5**).

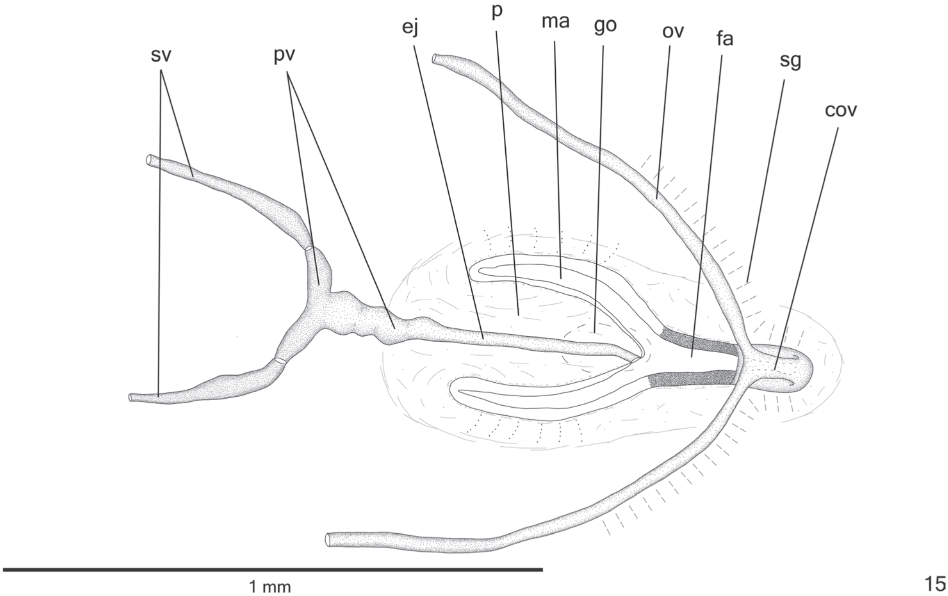
Table 2. Body height and cutaneous musculature in the median region of a transversal section of the pre-pharyngeal (PP) and cephalic (CE) regions, in micrometres, and ratio of the thickness of cutaneous musculature to the height of the body (*mc:h* index) of specimens of *Cratera cryptolineata* sp. n.

Measurement	Holotype MZUSP PL.1690		Specimen MZU PL.00217		Specimen MZU PL.00218
	PP	CE	PP	CE	PP
Dorsal cutaneous musculature	50	47	43	51	45
Ventral cutaneous musculature	58	55	41	50	53
Dorsal epidermis	9	9	12	9	12
Ventral epidermis	25	12	19	12	19
Body height	1252	794	1054	818	955
Mc:h (%)	9	13	8	12	10



Figures 6–13. *Cratera cryptolineata* sp. n., holotype, **6–7** anterior region, transverse section **8–11** pre-pharyngeal region, transverse sections: body margin (**9**), dorsal portion (**10**), ventral portion (**11**) **12** pharynx, sagittal section **13** ovary, sagittal section.





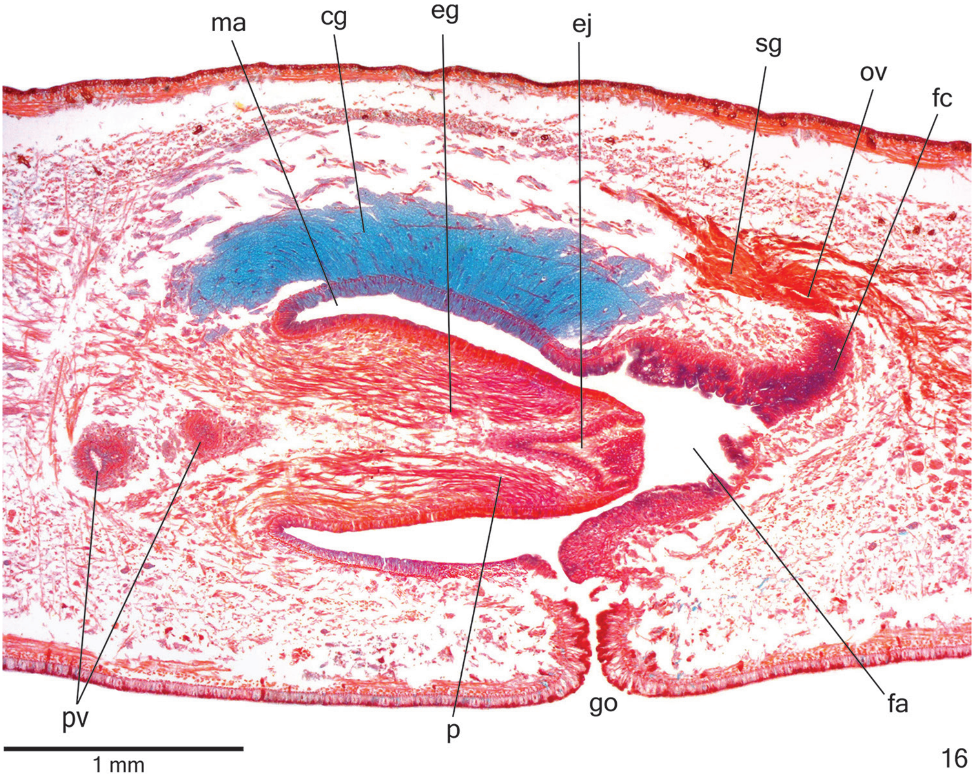
Figures 14–15. *Cratera cryptolineata* sp. n., **14** holotype, sagittal composite reconstruction of copulatory apparatus **15** specimen MZU PL.00219, horizontal composite reconstruction of copulatory apparatus.

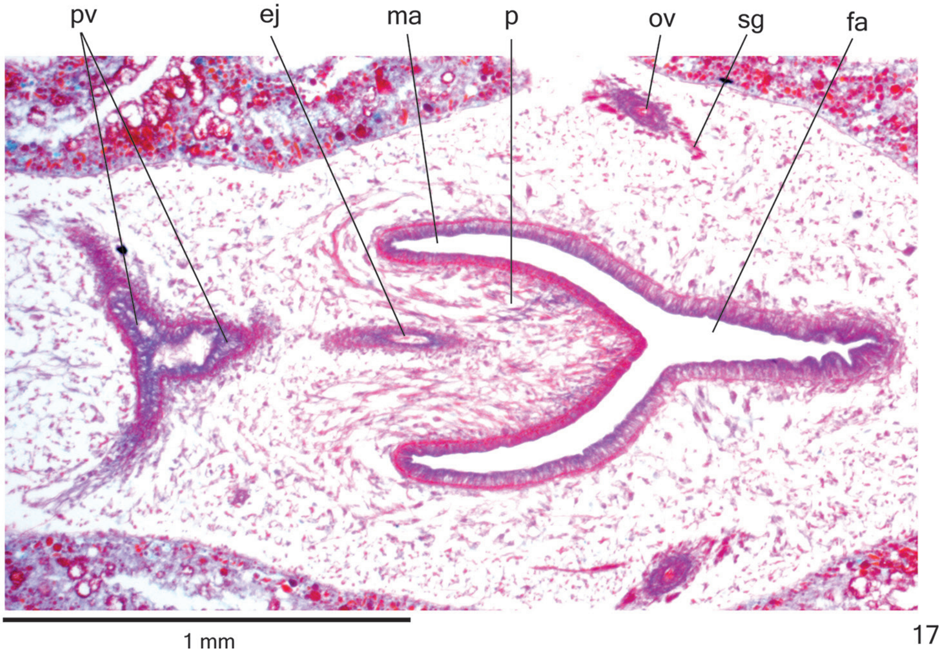
Digestive system. *Pharynx* cylindrical, nearly 5% of body length, occupies 81% of pharyngeal pouch. Pharyngeal dorsal insertion posteriorly shifted next to end of anterior third of pharyngeal pouch. Mouth slightly posterior to dorsal insertion (Fig. 12). Oesophagus short, with folded walls. Oesophagus: pharynx ratio 5%–9%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium, becoming squamous towards pharyngeal tip, with insunk nuclei. Pharyngeal glands constituted by four gland types: erythrophil glands of two types (with coarse and fine granular secretion); xanthophil glands with fine granular secretion and cyanophil glands with amorphous secretion. Outer pharyngeal musculature (4–8 µm thick) comprised of subepithelial layer of longitudinal fibres followed by layer of circular fibres. Inner pharyngeal musculature (30–40 µm thick) composed of thick subepithelial layer with circular fibres, followed by thin layer of longitudinal fibres. Both muscle layers become thinner towards pharyngeal tip. Oesophagus lined by ciliated, cuboidal to columnar epithelium with some insunk nuclei; Musculature of oesophagus (60–100 µm thick) composed of thick subepithelial layer with circular fibres, followed by thin layer with longitudinal fibres.

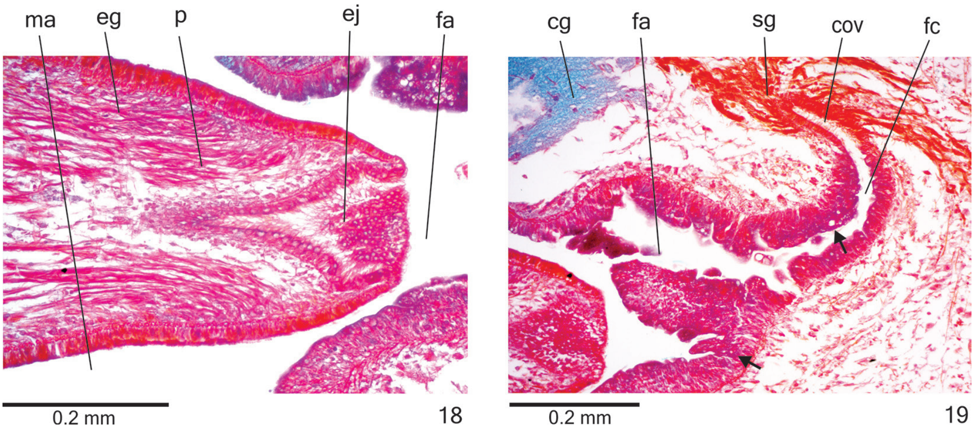
Reproductive organs. *Testes* in one irregular row on either side of body, located beneath dorsal transverse mesenchymal muscles, between intestinal branches (Figs 8, 10), begin slightly posteriorly to ovaries, in anterior fourth of body, and extend to near root of the pharynx (Table 1). Sperm ducts medial to ovovitelline ducts, under or among fibres of sub-intestinal transverse mesenchymal musculature, in pre-pharyngeal region (Fig. 11). They form spermiducal vesicles posteriorly to pharynx. Distally, spermiducal vesicles enter laterally into proximal portion of prostatic vesicle (Figs 14–15, 17). Extrabulbar prostatic vesicle, unpaired, located near common muscle coat, with proximal portion ample and distal portion tubular and sinuous. Proximal portion laterally expanded and T-shaped (Figs 15, 17), almost horizontal, but located closer to ventral epidermis than to dorsal epidermis (Figs 14, 16). Ejaculatory duct almost straight, opening through expansion at tip of penis papilla (Figs 14, 18). Male atrium without folds. Penis papilla conical and symmetrical, projecting into distal portion of female atrium (Figs 14–18, Table 1).

Sperm ducts lined with ciliated, cuboidal epithelium and coated with thin muscularis (approximately 2 µm thick) constituted of interwoven circular and longitudinal fibres. Prostatic vesicle lined with ciliated, tall columnar epithelium. Muscularis of prostatic vesicle (8–20 µm thick) comprises longitudinal and circular intermingled fibres. Ejaculatory duct lined with ciliated, columnar epithelium, with irregular height at expanded portion (Fig. 18). Muscle coat of ejaculatory duct thin (about 4 µm), mainly constituted of circular fibres. Numerous erythrophil glands with fine granular secretion as well as glands with amorphous cyanophil secretion open into both prostatic vesicle and ejaculatory duct. Penis papilla and male atrium columnar (nearly 40 µm thick) lined with non-ciliated, columnar epithelium. Xanthophil and erythrophil glands with fine granular secretion, besides glands with amorphous cyanophil secretion open through penis papilla and male atrium. Openings of cyanophil glands more numerous into male atrium and concentrated at dorso-lateral wall (Fig. 16). Muscula-





Figures 16–17. *Cratera cryptolineata* sp. n., **16** holotype, copulatory apparatus, sagittal section **17** specimen MZU PL.00219, copulatory apparatus, horizontal section.



Figures 18–19. *Cratera cryptolineata* sp. n., holotype, sagittal sections, **18** penis papilla **19** female organs. Arrows indicate lacunae.

ris of penis papilla (10–20 µm thick) and male atrium (6–10 µm thick) composed of subepithelial circular layer, followed by longitudinal layer.

Vitelline follicles (Figs 8, 10–11, 13) situated between intestinal branches. Ovaries ovoid (approximately 200 µm in diameter), dorsal to ventral nerve plate, in anterior fourth of body (Fig. 13, Table 1). Ovovitelline ducts emerge dorsally from median third of ovaries (Fig. 13) and run posteriorly immediately above nerve plate. Ascending portion of ovovitelline ducts located lateral to female atrium. Common glandular ovovitelline duct short, located dorsally to posterior third of female atrium (Figs 14–16, 19). Female genital duct dorso-anteriorly curved. Female atrium funnel-shaped without folds (Figs 16, 19), shorter than male atrium (Table 1).

Ovovitelline ducts and common ovovitelline duct lined with ciliated, columnar epithelium and covered with 5-µm-thick layer of intermingled circular and longitudinal muscle fibres. Numerous shell glands with erythrophil secretion empty into common glandular ovovitelline duct as well as into distal half of ascending portion of ovovitelline ducts (Figs 14–16, 19). Epithelial lining of female genital duct and atrium with irregular height (40–90 µm thick), stratified appearance; epithelial cells with some lacunae containing secretion (Fig. 19). Abundant cyanophil glands with amorphous secretion and erythrophil glands with fine granular secretion, as well as few xanthophil glands with fine granular secretion open into female duct and atrium. Muscularis of female duct and atrium (10–20 µm thick) composed of interwoven longitudinal and circular fibres. Specimens MZU PL.00218, MZU PL.00217 and MZU PL.00219 not fully mature, with poorly developed vitelline follicles, but showing shell glands opening into ovovitelline ducts and common glandular oviduct.

Male and female *atria* with ample communication, without separating folds (Figs 14–17). Common muscle coat thin along both male and female atria, thicker dorsally than ventrally, composed of circular, longitudinal and oblique fibres. Gonoduct vertical, lined with ciliated, columnar epithelium. Numerous cyanophil glands with amorphous secretion and rhabditogen glands with xanthophil secretion, as well as scarce erythrophil glands with fine granular secretion empty into gonoduct. Muscularis of gonoduct comprised of subepithelial layer of circular fibres, followed by longitudinal layer.

Etymology. The specific name is a composite of the Greek adjective *kryptós* (hidden) and the Latin noun *linea* (stripe), referring to the thin median stripe, visible only under the stereomicroscope.

Distribution. Known only from the type locality.

Cratera nigrimarginata sp. n.

http://zoobank.org/2EA2144E-2F1B-4752-9CBB-2AD34CA51633

Material examined. Holotype: MZUSP PL.1691: *leg*. I. Rossi, 18 July 2015, General Carneiro (Araucaria Natural Heritage Private Reserve), state of Paraná, Brazil – anterior region in three fragments on 114 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx: sagittal sections on 19 slides; copulatory apparatus: sagittal sections on 17 slides.

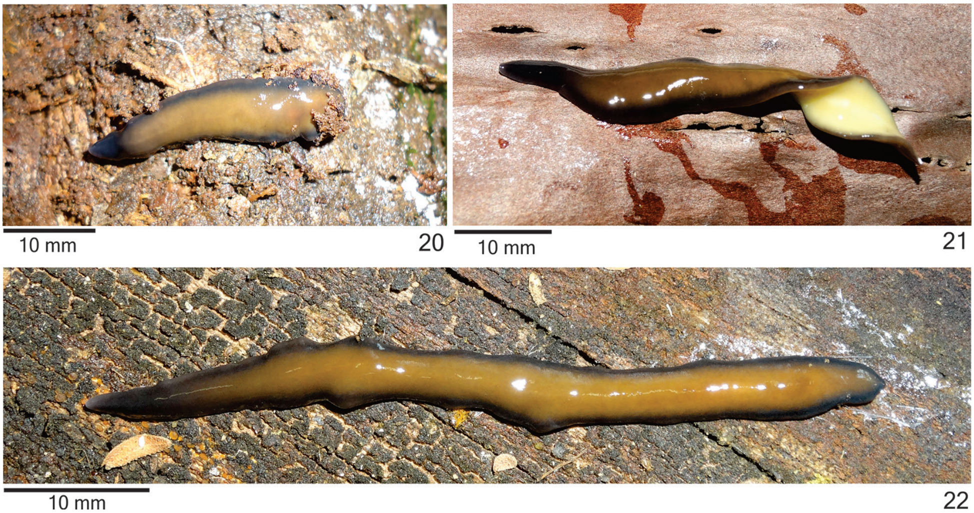
Other specimens: all specimens sampled in the same locality as the holotype. MZU PL.00220: *leg*. I. Rossi, 6 February 2015 – anterior tip: transverse sections on 24 slides; anterior region at the level of the ovaries: sagittal sections on 78 slides; pre-pharyngeal region: transverse sections on 16 slides; pharynx: sagittal sections on 40 slides; copulatory apparatus: sagittal sections on 33 slides. MZU PL.00221: *leg*. J. L. A. Braccini, 4 June 2015 – copulatory apparatus: horizontal sections on 20 slides.

Diagnosis. Species of *Cratera* with light-brownish dorsal colour bordered by dark margins; eyes dorsal with clear halos and bilobed appearance; pharynx cylindrical; prostatic vesicle with unbranched and dilated proximal portion; tip of penis papilla with infolds projecting into ejaculatory duct; cyanophil glands pierce male atrium evenly distributed.

Description. External features. *Body* elongate, flat and with parallel margins; anterior tip rounded and posterior tip pointed (Figs 20–22). When creeping, maximum length 57mm. After fixation, maximum length 47mm. Mouth and gonopore located at posterior fourth of body (Table 3).

Live animals with dorsal surface light-brownish, constituting broad band, bordered by greyish or black margins; cephalic region greyish (Figs 20–22). Ventral surface pale yellow (Fig. 21). Under stereomicroscope, dorsal band bordered by thin black paramarginal stripes. After fixation, besides dorsal band and paramarginal stripes, dorsal surface may contain inconspicuous median stripe (Fig. 23); ventral surface becomes whitish with greyish margins and anterior tip. In preserved specimens, dorsal band with maximum width of about two thirds of body width. Paramarginal stripes, with nearly 1/12th of body width, begin behind the cephalic region (approximately anterior 1/9th of body) and converge towards posterior tip (Figs 23, 25).

Eyes, initially uniserial and monolobate, surround anterior tip (Figs 24–25). After second millimetre of body, eyes become larger and with bilobated appearance (Fig. 27), spreading onto dorsal surface and occupying almost all body width in anterior

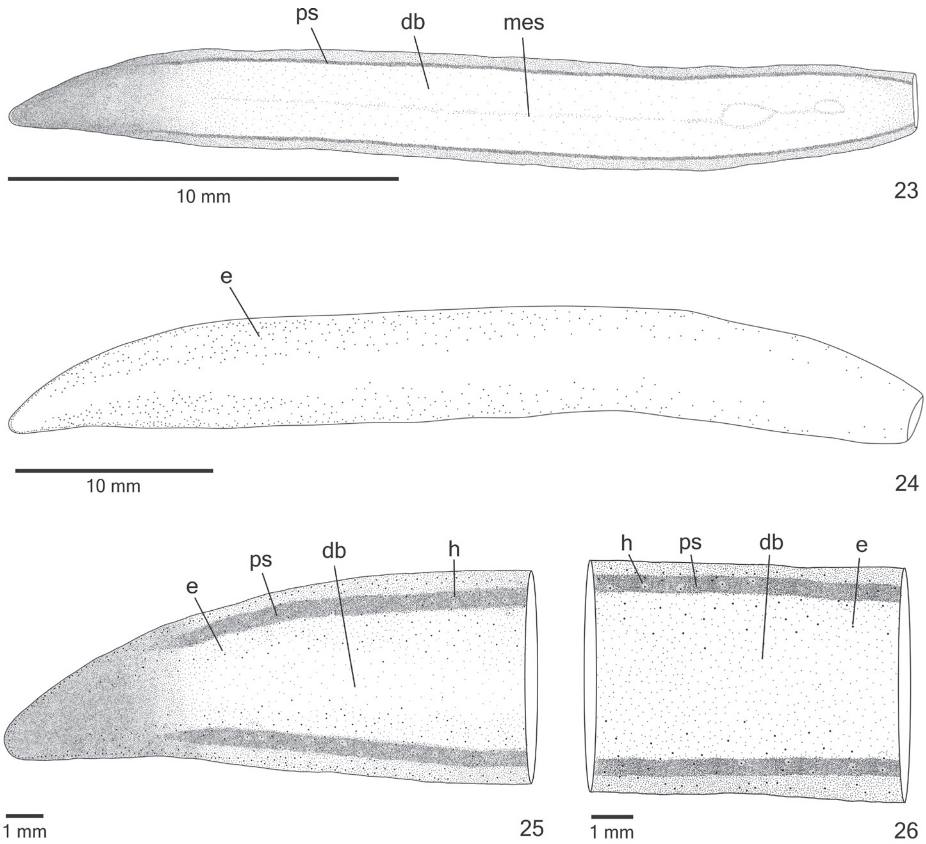


Figures 20–22. *Cratera nigrimarginata* sp. n., habitus, dorsal view, **20** holotype, anterior region **21** specimen MZU PL.00220 with part of ventral surface visible **22** specimen MZU PL.00221.

Table 3. Measurements, in mm, of specimens of *Cratera nigrimarginata* sp. n. Abbreviations: – not measured; * after fixation; DG distance of gonopore from anterior end; DM distance of mouth from anterior end; DMG distance between mouth and gonopore; DPVP distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

Measurement	Holotype MZUSP PL.1691	Specimen MZU PL.00220	Specimen MZU PL.00221
Maximum length in extension	55	57	55
Maximum width in extension	4	4	4
Length at rest	30	46	35
Width at rest	6	5	5
Length*	46	47	45
Width*	5	5.5	4.5
DM*	35.5 (77%)	37 (79%)	37 (82%)
DG*	43.5 (94%)	42 (89%)	41 (91%)
DMG*	8	5	4
DPVP*	0.7	0.6	0.8
Ovaries	10 (22%)	11.5 (24%)	–
Anteriormost testes	13 (28%)	15.5 (33%)	–
Posteriormost testes	30.5 (66%)	33.5 (71%)	–
Length of prostatic vesicle	0.8	0.8	0.7
Length of penis papilla	1.2	1.3	1
Length of male atrium	0.9	0.9	0.9
Length of female atrium	1.2	1.6	1.2

third of body (Fig. 24). Some eyes surrounded by inconspicuous small clear halos over paramarginal stripes (Figs 25–26). Eyes less numerous towards posterior tip. Diameter of pigment cups 15–40 µm.

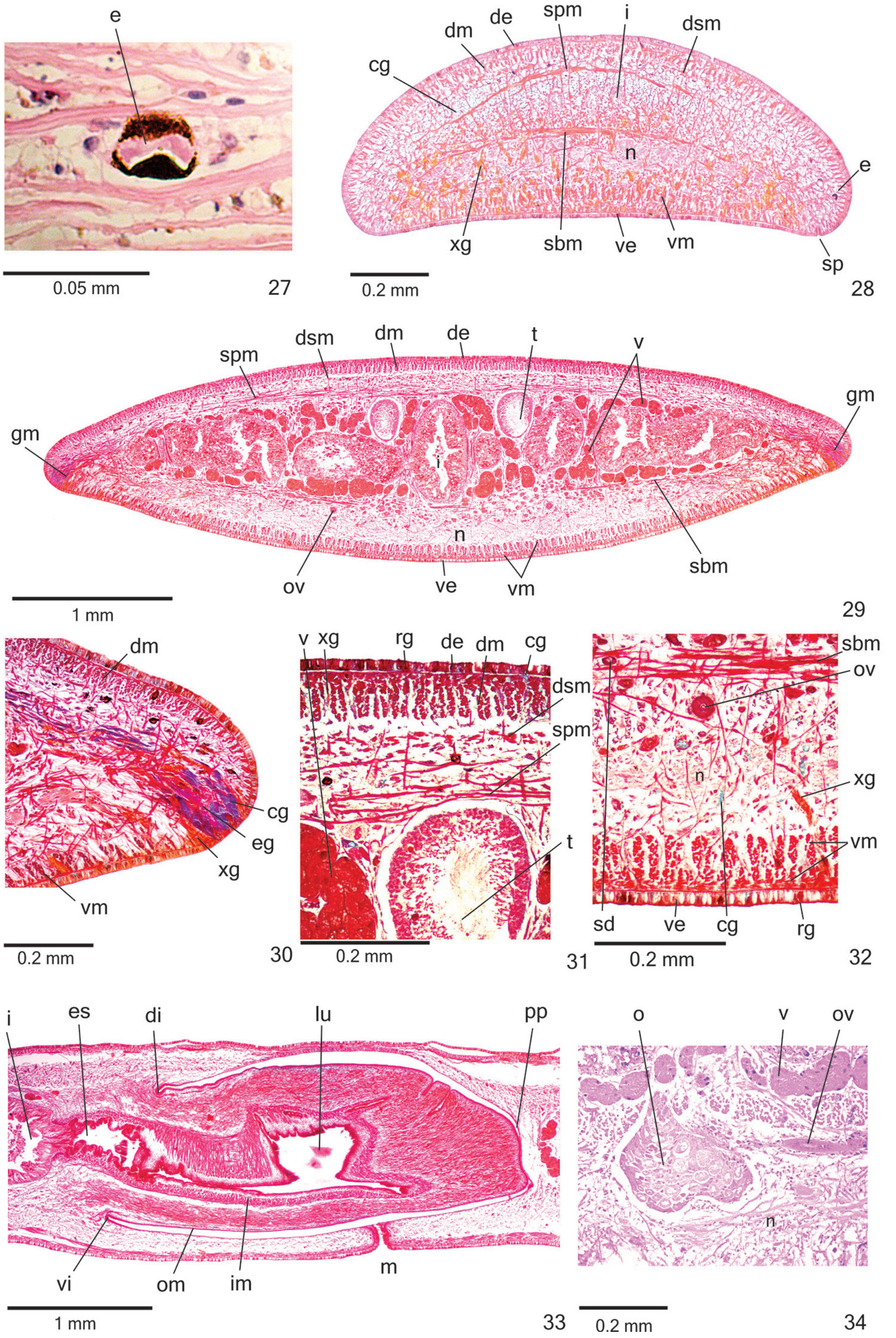


Figures 23–26. *Cratera nigrimarginata* sp. n., dorsal view, **23** holotype, pattern of pigmentation **24–26** specimen MZU PL.00220, eye pattern (**24**), anterior extremity (**25**) and median third of body (**26**).

Sensory organs, epidermis and body musculature. *Sensory pits* (Fig. 28), as simple invaginations (30–40 µm deep), contour anterior tip and occur ventromarginally in irregular, single row in anterior 1/8th of body. Creeping sole occupies 90% of body width in pre-pharyngeal region.

Three types of glands discharge through whole epidermis of pre-pharyngeal region: rhabditogen cells with xanthophil secretion (ventrally with smaller rhabdites), cyanophil glands with amorphous secretion and xanthophil glands with fine granular secretion (Figs 31–32). Few erythrophil glands with fine granular secretion open through ventral epidermis. Glandular margin conspicuous (Figs 29–30), after first millimetre of body. At least five types of glands constitute glandular margin: xanthophil glands with coarse granules of two types (heavily and slightly stained), cyanophil glands of two types (coarse granular and amorphous secretion) and erythrophil glands with coarse granules. Glands discharging through anterior tip of body similar to those of pre-pharyngeal region (Fig. 28).

Cutaneous musculature with usual three layers (circular, oblique and longitudinal layers); longitudinal layer with thick bundles (Figs 29–32, Table 4). Thickness of cu-



Figures 27–34. *Cratera nigrimarginata* sp. n., holotype, **27** dorsal eye, horizontal section **28** anterior region of body, transverse section **29–32** pre-pharyngeal region, transverse sections, body margin (**30**), dorsal portion (**31**), ventral portion (**32**) **33** pharynx, sagittal section **34** ovary, sagittal section.

Table 4. Body height and cutaneous musculature in the median region of a transverse section of the pre-pharyngeal (PP) and cephalic (CE) regions, in micrometres, and ratio of the thickness of cutaneous musculature to the height of the body (mc:h index) of specimens of *Cratera nigrimarginata* sp. n.

Measurement	Holotype MZUSP PL.1691		Specimen MZU PL.00220	
	PP	CE	PP	CE
Dorsal cutaneous musculature	71	37	72	35
Ventral cutaneous musculature	85	85	70	77
Dorsal epidermis	15	15	19	15
Ventral epidermis	22	15	22	15
Body height	1277	484	1104	484
Mc:h (%)	12	25	13	23

taneous musculature between two and four times that of epidermis (Table 4). Ventral musculature with similar thickness or slightly thicker than dorsal musculature at sagittal plane in pre-pharyngeal region (Table 4). Musculature becoming progressively lower towards body margins. In relation to body height, cutaneous musculature thinner in pre-pharyngeal region than in cephalic region, especially ventral musculature (Table 4, Fig. 28); thickness gradually diminishes towards anterior tip.

Mesenchymal musculature (Figs 28–29, 31–32) well developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to cutaneous musculature, with decussate fibres (6–10 fibres thick), (2) supra-intestinal transverse (8–14 fibres thick) and (3) sub-intestinal transverse (10–18 fibres thick). Mesenchymal musculature more developed in cephalic region (Fig. 28) than in pre-pharyngeal region, especially dorsal subcutaneous musculature (12–20 fibres thick).

Digestive system. *Pharynx* cylindrical, approximately 6% of body length, occupies 70% of pharyngeal pouch. Pharyngeal dorsal insertion slightly shifted posteriorly (Fig. 33); mouth in median third of pharyngeal pouch. Oesophagus short with folded wall. Oesophagus: pharynx ratio 4%–12%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium with insunk nuclei. Pharyngeal glands constituted by four secretory cell types: abundant erythrophil glands with fine granular secretion, xanthophil glands with coarse granular secretion, as well as two types of cyanophil glands (with amorphous and fine granular secretions). Outer pharyngeal musculature (10–30 µm thick) comprised of thin subepithelial layer of circular fibres, followed by thin layer of longitudinal fibres. Inner pharyngeal musculature (70–90 µm thick) comprises thick subepithelial layer of circular fibres, followed by thinner layer of longitudinal fibres. Outer and inner muscle layers gradually become thinner towards pharyngeal tip. Oesophagus lined by ciliated, cuboidal to columnar epithelium with insunk nuclei. Musculature of oesophagus (70–120 µm thick) composed of thick layer with circular fibres, followed by layer of longitudinal fibres.

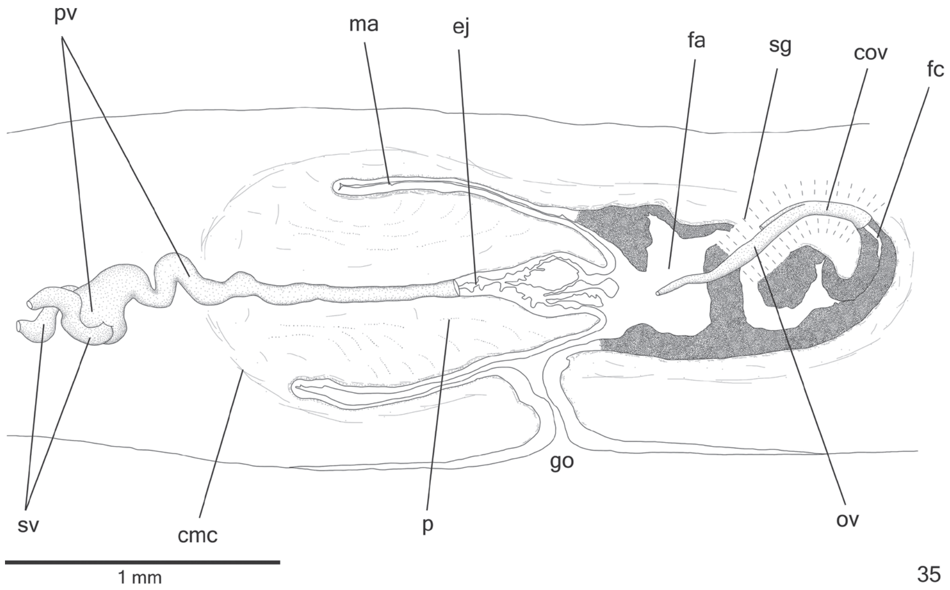
Reproductive organs. *Testes* in one irregular row on either side of body, located beneath dorsal transverse mesenchymal muscles (Figs 29, 31), begin slightly posteriorly to ovaries, in anterior third of body, and extend to near root of pharynx (Table 3).

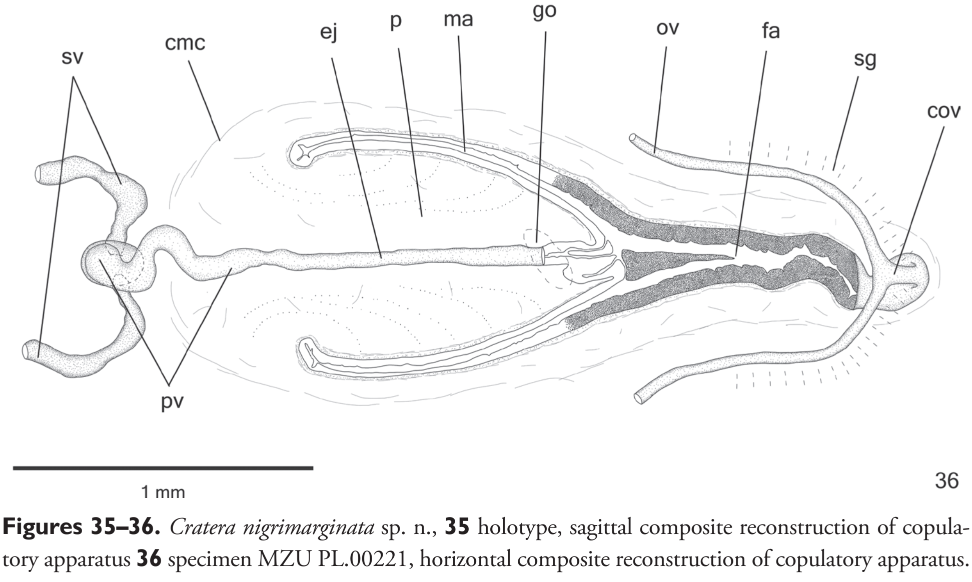
Sperm ducts medial to ovovitelline ducts, among fibres of sub-intestinal transverse mesenchymal musculature, forming spermiducal vesicles laterally to pharynx. Distally, spermiducal vesicles penetrate into lateral wall of proximal portion of prostatic vesicle (Figs 35–36, 38). Prostatic vesicle extrabulbar, unpaired, consisting of two portions: proximal portion short and dilated and distal portion tubular and sinuous. Proximal portion displaced ventrally in relation to distal portion and located closer to ventral epidermis than to dorsal epidermis (Figs 35, 37). Prostatic vesicle of specimen MZU PL.00220 showing larger lumen, filled with secretions. Ejaculatory duct almost straight, expanding at tip of penis papilla. Male atrium without folds. Penis papilla conical and symmetrical (Figs 35–38, Table 3). Tip of penis papilla occupying distal part of female atrium; with infolds projecting into ejaculatory duct (Fig. 40).

Lining epithelium of *sperm ducts* cuboidal and ciliated; thin muscularis (about 5 µm thick) constituted of interwoven circular and longitudinal fibres. Prostatic vesicle lined with ciliated, columnar epithelium. Muscularis of prostatic vesicle (20–40 µm thick) comprises mainly circular fibres mixed with longitudinal and oblique fibres (Fig. 39). Ejaculatory duct lined with ciliated, tall columnar epithelium (Fig. 40). Muscle coat of ejaculatory duct (5–10 µm) constituted of interwoven circular and longitudinal fibres. Erythrophil glands with fine granular secretion as well as cyanophil glands with amorphous secretion open into both prostatic vesicle and ejaculatory duct (Fig. 39). Penis papilla and male atrium lined with non-ciliated, columnar epithelium. Numerous cyanophil glands with amorphous secretion and few erythrophil glands with fine granular secretion open evenly distributed through penis papilla and male atrium. Muscularis of penis papilla (10–20 µm thick) and male atrium (6–10 µm thick) comprised of subepithelial layer of circular fibres, followed by layer of longitudinal fibres.

Vitelline follicles (Figs 29, 31, 34) situated between intestinal branches. Ovaries ovoid (approximately 200 µm in diameter) located dorsal to ventral nerve plate, in anterior fourth of body (Fig. 34, Table 3). Ovovitelline ducts emerge laterally from posterior half of ovaries and run posteriorly immediately above nerve plate. Ascending portion of ovovitelline ducts located lateral to female atrium. Common glandular ovovitelline duct short, located dorsally to posterior third of female atrium. Female genital duct dorso-anteriorly curved (Figs 35–37, 41). Female atrium oval-elongate with folded walls (Figs 35, 37), longer than male atrium (Table 3).

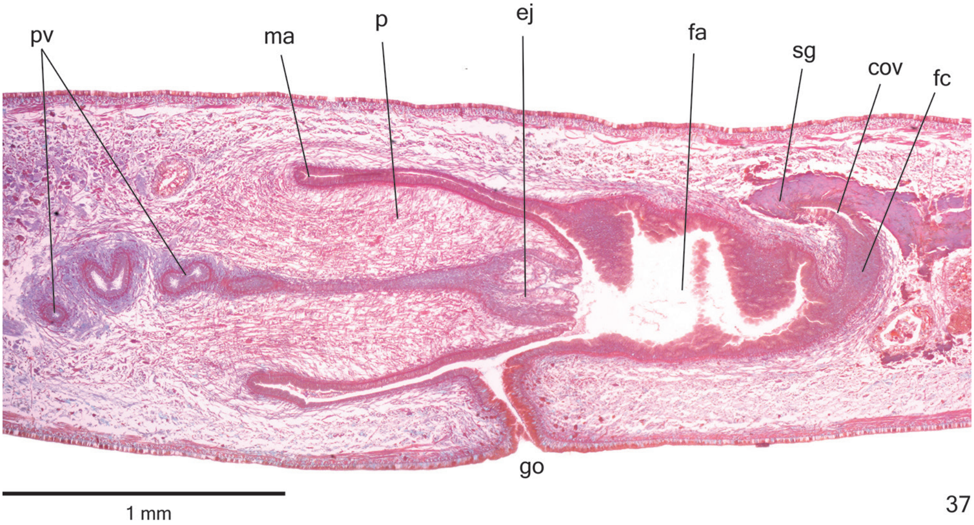
Ovovitelline ducts and common ovovitelline duct lined with ciliated, cuboidal to columnar epithelium and covered with intermingled circular and longitudinal muscle fibres (3–10 µm). Abundant shell glands with erythrophil secretion, besides cyanophil glands, empty into common glandular ovovitelline duct as well as into distal third of ascending portion of ovovitelline ducts (Figs 35–38, 41). Epithelial lining of female genital duct and atrium tall columnar, showing irregular height and sometimes stratified appearance (50–300 µm thick), ciliated in female duct. Epithelial cells with some lacunae containing cyanophil secretion (Figs 37, 41). Abundant cyanophil glands with amorphous secretion and less numerous erythrophil glands with fine granules open into female duct and atrium. Muscularis (10–20 µm thick) of female genital duct

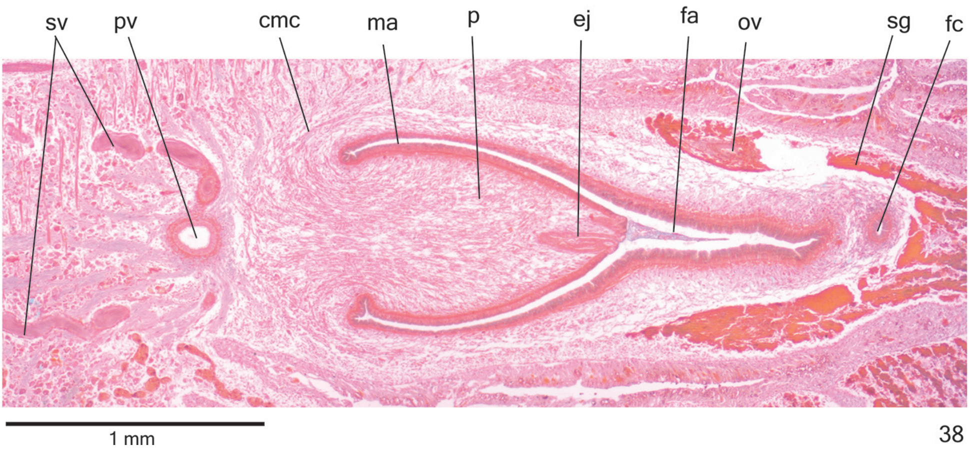




Figures 35–36. *Cratera nigrimarginata* sp. n., **35** holotype, sagittal composite reconstruction of copulatory apparatus **36** specimen MZU PL.00221, horizontal composite reconstruction of copulatory apparatus.

and atrium composed of interwoven circular and longitudinal fibres. Specimen MZU PL.00220 shows poorly developed vitelline follicles, but copulatory organs, including shell glands, fully developed.



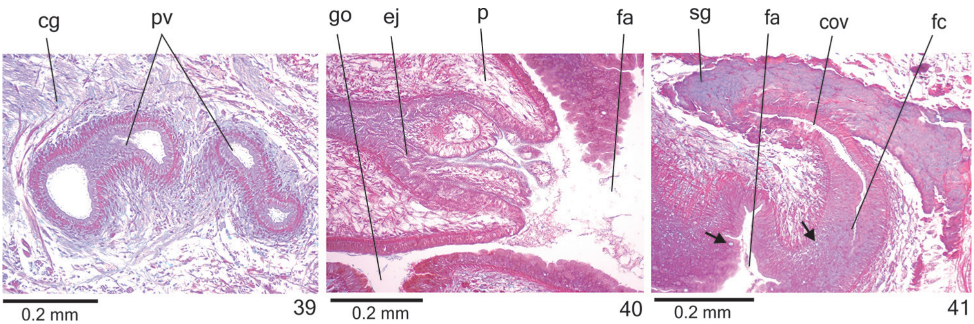


Figures 37–38. *Cratera nigrimarginata* sp. n., **37** holotype, copulatory apparatus, sagittal section **38** specimen MZU PL.00221, copulatory apparatus, horizontal section.

Male and female *atria* broadly communicated each other, without separating folds (Figs 35–38). Common muscle coat thin along both male and female atria, thicker dorsally than ventrally, composed of circular, longitudinal and oblique fibres. Gonoduct vertical, lined with ciliated columnar epithelium. Numerous cyanophil glands with amorphous secretion and rhabditogen glands open into gonoduct. Muscularis of gonoduct comprised of thin subepithelial layer of circular fibres, followed by thin layer of longitudinal fibres.

Etymology. The specific name is a composite of the Latin adjective *niger* (black) and the Latin noun *margo* (margin), referring to the colour pattern with dark margins.

Distribution. Known only from its type locality.



Figures 39–41. *Cratera nigrimarginata* sp. n., holotype, sagittal sections, **39** prostatic vesicle **40** penis papilla **41** female organs. Arrows indicate lacunae.

Cratera aureomaculata sp. n.

http://zoobank.org/E4F5F32E-D05F-49C7-92A6-670A85E1D052

Material examined. Holotype: MZUSP PL.1692: *leg*. J. L. A. Braccini, 3 June 2015, Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 19 slides; anterior region at the level of the ovaries: sagittal sections on 56 slides; pre-pharyngeal region: transverse sections on 14 slides; pharynx: sagittal sections on 35 slides; copulatory apparatus: sagittal sections on 31 slides.

Diagnosis. Species of *Cratera* with dorsal ground colour yellowish covered by brownish pigmentation in cephalic region and blackish pigmentation constituting irregular flecks over rest of dorsum; eyes dorsal with clear halos; pharynx cylindrical; prostatic vesicle unpaired with proximal portion displaced ventrally, laterally expanded and T-shaped; penis papilla conical and symmetrical with ventral insertion posteriorly displaced.

Description. External features. *Body* elongate with parallel margins and dorsal surface slightly convex; anterior tip rounded and posterior tip obtuse (Fig. 42). When creeping, maximum length 55mm. After fixation, maximum length 46mm. Mouth and gonopore located at posterior fourth of body (Table 5).

Live specimens with dorsal ground colour yellowish, covered by brownish pigmentation in cephalic region. Behind cephalic region, blackish pigmentation constitutes irregular flecks over dorsal surface, larger laterally and more concentrated towards posterior tip (Figs 42–43). Ventral surface light grey with yellowish margins; cephalic region (nearly anterior 1/8th of body length) brownish with darker margins.

Eyes monolobate, initially uniserial, surround anterior tip (Figs 44–45). After first millimetre of body, eyes become larger and spread onto dorsal surface, occupying maximum width of approximately one-third of body width on either side of body. Eyes remain dorsal, but less numerous towards posterior tip (Figs 44, 46). Some eyes over dorsal flecks surrounded by inconspicuous clear halos (Figs 45–46). Diameter of pigment cups 20–40 µm.

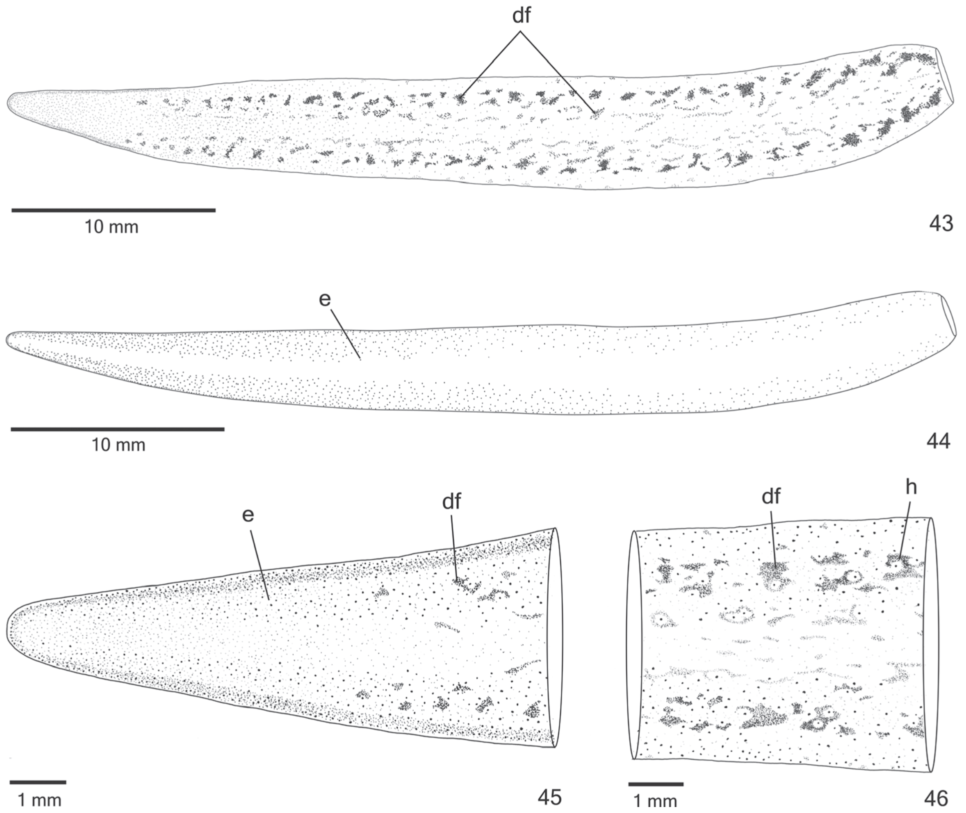
Figure 42. *Cratera aureomaculata* sp. n., holotype, habitus, dorsal view.

Table 5. Measurements, in mm, of the holotype of *Cratera aureomaculata* sp. n. Abbreviations: * after fixation; DG distance of gonopore from anterior end; DM distance of mouth from anterior end; DMG distance between mouth and gonopore; DPVP distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

Measurement	Holotype MZUSP PL.1692
Maximum length in extension	55
Maximum width in extension	4
Length at rest	30
Width at rest	6
Length*	46
Width*	5
DM*	35.5 (77%)
DG*	43.5 (95%)
DMG*	8
DPVP*	4
Ovaries	12.5 (27%)
Anteriormost testes	15.5 (34%)
Posteriormost testes	30 (65%)
Length of prostatic vesicle	0.3
Length of penis papilla	0.7
Length of male atrium	0.8
Length of female atrium	0.6

Sensory organs, epidermis and body musculature. *Sensory pits* (Figs 47–48), as simple invaginations (30–60 µm deep), contour anterior tip and occur ventromarginally in irregular, single row in anterior third of body. Creeping sole occupies whole body width in pre-pharyngeal region (Fig. 52).

Three types of *glands* discharge through whole epidermis of pre-pharyngeal region: abundant rhabditogen cells with xanthophil secretion (rhammites), cyanophil glands with amorphous secretion and xanthophil glands with fine granular secretion (Figs 51–52). Glandular margin (Figs 49–50) visible after anterior 1/16th of body. At least four types of glands constitute glandular margin: xanthophil and erythrophil glands, both

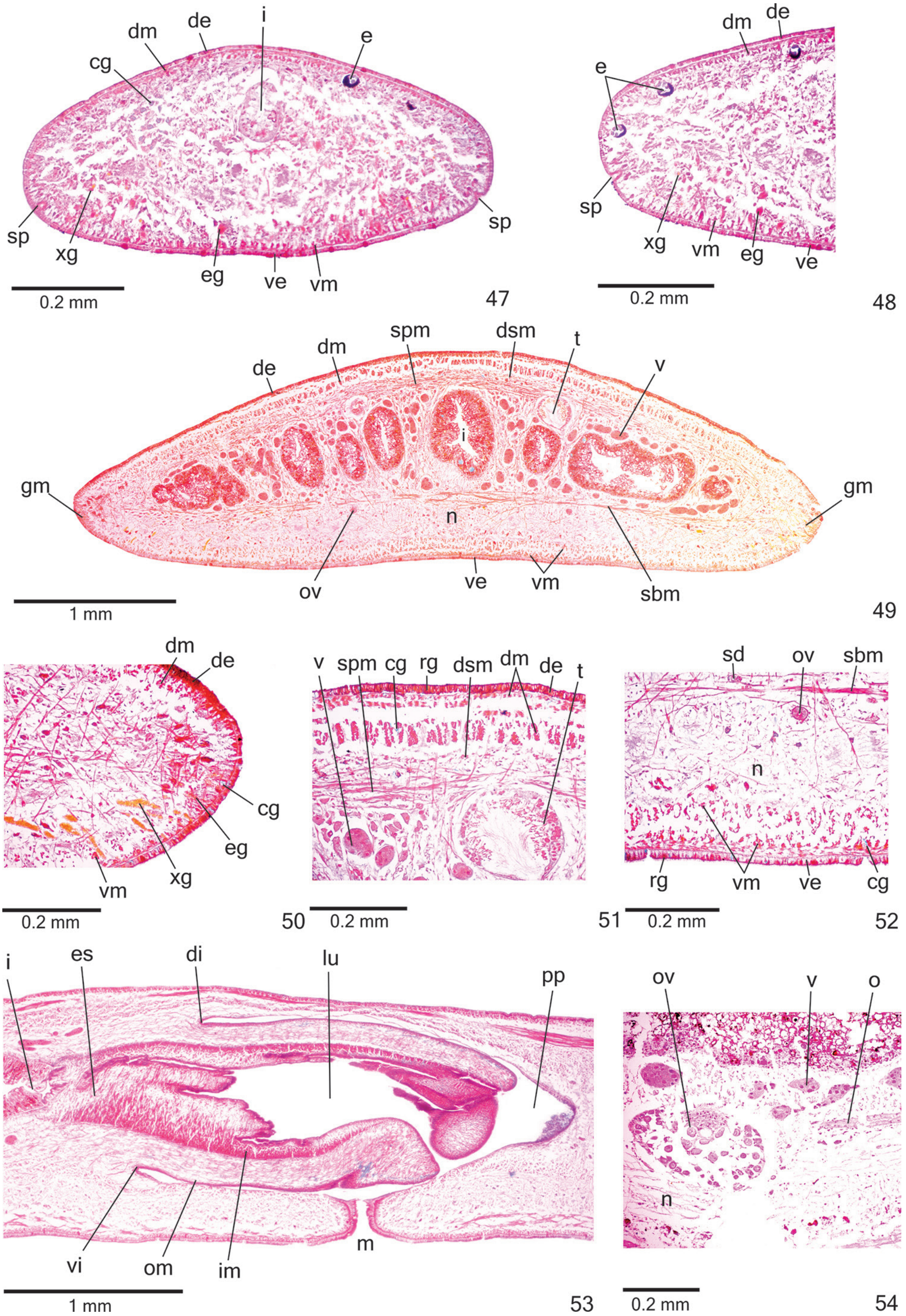


Figures 43–46. *Cratera aureomaculata* sp. n., holotype, dorsal view, **43** pattern of pigmentation **44** eye pattern **45–46** anterior extremity (**45**) and median third of body (**46**).

with coarse granular secretions, besides few xanthophil and cyanophil glands with fine granules. Glands discharging through anterior tip of body similar to those of pre-pharyngeal region (Figs 47–48).

Cutaneous musculature with usual three layers (circular, oblique and longitudinal layers); longitudinal layer with thick bundles (Figs 49–52, Table 6). Thickness of cutaneous musculature between four and five times that of epidermis (Table 6). Ventral musculature thicker than dorsal at sagittal plane in pre-pharyngeal region (Table 6). Musculature becoming progressively lower towards body margins. In relation to body height, cutaneous musculature slightly thinner in pre-pharyngeal region than in cephalic region (Figs 47–48), especially ventral musculature (Table 6); thickness gradually diminishes towards anterior tip.

Mesenchymal musculature (Figs 49, 51–52) well developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to cutaneous musculature, with decussate fibres variously oriented (3–5 fibres thick), (2) supra-intestinal transverse (8–14 fibres thick) and (3) sub-intestinal transverse (8–18 fibres thick). Mesenchymal musculature less developed in cephalic region (Fig. 47) than in pre-pharyngeal region.



Figures 47–54. *Cratera aureomaculata* sp. n., holotype, **47–48** anterior region, transverse section **49–52** pre-pharyngeal region, transverse sections: body margin (**50**), dorsal portion (**51**), ventral portion (**52**) **53** pharynx, sagittal section **54** ovary, sagittal section.

Table 6. Body height and cutaneous musculature in the median region of a transversal section of the pre-pharyngeal (PP) and cephalic (CE) regions, in micrometres, and ratio of the thickness of cutaneous musculature to the height of the body (mc:h index) of the holotype of *Cratera aureomaculata* sp. n.

Measurement	Holotype MZUSP PL.1692	
	PP	CE
Dorsal cutaneous musculature	57	42
Ventral cutaneous musculature	79	50
Dorsal epidermis	15	9
Ventral epidermis	22	12
Body height	1240	719
Mc:h (%)	11	13

Digestive system. *Pharynx* cylindrical, approximately 4% of body length, occupies 90% of pharyngeal pouch. Pharyngeal dorsal insertion slightly shifted posteriorly. Mouth in median third of pharyngeal pouch (Fig. 53). Oesophagus short with folded wall. Oesophagus: pharynx ratio 24%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium with insunk nuclei. Pharyngeal glands constituted by four secretory cell types: numerous erythrophil and xanthophil glands, both with fine granular secretion and cyanophil glands with amorphous secretion, as well as less numerous xanthophil glands with coarse granular secretion. Outer pharyngeal musculature (6–12 µm thick) comprised of thin subepithelial layer of longitudinal muscles, followed by layer of circular fibres. Inner pharyngeal musculature (60–110 µm thick) comprises thick subepithelial layer of circular fibres, followed by layer of longitudinal fibres. Outer and inner muscle layers gradually become thinner towards pharyngeal tip. Oesophagus lined by ciliated, cuboidal to columnar epithelium with insunk nuclei. Musculature of oesophagus (30–50 µm thick) composed of thick layer with circular fibres, followed by layer of longitudinal fibres.

Reproductive organs. *Testes* in one irregular row in either side of body, located beneath dorsal transverse mesenchymal muscles (Figs 49, 51), begin slightly behind anterior third of body and extend to near root of pharynx (Table 5). Sperm ducts medial to ovovitelline ducts, among fibres of sub-intestinal transverse mesenchymal musculature, form spermiducal vesicles posteriorly to pharynx. Distally, spermiducal vesicles bend to enter laterally into proximal expanded portion of prostatic vesicle (Fig. 55). Prostatic vesicle extrabulbar, unpaired, located near common muscle coat, with ample proximal portion and tubular distal portion. Proximal portion laterally expanded and T-shaped, displaced ventrally in relation to distal portion and located closer to ventral epidermis than to dorsal epidermis (Figs 55–57). Ejaculatory duct with slightly sinuous proximal portion and expanded distal portion (Figs 57, 58). Male atrium without folds. Penis papilla conical and symmetrical with ventral insertion posteriorly displaced (Figs 55–56, 58, Table 5).

Sperm ducts lined with ciliated, cuboidal epithelium and coated with thin muscularis (about 3 µm thick) constituted of interwoven circular and longitudinal fibres.

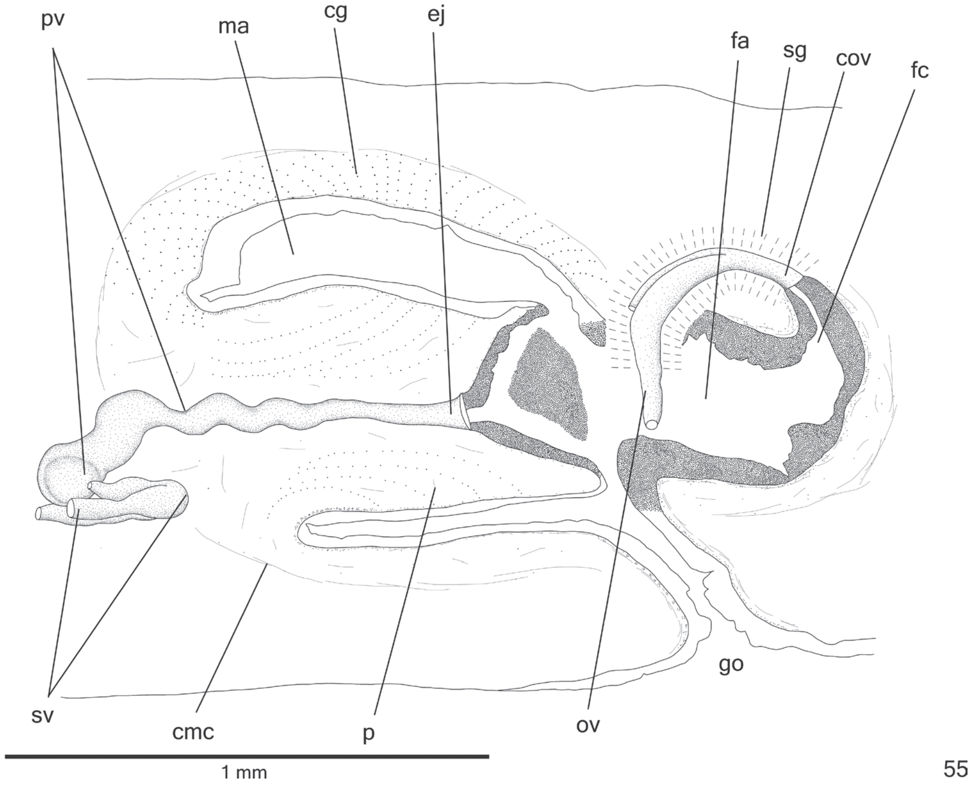
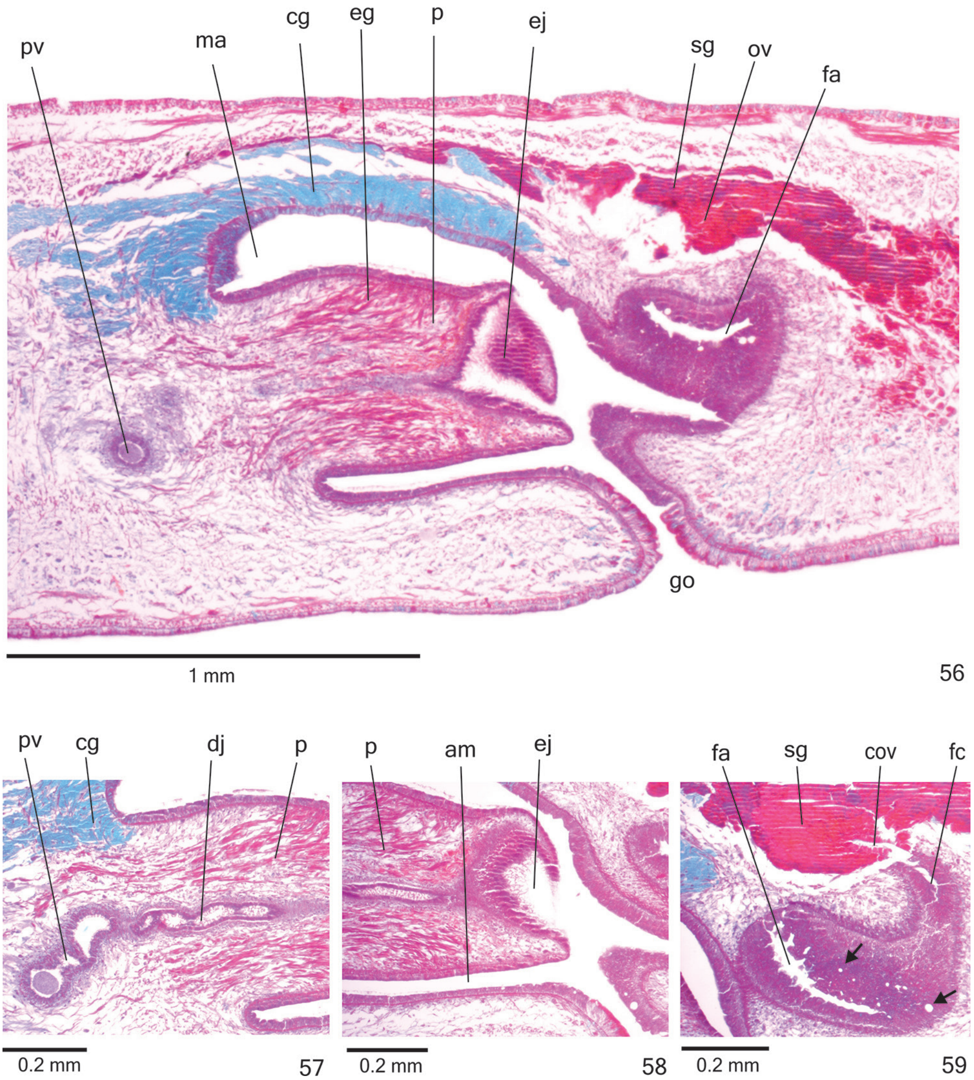


Figure 55. *Cratera aureomaculata* sp. n., holotype, sagittal composite reconstruction of copulatory apparatus.

Prostatic vesicle lined with ciliated, tall columnar epithelium (Fig. 57). Muscularis of prostatic vesicle (8–20 µm thick) comprises interwoven circular, longitudinal and oblique fibres. Ejaculatory duct lined with ciliated, columnar epithelium (Fig. 58). Muscle coat of ejaculatory duct thin (about 6 µm), mainly constituted of circular fibres. Numerous glands with fine granular, mixed secretion (cyanophil external part and erythrophil internal core) empty into both prostatic vesicle and ejaculatory duct; erythrophil glands with fine granules open into ejaculatory duct. Penis papilla and male atrium lined with non-ciliated, columnar or pseudostratified epithelium (approximately 40 µm thick). Erythrophil glands with fine granules, as well as cyanophil glands with amorphous secretion open through penis papilla and male atrium, besides xanthophil glands through penis papilla (Figs 56–58); cyanophil glands concentrate their numerous openings at dorso-lateral wall of male atrium (Figs 55–57). Muscularis of penis papilla (nearly 10 µm thick) and male atrium (5–10 µm thick) composed of subepithelial circular layer, followed by longitudinal layer.

Vitelline follicles (Figs 49, 51, 54) situated between intestinal branches. Ovaries oval-elongate (Fig. 54), two times longer than wide (approximately 200 µm in diameter), located dorsal to ventral nerve plate, in anterior third of body (Table 5).



Figures 56–59. *Cratera aureomaculata* sp. n., holotype, sagittal sections, **56** copulatory apparatus **57** prostatic vesicle **58** penis papilla **59** female organs. Arrows indicate lacunae.

Ovovitelline ducts emerge dorsally from median third of ovaries and run posteriorly immediately above nerve plate. Ascending portion of ovovitelline ducts located at level of gonopore. Common glandular ovovitelline duct short, located dorsally to median third of female atrium. Female genital duct dorso-anteriorly curved (Figs 55, 59). Female atrium funnel-shaped. Length of female atrium about half that of male atrium (Figs 55–56, 59, Table 5).

Ovovitelline ducts and common ovovitelline duct lined with ciliated, cuboidal to columnar epithelium and covered with intermingled circular and longitudinal muscle fibres (approximately 5 µm thick). Abundant shell glands with erythrophil secretion, besides cyanophil glands, empty into common glandular ovovitelline duct as well as into distal third of ascending portion of ovovitelline ducts (Figs 55–56, 59). Epithelial lining of female genital duct and atrium with irregular height and stratified appearance (30–120 µm thick); epithelial cells with some lacunae (Figs 56, 59). Abundant cyanophil glands with amorphous secretion and erythrophil glands with fine granules empty into female duct and atrium. Muscularis of female duct and atrium (8–20 µm thick) composed of interwoven circular and longitudinal fibres.

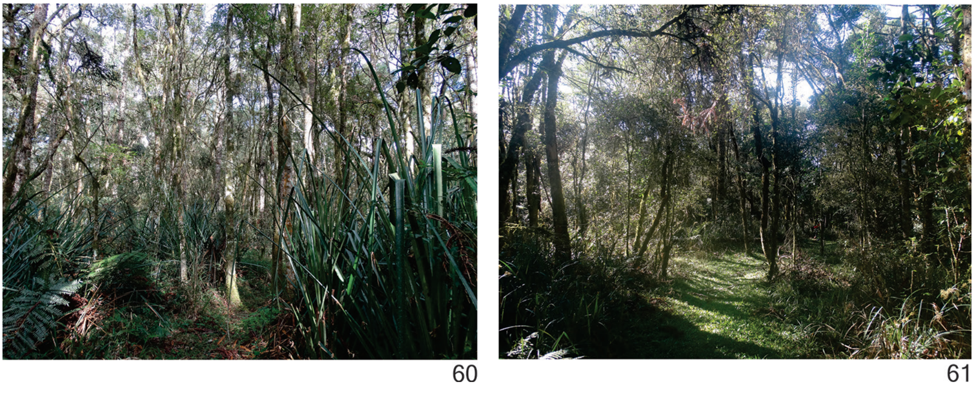
Male and female *atria* with ample communication, without separating folds (Figs 55–56). Common muscle coat thin along both male and female atria, thicker dorsally than ventrally, composed of circular, longitudinal and oblique fibres. Gonoduct anteriorly inclined, lined with ciliated columnar epithelium. Numerous cyanophil glands with amorphous secretion, besides rhabditogen glands, open into gonoduct. Muscularis of gonoduct comprised of subepithelial layer of circular fibres, followed by longitudinal layer.

Etymology. The specific name is a composite of the Latin adjective *aureus* (golden) and the Latin noun *macula* (spot), referring to the colour pattern with yellowish ground colour covered by black irregular flecks.

Distribution. Known only from its type locality.

Notes on ecology and distribution

Cratera cryptolineata and *Cratera aureomaculata* are sympatric in its type-locality, the Três Barras National Forest, in areas of *Araucaria* moist forest. They were recorded during night samplings in areas characterized by the dominance of *Bromelia antiachanta* Bentol. in the understorey (Fig. 60). *Cratera cryptolineata* showed high abundance in such areas, whereas *Cratera aureomaculata* was represented by a single specimen.



Figures 60–61. Sampling sites in type localities, **60** Três Barras National Forest, state of Santa Catarina, Brazil **61** Araucaria Natural Heritage Private Reserve, state of Paraná, Brazil.

Cratera nigrimarginata occurred only in its type-locality, the Araucaria Natural Heritage Private Reserve, in a site of *Araucaria* moist forest showing an initial stage of regeneration with poorly developed understorey (Fig. 61). The species showed low abundance during both day and night samplings.

Discussion

The three new species herein described can be easily assigned to the genus *Cratera* Carbayo et al., 2013, by presenting its diagnostic features, such as ejaculatory duct forming a distal cavity in the penis papilla, position of the ovovitelline ducts by approaching the female atrium and funnel-shaped female atrium.

By showing dorsal eyes and a cylindrical pharynx, the three species herein described share superficial similarities with four other species of *Cratera*: *C. joia* (Froehlich, 1956), *C. anamariae* Carbayo, 2015, *C. ochra* Rossi et al., 2015 and *C. viridimaculata* Negrete and Brusa, 2016 (Froehlich 1956, Carbayo and Almeida 2015, Rossi et al. 2015, Negrete and Brusa 2016), to which they are comparatively discussed.

Cratera cryptolineata sp. n.

Regarding the colour pattern, by having an almost homogeneous, dark brown dorsal surface with a thin median stripe, *Cratera cryptolineata* can be differentiated from *C. anamariae* and *C. ochra*, which show a yellowish ground colour with black pigmentation forming stripes or bands (Carbayo and Almeida 2015, Rossi et al. 2015), and from *C. viridimaculata,* with dorsal surface stippled with dark grey fine spots on a light olive green background. The colour pattern of *C. cryptolineata* is similar to that of *C. joia*, but the latter has a broader median stripe and conspicuous clear halos surrounding eyes, whereas in *C. cryptolineata* clear halos are inconspicuous (Froehlich 1956).

With respect to the copulatory apparatus, *C. cryptolineata*, showing a penis papilla tip slightly posterior to the gonoduct, can be differentiated from *C. joia*, in which the penis papilla is longer, occupying half of the female atrium length. In addition, *C. cryptolineata* differs from *C. joia*, *C. anamariae* and *C. viridimaculata* by having a prostatic vesicle unforked and an almost horizontal orientation, whereas in these three species it is curved ventrally, besides being forked in *C. anamariae* (Froehlich 1956, Carbayo and Almeida 2015, Negrete and Brusa 2016). By showing the penis papilla with both insertions at the same transversal level, *C. cryptolineata* can be distinguished from *C. ochra* and *C. viridimaculata*, which show the penis papilla with the ventral insertion posteriorly displaced (Rossi et al. 2015, Negrete and Brusa 2016). The anatomy of the female atrium of *C. cryptolineata*, ample and without folds, also differs from that of *C. anamariae*, which has lateral folds (Carbayo and Almeida 2015).

Cratera nigrimarginata **sp. n.**

By showing a light-brownish dorsal colour bordered by dark marginal stripes, *Cratera nigrimarginata* can be easily differentiated from *C. anamariae,* which has two broad lateral stripes, *C. ochra*, with dispersed pigmentation forming two broad bands, and *C. viridimaculata,* which show dispersed pigmentation without forming bands (Carbayo and Almeida 2015, Rossi et al. 2015, Negrete and Brusa 2016). *Cratera nigrimarginata* can also be differentiated from *C. joia* and *C. cryptolineata*, both with a light median stripe and the rest of the dorsum strongly pigmented (Froehlich 1956). In addition, *C. nigrimarginata* differs from their congeners by having dorsal eyes with a bilobated appearance, whereas other species show typical monolobated eyes along the body.

Regarding the copulatory apparatus, *C. nigrimarginata* shows an unbranched prostatic vesicle with dilated proximal portion, being differentiated from *C. cryptolineata*, *C. ochra* and *C. joia* with a prostatic vesicle showing proximal diverticula. In addition, it differs from *C. anamariae* and *C. viridimaculata*, which show a prostatic vesicle with forked proximal portions, C-shaped in *C. viridimaculata*. By having openings of cyanophil glands evenly distributed into the male atrium, *C. nigrimarginata* also differs from these species, in which the openings of cyanophil glands concentrate dorsolaterally into the male atrium.

Cratera aureomaculata **sp. n.**

Cratera aureomaculata shows a distinctive colour pattern, showing a blackish pigmentation constituting irregular flecks over the yellowish dorsal ground colour and a brownish pigmentation in the cephalic region. Thus, it differs from stripped species, such as *C. nigrimarginata* and *C. anamariae*, as well as from species showing a strongly pigmented dorsal surface with a light median stripe, such as *C. joia* and *C. cryptolineata* (E.M. Froehlich 1955, Froehlich 1956, Carbayo and Almeida 2015). It can also be distinguished from *C. ochra*, which shows dispersed pigmentation forming two broad bands, and from *C. viridimaculata* with dark grey body margins and cephalic region (Rossi et al. 2015, Negrete and Brusa 2016).

With respect to the copulatory apparatus, *C. aureomaculata* shows a prostatic vesicle with proximal portion laterally expanded and T-shaped, differing from *C. nigrimarginata*, which has a prostatic vesicle with dilated proximal portion, as well as from *C. anamariae* and *C. viridimaculata* which show a prostatic vesicle with forked proximal portions, C-shaped in the latter. By showing the penis papilla with the ventral insertion posteriorly displaced and the proximal portion of the prostatic vesicle ventrally displaced, *C. aureomaculata* differs from *C. cryptolineata* with both insertions at the same transversal level and prostatic vesicle almost horizontal. *C. aureomaculata* shows the penis papilla tip anterior to the gonoduct and a common ovovitelline duct dorsal to the female atrium, being differentiated from *C. joia*, in which the penis papilla is longer, occupying half of the female atrium length, and a common ovovitelline duct is absent. *C. aureomaculata* can be distinguished from *C. ochra* by the position of the proximal portion of the prostatic vesicle, which is more ventrally located in relation to the rest of the vesicle in *C. aureomaculata* than in *C. ochra*, in which the prostatic vesicle is almost horizontal.

Key to the species of the genus *Cratera* in the Neotropical region

1	Colour pattern with stripes or bands	**2**
–	Colour pattern without stripes or bands	**8**
2	Eyes spreading over the dorsal surface	**3**
–	Eyes exclusively on the margins or lateral parts of the body	**9**
3	Pharynx cylindrical	**4**
–	Pharynx bell-form	**12**
4	Prostatic vesicle with proximal portion laterally expanded and T-shaped	**5**
–	Prostatic vesicle with another form	**6**
5	Dark-brown dorsal colour, with a thin median stripe and greyish margins	***C. cryptolineata* sp. n.**
–	Yellow-ochre dorsal colour with dispersed greyish or greyish-brown pigmentation constituting two broad dorsal bands	***C. ochra* Rossi et al., 2015**
6	Unbranched prostatic vesicle with dilated proximal portion	***C. nigrimarginata* sp. n.**
–	Prostatic vesicle with forked proximal portion or with proximal diverticula	7
7	Dark-greyish dorsal colour with rusty median stripe, anterior tip and margins	***C. joia* (Froehlich, 1956)**
–	Yellow dorsal colour with two paramedian black stripes	***C. anamariae* Carbayo, 2015**
8	Prostatic vesicle tubular and C-shaped with forked proximal portion	***C. viridimaculata* Negrete & Brusa, 2016**
–	Prostatic vesicle with proximal portion laterally expanded and T-shaped	***C. aureomaculata* sp. n.**
9	Short and wide penis papilla with a large intra-penial cavity	***C. cuarassu* Carbayo & Almeida, 2015**
–	Conical and symmetrical penis papilla without intra-penial cavity	**10**
10	Pharynx bell-form	***C. steffeni* Rossi et al., 2014**
–	Pharynx cylindrical	**11**
11	Orange ground colour with a light median stripe and greenish pigmentation on the anterior tip	***C. yara* (Froehlich, 1955)**
–	Colour pattern with four thin, black stripes, besides orange marginal bands and thin median stripe	***C. pseudovaginuloides* (Riester, 1938)**
12	Black dorsal surface with a thin and light median stripe	***C. crioula* (Froehlich, 1955)**
–	Yellowish ground colour with brownish pigmentation forming bands	***C. tamoia* (Froehlich, 1955)**

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An early record of *Meloidogyne fallax* from Ireland

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Abstract

Root-knot nematodes, *Meloidogyne* spp., cause huge economic losses worldwide. Currently, three *Meloidogyne* spp. are present on the quarantine A2 list of EPPO, *M. chitwoodi*, *M. fallax* and *M. enterolobii*. As a quarantine organism, *M. fallax* has been detected in England and Northern Ireland on sport turf in 2011, and in England on leek in 2013. However, its presence in Ireland has probably been overlooked since 1965, when Mr. John F. Moore and Dr. Mary T. Franklin had detected a new *Meloidogyne* species for that time. While the relevant data was recorded and a preliminary manuscript describing the species was prepared but never submitted for publication, and together with the original slides, pictures and drawings, it was restudied recently. We compared the population of Irish *Meloidogyne* sp. to other similar *Meloidogyne* spp. Careful observation and comparison shows that it belongs to *M. fallax*. The characters found to be common for Irish *Meloidogyne* sp. and *M. fallax* are female stylet length (14.6 µm) with oval to rounded basal knobs, oval shaped perineal pattern with moderately high dorsal arch, slender stylet in males (18.5 µm) with set off and rounded basal knobs, slightly set off male head with one post-labial annule and incomplete transverse incisures, and second-stage juveniles with large and rounded stylet basal knobs, and a gradually tapering tail (46.9 µm) with a broadly rounded tip and a clearly delimitated smooth hyaline part sometimes marked by constrictions (12.9 µm). The host test and gall formation also correspond to *M. fallax*. The identification could not be additionally supported by molecular analysis, as we were unable to extract DNA from the old permanent slides. Nevertheless, our study reveals that the *Meloidogyne* species detected in Ireland in 1965 belongs to *M. fallax*.

Keywords

Root-knot nematode, Cork, morphology, morphometrics, host, characters

Introduction

Nematodes belonging to *Meloidogyne* spp. are among the most dangerous plant-parasitic nematodes worldwide and cause huge economic losses (Elling 2013). Out of more than 100 described *Meloidogyne* species (Hunt and Handoo 2009), three of them are present on the A2 list of EPPO at the moment, *M. chitwoodi*, *M. enterolobii* and *M. fallax* (EPPO/PQR 2014). There are very few records on the presence of root-knot nematodes in Ireland. However, they attracted attention after Entwistle (2003) described the process of developing yellow patches symptoms on golf courses throughout the UK and Ireland. These samples were positive for *M. naasi* and a new undescribed species which Karssen et al. (2004) described as *M. minor*. In 2011, *M. fallax* was detected on sport turf in Northern Ireland and England (EPPO 2013). There was a new record in 2013 in organic leeks (*Allium ampeloprasum* L.) in England with a very low risk to spread further. It was suspected that *M. fallax* was introduced with plant waste and soil of leeks produced in other EU member states (EPPO, OEPP/EPPO, 2013). Currently, *M. fallax* has been declared present with a restricted distribution in Northern Ireland and England (EPPO/PQR 2014). Nevertheless, the first presence of *Meloidogyne* spp. in Ireland has been overlooked. An annual report of Plant Sciences and Crop Husbandry Division (now named Teagasc) contains information about the *Meloidogyne* species attacking tomato (Moore 1965). In December 1965, the samples of galled tomato roots from an unheated greenhouse in Clonakilty, Cork were sent to the laboratory of Horticultural & Forestry Research Centre in Kinsealy for analysis. These galls contained visible *Meloidogyne* sp. females with well developed egg masses on the root surface. After extraction from the original tomato roots and from tomato roots grown in infested soil at the laboratory, all life stages of the nematode were obtained. In addition, the annual report of the Plant Sciences and Crop Husbandry Division includes a brief description and a host range test of this species (Moore 1966). Based on observations of Mr. John F. Moore and Dr. Mary T. Franklin (Rothamsted, UK) from 1965/66, it was marked as a new species which differed from all the known species at that time based on the male head and the unique perineal pattern in females. The name of the species was proposed, in an unpublished manuscript, as *Meloidogyne corkensis*, according to the county Cork where it had been found. In 1995, the Dutch NPPO received the original material of *Meloidogyne* sp. from Cork including permanent slides of 23 whole females, 18 males, 27 second-stage juveniles and 6 female perineal patterns, an unpublished manuscript, pictures and drawings. Based on our observations of this material, we hypothesize that it belongs to *M. fallax*, a quarantine species described five decades afterwards.

The main goal of our study was to compare the available original material of the population of *Meloidogyne* sp. detected in 1965 in Ireland to the type material of other similar *Meloidogyne* spp. Additionally, we tried to extract the DNA from the permanent slides originating from 1965 and 1966.

Materials and methods

Morphological and morphometrical analysis

In 1995, the Dutch NPPO received the original permanent slides of 23 whole females, 18 males, 27 second-stage juveniles and 6 female perineal patterns, including pictures, measurements and an unpublished manuscript. In 2005, all slides were re-mounted in glycerol.

Morphological observations of glycerine-embedded permanent slides of Irish *Meloidogyne* sp. were done using a compound light microscope (Zeiss Axio Imager 2). Pictures were obtained using a Leica DFC 450 digital camera. A compound light microscope (DM 2500, LEICA) equipped with differential interference contrast (DIC) was used for making drawings. Drawings and pictures were subsequently edited using GNU Image Manipulation Program (http://gimp.org). Permanent slides of the Irish *Meloidogyne* population were compared to type material (slides & living type populations) and reference populations, of similar *Meloidogyne* spp. (Table 1). See also Karssen (2002) and Karssen et al. (2004) for more details.

Host test

The original manuscript from 1966 describes in detail the conducted host range test of the Irish *Meloidogyne* sp.: Infested soil from the original site was placed together with a potential host plant species (seed/plant transplant) in 4-inch earthenware pots which were maintained in a glasshouse. The plant species used for the host range test are listed in Table 7. After 2 to 3 months, plants were removed from the pots and the root systems were examined for infections. The roots without visible galls were stained with cotton blue lactophenol to demonstrate if infection occurred. Infected plants are marked as a positive (+) and non-infected plants are marked as a negative (-).

Table 1. Populations of *Meloidogyne* spp. used for comparison to the original slides of an unknown Irish species.

Species	Material	Number	Male	Female	J2
M. chitwoodi	Type slides	(WT2076-WT2079)	2 paratypes	4 PP paraytpes	26 paratypes
	Reference live material	E7149	31	/	31
M. fallax	Type slides	WT3127-WT3130	2 paratypes	2 PP paratypes	5 paratypes
	Type live material	E6147	30	/	30
M. minor	Type slides	WT3371-WT3374	2 paratypes	2 PP paratypes	5 paratypes
	Type live material	F714-4	27	/	30
M. hapla	Reference live material	C3093	/	/	23
M. incognita	Reference live material	Rgi-23/42	30	/	30

Molecular analysis

DNA extraction

As only permanent slides of the Irish *Meloidogyne* sp. originating from 1966 were available, we attempted to extract the DNA from fixed nematodes based on Rubtsova et al. (2005). Briefly, slides of second-stage juveniles of Irish population were carefully broken with scalpel and T.A.F.-fixed specimens were transferred to staining blocks containing phosphate-buffered saline (PBS). Two protocols of DNA extraction were performed, i) with NaOH and Tween solution (Janssen et al. 2016), and ii) with Worm Lysis Buffer (20 mMTris-HCl, 100 mMKCl, 3.0 mM MgCl2, 2.0 mM DTT, 0.9% Tween 20) and Proteinase K (Rubtsova et al. 2005). As a positive control, DNA was extracted from three fresh second-stage juveniles of *M. fallax* (source ID: E6147; host: *Lycopersicon esculentum* L.).

PCR and gel electrophoresis

For amplification of a 120 bp region of COX1 gene, the forward primer, JB3 (5'-TTTTTTGGG CATCCTGAGGTTTAT-3') (Bowles et al. 1992), was used in combination with a newly developed reversed primer, COIR120 (5'-ATTGGTTTTATTGGTTGTTT-3'). The 23 µl of a master mix (10x PCR buffer, 10 mM dNTPs, 0.2 µl of forward primer (10 µM), 0.2 µl of reverse primer (10 µM) and 0.06 µl of ToptaqDNA polymerase (QIAGEN)) and 2 µl of extracted DNA were used per PCR reaction. PCR conditions were 94 °C for 4 min; 4 x (94 °C for 30 sec, 58-54 °C for 30 sec (annealing T dropped 1°C in each cycle), 72 °C for 2 min), 45 x (94 °C for 30 sec, 54 °C for 30 sec, 72 °C for 1 min); 72°C for 10 min. Amplified PCR products were visualized using gel electrophoresis (1% agarose gel stained with GelRed (Biotium, Hayward)). The GeneRuler 250 bp DNA ladder (Thermo Fisher Scientific) was used as a reference according to the manufacturer's instructions. The electrophoresis was run at 100V for 35 minutes. The pictures of gels were obtained after exposition to the UV light.

Results

Morphology

Morphological characters used for comparison in this study were selected according to Eisenback and Hirschmann (1981), Jepson (1987), Karssen (2002) and Karssen et al. (2004).

Females

Body shape and perineal pattern

Females of Irish *Meloidogyne* sp. show oval to pyriform shape.

The perineal pattern of females of Irish *Meloidogyne* population was used for comparison according to Jepson (1987), although it is not sufficient to distinguish *M. fallax* and *M. chitwoodi* from each other (Karssen 2002, Karssen 1996). The perineal patterns of Irish females and type material of *M. chitwoodi* and *M. fallax* are similar, having ovoid to oval shape and moderately high dorsal arch. Compared to the more rounded perineal pattern in type material of *M. minor* (Karssen et al. 2004), the correspondence of the Irish population with *M. chitwoodi* and *M. fallax* is more apparent (Figure 1).

Stylet

The stylet is slender with dorsally curved shaft. Stylet knobs are large, oval to rounded, slightly backwardly sloping, which corresponds to the original description of *M. fallax* (Karssen 1996). Table 2 clearly shows the greatest similarity between stylet knobs of the unknown Irish species and *M. fallax*. The stylet length, a supporting morphometrical character, is presented in Table 5 and Table 6.

Second-stage juveniles

The stylet knobs shape, tail shape and hyaline tail terminus are used for morphological observations of second-stage juveniles according to Jepson (1987) and Karssen (2002).

Stylet knob shape

Mr. Moore and Dr. Franklin described a slender stylet with rounded basal knobs. In specimens where it was possible to see, we observed large, rounded, set-off basal knobs that are characteristic for *M. fallax* (see Table 3).

Tail shape and hyaline tail terminus

Mr. Moore and Dr. Franklin observed a rounded tail with a clear hyaline tail terminus which is occasionally "swollen". Based on our observations (Table 3, Figure 2), the overall tail shape resembles the one originally described for *M. fallax* (Karssen 1996), i.e. gradually tapering tail with a broadly rounded tip and a clearly delimitated smooth hyaline part. Some specimens in the Irish slides also show irregular constrictions at the hyaline region (Figure 5).

Our observations disagree with those of Mr. Moore and Dr. Franklin regarding the hemizonid position; it is located at the same position of the excretory pore rather

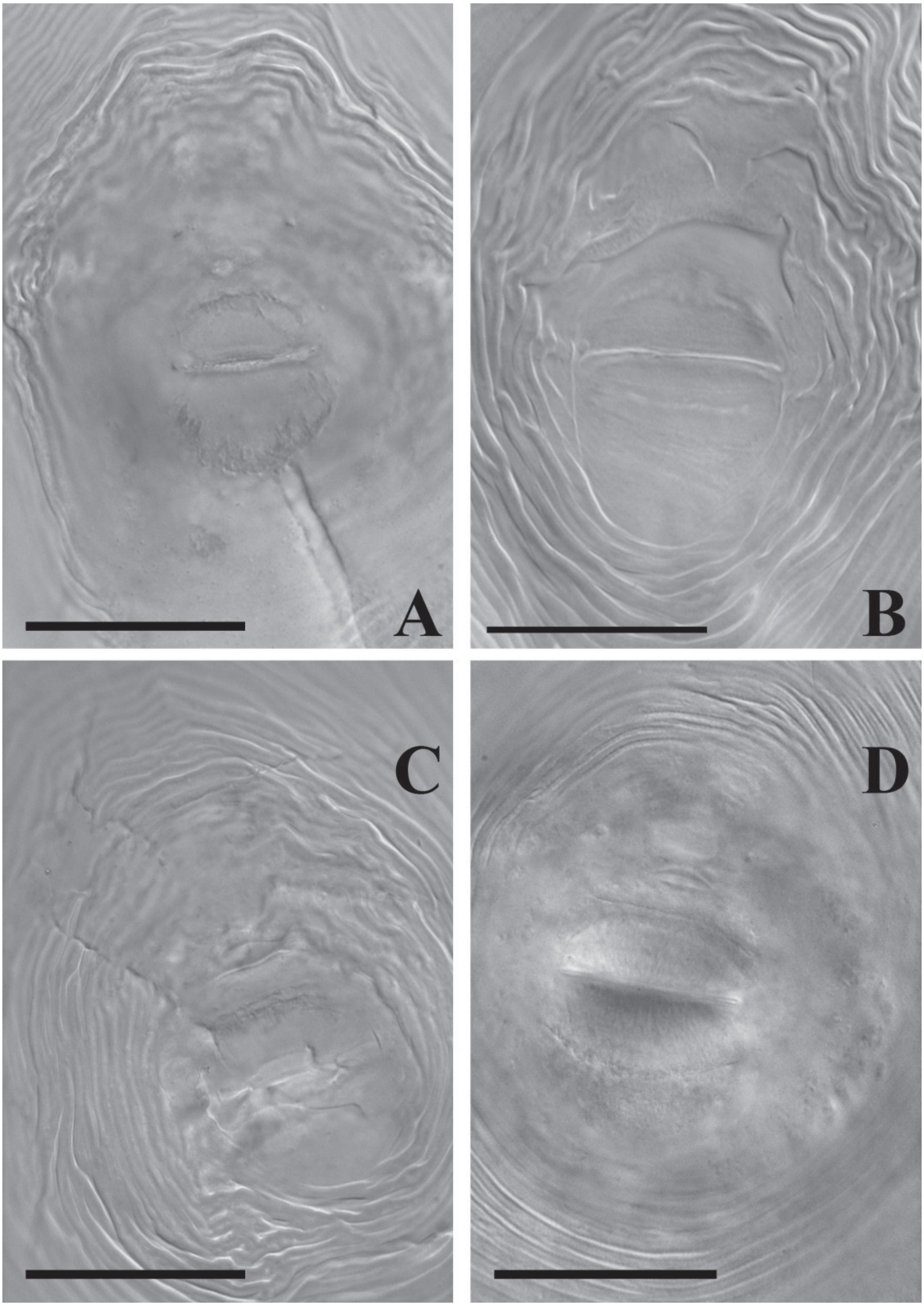

Figure 1. Comparison of perineal patterns in females. **A** Irish unknown *Meloidogyne* sp. **B** type material of *M. fallax*; **C** type material of *M. chitwoodi* **D** type material of *M. minor*. Scale bar = 20 µm.

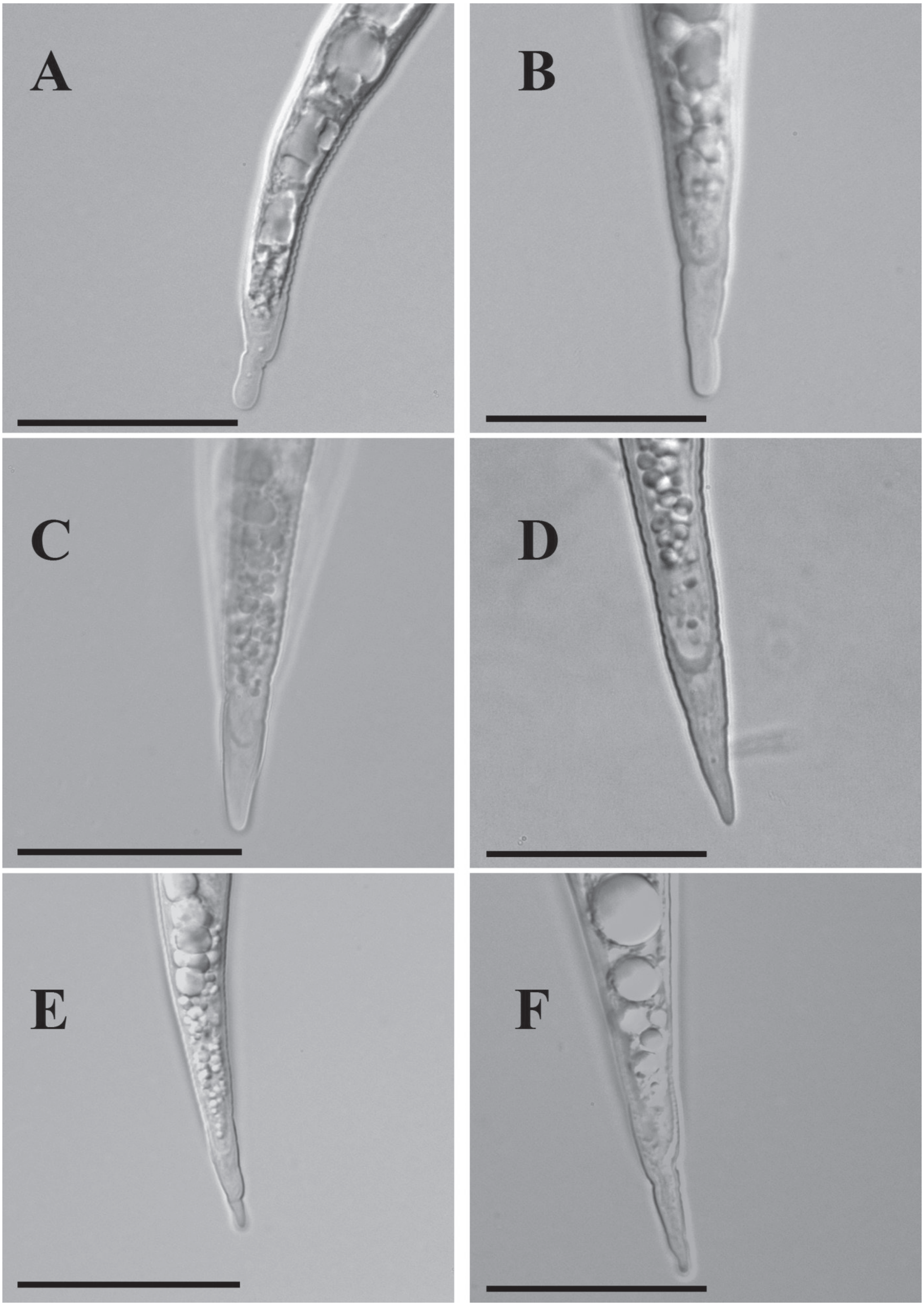

Figure 2. The comparison of tail and hyaline tail terminus shape in second-stage juveniles, lateral position. **A** an unknown Irish species **B** type material of *M. fallax* **C** type material of *M. chitwoodi* **D** type material of *M. minor* **E** reference material of *M. hapla*; **F** reference material of *M. incognita*. Scale bar = 20 µm.

Table 2. Differences in the stylet knob shape in females of compared *Meloidogyne* spp.

M & F + our observations	***M. fallax*** **(t.l.m. + o. d.)**	***M. chitwoodi*** **(r.l.m. + o. d.)**	***M. minor*** **(t.l.m. + o. d.)**	***M. hapla*** **(o. d.)**	***M. incognita*** **(o. d.)**
Large, rounded	Large, rounded	Small, irregular	Large, ovoid	Small, rounded	Large, broadly elongate

*M & F (Mr. Moore & Dr. Franklin), t. l. m. (type live material), r. l. m (reference live material), o. d. (original description)

than 1-2 annules above the excretory pore as they described. The position of the hemizonid at the same level of the excretory pore is characteristic for second-stage juveniles of *M. fallax* (Karssen 1996). The hemizonid position of second-stage juveniles of other examined species is usually above the excretory pore, except for *M. minor* where it is below the excretory pore (Karssen et al. 2004).

Males

Stylet knob and head shape are considered the most important characters for male identification according to Eisenback and Hirschman (1981), Jepson (1987) and Karssen (2002).

Stylet knob shape

As presented in Table 4, the stylet is slender with large and rounded stylet knobs, set off from the shaft, corresponding to those present in the type and reference material of *M. fallax*. The shape of stylet knobs (Figure 3) excludes both *M. minor*, with large, transversely ovoid stylet knobs slightly sloping backwardly (Karssen et al. 2004), and *M. chitwoodi*, with smaller stylet knobs of irregular shape sloping backwardly (Golden et al. 1980).

Head shape

Mr. Moore and Dr. Franklin described three annules in lateral view of the head. The first one is deeply pinched off and succeeded by two other faintly seen annules. Our observations resemble the male head shape of type and reference material of *M. fallax*. It is described as a slightly set off with a single post-labial annule usually subdivided with a transverse incisure (Karssen 1996). As Figure 3 shows, a labial disc is slightly elevated and typical for *M. fallax*.

Morphometrics

Males

The stylet length and stylet knob width, the most relevant morphometrical characters of males, were measured for populations of all observed species. Table 5 illustrates that

Table 3. Comparison of the most important morphological characters in second stage juveniles of the studied *Meloidogyne* spp.

	Irish *Meloidogyne* sp. (M. & F. + our observ.)	***M. fallax* (type material + o. d.)**	***M.chitwoodi* (type slides/r. l. m. + o. d.)**	***M. minor* (type material + o. d.)**	***M. hapla* (r. l. m. + o. d.)**	***M. incognita* (r. l. m. + o. d.)**
Stylet knob shape	Large, rounded	Prominent, rounded, set off	Small, irregular, sloping backwardly	Ovoid, slightly backwardly sloping	Small, rounded	Rounded, set off to transversely elongated, may indent anteriorly
Tail shape	Rounded to broadly rounded, gradually tapering until hyaline part	Gradually tapering until hyaline terminus, bluntly rounded tip	Conical, narrowly rounded tip	Gradually tapering until finely pointed tail tip, rectum weakly inflated	Short, narrow, difficult to delimitate it from hyaline region	Slightly tapering to subacute terminus
Hyaline tail terminus	Clear, rounded delimitation to the anterior, broadly rounded at the tip, sometimes with constrictions	Clearly delimitated, smooth hyaline part ending in a broadly rounded tip, faint constrictions	Short, clear rounded delimitation at the anterior end, narrowly rounded tip	Long, pointed terminus, rounded delimitation at the anterior region	Short, often irregularly shaped, delimitation at the anterior region difficult to observe	Pointed tip, clear delimitation at the anterior region

* M & F (Moore & Franklin), o. d. (original description), r. l. m. (reference live material)

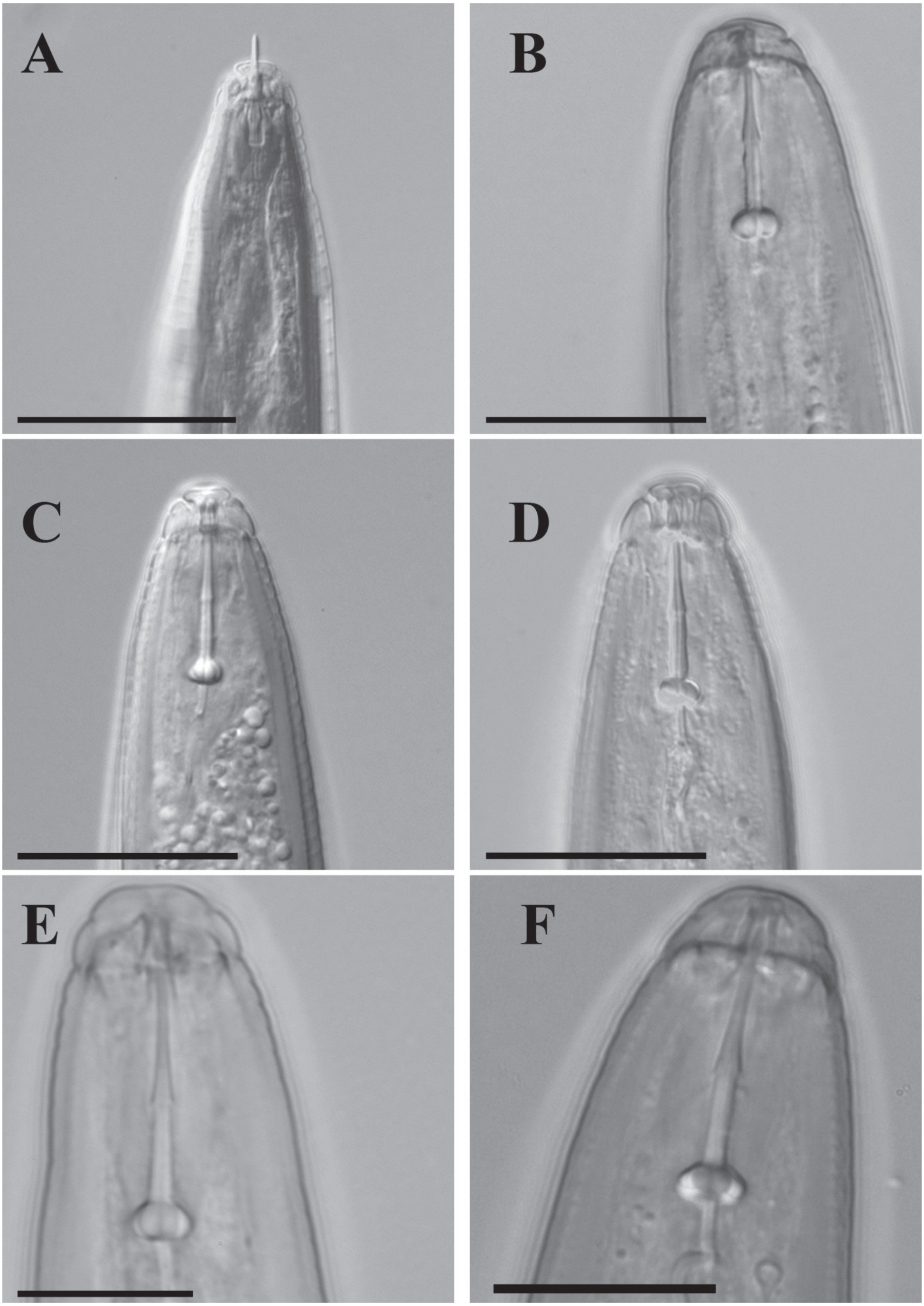

Figure 3. The comparison of anterior region in males of populations of observed *Meloidogyne* species. **A** an unknown Irish species (ventral position) **B** type material of *M. fallax* (lateral position); **C:** type material of *M. chitwoodi* (ventral position) **D** reference material of *M. hapla* (lateral position) **E** reference material of *M. incognita* (lateral position) **F** type material of *M. minor* (lateral position). Scale bar = 20 µm.

Table 4. Stylet knob and head shape in males of compared *Meloidogyne* spp.

	M & F + our observations	***M. fallax* (type material + o. d.)**	***M. chitwoodi* (type slides/r. l. m. + o. d.)**	***M. minor* (type material + o. d.)**	***M. hapla* (r. l. m. + o. d.)**	***M.incognita* (r. l. m. + o. d.)**
Stylet knob shape	Large, rounded, set off from the shaft	Large, rounded, set off from the shaft	Smaller, oval to irregularly shaped, backwardly sloping	Larger, ovoid, slightly backwardly sloping	Small, rounded, slightly backwardly sloping	Oval, angle between the shaft and knobs is more than 90°
Head shape	Labial disc elevated, head slightly set off with a post- labial annule, sometimes with an incomplete transverse incisure, as seen from the lateral view	Labial disc rounded and elevated, head slightly set off, one post-labial annule often with an incomplete transverse incisure	Labial disc not elevated, head not set off, no transverse incisures subdividing a single post-labial annule	Labial disc elevated, head not set off, one post-labial annule often with 1-2 transverse incisures	Labial disc elevated, head swollen, no transverse incisures on a post-labial annule	Labial disc not elevated, head slightly set off, incomplete transverse incisure on a post-labial annule

the average stylet length in Irish slides is 18.5 (17.0–20.0) μm with a smaller range than previously observed by Mr. Moore and Dr. Franklin, 18.0 (15.4–24.6) μm respectively. This is similar to the average stylet length in type material (type slides and type live material) of *M. fallax* (18.7 μm and 19.4 μm), and to type slides of *M. minor* (18.7 μm). The average stylet knob width of 3.9 μm in Irish slides is also within the range measured for *M. fallax* paratypes (Table 5).

Second-stage juveniles

The body length, tail length and hyaline tail length are considered the most reliable for morphometrical observation of second-stage juveniles. The body length range in our observations of Irish slides (280–410 μm) is narrower than observed by Mr. Moore and Dr. Franklin (361.5–432 μm) (Table 5). The tail length (46.9; 43.0–52.3 μm, noted by Mr. Moore and Dr. Franklin) is highly equivalent to that of *M. fallax*, 49.3; 46.1–55.6 (Karssen, 1996). It also matches the tail length measured in *M. fallax* paratypes (47.9; 41.0–54.0 μm). The hyaline tail length (12.9; 9.2–15.4 μm) is slightly lower than originally described, 13.5; 12.2–15.8 μm (Karssen, 1996) and when compared to the type live material of *M. fallax* (13.4; 10.5–15.0 μm). However, clearly delimitated hyaline tail terminus ending in a broadly rounded tip and often with constrictions in Irish specimens resembles the one characteristic for *M. fallax* (Figure 2).

Females

Although the female stylet length measured by Mr. Moore and Dr. Franklin is included in our study, it is considered unreliable as the length was measured from the anterior body end and not from the stylet tip. Therefore, the stylet length of the Irish *Meloido-*

Table 5. Morphometrical analysis of most important characters in females, males and second-stage juveniles {mean ± SD (range), all measurements in µm}.

Character	**Irish *Meloidogyne* sp. (M & F)**	**Irish *Meloidogyne* sp. (our observ.)**	***M. fallax***		***M. chitwoodi***		***M. minor***		***M. hapla* (r. l. m.)**	***M. incognita* (r. l. m.)**
			Type slides	**t. l. m.**	**Type slides**	**r. l. m.**	**t. l. m.**	**type slides**		
Female stylet length	18.0±2.4 (15.4–24.6)	14.6±0.5 (14.0–15.0)	/	/	/	/	/	/	/	/
Male stylet length	19.5±1.5 (17.0–24.6)	18.5±1.1 (17.0–20.0)	18.7±0.3 (18.5–19.0)	19.4±0.7 (18.0–21.0)	18.0±0.0	18.3±0.7 (17.0–19.0))	17.0±0.0	18.7±0.7 (17.0–20.0)	/	20.2±2.1 (18.0–25.0)
Male stylet knob width	/	3.9±0.5 (3.0–4.5)	4.2±0.3 (4.0–4.5)	4.6±0.4 (3.5–5.0)	3.75±0.3 (3.5–4.0)	3.75±0.3 (3.0–4.0)	4.0±0.0	4.0±0.3 (3.0–4.5)	/	4.1±0.8 (3.0–6.0)
J2 body length	406.1±16.1 (361.5–432.0)	358.6±27.6 (280–410)	347±7.5 (340–360)	384.3±22.3 (330–420)	371.5±10.5 (350–385)	371.9±15.9 (330–400)	347.8±17.4 (331.5–372.3)	369.0±32.5 (280–410)	364.2±31.3 (300–410)	379.2±20.0 (340.0–435.0)
J2 tail length	46.9±2.5 (43.0–52.3)	42.0±3.7 (33–50)	45.4±2.2 (43–49)	47.9±2.6 (41.0–54.0)	39.9±2.3 (36.0–43.5)	44.0±2.6 (40.0–49.0)	49.0±3.3 (45.5–53.0)	52.8±4.4 (46.0–62.0)	41.1±6.8 (31.0–50.0)	55.0±2.9 (48.0–61.0)
J2 hyaline tail length	12.9±1.8 (9.2–15.4)	11.3±1.8 (8.5–15.5)	13.1±0.8 (12.0–14.0)	13.4±1.3 (10.5–15.0)	9.6±0.8 (8.0–11.0)	12.2±1.0 (10.0–14.0)	13.8±1.9 (11.5–16.5)	16.9±1.6 (14.0–20.0)	8.8±1.2 (6.5–11.0)	12.2±1.7 (9.0–15.0)

Table 6. Comparison of female stylet length between Irish population and different *Meloidogyne* spp. {mean ± SD (range), all measurements in µm}

Species (females)	Unknown Irish sp. (Moore & Franklin)	Unknown Irish sp. (our observations)	*M. fallax* (original description)	*M. chitwoodi* (orig. descr.)	*M. hapla* (orig. descr.)	*M. minor* (orig. descr.)
Stylet length	18.0±2.4 (15.4–24.6)	14.6±0.5 (14.0–15.0)	14.5±0.4 (13.9–15.2)	11.9±0.3 (11.2–12.5)	13.0±/ (12.0–14.0)	14.2±1.1 (12.6–15.2)

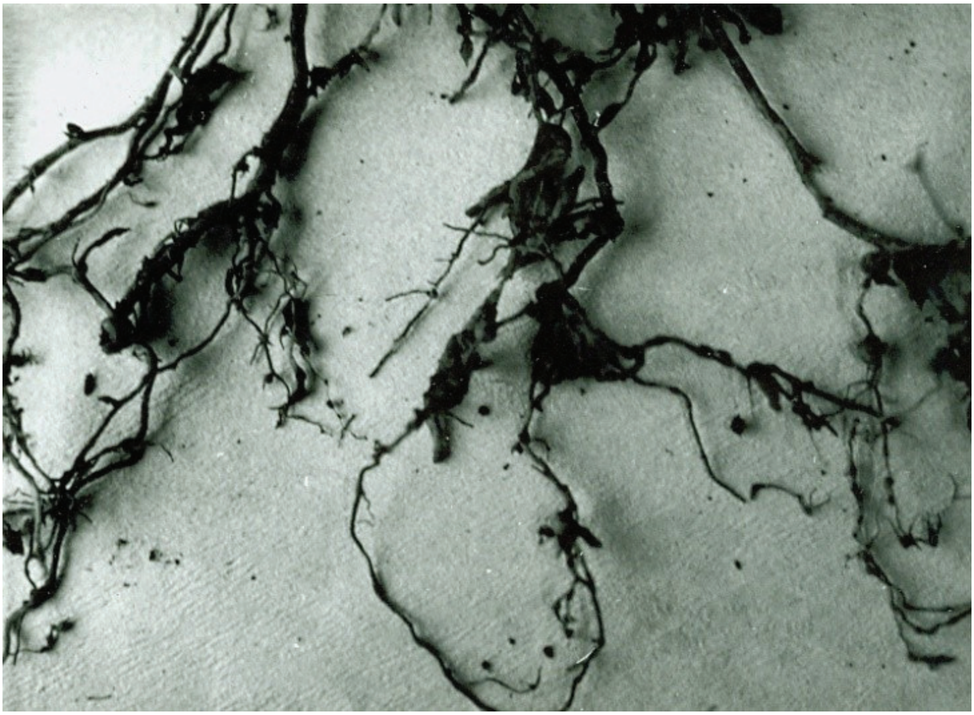

Figure 4. The tomato roots infected with a population of Irish *Meloidogyne* sp.

gyne sp. was compared to the one originally described for species used for comparison in this study. Based on our measurements (Table 6), the average female stylet length (14.6 µm) corresponds to *M. fallax* (14.5 µm).

Host test

The host-range test for Irish *Meloidogyne* sp. included both weeds and cultural plants belonging to mono- and dicots. The Table 7 shows that all tested plants were positive for the infection except for *Fumaria officinalis*. The original picture from 1966 shows relatively small galls on tomato roots caused by this species (Figure 4).

Table 7. The host test conducted in 1966 for a population of Irish *Meloidogyne* sp.

Family	Genus + species	Result
Chenopodiaceae	*Beta vulgaris* L. *S	+
	Chenopodium album L. *Pl	+
Compositae	*Matricaria matricarioides* (Less.) Porter *Pl	+
	Senecio jacobaea L. *Pl	+
	Sonchus sp. *Pl	+
	Lactuca sativa L.* S	+
Caryophyllaceae	*Stellaria media* L. *Pl	+
	Cerastium sp. *Pl	+
Polygonaceae	*Polygonum aviculare* L. *Pl	+
	Rumex sp. *Pl	+
Graminaceae	*Hordeum vulgare* L. *S	+
	Triticum aestivum L. *S	+
	Lolium multiflorum (Lam.) *S	+
Cruciferae	*Capsella bursa-pastoris* L. *Pl	+
	Brassica oleracea L. *var. capitata* *S	+
	Brassica napus L. *var. napobrassica* *S	+
Euphorbiaceae	*Mercuria lisannua* L. *Pl	+
Urticaceae	*Urticadioica* L. *Pl	+
Labiatae	*Lamium purpureum* L. *Pl	+
Umbelliferae	*Daucus carota* L. *S	+
Fabaceae	*Vicia faba* L. *S	+
Plantaginaceae	*Plantago major* L. *Pl	+
Rosacae	*Fragaria vesca* L. *Pl	+
	Potentilla erecta L. *Pl	+
Solanaceae	*Solanum tuberosum* L. (potato tuber)	+
Ranunculaceae	*Ranunculus repens* L. *Pl	+
	Ranunculus acris L. *Pl	+
Geraniaceae	*Erodium moschatum* L. *Pl	+
Amaranthaceae	*Spinacea oleracea*L. *S	+
Alliaceae	*Allium cepa* L. *S	+
Papaveraceae	*Fumaria officinalis* L. *Pl	–

*S = seed sown, Pl = plant transplants sourced local to the laboratory

Molecular analysis

The DNA extraction from glycerine-embedded nematodes in old slides was unsuccessful with both DNA extraction methods, as PCR product was not obtained. Contrastingly, the targeted region of COX1 gene was successfully amplified from all three fresh individuals of *M. fallax*. The primers used in this study have been designed to specifically amplify a short region of COX1 gene of *M. fallax* and *M. chitwoodi*, the two closely related species.

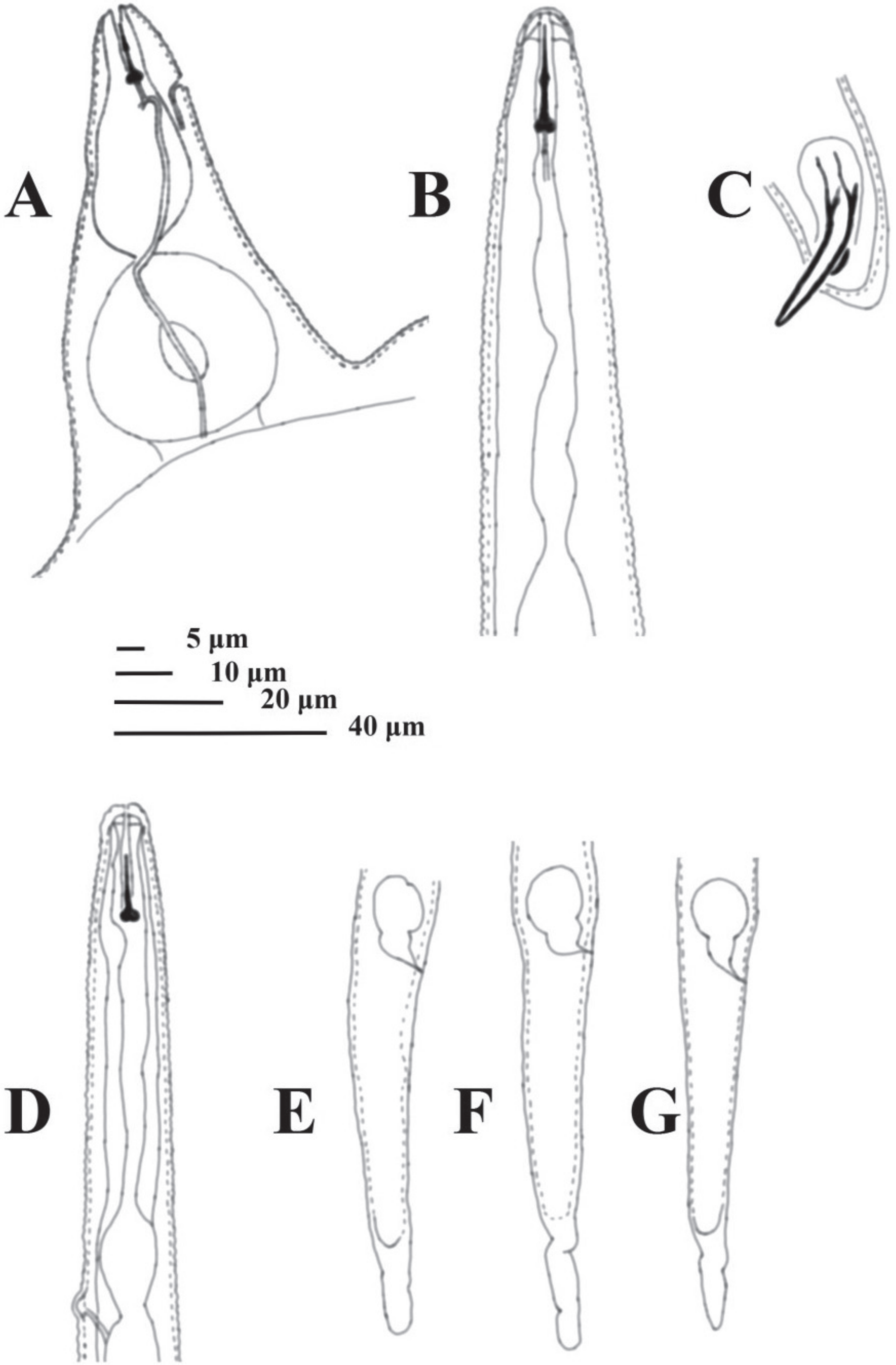

Figure 5. Irish population of *Meloidogyne* sp. (lateral position) from Ireland from 1965. **A** female anterior region **B** male anterior region **C** male – spicules **D** anterior region of the second-stage juvenile **E–G** tail variations in the second-stage juvenile.

Discussion

In the annual reports of Plant Sciences and Crop Husbandry Division from 1965 and 1966, a *Meloidogyne* species attacking tomato was recorded and briefly described by Mr. John F. Moore and Dr. Mary T. Franklin. Its host range was found to be very wide, including both dicots and monocots (Table 7). Some morphological characters, such as the position of anus and vulva on a marked protuberance, the posterior cuticular pattern of females, the unique male head and different characters of the second-stage juveniles, were considered important to characterize this putative new species. The differential diagnosis was mainly made to the species belonging to the former genus *Hypsoperine* (Sledge & Golden 1964) based on the posterior protuberance in females, although the perineal pattern was not comparable to other species. In addition, representatives of *Hypsoperine* sp., which was rejected as a valid genus (Plantard et al. 2007), were known to attack only monocots (Siddiqi 2000), while the host range of detected Irish population included both monocots and dicots.

Our observations show that the perineal pattern of Irish females greatly corresponds to the one originally described for *M. fallax* (Karssen 1996) and *M. chitwoodi* (Golden et al. 1980), making it difficult to decide if the striae are more or less coarse and belong to the former or to the latter. This is why Karssen (2002) and Karssen et al. (2004) did not use perineal pattern to differentiate *M. fallax* and *M. chitwoodi*, even though it is considered to be one of the most important diagnostic characters by Eisenback and Hirschmann (1981) and Jepson (1987). Importantly, the stylets of some females in Irish slides had remained intact and comparison showed a high similarity to those presented in the original description of *M. fallax* (Karssen 1996). None of measured female stylet lengths was within the range originally described for *M. chitwoodi* (Golden et al. 1980) indicating that the two species were not mixed together. According to Mr. Moore and Dr. Franklin, the duct of dorsal pharyngeal gland opens 4-6 µm behind the stylet base. We did not mark this character as diagnostic in females following Karssen (1996) and Jepson (1987), because a physical deformation of females in permanent slides made this distance variable among different specimens.

Males and second-stage juveniles appeared to have much more informative morphological and morphometrical characters for comparison with other similar species. Mr. Moore and Dr. Franklin described the male head with 3 annules where the first one is deeply pinched off and succeeded by two faintly seen annules. Contradictory to this, we observed one post-labial annule which is interrupted with 1-2 incomplete transverse incisures visible from the lateral view on dorsal and ventral sides. We also found a slightly set-off head region with a slightly elevated labial region as was originally described for males of *M. fallax* (Karssen 1996). To compare with, *M. chitwoodi* males have a flattened labial region.

Our careful observations show that the stylet length of males in Irish slides matches the one measured in paratypes of *M. fallax* and *M. minor*. Additionally, the stylet knob shape in Irish males, being rounded and set off from the shaft as originally described for *M. fallax*, mismatches ovoid and slightly backwardly sloping knobs characteristic for *M. minor* (Karssen et al. 2004).

The stylet length of second-stage juveniles was excluded from the basic comparison (Table 5) as it was difficult to accurately observe the stylet tip (Jepson 1987). However, large and rounded stylet knobs set off from the shaft in Irish specimens were comparable to the ones observed in type material of *M. fallax*, excluding both *M. minor* and *M. incognita* with stylet knobs slightly sloping posteriorly. On the contrary, body length of second-stage juveniles was easily observed. We noticed certain shrinkage of specimens in Irish slides compared to those observed by Mr. Moore and Dr. Franklin. This can be explained by the fact that up to 10% of shrinkage occurs after several years in slides mounted in both glycerol and lactophenol (Esser 1974). Nevertheless, the greatest correspondence of the mean body length in second-stage juveniles was found to the one described for paratypes of *M. fallax* (Karssen 1996). In addition, Eisenback and Triantaphyllou (1991) marked body length of second-stage juveniles as inadequate for species identification due to its high overlap between different species. Jepson (1987), on the other hand, included body length as important supplementary character in root-knot nematodes identification. Remarkably, in polyploid mitotic parthenogenetic *Meloidogyne* spp., the average body length is indeed not reliable for identification as there is a high variation of this character between different individuals of the same species. Also, a large body length seems to be correlated with increased chromosome number, e.g. tetraploidic forms of *M. microcephala* (Triantaphyllou and Hirschmann 1997) and polyploidic forms of *M. hapla* race B compared to haploid forms of *M. hapla* race A (Eisenback and Triantaphyllou 1991). In meiotic parthenogenetic species (e.g. *M. fallax* and *M. chitwoodi*) with haploid chromosome number of 18 and generally shorter body length compared to polyploidic species, high inter-specific and low intra-specific variation are sufficient enough to depict the body length as important diagnostic character.

It should also be pointed out that in Irish second-stage juveniles, a gradually tapering tail with bluntly rounded tip and a clearly delimitated hyaline part with broadly rounded tip and often constrictions resemble the tail and tail hyaline shape characteristic for *M. fallax* (Karssen 1996). Moreover, we observed the hemizonid in second-stage juveniles positioned at the same level as the E-S pore, rejecting the observation of the hemizonid position to be 1–2 annules above the E-S pore as marked by Mr. Moore and Dr. Franklin. In fact, until Karssen (1996) described *M. fallax*, the hemizonid had never been observed at the same level as the E-S pore. Current observation supports the taxonomic value of the hemizonid position, as it was clearly visible above the E-S pore in paratypes of *M. chitwoodi, M. hapla* and *M. incognita*, and bellow the E-S pore in *M. minor* paratypes.

The host test of Irish *Meloidogyne* sp. conducted in 1966 showed a wide host range which included both dicots and monocots. Although Castagnone-Sereno et al. (2013) do not consider the host test to be important for identification of certain species, we found the fact that *M. fallax* also parasitizes monocots and dicots (EPPO 2004) as important additional argument. The original pictures of galled tomato roots made in 1965 and 1966, show relatively small galls that are typical for the roots attacked by *M. fallax* (Karssen 1996).

An additional molecular support for our data is lacking as we were unable to extract DNA from the 50-year-old slides with both protocols used. It was confirmed by PCR amplification of COX1 gene, showing products only for fresh *M. fallax* specimens. The COX1 gene was chosen for analysis as it has been previously proven as a good marker for distinguishing closely related *Meloidogyne* species (Humphreys-Pereira and Elling 2015). It has the advantage of being maternally inherited and linked to an actual amino acid sequence. Moreover, there are many copies of this gene in a single specimen and targeting a very short region of a multi copy gene would increase a chance for its amplification from a damaged and fragmented DNA of Irish *Meloidogyne* sp. in the old slides.

In this study we showed a historical record of *M. fallax* in Ireland. It is not known which way it was introduced to the unheated glasshouse in the county Cork, either by infected tomato seedlings or by infested soil. Although EPPO (2004) described a direct evidence of the economic importance of *M. fallax* as lacking and obscured compared to its sister species *M. chitwoodi*, the fact that *M. fallax* was present in Ireland in '60s and again recorded in the sport turf in 2011 (Northern Ireland), indicates a continuous risk of introduction of this species in Ireland. Van der Gaag et al. (2011) relied on the matrix statistical model to assess the risk of introduction of *M. fallax* and *M. chitwoodi* into new countries. The outcome of this model showed a high risk of introduction of both species from France, Netherlands, Germany and UK via plant seedlings, dormant bulbs, tubers, tuberous roots, corms, crowns and rhizomes. Furthermore, a coarse soil texture in a combination with Irish climate provides good conditions for the establishment of both species.

Conclusion

To conclude with, observations of the original material of a population of *Meloidogyne* sp. from Ireland and its comparison to other similar *Meloidogyne* spp. indicate that it belongs to *M. fallax*.

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RESEARCH ARTICLE

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First report of the Gondwana genus *Beatogordius* from India, with further records of two *Chordodes* species (Gordiida, Nematomorpha)

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Abstract

Three horsehair worms (Nematomorpha) are recorded from Nagaland, India. The first species is *Chordodes combiareolatus*, which was recently described from Nagaland. The second is *Chordodes* cf. *furnessi*, earlier reported from Meghalaya, and some new observations are added. The third is a new species, described as *Beatogordius nagalandis* **sp. n.** This is the first report of the genus *Beatogordius* in India and this observation fits well into the Gondwana distribution of the genus. The species is quite similar to *B. chinensis*, reported from South China.

Keywords

Beatogordius, biodiversity, *Chordodes*, Gordiida, Nematomorpha, new species

Introduction

Horsehair worms (Nematomorpha) are parasitic worms with a free-living phase in the life cycle. Thirteen species of horsehair worms (Nematomorpha: Gordiida) from five genera (*Gordius*, *Chordodes*, *Parachordodes*, *Paragordius*, *Gordionus*) have been reported from India until 1912 (see Schmidt-Rhaesa and Yadav 2004). Recently, this group of parasitic worms has achieved new attention in India, and since 2004 seven further species were newly described or newly recorded (Schmidt-Rhaesa and Yadav 2004, 2013, Schmidt-Rhaesa and Lalramliana 2011, 2016, Schmidt-Rhaesa et al. 2015). Six of these newly reported species belong to the genus *Chordodes*, and one to the genus *Acutogordius*, which had not been reported from India before.

In the present study a representative of the genus *Beatogordius* is described. This is the first species of this genus reported from India. Representatives of this genus are characterized by an arrangement of areoles, fine cuticular elevations, which are characteristic cuticular substructures in nematomorphs, arranged in longitudinal lines. The genus was erected by Heinze (1934) and has an interesting geographic distribution. All the 21 species currently known under the genus are mostly distributed in South America (De Villalobos et al. 2003), Africa (Schmidt-Rhaesa and de Villalobos 2002), and Madagascar (Schmidt-Rhaesa and Bryant 2004), with further reports from Australia (Schmidt-Rhaesa and Bryant 2004) and China (Schmidt-Rhaesa 2011). With the exception of China, the genus shows a perfect Gondwana distribution and the long time suspected occurrence in India is now confirmed.

In addition, new records are reported for the species *Chordodes combiareolatus* Schmidt-Rhaesa, Limatemjen & Yadav, 2015, and *C.* cf. *furnessi* Montgomery, 1898, and supplement the previous documentations by new images.

Material and methods

The specimens for the present study were collected by hand (for exact locations see below) and preserved in 70% ethanol. Pieces 2–3 mm long from the middle region of body, the anterior, and the posterior ends of body were processed for Scanning Electron Microscopy (SEM). The material was dehydrated in an increasing ethanol series, critically-point dried, and coated with gold in a sputter coater. Observations were made using LEO SEM 1524 at the University of Hamburg. Digital images of various body regions were taken.

Results

***Beatogordius nagalandis* sp. n.**

http://zoobank.org/0522D719-7394-4017-9214-4A14F14C4F90
Figs 1, 2

Type locality. P-Khel Viswema Village, Kohima, Nagaland, India, from the community tap water. Collected by Mrs Bazule Toso.

Holotype. A single male specimen from the type locality; alcohol preserved pieces of holotype deposited in the Museum of Department of Zoology, NEHU, Shillong with the accession no. MDOZ/NEHU/INV/112.

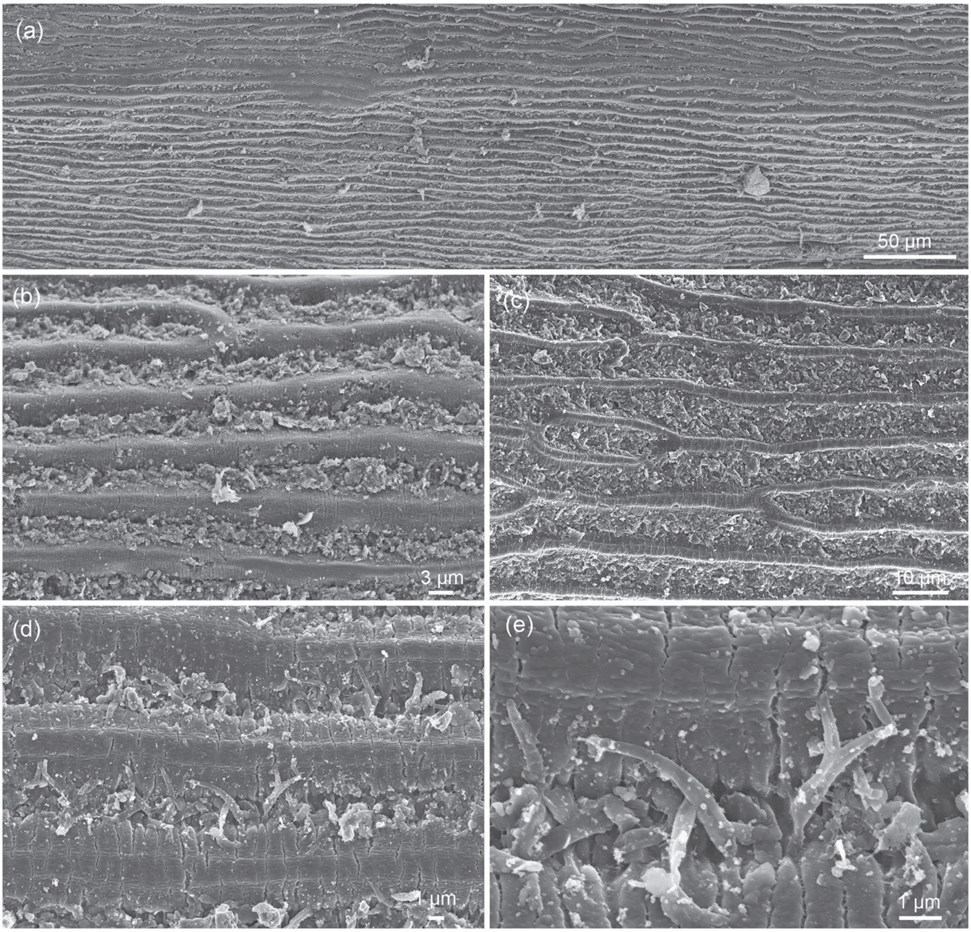

Figure 1. *Beatogordius nagalandis* sp. n., cuticular pattern: **a** cuticular pattern at lower magnification **b, c** branching and fusion of "bands", space between bands is filled with debris **d, e** spines are present between the bands, fine cracks structure the bands stronger (d) or weaker (e) into subunits.

Host. unknown

Etymology. The species has been named after one of the Indian states, Nagaland, from where the specimen was collected.

Diagnosis. Cuticle in midbody with longitudinal elevated ridges, which sometimes branch and fuse. Ridges highest in the central part, lower in lateral parts. Surface of ridges with fine cracks perpendicular to longitudinal axis. Cuticle between the ridges with 4 µm long spines. Ridges change to isolated areoles towards the anterior, anterior tip free of cuticular structures. Posterior end of male with two tail lobes, each lobe about three times as long as wide. Cloacal opening surrounded by spines, further spines in the region posterior of the cloacal opening. Anterolateral bristlefields are likely present. Adhesive warts with a keel are present on the ventral side anterior of the cloacal opening.

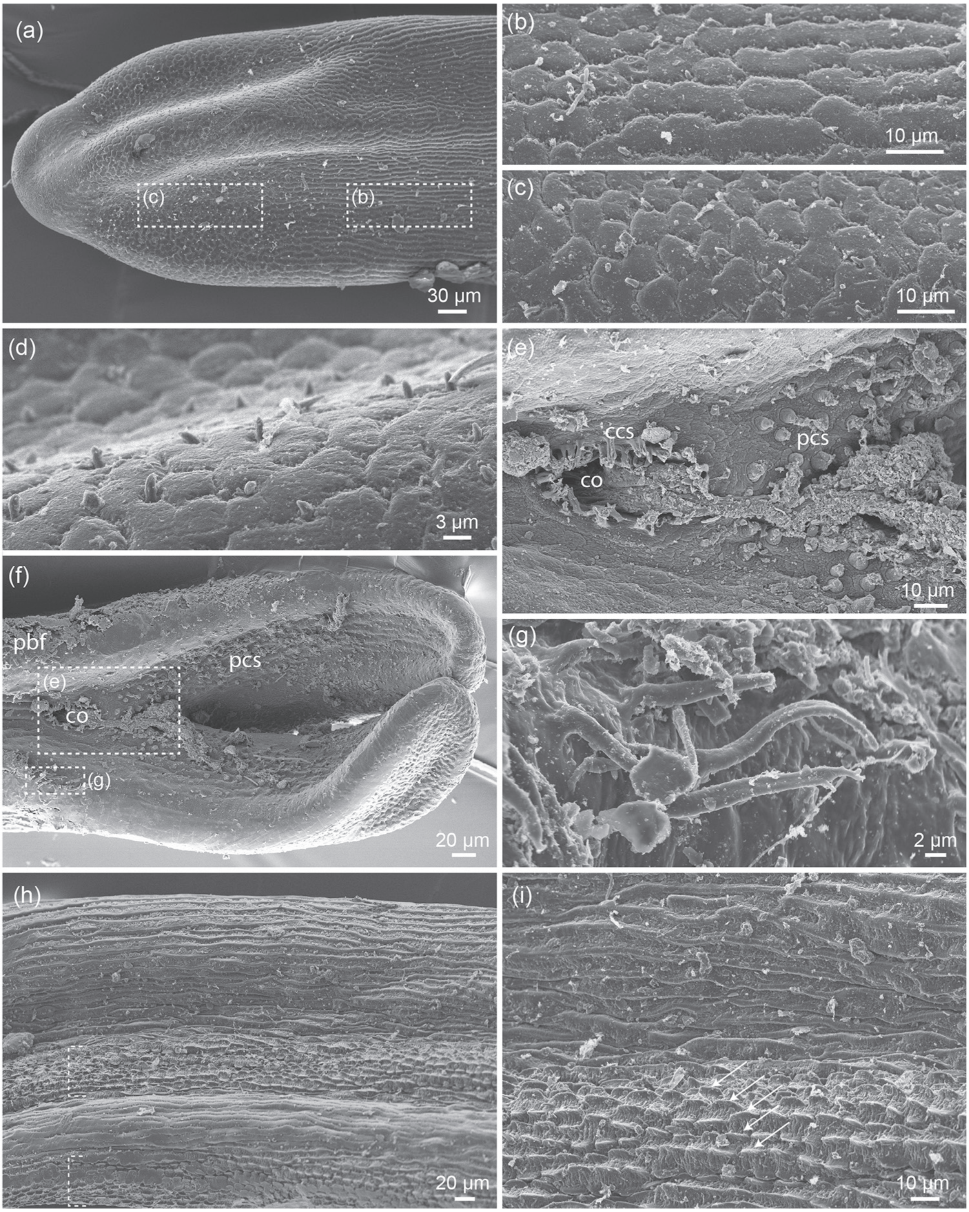

Figure 2. *Beatogordius nagalandis* sp. n., anterior and posterior end: **a** anterior end in low magnification, approximate positions of images b and c are indicated **b, c** transition of "bands" into areoles in two different regions as indicated in fig. a **d** fine spines are present between areoles in the anterior end **e** region of the cloacal opening (**co**) with circumcloacal spines (**ccs**) and postcloacal spines (pcs) **f** ventral view on posterior end with cloacal opening (**co**), postcloacal spines (**pcs**) and precloacal bristle fields (**pbf**). Approximate positions of images e and g are indicated by dotted rectangles **g** some bristles from the precloacal bristle fields **h** position of the broad rows of keeled structures (see dashed markings) on the ventral side of the animal **i** magnification of keeled structures (arrows).

Description. The holotype is 130 mm long, with a diameter of 0.37 mm in the midbody region. The specimen is very light (yellowish-white) in colour. The posterior end has two tail lobes.

The cuticle is structured by long elevated bands or ridges running parallel to the longitudinal axis of the animal (Fig. 1a–d). These bands sometimes branch or fuse, and branches may end blindly (Fig. 1b, c). In most observed regions the bands are composed of a higher central part and lateral lower parts (Fig. 1d, e). In some regions the lower parts were not clearly observed, but this may be due to a covering of dirt in the region between the bands. The surface of the bands contains numerous fine cracks orientated perpendicular to their axis (Fig. 1d). In some regions these cracks appear without a certain pattern and are only partial (Fig. 1e), in others they appear to separate the band into numerous subunits (Fig. 1d). The region between the bands is often filled with debris, in parts where the dirt could be removed there are bristles or spines which are up to 4 µm long (Fig. 1d, e).

In the anterior end, approximately 270 µm from the anterior tip, the longitudinal bands gradually turn into polygonal or irregularly shaped individual areoles (Fig. 2a–c). Between these areoles solid spines of 1–2 µm length are scattered (Fig. 2d). The anterior most 75 µm are free of cuticular structures (Fig. 2a).

The posterior end has two tail lobes and contains the ventral cloacal opening (Fig. 2f). This is oval, approximately 20 × 45 µm in size and surrounded by circumcloacal spines (Fig. 2e). These spines are stout, approximately 5 µm long, which are apically branched. The cloacal opening is 30–35 µm from the point where the separation into two tail lobes starts. The tail lobes are approximately three times as long as broad (roughly 300 × 100 mm). Posterior to the cloacal opening, extending onto the inner side of the tail lobes, are conical spines, which decrease in size posteriorly (Fig. 2e, f). Anterolaterally of the cloacal opening are structures which could represent parts of anterocloacal bristle fields, but as there is some dirt and suboptimal cuticle preservation in this region, this observation has to be taken with caution (Fig. 2f, g).

In the ventral region anterior of the posterior end, modifications of the cuticle were observed. There are paired stripes, in which elevation with longitudinal keels of 7.5 to 10 µm occur (Fig. 2h, i). Eight rows of such keeled structures occur in one stripe.

Taxonomic remarks. In most *Beatogordius* species, areoles are clearly recognized as the basic elements, which form the longitudinal lines, and should be considered a characteristic of the genus (De Villalobos et al. 2003, Schmidt-Rhaesa and de Villalobos 2002). More or less continuous lines occur in *B. sankurensis* Sciacchitano, 1958 and *B. wilsoni* Sciacchitano, 1958 from Africa (see Schmidt-Rhaesa and de Villalobos 2002), *B. lineatus* Schmidt-Rhaesa & Bryant, 2004 from Australia (Schmidt-Rhaesa and Bryant 2004), and *B. chinensis* Schmidt-Rhaesa, 2011 from China (Schmidt-Rhaesa 2011). Among all the above-mentioned species, *B. chinensis* is the only species to display long spines in the region between the longitudinal bands. The difference from the Indian *Beatogordius* is that in *B. chinensis* the bands have a “pearl-collar” appearance, which means that they usually have broader and narrower regions, whereas in the

Indian *Beatogordius* the bands are of continuous thickness. It might be supposed that the Indian and the Chinese species are closely related. *Beatogordius chinensis* is the only species reported outside former Gondwana continents, but the collecting locality in Yunnan province is very close to Nagaland province, approximately 300–400 km east.

Chordodes combiareolatus Schmidt-Rhaesa, Limatemjen & Yadav, 2015

Figure 3a–c

Locality. Tzuden stream of Longjang village, Mokokchung district, Nagaland, India. Collected by Mr & Mrs Lanu Pongen.

Description. Female specimen of 260 mm length and 1.5 mm in diameter. The cuticular characters correspond to the ones previously described by Schmidt-Rhaesa et al. (2015) for this species; the characteristic combination of simple and tubercular areoles are present (Fig. 3c). Crowned areoles were observed in different grades of completeness (Fig. 3a, b). The species had been described on the basis of a female specimen from Nagaland (Schmidt-Rhaesa et al. 2015); the male remains unknown.

Chordodes cf. *furnessi* (Montgomery, 1898)

Figs 3d–h, 4

Locality. Tzuden stream of Longjang village, Mokokchung district, Nagaland, India. Collected by Mr & Mrs Lanu Pongen.

Description. Female specimen of 264 mm length and 1.5 mm diameter. Simple areoles are flat and have fine bristles on top (Fig. 3d, f). Tubercle areoles and thorn areoles are present, but rare (Fig. 3e, f). Bulging areoles occur, mostly in pairs (Figs 3d, g, 4b, c). There is no distinction between crowned areoles and circumcluster areoles, all such areoles within one cluster have a similar shape and differ only slightly in size (Figs 3g, h, 4a). Crowned areoles with long apical filaments are present and restricted to a paired longitudinal line, presumably on the ventral side of the animal (Fig. 4b, c). Towards the anterior end there is a transition of the cuticular pattern; only one type of areole is present in the anterior end, which resembles simple areoles, is moderately elevated and has numerous bristles on top (Fig. 4d-f). Scattered spines are present between these areoles (Fig. 4f).

Taxonomic remarks. In most *Chordodes* species, there are clusters composed of central crowned areoles and surrounding circumcluster areoles. These two types differ in structure but in some species, clusters are composed of areoles of the same type: *Chordodes furnessi* Montgomery, 1898 is one such species, first described from Borneo (Indonesia). Schmidt-Rhaesa and Yadav (2004) found specimens from Shillong, India which is very similar to this species, but, because of slight differences observed, it was recorded as *Chordodes* cf. *furnessi*. This newly reported specimen corresponds to the description given by Schmidt-Rhaesa and Yadav (2004), but adds some further observations. Bulging areoles were not previously reported, but they are figured (Fig. 1b

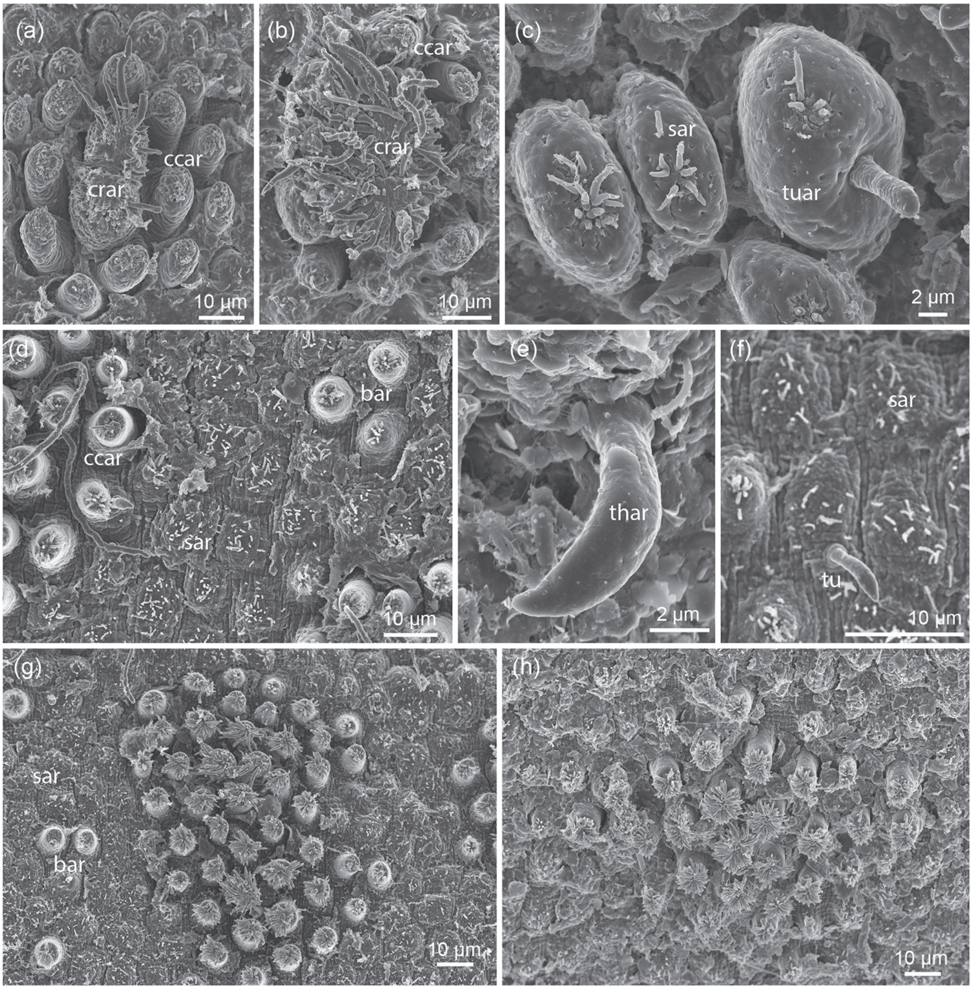

Figure 3. a–c *Chordodes combiareolatus*: **a, b** cluster of crowned areoles (**crar**) and circumcluster areoles (**ccar**) in different degrees of preservation of the apical filaments **c** simple areoles (**sar**) and the combined simple/tubercle areoles (**tar**) characteristic for this species **d–h** *Chordode*s cf. *furnessi*: **d** simple areoles (**sar**) and bulging areoles (**bar**) between clusters of crowned areoles (**ccar** = circumcluster areoles) **e** thorn areole (**thar**) **f** tubercle (**tu**) **g, h** two different clusters of crowned and circumcluster areoles, where no differences between these two types of areoles are visible.

in Schmidt-Rhaesa and Yadav 2004). Solid thorns have not been reported previously from this species, but are present in the newly reported specimen. Tubercles occur usually in the genus *Chordodes* as tubercle areoles, i.e., as areoles with a central tubercle on top, but in the present specimen, they occur near the base of simple areoles, as reported by Schmidt-Rhaesa and Yadav (2004). Due to these observations it is assumed that the specimens reported by Schmidt-Rhaesa and Yadav (2004) and in this work belong to the same species, which may or may not be *Chordodes furnessi*. The lack of rare structures such as thorns may be due to the scarceness of these structures.

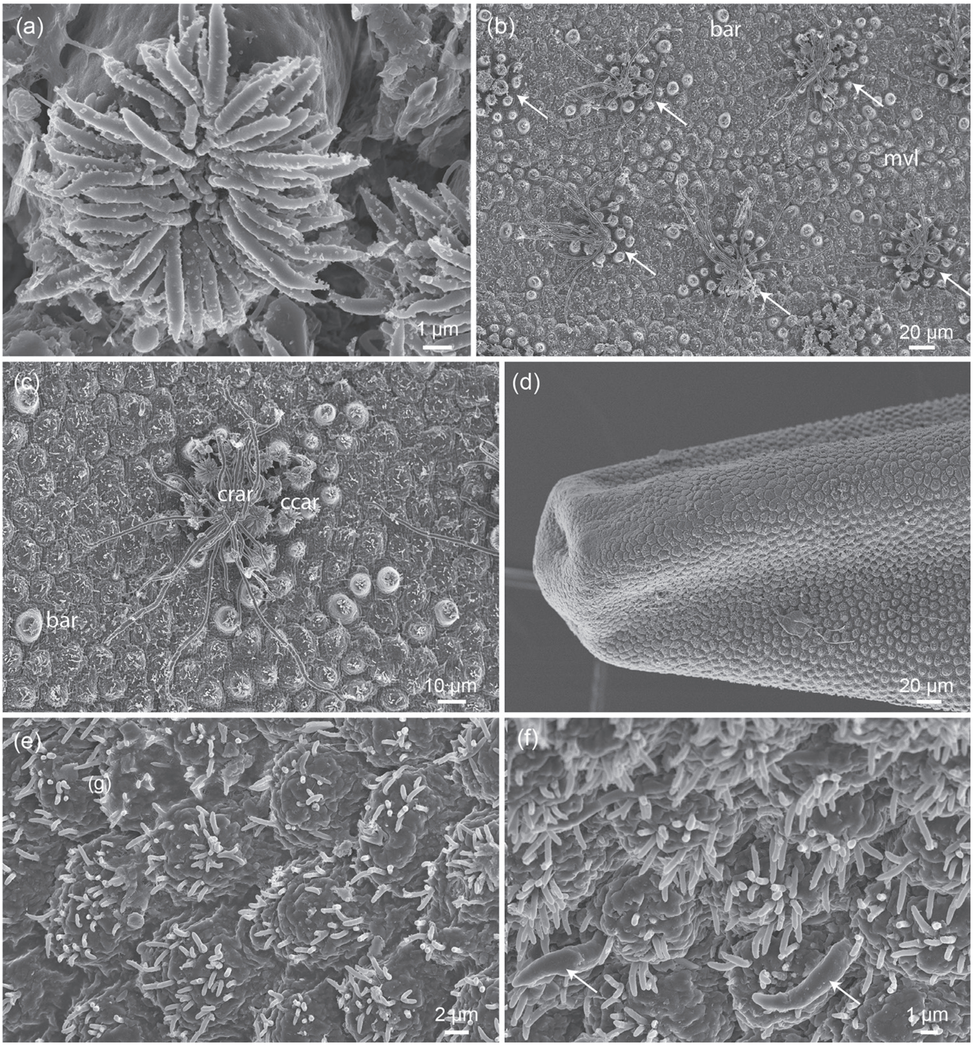

Figure 4. *Chordodes* cf. *furnessi*: **a** fine structure of areole from a crowned areole cluster **b** clusters containing crowned areoles with long apical bristles (arrowed) along the assumed midventral line (**mvl**) **c** in such clusters crowned areoles (**crar**) and circumcluster areoles (**ccar**) can be distinguished (bar = bulging areoles) **d** anterior end of the animal **e** fine structure of areoles in the anterior end **f** some spines (arrowed) are present between the areoles in the anterior end.

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RESEARCH ARTICLE

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Four new species of the trapdoor spider genus *Conothele* Thorell, 1878 from Mainland China and Laos (Araneae, Ctenizidae)

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Abstract

Here for the first time the presence of the trapdoor spider genus *Conothele* Thorell 1878 (Araneae: Ctenizidae) is reported from mainland China and Laos. Four *Conothele* species collected from the regions are described as new to science, based on the female genital morphology: *C. baiyunensis* Xu, Xu & Liu, **sp. n.** (Guangdong Province), *C. daxinensis* Xu, Xu & Li, **sp. n.** (Guangxi Province), *C. sidiechongensis* Xu, Xu & Liu, **sp. n.** (Yunnan Province, China and Vietnam), *C. yundingensis* Xu, Xu & Li, **sp. n.** (Yunnan Province).

Keywords

Araneae, China, *Conothele*, Ctenizidae, Laos, taxonomy

Introduction

The family Ctenizidae belongs to the suborder Opisthothelae and the infraorder Mygalomorphae. Ctenizids are widely distributed, and mainly found across Asia (China, India, Japan, Laos, Myanmar, Sumatra, Thailand, Vietnam), the Americas (North and South America), the Mediterranean, South Africa and Australia (World Spider Catalog 2016). Ctenizidae is a dispersal-limited, ground-dwelling lineage, members of which usually build underground silk lined burrows opening to the surface with a trapdoor. The trapdoor is sometimes covered with a layer of leaf litter or moss, which blends well with the surrounding environment, thus making it difficult to spot in the field (Gertsch and Wallace 1936; Hunt 1976; Platnick and Gertsch 1976; Bond and Coyle 1995).

Ctenizids were traditionally divided into two subfamilies based on morphological features, Ctenizinae and Ummidiinae (Raven 1985; Ortiz 2007). Ctenizinae includes six genera, *Bothriocyrtum* Simon, 1891, *Cteniza* Latreille, 1829, *Cyclocosmia* Ausserer, 1871, *Cyrtocarenum* Ausserer, 1871, *Latouchia* Pocock, 1901, and *Stasimopus* Simon, 1892. However, this grouping is not supported by any identified synapomorphies (Raven 1985). Ummidiinae contains three genera, *Conothele* Thorell, 1878, *Hebestatis* Simon, 1903, and *Ummidia* Thorell, 1875 and it is diagnosed by the presence of a saddle depression on the tibia III that may serve as an anchor in the burrow (Gertsch 1979; Coyle 1981, 1985). Recently Decae (2010) removed *Hebestatis* from this subfamily based on some distinct morphological characteristics, including the presence of lateral sternal sigilla, a less pronounced and glabrous dorsal saddle on the tibia III, an absence of curvy spines, a lack of tarsal clavate trichobothria, and the absence of centrally sclerotized spermathecae, which are not present in the other genera of Ummidiinae. Therefore, the taxonomic position of *Hebestatis* remains uncertain (but see Garrison et al. 2016). The phylogenetic structure of the family Ctenizidae is also contentious. Recent phylogenetic studies have recovered neither the monophyly of Ctenizidae nor of Ctenizinae (Hedin and Bond 2006; Ayoub et al. 2007; Bond et al. 2012; Opatavo et al. 2013), even though Garrison et al. (2016) recovered *Cyclocosmia* and *Hebestatis* as monophyletic. Most importantly, *Cteniza*, the type genus of the family, which is consistently supported as sister to the other Mediterranean genus *Cyrtocarenum*, is never recovered as closely related to any of the remaining genera of the family (Raven 1985; Decae 1996; Opatova 2013). Similarly, the genus *Stasimopus* never clusters with any other Ctenizidae genus (Hedin and Bond 2006).

The genus *Conothele* is represented by 20 species distributed in the Oriental and Australian regions. The taxonomic status of *Conothele* has been a matter of debate. The genus is closely related to *Ummidia,* from which it only differs by characters of uncertain generic significance (Main 1957; Siliwal et al. 2009; Opatova et al. 2013), which has led some authors to consider *Conothele* as a junior synonym. However, the two genera show some differences in distribution and burrow architecture. Unlike

Conothele, *Ummidia* is restricted to the New World and the Mediterranean region (Main 1957; Bond et al. 2012; Siliwal et al. 2015; World Spider Catalog 2016). In addition to the different geographical distributions, the burrowing habits of both genera are also entirely different: *Conothele* constructs a short superficial burrow (arboreal or ground) parallel to the ground surface, while *Ummidia* digs a deeper and longer burrow (Main 1957; Haupt 2006; Siliwal et al. 2009). To date, only eight *Conothele* species have been described from Asia, i.e., *C. birmanica* Thorell, 1887 (juvenile) from Myanmar, *C. cambridgei* Thorell, 1890 (juvenile) from Indonesia (Sumatra), *C. fragaria* (Dönitz, 1887) (♂♀) from Japan, *C. taiwanensis* (Tso, Haupt & Zhu, 2003) (♂♀) from Taiwan, *C. giganticus* Siliwal & Raven, 2015 (♀), *C. khunthokhanbi* Kananbala, Bhubaneshwari & Siliwal, 2015 (♀), *C. vali* Siliwal et al., 2009 (♀) and *C. varvarti* Siliwal et al., 2009 (♀) from India.

In this study, four new *Conothele* species are diagnosed and described based on the morphology of female specimens collected from mainland China and Laos, where the genus had not been reported before. Although, ideally both male and female characters should be included in the description of new species, in trap-door spiders obtaining males is extremely difficult and indeed we were unable to obtain male *Conothele* specimens in this study. Direct collection by searching and digging burrows primarily results in either females or immature specimens. Males are short lived and leave the burrow immediately after the adult moult to search for females (Haupt and Shimojana 2001; Haupt 2003). Therefore, collecting males is only possible at certain times of the year, and thus is not feasible during all collection trips.

Materials and methods

Specimens were examined under an Olympus SZX16 stereomicroscope, and photographed using an Olympus BX51 compound microscope. Genitalia were cleaned in boiling KOH for a few minutes to dissolve soft tissues. All the specimens were deposited at the Centre for Behavioural Ecology and Evolution (CBEE), College of Life Sciences, Hubei University, Wuhan, China. All measurements are in millimetres. Leg and palp measurements are given in the following order: total length (femur + patella + tibia + metatarsus + tarsus).

Abbreviations used are:

ALE	anterior lateral eye;
AME	anterior median eye;
PLE	posterior lateral eye;
PME	posterior median eye;
MOA	median ocular area,
PMS	posterior median spinneret,
PLS	posterior lateral spinneret.

Taxonomy

Genus *Conothele* Thorell, 1878

Type. *Conothele malayana* (Doleschall 1859): 5, pl. 5, figure 8 (described female).

Diagnosis. The genus *Conothele* can be distinguished from the genus *Ummidia* by their burrowing habits, the former constructs a short, parallel to the surface of ground, superficial burrow, whereas the latter digs a several centimeters long burrow in the soil (Haupt 2006). Moreover, *Conothele* distributes in the Oriental and Australian regions, whereas *Ummidia* is only found from New World and the Mediterranean region (Haupt 2006; Siliwal et al. 2009; World Spider Catalog 2016).

Conothele baiyunensis Xu, Xu & Liu, sp. n.

http://zoobank.org/EBF1B2F7-6A80-4A4D-8FC6-4848E9195F81
Fig. 1

Holotype. Female (XUC-2014-062), Mt. Baiyun, Guangzhou City, Guangdong Province, China, 23.294°N, 113.484°E, 20 June 2014, collected by F.X. Liu, C. Xu and Z.T. Zhang. No male found.

Etymology. 'Baiyun' refers to the type locality of this species.

Diagnosis. Female of *C. baiyunensis* sp. n. can be distinguished from other species of *Conothele* by the slightly globular lobes of spermathecae in the terminal part; stalks with sclerotized and inward-bent distal part; stalk terminal parts relatively short, simple and direct (Fig. 1E).

Description. Total length, including chelicerae, 12.50; carapace 4.80 long, 4.40 wide; opisthosoma 6.70 long, 5.20 wide. Carapace black brown, glabrous, with a few slender setae on eye tubercle and its back (Fig. 1A). Caput arched. Fovea deep and darker, strongly procurved and U-shaped (Fig. 1A). Eye tubercle low and black on its margin. Eight eyes in two rows, with the anterior eye row slightly procurved and posterior row straight from above (Fig. 1A); eye group 0.70 long, 1.25 wide; ALE-AME 0.20, AME-AME 0.10, PLE-PME 0.05, PME-PME 0.45; MOA 0.55 long, front width 0.40, back width 0.95; ALE: AME: PLE: PME (0.30: 0.15: 0.20: 0.25). Clypeus width 0.30. Chelicerae black; inner margin with 4 teeth, outer margin with 8 teeth. Labium, coxae of palp (maxillae) and sternum yellow brown (Fig. 1B). Labium 0.70 long, 0.80 wide, with 3 conspicuous cuspules (one absent). Coxae of palp (maxillae) 1.65 long, 1.10 wide, with about 21 conspicuous cuspules ventrally. Sternum 3.00 long, 2.40 wide, with large, irregularly shaped sigilla in the centre (Fig. 1B).

Legs black brown, light-coloured ventrally, with long and short brown sparse setae, short thorn-like and normal spines. Tibia III with a saddle-like depression dorsally on the basal part (Fig. 1C). Metatarsus III with three prolateral spines. Femur III thickest. Scopulae and claw tufts absent on trasi of all palp and legs. Palpal claw with a single branched tooth; legs each with three tarsal claws, paired claws with two denticles. Leg formula: 4123. Measurements of palp and legs: palp 7.35 (2.80 + 1.25 + 1.75 + 1.55),

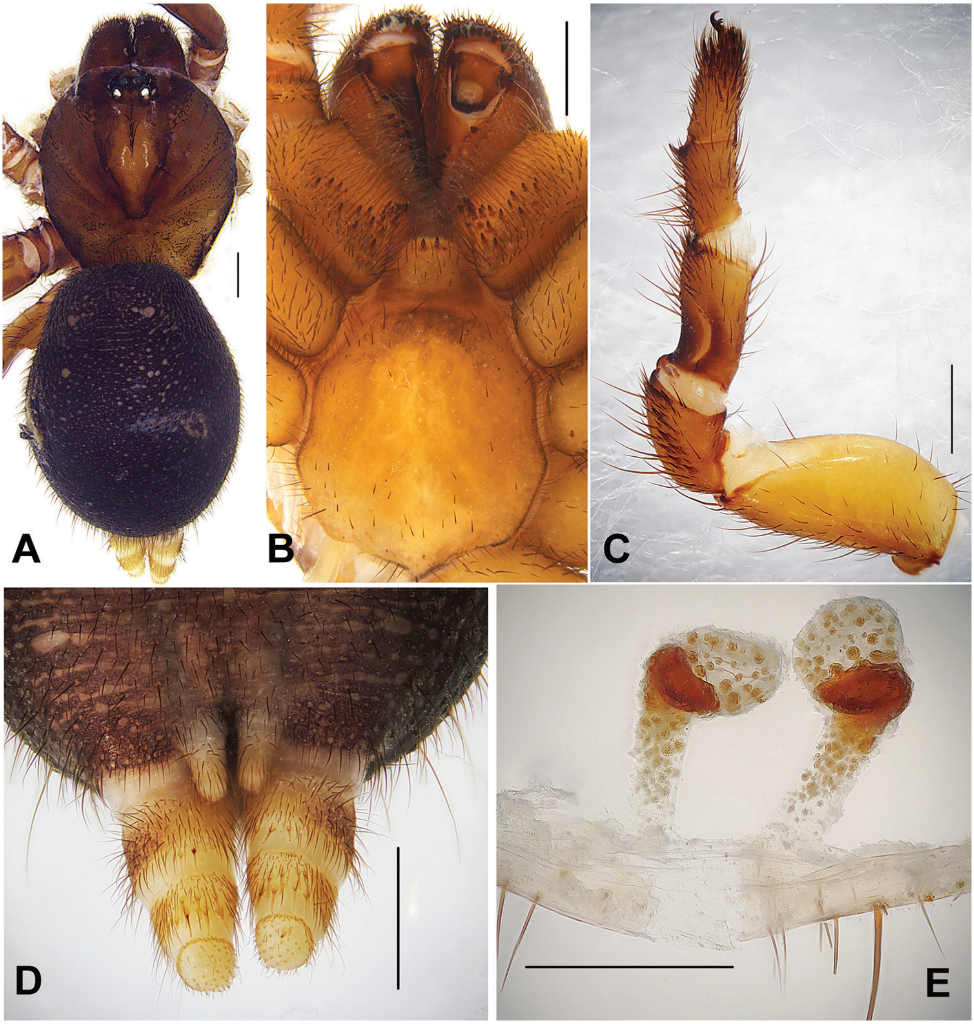

Figure 1. A General somatic morphology of *Conothele baiyunensis* Xu, Xu & Liu, sp. n. (female, XUC-2014-062) **B** chelicerae, labium, coxae of palp and sternum, ventral view **C** left leg III, prolateral view **D** spinnerets, ventral view **E** female genitalia, dorsal view. Scale bars: **A–D** 1 mm, **E** 0.5 mm.

leg I 8.30 (3.10 + 1.55 + 1.75 + 0.95 + 0.95), leg II 7.50 (2.50 + 1.25 + 1.75 + 1.05 + 0.95), leg III 7.35 (2.50 + 0.90 + 1.65 + 1.10 + 1.20), leg IV 9.70 (3.25 + 1.25 + 2.00 + 1.90 + 1.30).

Opisthosoma black, scattered with thick and slender black setae. Spinnerets brownish yellow, PMS one-segmented, 0.60 long, PMS-PMS 0.10; PLS three-segmented, 1.85 long, covered with brown spines, apical segment dome-shape (Fig. 1D). Genitalia with paired slightly globular lobes of spermathecae in the terminal part, each stalk slender, long, at the distal part is sclerotized and bent, yet the bending is relatively short and looks more simple and direct (Fig. 1E).

Distribution. Guangdong Province (Mt. Baiyun, Guangzhou), China.

Conothele daxinensis **Xu, Xu & Li, sp. n.**

http://zoobank.org/2D68220C-B6AA-4C0D-86B6-D796DB366FD4
Fig. 2

Holotype. Female (XUC-2014-002+), Minghua Village, Daxin Town, Chongzuo City, Guangxi Province, China, 23.320°N, 107.728°E, 22 December 2014, collected by D. Li, F.X. Liu and X. Xu. No male found.

Etymology. 'Daxin' refers to the type locality of this species.

Diagnosis. Female of *C. daxinensis* sp. n. differs from other species of *Conothele* by the spermathecae with face to face, bowl-shape lobes (Fig. 2E); each stalk slender, long, slightly broader at base, distally sclerotized and incurved (ca. 110°) (Fig. 2E).

Description. Total length, including chelicerae, 10.40; carapace 4.00 long, 4.60 wide; opisthosoma 5.60 long, 4.90 wide. Carapace light brown, glabrous, with 3 slender setae on eye tubercle and 4 on its back (Fig. 2A). Caput arched. Eye tubercle black brown. Fovea deep, strongly procurved and U-shaped (Fig. 2A). Eight eyes in two rows, with both the anterior and posterior rows straight from above (Fig. 2A);

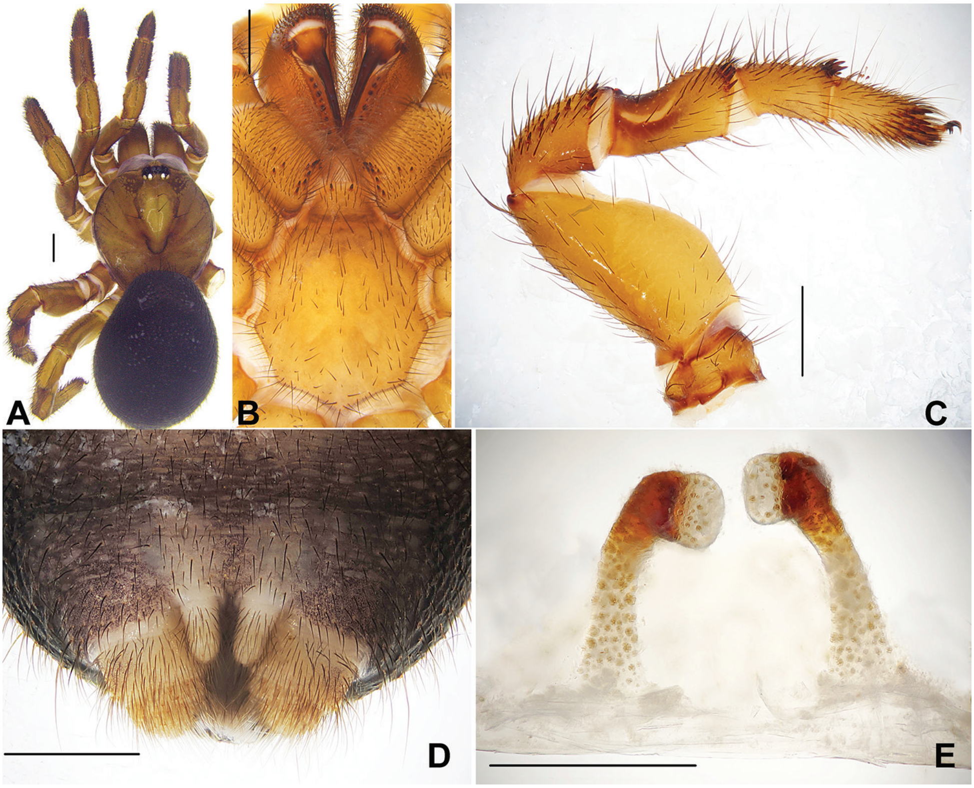

Figure 2. A General somatic morphology of *Conothele daxinensis* Xu, Xu & Li, sp. n. (female, XUC-2014-002+) **B** chelicerae, labium, coxae of palp and sternum, ventral view **C** left leg III, prolateral view **D** spinnerets, ventral view **E** female genitalia, dorsal view. Scale bars: **A–D** 1 mm, **E** 0.5 mm.

eye group 0.60 long, 1.10 wide; ALE-AME 0.10, AME-AME 0.05, PLE-PME 0.04, PME-PME 0.30; MOA 0.55 long, front width 0.45, back width 0.96; ALE: AME: PLE: PME (0.30: 0.20: 0.13: 0.33). Clypeus width 0.25. Chelicerae black, inner margin with 4 teeth, outer margin with 9 teeth. Labium, coxae of palp (maxillae) and sternum light brown (Fig. 2B). Labium 0.55 long, 0.85 wide, with 2 conspicuous cuspules. Coxae of palp (maxillae) 1.95 long, 0.95 wide, with about 10 conspicuous cuspules ventrally. Sternum 3.35 long, 2.85 wide, with irregularly shaped sigilla in the centre (Fig. 2B).

Legs light brown, light-colored ventrally, with long and short brown sparse setae. All tarsi with tadpole-shaped trichobothrial hairs besides the normal ones. Basal part of tibia III with a saddle-like depression dorsally (Fig. 2C). Tibia and tarsus of palp, distal three segments of legs I and II with bands of short thorn-like spines laterally; tibia III with 4 short spines distally (Fig. 2C). Femur III thickest. Scopulae and claw tufts absent. Palpal claw with a single branched tooth; legs each with 3 tarsal claws, paired claws with two denticles. Leg formula: 4132. Measurements: palp 6.90 (2.70 + 1.10 + 1.60 + 1.50), leg I 8.00 (3.00 + 1.40 + 1.85 + 1.00 + 0.75), leg II 7.55 (2.50 + 1.60 + 1.60 + 0.85 + 1.00), leg III 7.95 (2.75 + 1.50 + 1.45 + 1.00 + 1.25), leg IV 10.30 (3.15 + 1.80 + 1.90 + 1.90 + 1.55).

Opisthosoma black, scattered with thick and slender black setae. Spinnerets brownish, PMS one-segmented, 0.50 long, PMS-PMS 0.20; PLS three-segmented, 0.60 long, thicker and shorter (Fig. 2D). Genitalia with a pair of spermathecae, each stalk slender, long, broader towards the base, distally gradually sclerotized and incurved around 110°, terminating with face-to-face bowl-shaped lobes (Fig. 2E).

Distribution. Guangxi Province (Chongzuo), China.

Conothele sidiechongensis Xu, Xu & Liu, sp. n.

http://zoobank.org/D872AF4D-CD97-487B-8DF6-A5DAE371887B
Fig. 3

Holotype. Female (C-YN-005), Sidiechong, Mojiang County, Yunnan Province, China, 23.420°N, 101.676°E, 5 August 2013, collected by D. Li, F.X. Liu and X. Xu.

Paratypes. One female (C-YN-003) collected at Baka Village, Menglun Town, Mengla City, Xishuangbanna, Yunnan Province, China, 21.968°N, 101.210°E, 13 July 2013, collected by F.X. Liu and X. Xu. One female (C-Laos-001), Oudomxay Province, Laos PDR, 27 July 2013, collected by D. Li, F.X. Liu and X. Xu. No male found.

Etymology. 'Sidiechong' refers to the type locality of the holotype specimen of this species.

Diagnosis. Female genitalia of *C. sidiechongensis* sp. n. resembles to *C. taiwanensis* (Tso, Haupt & Zhu, 2003), but can be distinguished from the latter by more or less upwards oriented bowl-shape lobes and stalk bent in zigzag pattern distally (Fig. 3E).

Description. Total length, including chelicerae 25.50; carapace 9.00 long, 9.30 wide; opisthosoma 13.50 long, 10.80 wide. Carapace black brown, black on its margin; with

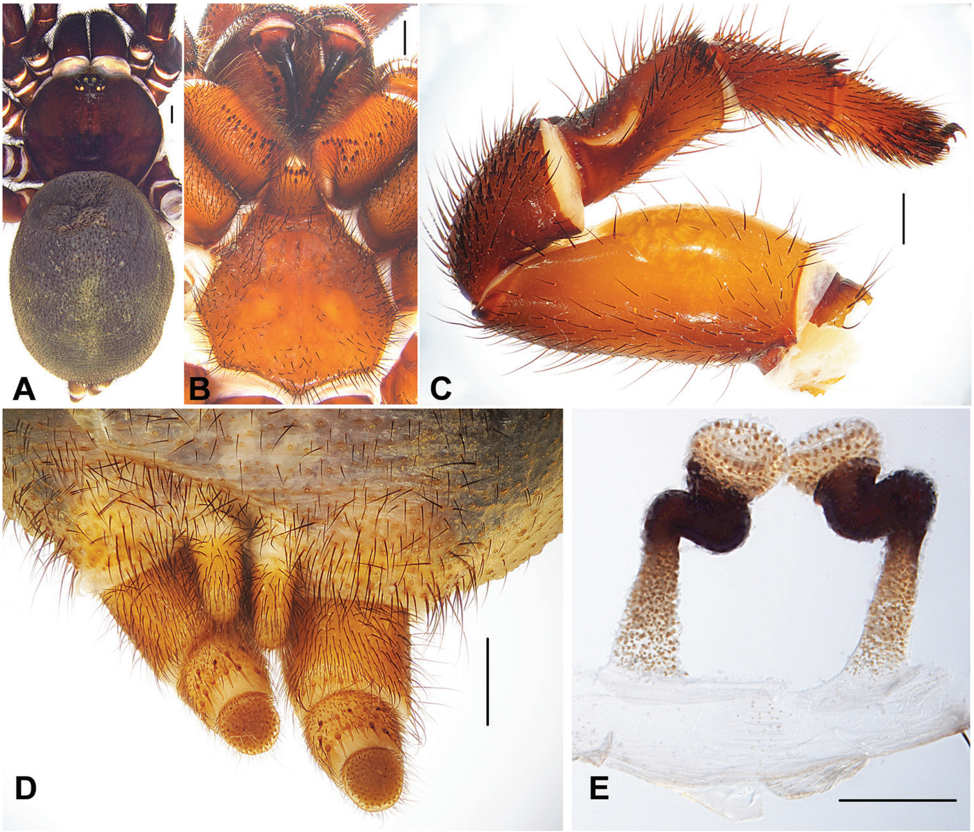

Figure 3. A General somatic morphology of *Conothele sidiechongensis* Xu, Xu & Liu, sp. n. (female, C-YN-005) **B** chelicerae, labium, coxae of palp and sternum, ventral view **C** left leg III, prolateral view **D** spinnerets, ventral view **E** female genitalia, dorsal view. Scale bars: **A–D** 1 mm, **E** 0.5 mm.

4 slender setae on eye tubercle and 5 on its back. Caput arched. Fovea deep, strongly procurved and U-shaped (Fig. 3A). Eye tubercle black and low. Eight eyes in two rows, with the anterior eye row slightly procurved and posterior row straight from above (Fig. 3A); eye group 0.60 long, 1.10 wide; ALE-AME 0.25, AME-AME 0.20, PLE-PME 0.05, PME-PME 0.85; MOA 0.60 long, front width 0.86, back width 1.55; ALE: AME: PLE: PME (0.65: 0.33: 0.45: 0.35). Clypeus width 0.45. Chelicerae black, inner margin with 11 teeth, outer margin with 8 teeth. Labium, coxae of palp (maxillae) and sternum black brown (Fig. 3B). Labium 1.60 long, 1.90 wide, with 7 conspicuous cuspules. Coxae of palp (maxillae) 3.60 long, 1.75 wide, with about 29 conspicuous cuspules ventrally. Sternum 5.25 long, 5.90 wide, with large, irregularly shaped sigilla in the centre (Fig. 3B).

Legs black brown, light-coloured ventrally, with long and short brown sparse setae. All tarsi with tadpole-shaped trichobothrial hairs besides the normal ones. Tibia III with a saddle-like depression dorsally (Fig. 3C). Tibia and tarsus of palp, distal three segments of legs I and II with bands of short thorn-like spines laterally; tibia III with 4 short thorn-like spines distally (Fig. 3C); femur III thickest. Scopulae and claw tufts absent. Palpal claw with a single branched tooth; legs each with 3 tarsal claws, paired claws with two denticles, one big and one small. Leg formula: 4123. Measurements: palp 14.88 (5.00 + 2.53 + 3.75 + 3.60), leg I 16.85 (6.00 + 3.00 + 4.00 + 2.10 + 1.75), leg II 16.05 (5.80 + 2.50 + 3.75 + 2.15 + 1.85), leg III 15.80 (4.90 + 3.50 + 3.10 + 2.10 + 2.20), leg IV 19.90 (6.10 + 3.50 + 4.10 + 4.10 + 2.10).

Opisthosoma black, scattered with thick and slender black setae. Spinnerets brownish, PMS one-segmented, 1.20 long, PMS-PMS 0.10; PLS three-segmented, 2.50 long, thicker, covered with brown spines, apical segment dome-shape (Fig. 3D). Genitalia with a pair of spermathecae, with bowl-shaped lobes facing up at the terminal part, long stalks, slightly broader at the basal part, strongly sclerotized and bent in zigzag pattern at the distal part (Fig. 3E).

Distribution. Yunnan Province (Mojiang, Mengla), China; Oudomxay Province, Laos.

Conothele yundingensis **Xu, Xu & Li, sp. n.**

http://zoobank.org/3D374070-85D6-4177-82E1-A575F883D74D
Fig. 4

Holotype. Female (XUC-2014-001+), Mt. Yunding, Tengchong City, Yunnan Province, China, 25.805°N, 98.800°E, 16 December 2014, collected by D. Li, F.X. Liu and X. Xu. No male found.

Etymology. 'Yunding' refers to the type locality of this species.

Diagnosis. Female of *C. yundingensis* sp. n. can be distinguished from *C. daxinensis* sp. n. by the slightly upwards and globular lobes terminally (Fig. 4E), can be distinguished from *C. baiyunensis* sp. n. by the distal part of stalks bent towards inside about 90° (Fig. 4E).

Description. Total length, including chelicerae 18.30; carapace 7.00 long, 6.80 wide; opisthosoma 9.10 long, 7.90 wide. Carapace black brown, black on its margin; with 9 slender setae on eye tubercle and 3 on its back (Fig. 4A). Caput arched. Fovea deep, strongly procurved and U-shaped (Fig. 4A). Eye tubercle black and low. Eight eyes in two rows, with the anterior eye row procurved and posterior row straight from above (Fig. 4A); eye group 0.8 long, 1.8 wide; ALE-AME 0.35, AME-AME 0.20, PLE-PME 0.10, PME-PME 0.65; MOA 0.70 long, front width 0.75, back width 1.45; ALE: AME: PLE: PME (0.65: 0.25: 0.30: 0.40). Clypeus width 0.40. Chelicerae black, inner margin with 4 teeth, outer margin with 8 teeth. Labium, coxae of palp (maxillae) and sternum black brown (Fig. 4B). Labium 1.40 long, 1.30 wide, with 7 conspicuous cuspules. Coxae of palp (maxillae) 2.35 long, 1.40 wide, with about 45 conspicuous cuspules ventrally. Sternum 3.95 long, 4.10 wide, with irregularly shaped sigilla in the centre (Fig. 4B).

Legs black brown, light-coloured ventrally, with long and short brown sparse setae. All tarsi with tadpole-shaped trichobothrial hairs besides the normal ones. Tibia III with a saddle-like depression dorsally (Fig. 4C). Tibia and tarsus of palp, distal

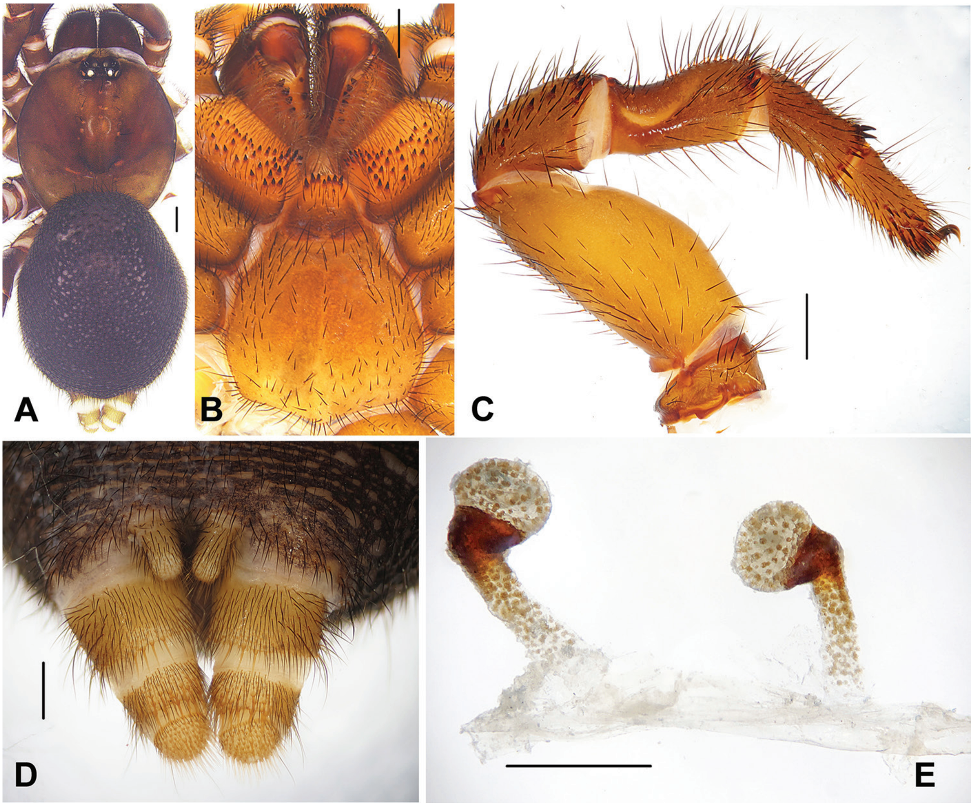

Figure 4. A General somatic morphology of *Conothele yundingensis* Xu, Xu & Li, sp. n. (female, XUC-2014-001+) **B** chelicerae, labium, coxae of palp and sternum, ventral view **C** left leg III, prolateral view **D** spinnerets, ventral view **E** female genitalia, ventral view. Scale bars: **A–D** 1 mm, **E** 0.5 mm.

three segments of legs I and II with bands of short thorn-like spines laterally; metatarsus III with 6 and tibia III with 2 short thorn-like spines distally (Fig. 4C); femur III the thickest. Scopulae and claw tufts absent. Palpal claw with a single branched tooth; legs each with 3 tarsal claws, paired claws with two denticles. Leg formula: 4132. Measurements: palp 11.40 (4.25 + 1.50 + 3.00 + 2.65), leg I 13.00 (4.25 + 2.00 + 3.10 + 1.75 + 1.90), leg II 12.25 (3.75 + 1.75 + 3.00 + 1.85 + 1.90), leg III 12.50 (4.00 + 2.50 + 2.50 + 1.75 + 1.75), leg IV 13.50 (4.50 + 2.50 + 2.90 + 2.10 + 1.50).

Opisthosoma black, scattered with thick and slender black setae. Spinnerets brownish, PMS one-segmented, 0.40 long, PMS-PMS 0.15; PLS three-segmented, 2.00 long, thicker, covered with brown spines, apical segment dome-shape (Fig. 4D). Genitalia with a pair of spermathecae with slightly upwards oriented globular lobes terminally, each stalk slender, sclerotized and bent towards inside about 90° distally (Fig. 4E).

Distribution. Yunnan Province (Mt. Yunding, Tengchong), China.

Acknowledgements

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RESEARCH ARTICLE

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Trapdoor spiders of the genus *Cyclocosmia* Ausserer, 1871 from China and Vietnam (Araneae, Ctenizidae)

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Abstract

A species of the genus *Cyclocosmia* Ausserer, 1871 collected from Guizhou Province, China is diagnosed and described as new to science: *C. liui* Xu, Xu & Li, **sp. n.** (♀). New records of *C. latusicosta* Zhu, Zhang & Zhang, 2006 (♀) from China (Yunnan Province) and Vietnam (Vinh Phuc Province, Ninh Binh Province), and *C. ricketti* (Pocock, 1901) collected from Jiangxi Province, China are also reported in this study.

Keywords

China, *Cyclocosmia*, taxonomy, trapdoor spider, Vietnam

Introduction

The mygalomorph family Ctenizidae is ancient, long-lived, regionally endemic and dispersal-limited, and thus is of long-standing and persistent conservation significance in many regions of the world (Zhu et al. 2006; Opatova et al. 2013, 2016). Ctenizids are widely distributed in east and southeast Asia, north and south America, the Mediterranean region, southern Africa and Australia (World Spider Catalog 2016). These medium-sized, ground-dwelling spiders usually construct silk-lined burrows underground, which open to the surface with a trapdoor. Trapdoors covered with a layer of leaf litter and/or a sheet of moss match the background well, thus making them very difficult to spot in the field (Gertsch and Wallace 1936; Gertsch and Platnick 1975; Hunt 1976; Bond and Coyle 1995).

Despite being present across much of the world, Ctenizidae is represented by only approximately 130 extant species-level taxa (World Spider Catalog 2016). These taxa are conventionally divided into nine genera and two subfamilies, Ctenizinae and Ummidiinae (Ortiz 2007). Ummidiinae includes three genera, *Conothele* Thorell, 1878, *Hebestatis* Simon, 1903 and *Ummidia* Thorell, 1875. *Hebestatis* was recently removed from this subfamily since it posseses lateral sternal sigilla and a less pronounced and glabrous dorsal saddle on the tibia III, but lacks curvy spines, tarsal clavate trichobothria and centrally sclerotized spermathecae (Decae 2010). The taxonomic position of *Hebestatis* therefore remains unclear (but see Garrison et al. 2016). The subfamily Ctenizinae contains six genera, *Bothriocyrtum* Simon, 1891, *Cteniza* Latreille, 1829, *Cyrtocarenum* Ausserer, 1871, *Cyclocosmia* Ausserer, 1871, *Latouchia* Pocock, 1901, and *Stasimopus* Simon, 1892. However, this grouping currently lacks the support of any identified synpomorphies (Raven 1985).

Despite being a small genus, *Cyclocosmia* contains some of the most fascinating spiders in the world (Buchli 1969; Gertsch and Platnick 1975). Their abdomens are abruptly truncated and finish in a hard, heavily sclerotized disc that is enhanced by a series of raised ribs separated by narrow grooves (Gertsch and Platnick 1975). This acts as a distinctive morphological defence to cope with intruders, such as predators, into the borrow. When the spider retreats head-first into its burrow, the abdominal disc fits tightly against the round walls of the burrow and forms an impenetrable false trapdoor (Gertsch and Wallance 1936; Gertsch and Platnick 1975). *Cyclocosmia* spiders usually build their burrows in steeply sloping banks of sandy clay (Fig. 1A). The trapdoor is usually made of silk mixed with soil and covered with a layer of leaf litter and/or moss. Like many other ctenizids, *Cyclocosmia* spiders are very difficult to find in the field because the remarkably effective camouflage of their trapdoors. Therefore, they are often regarded as one of the rarest spiders (Gertsch and Wallance 1936; Gertsch and Platnick 1975; Zhu et al. 2006).

Cyclocosmia is currently represented by seven nominal species: two in the USA (*C. torreya* Gertsch & Platnick, 1975 and *C. truncata* (Hertz, 1841)), one in Mexico and Guatemala (*C. loricata* (C. K. Koch, 1842)) and four in East and Southeast Asia (*C. latusicosta* Zhu, Zhang & Zhang, 2006 and *C. ricketti* (Pocock, 1901) in China, *C. lannaensis* Schwendinger, 2005 in China and Thailand, *C. siamensis* Schwendinger, 2005 in Thailand and Laos) (World Spider Catalog 2016). In this study, three *Cyclocosmia* species collected from China and Vietnam are diagnosed and described, one of which is believed to be new to science. One of the species is a new record from China (Yunnan) and Vietnam, based on the morphology of female specimens. Ideally, both male and female specimens would be included in the description of new species; however, it is extremely difficult to obtain adult males of trapdoor spiders. Indeed, we were unable to obtain male *Cyclocosmia* specimens in this study. By searching and digging burrows, we obtained a few immature and/or adult female specimens. Males are short-lived and leave the burrow immediately after they reach maturity to search for females (Haupt and Shimojana 2001; Haupt 2003). Therefore, collecting males is only possible at certain times of the year, and therefore is not feasible during each field trip.

Materials and methods

Specimens were studied using an Olympus SZX16 stereomicroscope. Anatomical details were examined and photographed with on Olympus BX51 compound microscope and a Canon 7D camera. Genitalia were cleared in boiling KOH for a few minutes to dissolve soft tissues. All the specimens were deposited at the Centre for Behavioural Ecology and Evolution (CBEE), College of Life Sciences, Hubei University, Wuhan, China. All lengths are given in millimetres. Leg and palp measurements are given in the following order: total length (femur + patella + tibia + metatarsus + tarsus).

Abbreviations used:

ALE	anterior lateral eye;
AME	anterior median eye;
PLE	posterior lateral eye;
PME	posterior median eye;
MOA	median ocular area;
PMS	posterior median spinneret;
PLS	posterior lateral spinneret.

Taxonomy

Genus *Cyclocosmia* Ausserer, 1871

Cyclocosmia Ausserer, 1871, type species *Cyclocosmia truncata* (Hentz, 1841): 144.
Chorizops Ausserer, 1871, type species by original designation *Actinopus loricatus* C. L. Koch, 1842, synonymised by Gertsch & Platnick, 1975: 15.

Diagnosis. The genus *Cyclocosmia* differs from all the other genera of Ctenizidae by the abruptly truncated abdomen forming a heavily sclerotized disc that is enhanced by a series of raised ribs and grooves (Gertsch and Platnick 1975) (Fig. 1B-D, I-L). Genera *Galeosoma* and *Idiosoma* of the family Idiopidae have the similar abdominal form as *Cyclocosmia*, but the genus *Galeosoma* can be distinguished from *Cyclocosmia* by the distinctly truncated abdomen without ribs or grooves, and the genus *Idiosoma* can be distinguished from *Cyclocosmia* by the moderately truncated abdomen, even though with ribs or grooves. Moreover, the arrangement of eyes is also different, *Cyclocosmia* with two eye rows, yet the two genera of Idiopidae with three eye rows (Gertsch and Platnick 1975; Zhu et al. 2006).

Cyclocosmia ricketti (Pocock, 1901)

Fig. 1

Halonoproctus ricketti Pocock, 1901: 209, pl. 21, f. 1 (described female of Pocock, 1901 was not examined).

Cyclocosmia ricketti Simon, 1903: 887, f. 1044–1047; Gertsch & Platnick, 1975: 18, f. 28–29, 32, 36; Song, Zhu & Chen, 1999: 36, f. 16H, K–L; Schwendinger, 2005: 227, f. 2–8, pl. 1D; Zhu, Zhang & Zhang, 2006: 120, f. 1, 2A–E; Zhang, Gao & Li, 2007: 385, f. 101; Yin et al., 2012: 134, f. 13a–e.

Material examined. Female (XUC-2013-013), Mt. Nan, Ciping Town, Jinggangshan City, Jiangxi Province, China, 26.56892°N, 114.16350°E, 22 October 2013, collected by F.X. Liu, X. Xu and C. Xu; 1 Juvenile (C-2016-001), Cemetery of Jinggangshan Revolutionary Martyrs, Ciping Town, Jinggangshan City, Jiangxi Province, 26.57873°N, 114.15960°E, 31 August 2016, collected by X. Xu; 1 female (Tianzishan-2016), Mt. Tianzi, Zhangjiajie, Hunan Province, China, 29.40°N, 110.44°E, 10 March 2016, collected by S.F. Peng.

Diagnosis. *Cyclocosmia ricketti* differs from other species of *Cyclocosmia* by the character of 23-33 radiating ribs on each side of abdominal disc (Fig. 1I-L), and parallel-sided spermathecae (Fig. 1G, H). It can be distinguished from *C. latusicosta* by the lack of the elevated central zone inside the upper pair of muscle impressions (Fig. 1I-L). More details see Zhu et al. 2006.

Description. Female (XUC-2013-013). Total length, including chelicerae, 14.00; carapace 6.50 long, 5.40 wide; abdomen 6.50 long, 9.30 wide. Carapace red-brown and smooth, with a few marginal hairs in the front of ocular area, four long bent bristles in longitudinal row running through ocular area, the posterior two bristles have been damaged (Fig. 1D). Ocular area with a black ring around each eye of the anterior eye row and a black band in front of fovea. Cervical groove and radial furrows distinct. Fovea deep and procurved, U-shaped, its greatest width occupying one fourth of carapace width at that point. Eyes set on low tubercle, ocular 0.70 long, 1.70 wide anteriorly, 1.70 wide posteriorly. Clypeus height 0.60. Anterior eye row straight and posterior eye row recurved, both rows almost equal in length. Ratio of eyes, ALE: AME: PLE: PME (0.40: 0.25: 0.30: 0.20). ALE-AME 0.25, AME-AME 0.20, PLE-PME 0.10, PME-PME 0.70. MOA 0.70 long, 0.70 wide in front, 1.10 wide at back. Chelicerae red-brown, inner margin with eight teeth and six denticles, outer margin with seven teeth and four denticles. Rastellum raised on prominent angled projection and consisting of many short black teeth. Labium yellow-brown, 1.10 long, 1.20 wide, with three black cuspules anteriorly. Maxilla yellow-brown, 2.30 long, 1.50 wide, with a few black cuspules at base.

Legs yellow-brown. Tibiae and tarsi of pedipalps, and distal three segments of legs I and II with numerous horn-like spines, metatarsus III with a few short dorsal and two ventrally spines, tarsus III with a few dark spines prolaterally and ventrally. Legs each with three tarsal claws, paired claws with a single large tooth, unpaired claw lacking

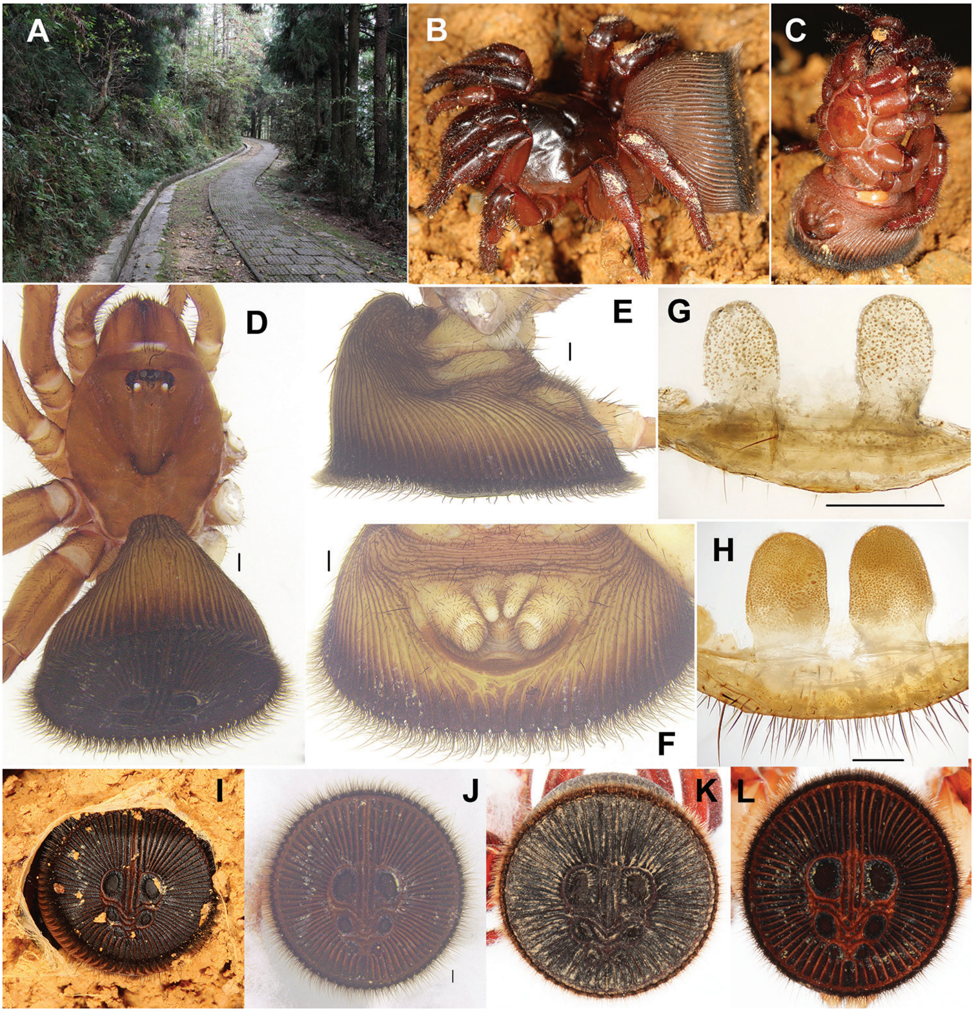

Figure 1. *Cyclocosmia ricketti* (Pocock, 1901) **A** habitat **B–D** habitus of female (XUC-2013-013) **E** abdomen, lateral view **F** posterior portion of opisthosoma, ventral view, showing spinnerets **G** female genitalia (XUC-2013-013), dorsal view **H** female genitalia (tianzishan-2016), dorsal view **I** showing opisthosomal disc in plugging position (XUC-2013-013) **J–L** abdomen, caudal view (**J** XUC-2013-013 **K** Tianzishan-2016 **L** C-2016-001). Scale bars 0.5 mm.

tooth. Palp with a single claw bearing one tooth. Measurements: palp 10.06 (4.00 + 2.10 + 2.30 + 2.20), leg I 11.00 (4.20 + 1.60 + 2.50 + 1.50 + 1.20), leg II 8.60 (3.20 + 1.40 + 1.50 + 1.40 + 1.10), leg III 8.50 (3.50 + 1.30 + 1.20 + 1.00 + 1.50), leg IV 12.20 (4.00 + 2.60 + 2.10 + 2.00 + 1.50). Formula: 4123.

Abdomen funnel-shaped and dark yellow-brown (Fig. 1D). Caudal disc slightly concave, 8.80 in transversal diameter and 8.50 in longitudinal diameter, with two rids running dorso-ventrally and 32/33 (XUC-2013-013), 29/28 (Tianzishan-2016), 33/33 (C-2016-001) radiating ribs on each side. Abdominal disc with six well-marked muscle impressions (Fig. 1I-L). Four spinnerets, with inner pair small and one-segmented, and outer pair slightly longer and three-segmented (Fig. 1F). Genitalia with paired spermathecae (Fig. 1G, H), sack-like, parallel-sided, the length of each one is more or less one and a half times its width: length = 0.55 mm, width = 0.33 mm (XUC-2013-013); length = 1.17 mm, width = 0.78 mm (Tianzishan-2016).

Distribution. China (Fujian, Hunan, Jiangxi, Zhejiang, Sichuan).

Remarks. *Cyclocosmia ricketti* was diagnosed and described based on the holotype female collected from Fujian, and since then, according to the character of the abdominal disc with 23-33 ribs on each side, researchers have identified specimens collected from Hunan, Zhejiang, Sichuan as *C. ricketti*. Here, a specimen collected from Jiangxi is also identified as *C. ricketti* on the basis of this character; *C. ricketti* was not recorded in Jiangxi before. Males remain unknown.

Cyclocosmia latusicosta Zhu, Zhang & Zhang, 2006
Fig. 2

Cyclocosmia latusicosta Zhu, Zhang & Zhang, 2006: 121, f. 4, 5A–D, 6A–J (described female of Zhu, Zhang & Zhang, 2006 was not examined); Zhang, Gao & Li, 2007: 385, f. 1–100.

Material examined. Female (17-1-2013), Tam Dao Town, Tam Dao National Park, Vinh Phuc Province, Vietnam, 21.45847°N, 105.64834°E, 17 January 2013, collected by D. Li, F.X. Liu and X. Xu; Female (XUC-2016-017), Cuc Phuong National Park, Nho Quan, Ninh Binh Province, Vietnam, 20.34915°N, 105.59927°E, 31 May 2016, D. Li, F. Li and F.X. Liu; 3 females and 1 juveniles (LH-2016-(002-005)), rubber plantation, Hekou City, Yunnan, 22.537°N, 103.942°E, 15 September 2016, F. Li, F.X. Liu and L. Yu.

Diagnosis. Females of *C. latusicosta* are different from other *Cyclocosmia* species by their abdominal disc with 22–27 wide ribs on each side, each upper muscle impression with an elevated zone connected to the outer rim of each upper muscle impression, and the length of spermathecae being more or less 1.7–2.0 times longer than width.

Description. Female. Total length, including chelicerae, 17.10–30.20; chelicerae 2.60–4.90 long; carapace 7.50–14.50 long, 6.71–12.50 wide; abdomen 9.30–11.60 long, 12.20–18.50 wide. Carapace red-brown and smooth, with a few marginal hairs and a long bristle in front of ocular area, six long bent bristles in longitudinal row and two bristles in latitudinal running through ocular area (Fig. 2A). Ocular area black, with a black band in front of fovea and beside ocular area respectively. Cervical groove and radial furrows distinct. Fovea deep and procurved, U-shaped, its greatest width occupying one fifth of carapace width at that point. Eyes set on low tubercle, ocular 0.90 long, 2.00 wide anteriorly, 2.00 wide posteriorly, occupying one fourth of carapace width at that point. Clypeus height 2.40. Both anterior and posterior eye rows straight and almost equal in length. Ratio of eyes, ALE: AME: PLE: PME (0.38: 0.25: 0.20:

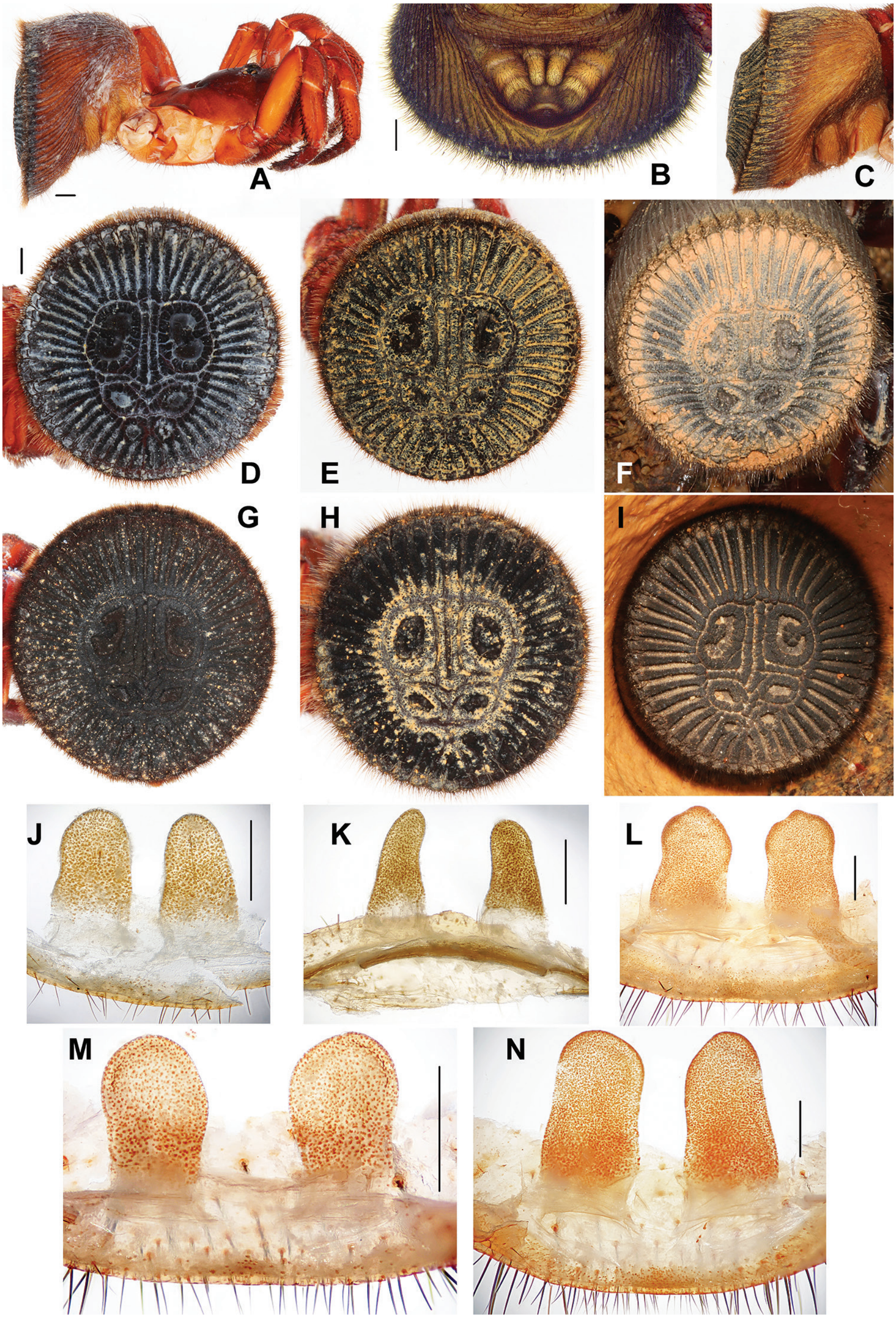

Figure 2. *Cyclocosmia latusicosta* Zhu, Zhang & Zhang, 2006 **A** habitus of female, lateral view (17-I-2013) **B** posterior portion of opisthosoma, ventral view, showing spinnerets **C** abdomen, lateral view (XUC-2016-017) **D–I** abdomen, caudal view (**D** 17-I-2013 **E** XUC-2016-017 **F** LH-2016-002 **G** LH-2016-003 **H** LH-2016-004 **I** LH-2016-005) **J–N** female genitalia (**J** 17-I-2013 **K** XUC-2016-017 **L** LH-2016-003 **M** LH-2016-004 **N** LH-2016-005), dorsal view. Scale bars **A, B, D** 1mm, **J–N** 0.5 mm.

0.20). ALE-AME 0.28, AME-AME 0.28, PLE-PME 0.02, PME-PME 0.80. MOA 0.80 long, 0.78 wide in front, 1.20 wide at back. Chelicerae red-brown, inner margin with six teeth and one denticle, outer margin with seven teeth and four denticles. Rastellum raised on prominent angled projection and consisting of many short black teeth. Labium yellow-brown, 1.40 long, 1.40 wide, with three black cuspules anteriorly. Maxilla yellow-brown, 15.90 long, 1.20 wide, with a few black cuspules at base.

Legs yellow-brown. Tibiae and tarsi of pedipalps, and distal three segments of legs I and II with numerous horn-like spines, metatarsus III with a few short dorsal and ventrally spines, tarsus III with a few dark spines prolaterally and ventrally. Legs each with 3 tarsal claws, paired claws with a single large tooth, unpaired claw lacking tooth. Palp with a single claw bearing one tooth. Measurements: palp 12.10 (4.20 + 1.80 + 3.10 + 3.00), leg I 14.00 (5.00 + 2.00 + 3.00 + 2.00 + 2.00), leg II 11.20 (4.10 + 2.00 + 2.00 + 1.50 + 1.60), leg III 11.00 (4.10 + 1.00 + 2.20 + 1.70 + 2.00), leg IV 15.20 (5.00 + 3.00 + 2.50 + 2.50 + 2.20). Formula: 4123.

Abdomen funnel-shaped and dark yellow-brown (Fig. 2A). Caudal disc slightly convex, 11.00 in transversal diameter and 10.20 in longitudinal diameter, with two rids running dorso-ventrally (with small interrupt at the groove outer the upper pair muscle impressions) and 24/25 (17-1-2013), 23/25 (XUC-2016-017), 24/23 (LH-2016-002), 28/26 (LH-2016-003), 23/24 (LH-2016-004), 22/23 (LH-2016-005) radiating ribs on each side (Fig. 2D-I). Abdominal disc with six well-marked muscle impressions. All rims within the muscle impression zone with distinct granular structures in different sizes (Fig. 2D-I). Four spinnerets, with inner pair small and one-segmented, and outer pair longer and three-segmented. Paired spermathecae sack-like, parallel-sided, each one with a length 1.7-2.0 times its width (Fig. 2J-N), length = 0.86 mm, width = 0.43 mm (17-1-2013), length = 0.94 mm, width = 0.39 mm (XUC-2016-017), length = 1.42 mm, width = 0.83 mm (LH-2016-003), length = 0.73 mm, width = 0.40 mm (LH-2016-004), length = 1.67 mm, width = 0.83 mm (LH-2016-005).

Distribution. China (Guangxi, Yunnan), Vietnam (Vinh Phuc, Ninh Binh).

Remark. *Cyclocosmia latusicosta* was diagnosed based on the holotype female collected from Guangxi Province, China, near the border to Vietnam. This study provides a new record from China (Yunnan) and Vietnam. We preliminarily treated the differences in the spermathecae among five specimens (17-1-2013, XUC-2016-017, LH-2016-003/004/005) as the intraspecific variation according to the work of Zhang et al. (2007). Males remain unknown.

Cyclocosmia liui Xu, Xu & Li, sp. n.

http://zoobank.org/BAD72BC4-CC48-44DF-BA1A-138BAAB915B5
Fig. 3

Holotype. Female (C-XUX-2015), Mt. Fanjing, Taiping Town, Jiangkou County, Tongren City, Guizhou Province, China, 27.8513°N, 108.7779°E, 25 May 2015, collected by Z.Q. Li, F.X. Liu and M. Yan.

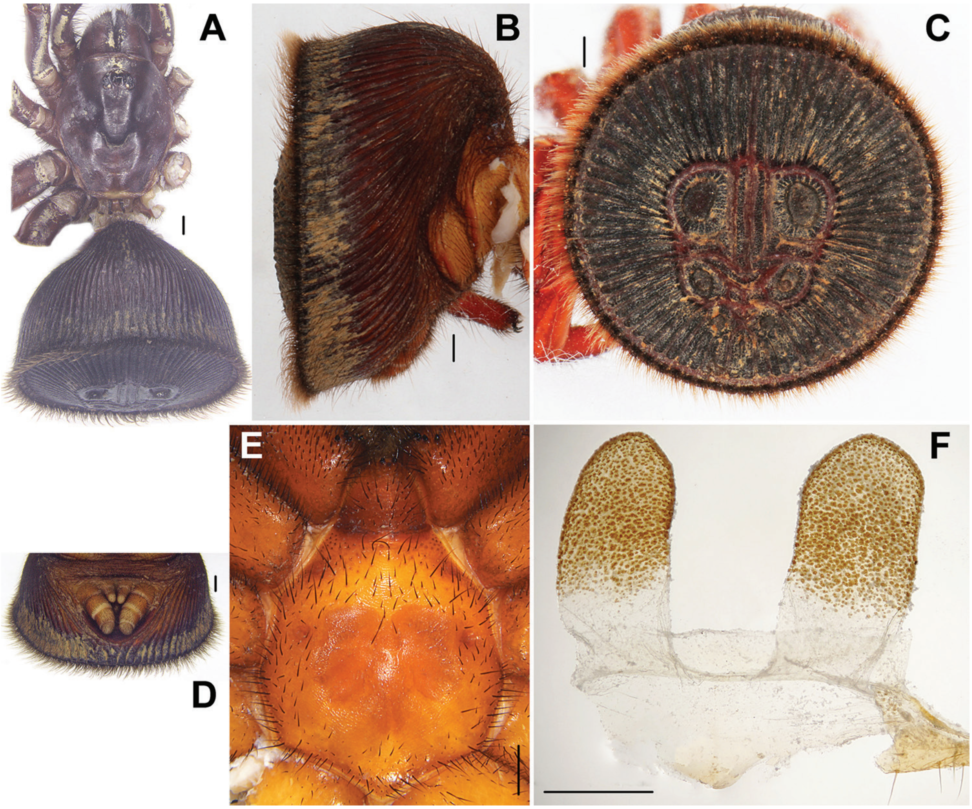

Figure 3. *Cyclocosmia liui* Xu, Xu & Li sp. n. **A** habitus of female (C-XUC-2015) **B** abdomen, lateral view **C** abdomen, caudal view **D** posterior portion of opisthosoma, ventral view, showing spinnerets **E** sternum, ventral view **F** female genitalia, ventral view. Scale bars **A–E** 1 mm, **F** 0.5 mm.

Etymology. The specific name is taken from the family name of the collector Fengxiang Liu, who joined all collecting trips and has worked on spiders for a few decades.

Diagnosis. Female of *C. liui* sp. n. can be distinguished from *C. ricketti* by abdominal disc with 33/34 ribs on each side, the rims of the upper pair muscle impressions with distinct granular structures in almost same size, the groove around the six well-marked muscle impressions dark red colour, and the middle pair muscle impressions with an elevated central zone connected to the inner rim of muscle impression (Fig. 3C). It is similar to *C. latusicosta* in the shape of spermathecae, but can be distinguished from the latter by abdominal disc with 33/34 ribs on each side (Fig. 3C).

Description. Total length, including chelicerae, 22.50; chelicerae 3.40 long; carapace 9.70 long, 8.00 wide; abdomen 11.50 long, 14.20 wide. Carapace dark brown and smooth, with a few marginal hairs and a long bristle in the front of ocular area, three long bent bristles in longitudinal row running through ocular area (Fig. 3A). Carapace widest at coxae II. Ocular area black. Cervical groove and radial furrows distinct.

Fovea deep and procurved, U-shaped, its greatest width occupying one fourth of carapace width at that point. Eyes set on low tubercle, ocular 1.00 long, 2.24 wide anteriorly, 2.20 wide posteriorly, occupying one fourth of carapace width at that point (Fig. 3A). Clypeus height 2.10. Both anterior and posterior eye rows straight and almost equal in length. Ratio of eyes, ALE: AME: PLE: PME (0.53: 0.34: 0.43: 0.33). ALE-AME 0.35, AME-AME 0.25, PLE-PME 0.05, PME-PME 1.00. MOA 1.00 long, 0.93 wide in front, 1.66 wide at back. Chelicerae red-brown, inner margin with eight teeth and six denticles between, outer margin with seven teeth and three denticles between. Rastellum raised on prominent angled projection and consisting of many short black teeth. Labium brown, 1.90 long, 1.70 wide, with three black cuspules anteriorly. Maxilla dark-brown, 18.5 long, 1.5 wide, with a few black cuspules at base. Sternum 5.50 long, 5.00 wide, with large, irregularly shaped sigilla in the centre (Fig. 3E).

Legs brown. Tibiae and tarsi of pedipalps, and distal three segments of legs I and II with numerous horn-like spines, metatarsus III with a few short dorsal ventrally spines, tarsus III with a few dark spines prolaterally and ventrally. Legs each with three tarsal claws, paired claws with a single large tooth, unpaired claw lacking tooth. Palp with a single claw bearing one tooth. Measurements: palp 14.50 (5.60 + 2.30 + 3.00 + 3.60), leg I 17.50 (6.00 + 3.00 + 3.70 + 2.80 + 2.00), leg II 14.40 (5.10 + 2.30 + 3.00 + 2.20 + 1.80), leg III 14.80 (5.20 + 3.00 + 2.30 + 2.20 + 2.10), leg IV 17.70 (5.50 + 3.20 + 3.50 + 3.10 + 2.40). Formula: 4132.

Abdomen funnel-shaped and dark brown. Caudal disc slightly convex (Fig 3A, B), 13.20 in transversal diameter and 12.30 in longitudinal diameter, with two rids running dorso-ventrally (with small interrupt at the groove outer the upper pair muscle impressions) and 34/35 radiating ribs on each side (Fig 3C). Abdominal disc with six well-marked muscle impressions, the middle pair muscle impression with an elevated central zone connected to the inner rim of muscle impression. Four spinnerets, with inner pair small and one-segmented, and outer pair much longer and three-segmented (Fig. 3D). Paired spermathecae sack-like, parallel-sided, the length of each one is more or less two times its width (Fig. 3F), length = 1.09 mm, width = 0.56 mm.

Male. Unknown.

Distribution. China (Guizhou).

Acknowledgements

This study was supported by the grants from the National Natural Sciences Foundation of China (NSFC-31601850 and NSFC-31272324) and the Singapore Ministry of Education (MOE) AcRF Tier 1 grant (R-154-000-638-112). We value the positive feedback from Hirotsugu Ono and Vera Opatova and thank them for their insightful and constructive comments on the manuscript. We are grateful to Ziqi Li, Mu Yan, Zengtao Zhang, and Shuaifu Peng for their field work. We also thank the staff of the Centre for Behavioural Ecology and Evolution (CBEE, Hubei University) for all their help and support throughout this study.

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The unexpected finding of *Parapholidoptera castaneoviridis* in south-eastern Romania (Insecta, Orthoptera, Tettigoniidae)

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Abstract

The Balkano-Anatolian genus *Parapholidoptera* comprises 21 species and the westernmost one, *Parapholidoptera castaneoviridis*, previously recognized to occur in western Turkey, north-eastern Greece and south-eastern Bulgaria is recorded for the first time from south-eastern Romania, almost 300 km away from the closest known locality. Illustrations and measurements of morphological characters are given and the male calling song from this new, northernmost population is described.

Keywords

Balkan Peninsula, bush-cricket, calling song, relict population, distribution

Introduction

With 21 described species, the genus *Parapholidoptera* Mařan is distributed from the Caucasus in the east, to the Balkans in the west, across the Anatolian Plateau and reaching Israel and Jordan in the south (Çiplak 2000, Katbeh Bader and Massa 2001, Çiplak 2004, Eades et al. 2016). Within the genus, Çiplak (2000) defines two clades: *P. castaneoviridis* group with a south-western distribution and *P. distincta* group, occurring in north-eastern Anatolia and the Caucasus. It seems that the Anatolian mountain ranges played an important role in the speciation and distribution of the genus (Çiplak 2004), 16 species being endemic to various localities and mountains in Turkey, Georgia, Armenia and Jordan.

Parapholidoptera castaneoviridis (Brunner von Wattenwyl, 1882) is one of the most widely distributed species of this genus, known to occur from the north Aegean region of Turkey, Samothrace Island, Macedonia and Thrace regions in Greece, Strandzha Mountains and the territory eastwards from Eastern Rhodopes in Bulgaria (Ingrisch and Pavićević 1985, Çiplak 2000, Popov and Chobanov 2004). The species is characterized by a cylindrical pronotum, extended metazona, male with last tergum light-colored, with two small, straight processes and females with a long, straight ovipositor and subgenital plate narrowly rounded, with a deep groove (Çiplak 2000).

The finding of *Parapholidoptera castaneoviridis* in Romania, the northernmost known location in the distribution of this species, is discussed.

Material and methods

The first specimens of *Parapholidoptera castaneoviridis* were found while actively searching for orthopterans in the forest clearings near Ciucurova village (Tulcea county, south-eastern Romania), in the summer of 2016. The bush-crickets were collected at daytime, in xerophytic *Quercus* forest clearings with scrub, at altitudes of approximately 200 m ASL. Individuals were identified according to genital morphology and calling song oscillographic structure. Photos were taken with a Canon EOS 6D DSLR camera and a Canon EF 180 mm f3.5 macro lens. Acoustic recordings were made at night, in laboratory conditions with an Edirol R-09HR digital recorder (sampling rate 96 kHz, 24-bit amplitude resolution, microphone response frequency up to 45 kHz) and a Knowles electret condenser microphone connected to a PC through a TransitUSB external sound card (48 kHz, 16-bit) and the sound analysis was run with Audacity 2.1.2 and Batsound 4 software. As song element durations are usually temperature dependent, the ambient air temperature was measured during these recordings.

The bioacoustic terminology is adopted mainly from Ragge and Reynolds (1998): *calling song* – song produced by an isolated male; *syllable* – the song produced by one to-and-fro movement of the tegmina; *hemisyllable* – the song produced by one unidirectional movement of the tegmina (opening or closing); *echeme* – a first order assemblage of syllables; *echeme sequence* – a first order assemblage of echemes. The following oscillographic characters were measured in the songs of three males from Ciucurova and compared to previously known results (Heller 1988, Sevgili et al. 2011): *duration of an echeme* (DE) – the time elapsed from the beginning of first syllable to the end of last syllable of an echeme; *duration of a syllable* (DS) – the time elapsed from the beginning of first impulse to the end of last impulse of a syllable; *duration of an opening hemisyllable* (DOH) – the time elapsed from the beginning of first impulse to the end

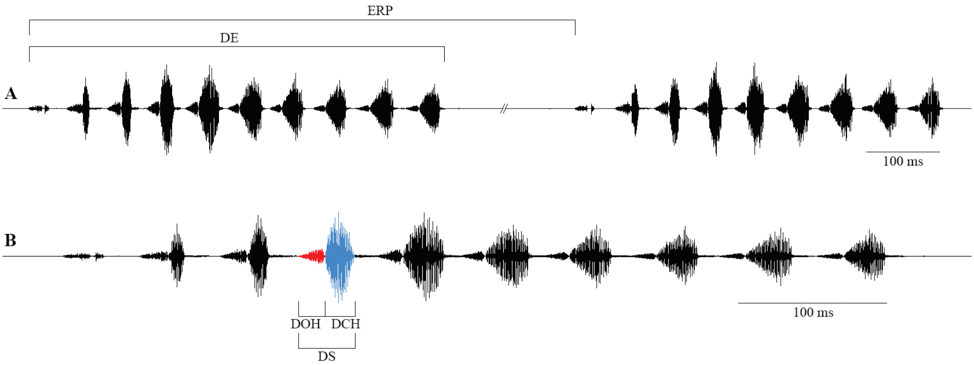

Figure 1. Studied male calling song oscillographic characters in *Parapholidoptera castaneoviridis*: **A** sequence of two echemes **B** detailed echeme (red – opening hemisyllable, blue – closing hemisyllable). Abbreviations: **ERP** – echeme repetition period; **DE** – duration of an echeme; **DOH** – duration of an opening hemisyllable; **DCH** – duration of a closing hemisyllable; **DS** – duration of a syllable.

of last impulse of an opening hemisyllable; *duration of a closing hemisyllable* (DCH) – the time elapsed from the beginning of first impulse to the end of last impulse of a closing hemisyllable; *echeme repetition period* (ERP) – the time elapsed from the beginning of an echeme to the beginning of the next echeme (Fig. 1); *echeme recurrence rate* – density of echemes repetition in a specific time period.

Examined material: 2 ♂♂ 2 ♀♀, 2016.07.03, forest clearing north of Ciucurova village, Tulcea county, Romania, 44.9576°N 28.5245°E, 190 m ASL (leg. I. Ș. Iorgu); 1 ♂ 2 ♀♀, 2016.07.30, same locality (leg. I. Ș. Iorgu & E. I. Iorgu) (Fig. 2A, B).

Other material recorded in the Balkan Peninsula: see Suppl. material 1 and Figure 3.

Acoustic recordings: 2 ♂♂, 2016.07.03, Ciucurova, Romania (temperature 26°C); 1 ♂, 2016.07.30, same locality (28°C); 1 ♂, 2007.06.26, Strandzha Mountains, Malko Turnovo - Gradishteto hill, Bulgaria, 41.965°N 27.491°E, 650 m ASL, temperature 25°C (leg. D. P. Chobanov & M. Ilieva).

Calling song

Typically, the males produce their calling song in the evening and at night. In the recorded males, the calling song consists of a long series of echemes, each echeme lasting for 426–583 ms (mean ± SD 503.87 ± 46.81 ms) and containing 8–10 syllables (mean ± SD 8.49 ± 0.52) (Fig. 4). The recurrence rate is fairly 20–30 / minute, the echeme repetition period being 2122–2760 ms (mean ± SD 2408.28 ± 197.71 ms). Syllables are quite short (39–54 ms, mean ± SD 47.28 ± 4.1 ms) and consist of two distinct parts: a shorter, lower amplitude opening hemisyllable (15–20 ms, mean ± SD 17.55 ± 1.64 ms) and a longer, higher amplitude closing hemisyllable (20–39 ms, mean ± SD 29.73 ± 4.49 ms). Acoustic signal amplitude modulation pattern is increasing and de-

Figure 2. The habitus of *Parapholidoptera castaneoviridis* in the northernmost known location: **A** male **B** female (Romania, Ciucurova, 2016.07.03).

Table 1. Descriptive statistics of male calling song parameters in *Parapholidoptera castaneoviridis* from south-eastern Romania (n = 100 measurements/male from 3 males). All values are given in milliseconds. Abbreviations: **DE** – duration of an echeme; **DS** – duration of a syllable; **DOH** – duration of an opening hemisyllable; **DCH** – duration of a closing hemisyllable; **ERP** – echeme repetition period.

	DE	DS	DOH	DCH	ERP	Number of syllables / echeme
Max.	583	54	20	39	2760	10
Min.	426	39	15	20	2122	8
Mean	503.87	47.28	17.55	29.73	2408.28	8.49
SD	46.816	4.102	1.641	4.49	197.719	0.522

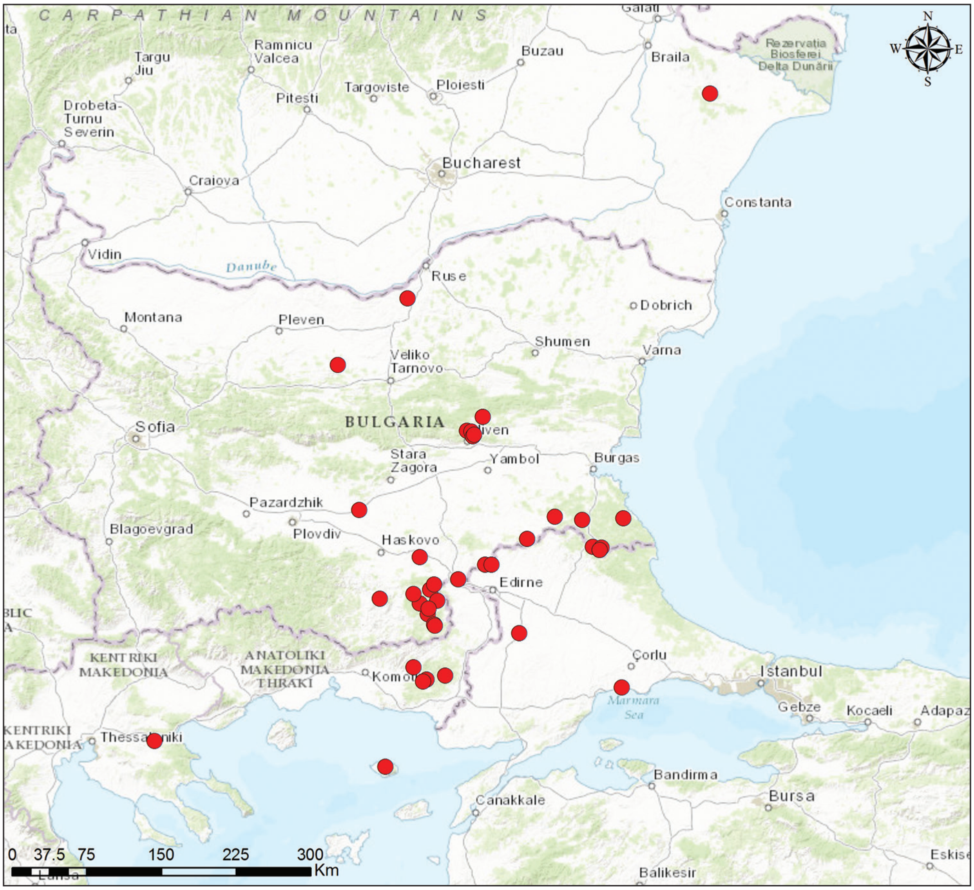

Figure 3. Geographic distribution of *Parapholidoptera castaneoviridis* in the Balkan Peninsula (for a complete list of localities see the Suppl. material 1).

creasing in both the opening and closing hemisyllables. The detailed descriptive statistics of the song characters are presented in Table 1. The calling song has the dominant frequency components between 7 kHz and up to more than 45 kHz (45 kHz being the upper limit of microphone frequency response in our recordings), with a main peak at about 12 kHz in both opening and closing hemisyllables (Fig. 5).

Discussion

Although recently *Parapholidoptera* was divided in two groups relying exclusively on morphological traits (Çiplak 2000), the genus is characterized by a more or less uniform calling song, especially among the *Parapholidoptera castaneoviridis* group – multisyllabic echemes separated by silent intervals lasting for several seconds, with differences in the syllable number per echeme (Heller 2006).

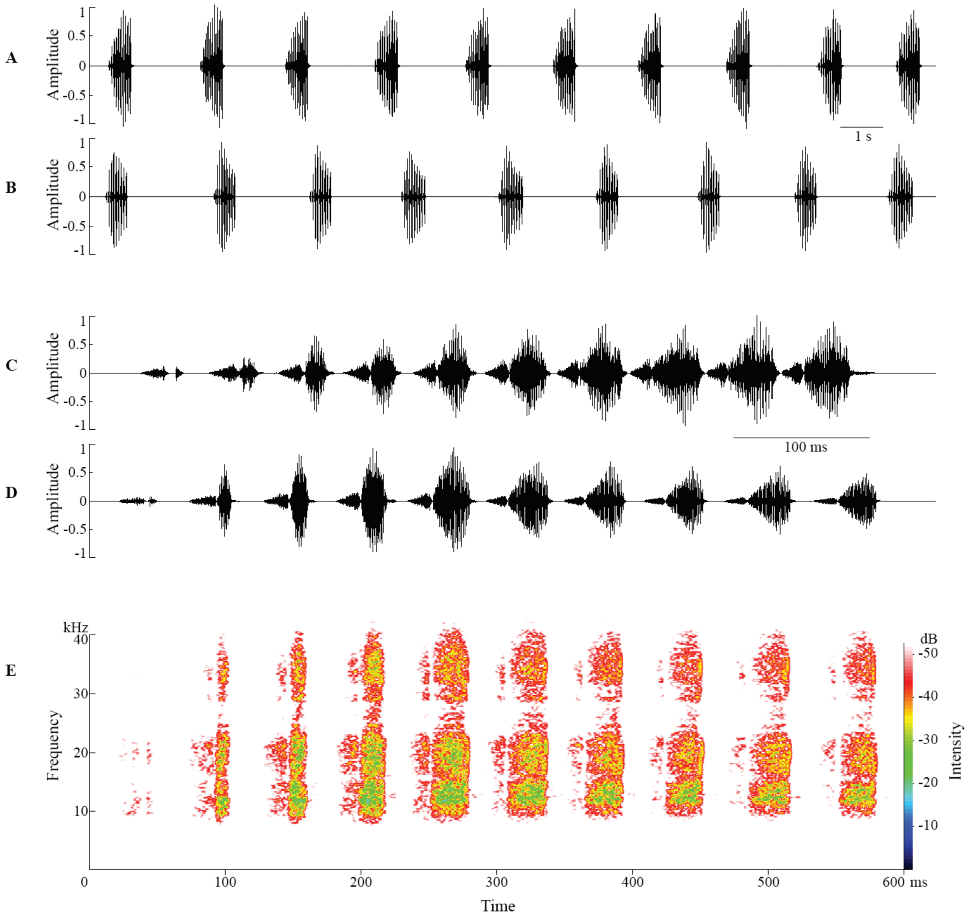

Figure 4. *Parapholidoptera castaneoviridis* male calling song: **A, B** oscillographic representation of an echeme sequence **C, D** detailed echeme **E** spectrogram (referring to the detailed echeme **D**). **A, C** Bulgaria, Strandzha Mts., Malko Turnovo, 25°C **B, D, E** Romania, Ciucurova, 26°C.

The males discovered in Romania are characterized by a homogeneous song pattern with the specimens from Strandzha Mountains and Turkey: variable echemes consisting of 5–8 (Sevgili et al. 2011), 10–12 or 11–13 syllables (Heller 1988, 2006), lasting for 268–420 ms (Sevgili et al. 2011) or more than 500 ms (see oscillogram in Heller 1988) and repeated at 1.5–3 s (Sevgili et al. 2011) or up to 2.78–4.20 s (Heller 1988) are produced by individuals from the core area of species distribution (south-eastern Balkans and western Asia Minor). Having 8–10 syllables/echeme and lasting for 426–583 ms, with a repetition period of 2.1–2.7 s, the song of Romanian specimens fits well within this description. Moreover, the morphological structures and measurements of specimens from the newly found population (Fig. 6) are very similar with the ones described by Çiplak (2000) (for a complete numeric comparison, see Table 2).

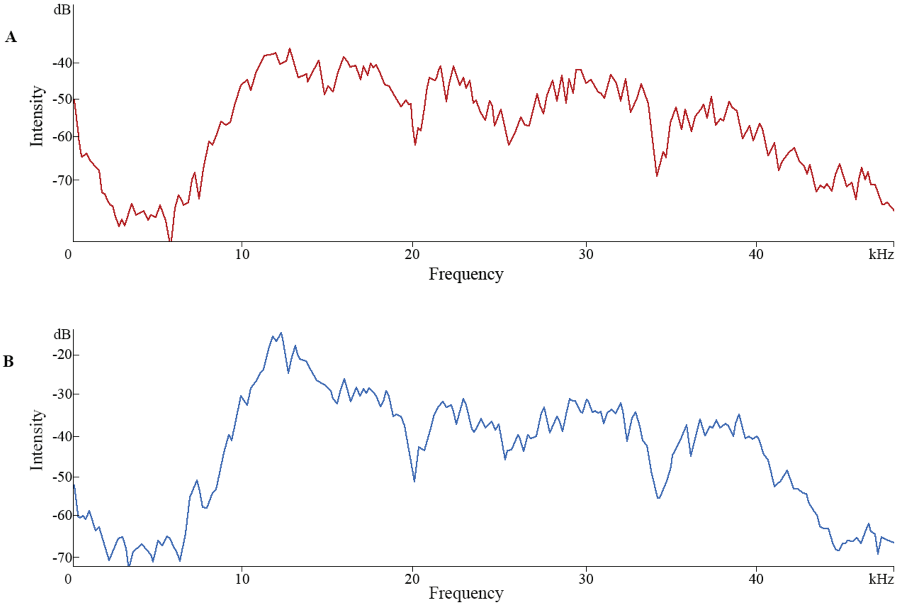

Figure 5. *Parapholidoptera castaneoviridis* male calling song spectrum: **A** opening hemisyllable frequencies (red) **B** closing hemisyllable frequencies (blue). Function: Hanning window; size: 512 (Romania, Ciucurova, 26°C).

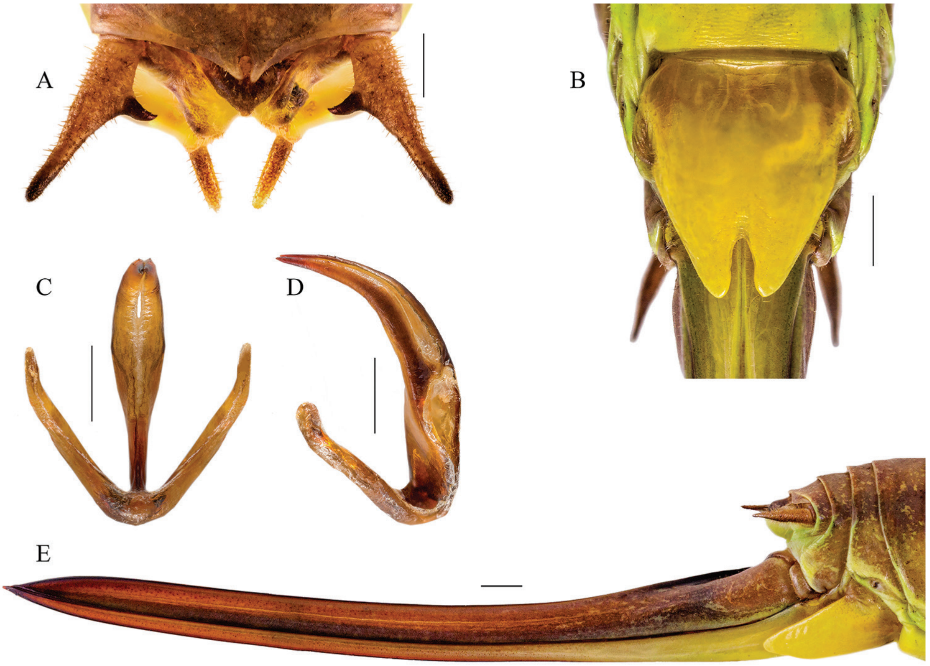

Figure 6. The eidonomy of *Parapholidoptera castaneoviridis* specimens from south-eastern Romania: **A** male cerci **B** female subgenital plate **C, D** titillator **E** ovipositor. Scale bar 1 mm.

Table 2. Morphological measurements in *Parapholidoptera castaneoviridis* from south-eastern Romania (n = 3♂♂ 4♀♀), compared with Turkey and Greece (data from Çiplak 2000). All values are given in millimetres.

		Vertex	Scapus	Pronotum	Body	Hind femur	Ovipositor
Romania	♂	2–2.3 (mean 2.16±0.15)	0.9–1 (mean 1±0.1)	7.7–8.7 (mean 8.2±0.5)	23–25 (mean 24±1)	23–24 (mean 24±1)	–
	♀	2–2.3 (mean 2.2±0.1)	0.9–1 (mean 1±0.1)	8.4–8.8 (mean 8.6±0.2)	22–25 (mean 23±1)	26.5–27.5 (mean 27.1±0.42)	21–22 (mean 22±1)
Turkey, Greece	♂	1.8–2.2	0.9–1	8.4–10.8	19–26	21–24.5	–
	♀	2.2–2.3	0.9–1.1	8.3–10.5	20–27	24–28	19–26

Parapholidoptera castaneoviridis is a micropterous insect with limited dispersal abilities. The newly discovered population from the northern area of Dobrogea most likely persisted as relict from a previously wider distribution when the forest habitat was continuous, rather than being the result of a recent expansion. This hypothesis may be supported by the isolated former findings of this species in northern Bulgaria, while recently it has not been found in this area, regardless of the significant collecting efforts that have been made in the northeastern part of this country. Currently, the species is common in south-eastern Bulgaria (D. Chobanov, pers. obs.; fig. 3), where it can be found both in natural and agricultural areas, mostly tolerating dry warm microclimate in scrub-grass associations or thermophilic sparse forests.

Numerous recent studies targeting the Balkan orthopteran fauna confirmed the need of exploring unknown territories for obtaining a complete picture of the species geographic ranges (eg. Çiplak et al. 2007, Chobanov and Heller 2010, Chobanov et al. 2013, Chobanov 2014, Kaya et al. 2015, Iorgu et al. 2016). The finding of the only Balkan *Parapholidoptera* species in Romania is just another example of how exhaustive field work in a previously poorly studied area could produce unexpected results, providing a better understanding of the currently interrupted distribution area of a formerly wider spread species.

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Supplementary material 1

Occurrence records of *Parapholidoptera castaneoviridis* in the Balkan Peninsula

Authors: Ionuț Ștefan Iorgu, Dragan Petrov Chobanov, Elena Iulia Iorgu

Data type: occurrence

Explanation note: The occurrence records of *Parapholidoptera castaneoviridis* in the Balkan Peninsula, from literature and collected by the authors.

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http://zookeys.pensoft.net

RESEARCH ARTICLE

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A new highly cave-adapted trechine genus and species from northern Guizhou Province, China (Coleoptera, Carabidae, Trechinae)

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Abstract

A remarkable aphaenopsian beetle, a sympatric species of *Qianotrechus tenuicollis* Uéno, 2000, was newly discovered in Cave Mahuang Dong of Shuanghe Dong cave system, the longest cave system of China in Suiyang County, northern Guizhou Province. To categorize this striking but still unknown species, a new genus and species are proposed: *Shuangheaphaenops elegans* **gen. n.**, **sp. n.** Relationships of *Shuangheaphaenops* and other highly modified aphaenopsian genera from southern China Karsts are discussed.

Keywords

aphaenopsian, ground beetle, hypogean, southern China Karsts

Introduction

Guizhou Province and Guangxi Zhuang Autonomous Region of southern China hold the largest karstic landscapes in the world (Waltham 2009), and the richest cave beetle fauna, too, at least at generic level. Of 120 hypogean trechine species belonging to 48 genera known in China (Deuve and Tian 2016; Fang et al. 2016; Tian et al. 2016; Zhao and Tian 2016), 72 species in 23 genera are reported from Guizhou and Guangxi karsts. Furthermore, all highly modified aphaenopsian genera are distributed only in southern Guizhou and northern Guangxi, such as *Sinaphaenops* Uéno & Wang, 1991, *Dongodytes* Deuve, 1993, *Giraffaphaenops* Deuve, 1992, *Pilosaphaenops* Deuve & Tian, 2008 and *Uenotrechus* Deuve & Tian, 1999 (Deuve et al. 1999).

Members of *Sinaphaenops* are endemic to southern parts of Guizhou, with only an exception for *S. wangorum* Uéno & Ran, 1998 which is recorded from both Libo (southernmost Guizhou) and Huanjiang (northernmost Guangxi) counties (Uéno and Wang 1991; Uéno and Ran 1993; Uéno 2002; Tian et al. 2010; Deuve and Tian 2014; Tian and Huang 2015). All species of both *Pilosaphaenops* and *Uenotrechus*, together with three species of *Sinaphaenops*, are occurring in a narrow area between southernmost Guizhou and northernmost Guangxi, viz. Maolan, Mulun and adjacent karstic areas, where the largest and most primitive karstic forest in southern China is maintained (Deuve et al. 1999; Deuve and Tian 2008; Uéno 2009; Tian 2010, 2011). The genera *Dongodytes* and *Giraffaphaenops* are endemic to northern Guangxi, but not sympatric with above three genera (Deuve 1993, 2002; Uéno 1998, 2005; Tian and Luo 2015).

In October, 2016, a cave biodiversity survey was carried out by our team in several counties of Zunyi and Tongren Districts, northern and northeastern Guizhou, in order to collect species of the genera *Qianaphaenops* Uéno, 2000 and *Qianotrechus* Uéno, 2000. The result was very satisfactory, leading to increase the number of almost all of the Uéno's species recorded from these areas. In addition, an unexpected single male beetle and an elytral debris of the same species were collected in a limestone cave belonging to Shuanghe Dong cave system, Suiyang County. This extremely troglobiomorphic aphaenopsian beetle looks like a *Uenotrechus* species at first sight on its fore body (head and thorax), but the hind part (elytra) is more likely similar to a *Dongodytes* (*s. str.*) species. It has three pairs of supraorbital setiferous setae on head and lacks lateromarginal setae on pronotum. It is also different from *Sinaphaenops* Uéno & Wang although its first and second male protarsomeres are dilated and spurred inward apically, a sexual modification appeared also in some *Sinaphaenops* species. Far more important, its elytral chaetotaxy is not similar to any other aphaenopsian genera mentioned above. Therefore, this interesting beetle represents a peculiar lineage within Chinese hypogean trechines. Here we describe this remarkable species, the first highly cave-adapted aphaenopsian species from northern Guizhou Province.

As the longest cave system in China, Shuanghe Dong is connected by 42 caves or entrances, with total length coming up to 186.33 km (He et al. 2016). But the major part of this cave system is still not investigated regarding cave biodiversity. Hence, it is expected that more hypogean trechine beetles would be discovered from this cave system in future.

Materials and methods

The single blind beetle and the elytral debris for this study were collected by the naked eye using an aspirator inside the cave Mahuang Dong, and kept in 50% ethanol before study. Other cave beetles used for comparing were dry and mounted specimens of the insect collection of South China Agricultural University, Guangzhou, China (SCAU).

Dissections and observations were made under a Leica S8AP0 microscope. Dissected genital pieces, including the median lobe and parameres of the aedeagus, were glued onto small transparent plastic plates and pinned under the specimen. Habitus pictures were taken by means of a Keyence VHX-5000 digital microscope. Genital pictures were taken using a Canon EOS 40D camera connected to a Zeiss AX10 microscope, and then stacked and processed using Adobe Photoshop CS5 software. Distribution maps were drawn using Mapinfo software.

The length of the body was measured from the apex of the right mandible (in open position) or from labrum to the elytral apex; the width of the body was taken as the maximum width of the elytra.

Abbreviations of other measurements used in the text are as follows:

HLm	length of head including mandibles, from apex of right mandible to neck constriction
HLl	length of head excluding mandibles, from front of labrum to occipital suture
HW	maximum width of head
PrL	length of prothorax, along the median line
PnL	length of pronotum, as long as PrL
PrW	maximum width of prothorax
PnW	maximum width of pronotum
PfW	width of pronotum at front
PbW	width of pronotum at base
EL	length of elytra, from base of scutellum to elytral apex
EW	maximum width of combined elytra

Taxonomic treatment

Genus *Shuangheaphaenops* gen. n.

http://zoobank.org/0E7CBC8A-C92E-426F-A331-93156EE2B0A8

Type species. *Shuangheaphaenops elegans* sp. n.

Diagnosis. Large sized blind beetles, fore body evidently elongated and as long as elytra, shape intermediate between *Uenotrechus* and *Dongodytes* species, presence of three pairs of supraorbital setae on head, two dorsal and preapical pores on elytra, the first and second protarsomeres in male distinctly modified.

Generic characteristics. Highly modified aphaenopsian trechines, fore part (head and thorax) of the body somewhat similar to *Uenotrechus* Deuve & Tian, 1999, while hind part (elytra) to *Dongodytes* Deuve, 1993; large sized, with body and appendages thin and very elongate, fore body almost as long as hind part; three pairs of supraorbital setiferous pores present on head, with the posterior two pairs very close to each other; mandibles thin and elongated, feebly curved apically, longer than width of head, right mandible edentate though two vanished teeth can be faintly traced; labial suture moderately defined, separating of mentum and submentum, with the former bisetose and the latter 6-setose; mental tooth simple and thin, basal foveae quite narrow; antennae very long, the 10th and 11th antennomeres extending over apical margin of elytra. Prothorax dolioform, propleura distinctly tumid at basal half, evidently visible from above; pronotum barrel-shaped, distinctly elongated, longer than head excluding mandibles, narrower than head; without lateromarginal setae. Elytra similar to those of *Dongodytes* (*s. str.*) Deuve, 1993, narrowed anteriorly and dilated posteriorly, side margins narrowly bordered throughout, shoulders lacking; striae lacking though easily traceable; presence of two dorsal and preapical setiferous pores; the 1st pore in the humeral group of the marginal umbilicate series not transversely and backwardly shifted, the 5th and 6th pores in the middle group widely spaced. Protibia smooth, without longitudinal sulcus; the 1st and 2nd protarsomeres in male dilated and inwardly spurred at apices. Abdominal ventrites sparsely pubescent, each of ventrites IV-VII in male bisetose apically. Male genitalia moderately sclerotized, small, strongly curved ventrally in lateral view, with a quite large sagittal aileron; apical lobe very thin in dorsal view; parameres well developed, but much shorter than median lobe.

Discussion. *Shuangheaphaenops* can not be included in any lineage of the highly modified aphaenopsian genera known in southern China regarding to the peculiar morphological characteristics mentioned above, such as the peculiar facies and configuration of the body (which is more or less similar to *Uenotrechus* Deuve & Tian, 1999 in fore body, but to *Dongodytes* Deuve, 1993 in elytra), vanished bidentate right mandible, and chaetotaxal patterns in which there are three pairs of supraorbital setiferous pores on head, lack of lateromarginal setae on pronotum, and unique pattern on elytral marginal umbilicate series, in particular, the humeral and middle groups.

Apart from the similarity in elytra and antennae between *Shuangheaphaenops* and *Dongodytes* (*s. str.*) which occurs only in northern Guangxi where is far distant from Cave Shuanghe Dong in northern Guizhou, the following characteristics are different: (1) head subparallel-sided, with three pairs of supraorbital setiferous pores, right mandibular teeth bidentate but almost vanished in *Shuangheaphaenops* (versus triangular shaped in general, presence of two pairs of supraorbital pores, and well-marked tridentate teeth on right mandible in *Dongodytes*); (2) the 1st and 2nd protarsomeres of male distinctly modified in *Shuangheaphaenops* (indistinctly or not modified in *Dongodytes*); (3) pronotum much slender and lack of lateromarginal setae in *Shuangheaphaenops* (versus stouter and presence of lateromarginal setae in *Dongodytes*); and (4) the middle group (the 5th and 6th pores) of the marginal unbilicate series on elytra widely spaced each other in *Shuangheaphaenops* (versus close to each other in *Dongodytes*).

The fore body of this new genus is more or less similar to that of *Uenotrechus* Deuve & Tian, 1999, but *Shuangheaphaenops* has a slenderer head bearing three pairs of supraorbital setiferous pores, reduced bidentate teeth of right mandible, and much longer antennae (versus bearing two pairs of supraorbital setiferous pores, mandibular teeth well-defined and clearly tridentate, and shorter antennae in *Uenotrechus*), and pronotum without lateromarginal setae (versus with pair of lateromarginal setae in *Uenotrechus*). In addition, head and elytra are glabrous in *Shuangheaphaenops* (versus whole body densely pubescent in *Uenotrechus*), the 1st pore of the marginal umbilicate series is located before the 2nd in *Shuangheaphaenops* (versus transversely shifted inwards and backwards, at level behind the 2nd pore in *Uenotrechus*), and both the 1st and 2nd protarsomeres in male are modified in *Shuangheaphaenops* (versus not modified in *Uenotrechus*).

Etymology. "Shuanghe + Aphaenops". To indicate that the highly modified trechine genus occurs in Shuanghe Dong, the longest cave system in China.

Generic range. Guizhou (Suiyang) (Fig. 1).

Shuangheaphaenops elegans sp. n.

http://zoobank.org/392C6535-5261-47FF-A2F7-D7123A5D9DFA
Figs 1–6

Holotype. Male, Cave Mahuang Dong, Shuanghe Dong cave system, Wenquan Zhen, Suiyang County, 28°14'32"N, 107°17'24"E, 720 m, X-18-2016, leg. Wenbo Li, deposited in the insect collection of South China Agricultural University, Guangzhou, China (SCAU); additional material: an elytral debris, same cave and collecting date as above, leg. Mingruo Tang, in SCAU.

Diagnosis. A large-sized, eyeless cave trechine beetle, highly modified in morphology, with very elongated and slender body which is about four times longer than wide, fore body about as long as elytra, antennae as long as body including mandibles, extending beyond elytral apex; body glabrous, except for basal half of pronotum which is covered with erected setae.

Description. Length: 7.9 mm (from apex of right mandibles to elytral apex) or 7.2 mm (from labrum to elytra); width: 1.79 mm. Fore body (including mandibles) longer than elytra, (HLm+PrL)/EL = 1.07. Habitus as in Fig. 2.

Yellowish brown, a little darker on head, pronotum and basal half of elytra, pale on antennae, mouthparts, palps and tarsi. Moderately shiny. Head and elytra glabrous, pronotum glabrous on apical half (but with two or three short setae near middle of frontal margin) but pubescent on basal half. Underside of head and prothorax glabrous (Fig. 3b), abdominal ventrites sparsely pubescent. Microsculptural engraved meshes more or less isodiametric on head and elytra, but transverse striate on pronotum.

Head (Fig. 3a) elongate quadrate, much longer than wide (HLm/HW = 2.74, HLl/HW = 1.87); genae fairly developed, hardly dilated laterally, suddenly constricted posteriorly before occipital suture, making a well-marked but short neck constriction; subparallel-sided, widest at about middle from labrum to base of head; frons and vertex moderately convex, frontal furrows deep and well-marked, subparallel-sided, ended just behind the level of anterior supraorbital pores; clypeus transverse, 4-setose; labrum transverse, with frontal margin slightly protruding medially, 6-setose; anterior supraorbital setiferous pores located at about middle from frontal margin of labrum to base of head, while the posterior two pairs (which are very close to each other) at about 1/4 of head from base; palps long, slender and glabrous except for the 2nd labial palpomere

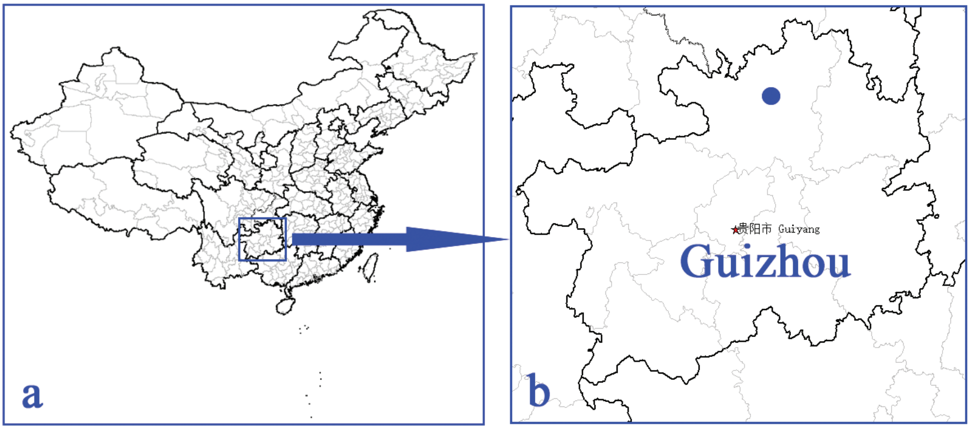

Figure 1. Distribution of *Shuangheaphaenops elegans* gen. n., sp. n. **a** map of China showing the location of Guizhou Province **b** map of Guizhou Province, the location of Shuanghe Dong cave system shown by blue point.

which is bisetose on inner margin; the 2nd labial palpomere 1.35 times longer than the 3rd; the 3rd maxillary palpomere 1.25 times longer than the 4th; suborbital pores at about middle from base to labial suture. Antennae thin and long, the 1st antennomere stouter than other, and the shortest, the 3rd the longest; the comparative length ratio of each antennomeres as: the 1st (8.5), 2nd (11.0), 3rd (18.0), 4th (17.0), 5th (17.0), 6th (16.5), 7th (13.5), 8th (11.0), 9th (11.0), 10th (10.0) and 11th (10.5).

Prothorax shorter than head including mandibles (PrL/HLm = 0.80), but longer than head excluding mandibles (PrL/HLl = 1.17), widest at about 1/4 from base, twice as long as wide (PrL/PrW = 2.10), slightly wider than head (PrW/HW = 1.09), evidently wider than pronotum (PrW/PnW = 1.22), half as wide as elytra (PrW/EW = 0.49). Pronotum elongate, dolioform, two and half times longer than wide (PnL/PnW = 2.46), evidently narrower than head (PnW/HW = 0.89), base slightly wider than front (PbW/PfW = 1.09); lateral sides finely bordered throughout, base and front unbordered; nearly parallel-sided, fairly expanded at the widest part which is at about 3/7 from base, slightly sinuate before hind angles which are nearly rectangular, fore angle obtuse; median line well-marked, basal transversal impression very short; front slightly convex, base feebly concave. Scutellum fairly small.

Elytra (Fig. 4) elongate ovate, much longer than pronotum (EL/PnL = 2.12), about twice as long as wide (EL/EW = 2.08); distinctly dilated posteriorly, widest at about apical 3/7 of elytra, lateral sides smooth, not ciliate, finely bordered throughout; distinctly convex; striae easily traceable though devoid, intervals moderately convex. Chaetotaxy: basal pore at subequal distant from scutellum to marginal gutter; two dorsal setiferous pores present on the 3rd stria at about 1/3 and 2/3 from base respectively; the preapical pore closer to suture than to apical margin; the marginal umbilicate pores not aggregated, the 8th pore near marginal gutter, the 4th and 5th pores distant from the gutter, others intermediary located; the humeral groups separately spaced, the 1st and

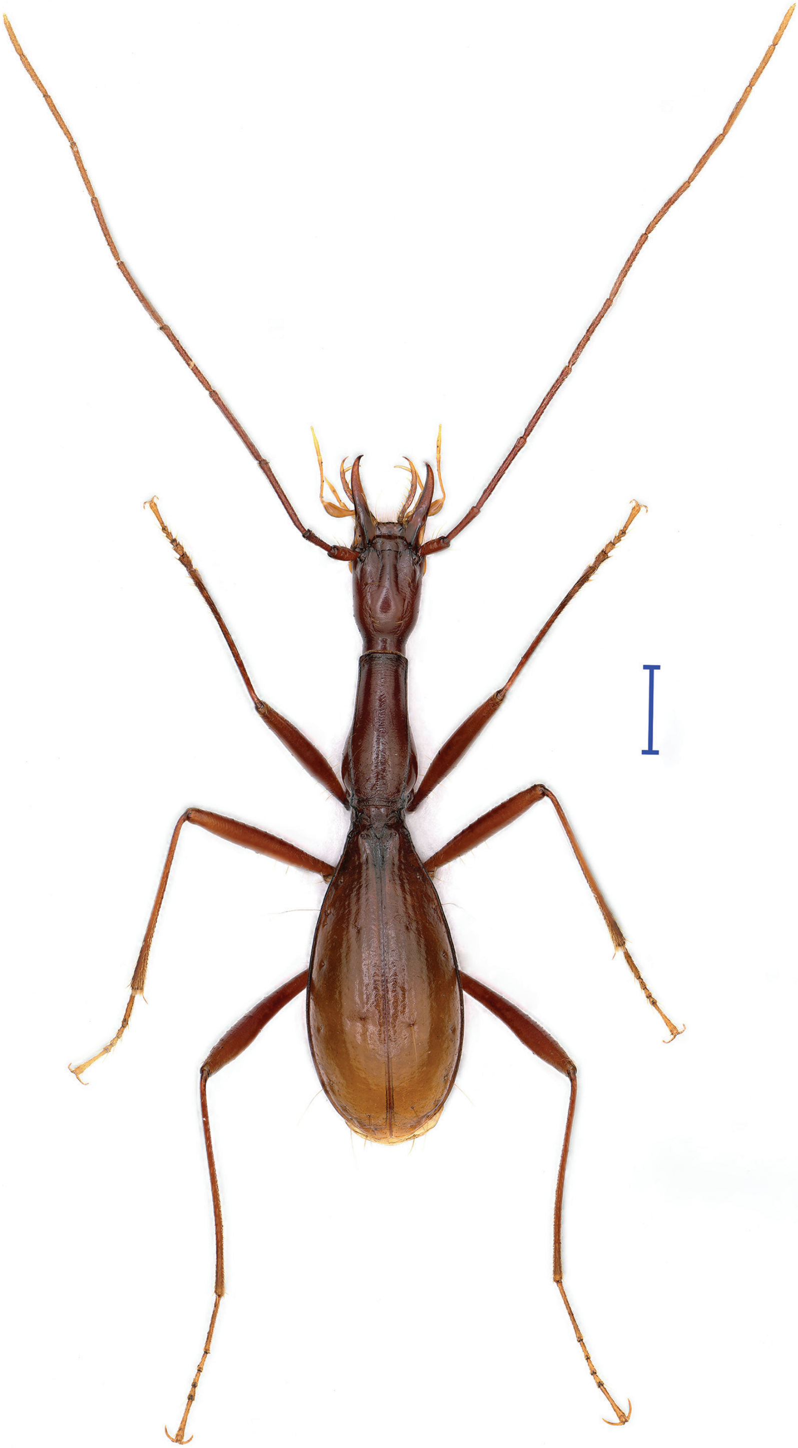

Figure 2. Habitus of *Shuangheaphaenops elegans* gen. n., sp. n., holotype, male. Scale bar 1 mm.

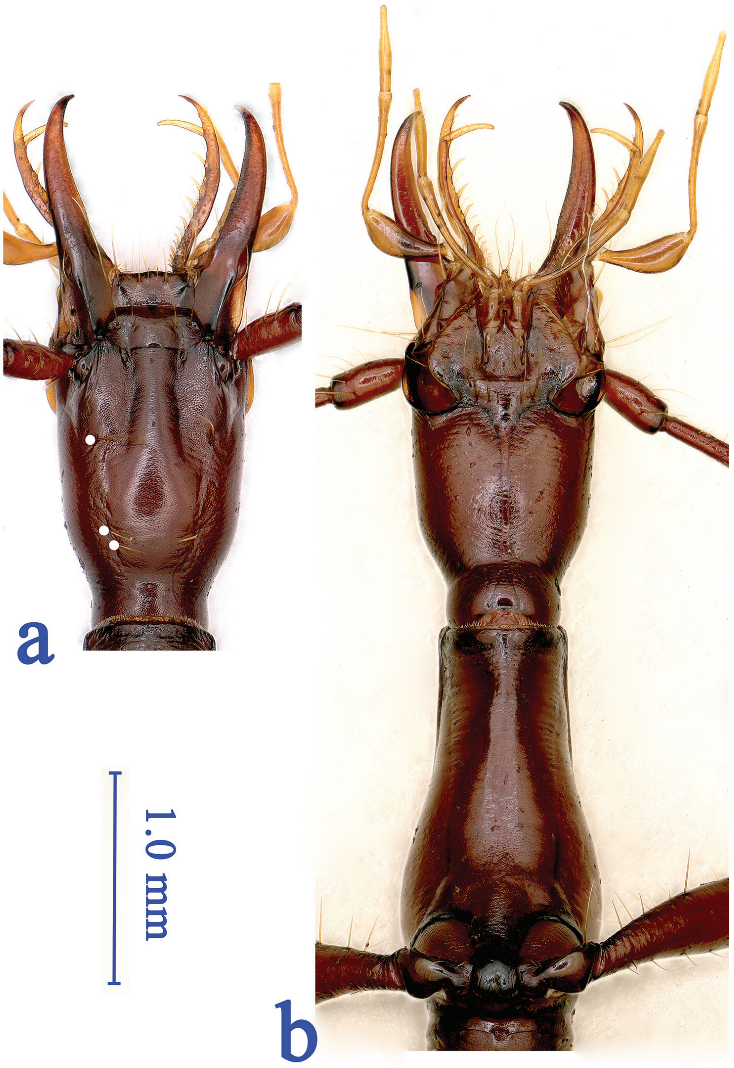

Figure 3. Head and prothorax of *Shuangheaphaenops elegans* gen. n., sp. n. **a** head, dorsal view, supraorbital setiferous pores on the left side shown by white points **b** head and prothorax, ventral view.

4th pores distant from the 2nd and 3rd pores respectively which are close to each other, distance from the 1st pore to the 3rd slightly shorter than that from the 2nd to 4th; the middle group widely spaced, making distance of the 5th pore and 4th subequal to that of the 5th and 6th; apical group composed of three pores.

Legs thin and long, bearing short pubescence; fore- and middle femora sparsely setose; fore tibia smooth, without longitudinal furrow or sulcus; the 1st tarsomere shorter than, as long as, and longer than the 2nd–4th tarsomeres together in fore, middle, and hind legs, respectively.

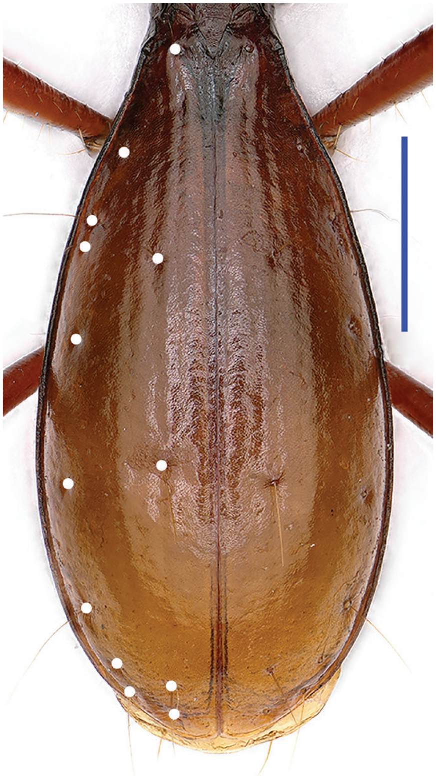

Figure 4. Elytra of *Shuangheaphaenops elegans* gen. n., sp. n., holotype, male, chaetotaxal pattern shown by white points. Scale bar 1 mm.

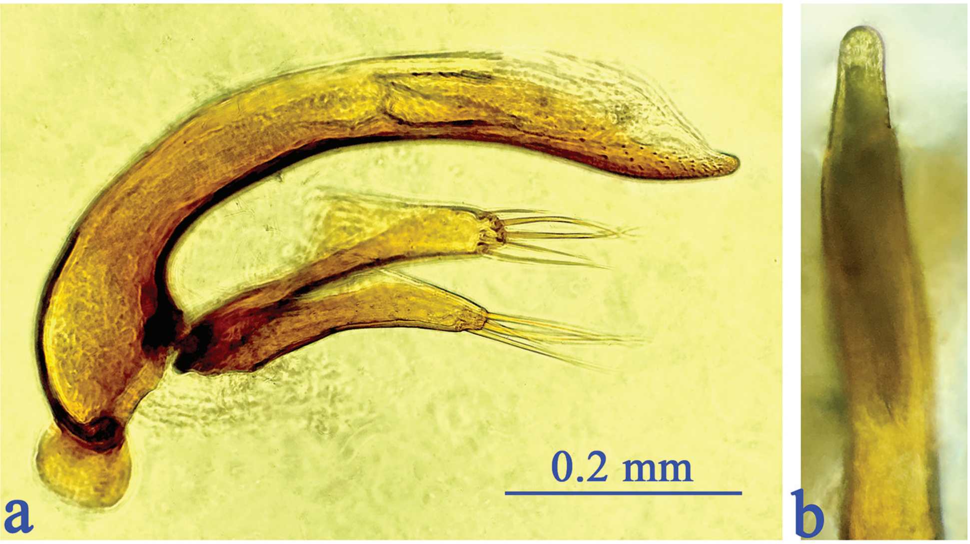

Figure 5. Male genitalia of *Shuangheaphaenops elegans* gen. n., sp. n. **a** median lobe and parameres, lateral view **b** apical lobe, dorsal view.

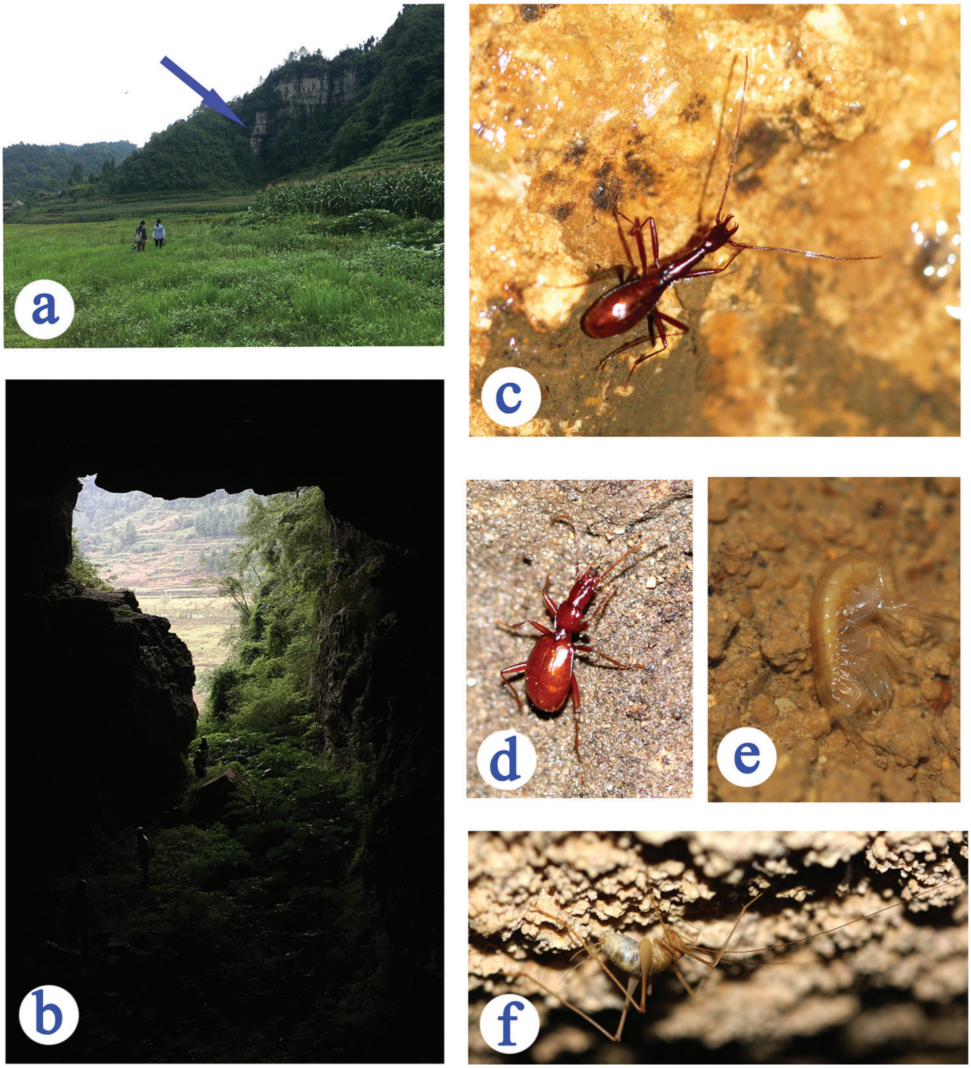

Figure 6. Cave Mahuang Dong, the type locality of *Shuangheaphaenops elegans* gen. n., sp. n., and some sympatric cave animals **a** opening, showed by arrowhead **b** entrance **c** *Shuangheaphaenops elegans* **d** a *Qianotrechus tenuicollis* Uéno, 1998 **e** amphipod **f** cave cricket.

Male genitalia (Fig. 5): Aedeagus moderately sclerotized, quite small and short; distinctly curved at about basal 1/3 in lateral view, feebly curved toward subapex, then broadly ended at apex; inner sac armed with a fairly large copulatory piece which is about 1/4 as long as the median lobe; base moderately sized, opened ventrally; in dorsal view the apical part of aedeagus thin, slightly sinuate from middle to apex, apical lobe narrow, much longer than wide, gradually constricted towards the rounded apex. Parameres short and quite stout, right and left parameres bearing five and four long apical setae respectively.

Etymology. To indicate the slender shape of this beautiful aphaenopsian beetle.

Distribution. China (Guizhou: Suiyang) (Fig. 1). Known only from Cave Mahuang Dong, the type locality.

Mahuang Dong (Figs 6a, b) is one of the most important caves in Shuanghe Dong cave system (Li et al. 2008). It is opened along the main road of the Shuanghe Dong National Geopark on north, and is about 1.2 km long. The beautiful beetle, together with the elytral debris, were collected in a small chamber of the labyrinthic part at about 100 m from the entrance. Other cave animals found also in Mahuang Dong were the semi-aphaenopsian beetle *Qianotrechus tenuicollis* Ueno, 1998, an amphipod, a cave cricket (Figs 6c-f), a pseudoscorpion and two millipedes.

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The Amphibians of Mount Oku, Cameroon: an updated species inventory and conservation review

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Abstract

Amphibians are a disproportionately threatened group of vertebrates, the status of which in Sub-Saharan Africa is still uncertain, with heterogeneous fauna punctuated by mountains. Mount Oku, Cameroon is one such mountain, which holds many endemic and restricted-range species. The history of amphibian research on Mt Oku, current knowledge on biogeography and conservation biology is reviewed, including recent findings. This updated inventory adds 25 further species, with 50 species of amphibian so far recorded to the Oku Massif (c. 900 to 3,011 m). This includes 5 endemic to Mt Oku, 7 endemic to the Bamenda Highlands, 18 restricted to the highlands of Cameroon and Nigeria, and 20 with broader ranges across Africa. This includes a new mountain locality for the Critically Endangered *Leptodactylodon axillaris*. Among others, the first record of *Phrynobatrachus schioetzi* and *Ptychadena taenioscelis* from Cameroon are presented. The uncertainty of habitat affinities and elevational ranges are discussed. The proportion of threatened species on Mt Oku is 44.2%, but projected to increase to 47.9% due to new species descriptions and recent dramatic declines. The natural habitats of Mt Oku are irreplaceable refuges for its endemic and restricted-range amphibian populations under severe pressure elsewhere in their range. Threats to this important amphibian fauna are increasing, including agricultural encroachment, expanding aquaculture, livestock grazing, pollution, invasive species, forest loss and degradation. Past, present and desired conservation interventions to address these threats are discussed.

Keywords
Biodiversity, caecilians, Cameroon Volcanic Line, Central Africa, frogs, Lake Oku, montane forests and grasslands

Introduction

Amphibians are among the most threatened animal group worldwide, with threats ranging from habitat loss, emerging infectious disease, climate change, overexploitation (for food and/or pet trade), invasive alien species and pollution (Stuart et al. 2004; Beebee and Griffiths 2005). Knowledge on the distribution and status of Africa's amphibians and threats is patchy and generally lags behind the rest of the world (Lawson and Klemens 2001; Schiesari et al. 2007; Gardner et al. 2007; Brito 2008). Cameroon has been comparatively well explored for amphibians in the 20th Century (Amiet 2008). The heterogeneous topography of Cameroon however makes detailing biodiversity distribution difficult.

The very first sampling of amphibians from the Bamenda region was conducted by Lt. Adametz in the early 20th Century (Nieden 1910). However, Mt Oku was apparently not systematically surveyed for amphibians until the 1960s (Amiet 1971). Prior to this study, some amphibians from the general Bamenda Highlands (sometimes referred to as the Bamenda-Banso Highlands) were also sampled by Perret (1966), including the description of *Leptopelis nordequatorialis* Perret, 1966, a species widespread in the highlands of Cameroon and Nigeria. Work was to be followed by Amiet in the 1970s to 1980s, which included the descriptions of many of the restricted range montane species found on Oku. This was followed by the International Council for Bird Preservation (UK) expedition in 1984 (Stuart 1986), which made a preliminary conservation assessment of the herpetofauna of Oku and other mountains in Cameroon (Gartshore 1986). Other herpetologists have subsequently made contributions: an updated inventory was published by Wild (1994); for caecilians (Scolecomorphidae: *Crotaphatrema*) (Nussbaum 1981; Doherty-Bone et al 2011a); Clawed Frogs (Pipidae; *Xenopus*) (Loumont and Kobel 1991; Blackburn et al. 2010a; Evans et al. 2015); Wolterstorff toads (Bufonidae; *Wolterstorffina*) (Perret 1972; Boistel and Amiet 2001); Squeaker Frogs and kin (Arthroleptidae; *Arthroleptis*, *Cardioglossa*) (Blackburn 2008a, b; Blackburn et al. 2010b); Puddle Frogs (Phrynobatrachidae; *Phrynobatrachus*) (Zimkus 2009; Zimkus and Gvoždík 2013).

Here the current knowledge of the amphibians of the Oku Massif is summarised, including results of more recent, mostly unpublished fieldwork by the present authors since 2005. This includes an appraisal of habitats, biogeography, endemic species and their origins. Habitat affinities are reviewed, and the issues affecting their conservation in the near future, including past, present and future interventions to prevent extinctions. Conservation statuses are also proposed for those species newly described. This review and update is expected to act as a primer for more dedicated research and conservation practise on this and other mountains in Cameroon.

Methods

Description of study area

Mount Oku does not form a clear mountain as it occurs on the Bamenda Plateau. Mt Oku is here defined by the boundary of its lower localities within the Oku Massif, such as Big Babanki (1200–1300 m), Bamo Forest west of Big Babanki (900 m), Babungo (1770 m), Ibal (1380 m), Belo (1530 m) and the Mbi Crater (2010 m) (Fig. 1). The term "Mt Oku" used throughout this paper does not exclusively address land controlled by the Oku community, but land also controlled by other communities, such as the Banso, Mbessa, Kedjom-Keku, Fulani and Kom, the latter for example primarily controlling the Ijim Ridge and forest. TMD-B and VG have sampled Elemighong, Anyafouma Forest, Abuh village, Abuh Forest, "Mount Ijim", Emfveh-mi Forest, Elak-Oku, Kissotin, the Kedzem-Mawes Forest, Afua Swamp, the Mt Oku summit grasslands, Lake Oku and surrounding forest (the Kilum-Ijim Plantlife Sanctuary), Mbi Crater, Bambui, Mendong Buo, Kedjom-Keku village, Mejung village, Bamo Forest (Fig. 1).

Habitats on Mt Oku consist of (as summarised by Forboseh et al. 2003): montane forest; high altitude *Podocarpus* forest; woodland with *Gnidia glauca* and *Maesa lanceolata*; mature bamboo forest; scrubland dominated by *Erica manni*; montane grassland; and subsistence agriculture. Land use in subsistence farms consists of sun crops predominately maize grown with beans, pumpkins and shade crops of coffee, banana, kola nut, avocado, sour plum and other trees. Most farms rear poultry, goats, sheep, pigs and fish (the latter two sometimes combined with pig sheds above fish ponds). Grasslands are often used for grazing of livestock (cattle, sheep, goats).

Sampling

Amphibian species records from the localities mentioned above were listed from published data and unpublished field studies by the authors. The latter field survey data is presented during the following dates: November to December 2005; June to September 2006; September to December 2008; April to June 2009; November 2009; May 2010; September 2010; May to September 2012; October 2013; December 2014; April 2015. Survey methods during all field seasons typically involved visual and acoustic encounter surveys (searches with surveyor effort recorded) or opportunistic searches (or incidental observations) of habitats during both daylight and night time. Other techniques included pitfall bucket traps (2006 and 2012), dip-netting and aquatic funnel traps in ponds, streams and lakes. Local people also presented animals on an *ad hoc* basis. In addition, TMD-B has recruited and trained local technicians to record observations throughout the year at specific localities (notably Lake Oku) since 2006. In addition to the literature, we re-examined specimens from Mt Oku deposited in the Natural History Museum, London (Collection IDs: *Asytlosternus* – BM1984.356-357; *Phrynobatrachus* – BM1984.445-472; *Xenopus* – BM1984.194-236). Records include tadpoles as well as post-metamorphic individuals.

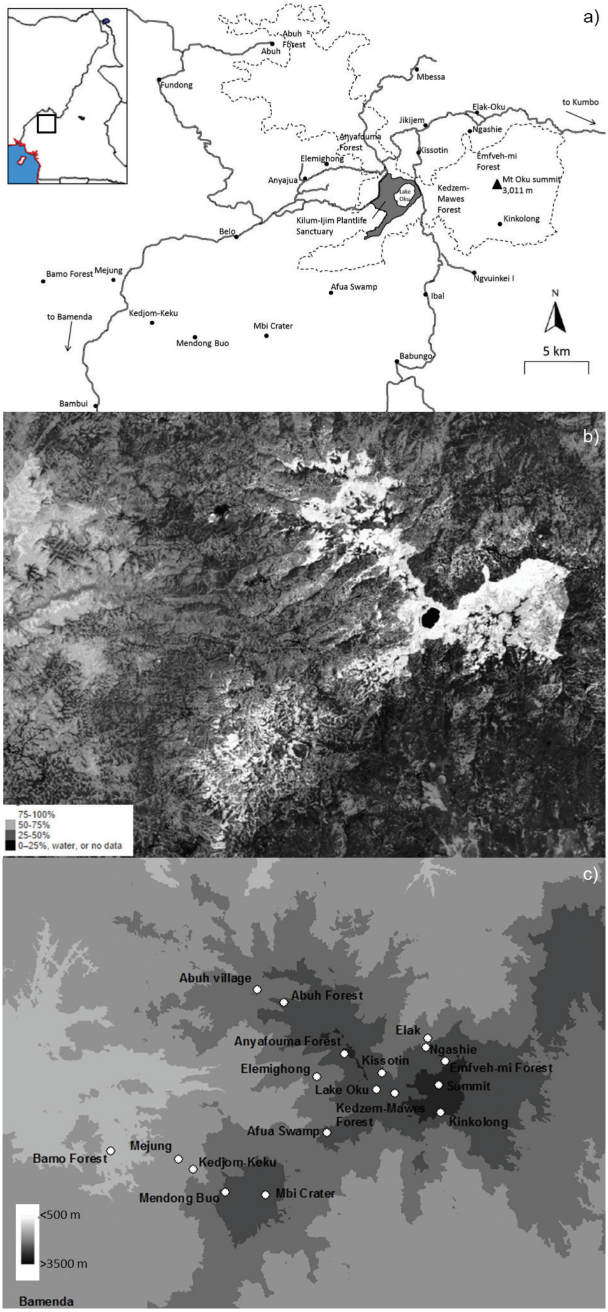

Figure 1. Maps: **a** Main localities on Mt. Oku and surrounding areas (= Oku Massif), including those sampled for amphibians. Dashed line indicates the boundary of the Kilum-Ijim Forest. The grey shaded area shows the Kilum-Ijim Plantlife Sanctuary government protected area. Road (solid lines) bisecting the Kilum-Ijim forest (dashed line) and larger urban centres (Belo, Fundong, Ibal) are also shown **b** forest cover indicated by white shading, from Hansen et al . (2013) (downloaded from https://earthenginepartners.appspot.com/science-2013-global-forest) **c** topographic map showing elevation.

Threats to the conservation of various species were characterised from IUCN assessments, and from updated observations by the authors, such as incidents of new invasive alien species when encountered or changes in land use. The proportion of threatened species for Mt Oku was calculated following Böhm et al. (2013) by dividing the total species classified as CR, EN, VU by the total number of species already assessed by the IUCN, so excluding DD and Not Evaluated species. For those species that have recently been described, conservation status was proposed based on IUCN criteria (IUCN Standards and Petitions Subcommittee 2016) and the proportion of species threatened on Mt Oku calculated as above.

Results

The updated species inventory for Mt Oku found 50 species, of which five are endemic to Mt Oku (pictured in Figure 2), seven endemic to the Bamenda Highlands, 18 restricted to the highlands of Cameroon and Nigeria, 12 with broader ranges across West and Central Africa and eight with Pan-African distributions (Table 1). Contemporary fieldwork added 25 species to the list obtained from published sources, in addition to the proposed new *Phrynobatrachus* discussed below (see Supplemental Table 1 for museum accession numbers). This includes Schiøtz's Puddle Frog, *Phrynobatrachus schioetzi* Blackburn & Rödel, 2011, representing the first record of this species to Cameroon. This species was described from collections made on the Obudu Plateau, Nigeria from the 1950s and mid 2000s as a possible Nigerian endemic. Cameroonian specimens of this species had been collected on Oku by TMD-B, originally thought to be *P. werneri* until its description as a valid species in 2011 (Blackburn and Rödel 2011). Further specimens have been observed in Mendong Buo in an open grassland area close to a small forest patch at 2,200 m in 2005, two in Abuh Forest and Elemighong in 2006, two in the Kilum-Ijim Plantlife Sanctuary in 2008, one at the same locality in 2009, two from Afua Swamp in 2009 and one at the Mbi Crater in June 2012. As it has only recently been described, it has yet to be assessed for conservation status by the IUCN. Considering only scarce findings of this species over the years of surveys, but evidently having a wider range as the Obudu and Oku are ca 120 km far from each other, it is likely this species will be listed as Endangered A2(a), B1(b),B2(b): its Estimate of Occurrence (EOO)=1,468 km^2 and Area of Occupany (AOO)=32km^2. The identification of *Ptychadena taenioscelis* Laurent, 1954 (specimen accession #: BM2008.476, 16S barcode corresponding to DQ525943, Kenya, Measey et al., 2007) from Afua Swamp is also a new, extreme north-westerly record for this species. Other species new to the published list for Mt Oku include: *Arthroleptis* cf. *perreti* Blackburn, Gonwouo, Ernst and Rödel, 2009 (previously referred to as *A. variabilis* Matschie, 1893 in Doherty-Bone et al. 2008), *Cardioglossa leucomystax* (Boulenger 1903), *Leptopelis modestus* (Werner, 1898), *L. notatus* (Peters, 1875), *Astylosternus* cf. *diadematus* Werner, 1898, *A. montanus* Amiet, 1977, *Leptodactylodon axillaris* Amiet, 1971, *Leptodactylodon bicolor* Amiet, 1971, *Trichobatrachus robustus* Boulenger, 1900, *Sclerophrys*

Figure 2. Endemic amphibians of Mt Oku. Clockwise from top-left: Lake Oku Clawed Frog, *Xenopus longipes*; Mount Oku Subalpine Toad, *Wolterstorffina chirioi*; Mount Oku caecilian *Crotaphatrema lamottei*; Lake Oku Puddle Frog, *Phrynobatrachus njiomock* found almost exclusively in forest around Lake Oku; Puddle Frog *Phrynobatrachus* sp. aff. *werneri*; Spiny Puddle Frog, *Phrynobatrachus chukuchuku*.

latifrons (Boulenger, 1900), *S. maculatus* (Hallowell, 1855), *S. regularis* (Reuss, 1833), *S. superciliaris* (Boulenger, 1888), *S. villiersi* (Angel, 1940), *Hyperolius ademetzi* Ahl, 1931, *H. igbettensis* Schiotz, 1963, *H. nitidulus* Peters, 1875, *Petropedetes* sp. aff. *parkeri*, *Phrynobatrachus calcaratus* (Peters, 1863), *P. cricogaster* Perret, 1957, *P. natalensis* (Smith, 1849), *Ptychadena* cf. *mascareniensis* “D” (OTU 6; sensu Zimkus et al. 2017) and *P.* cf. *oxyrhynchus* (Smith, 1849).

Should one *Phrynobatrachus* species be confirmed to be new to science, this would make this 51 species to the mountain, of which 6 will be endemic. This potentially new, undescribed, endemic puddle frog, *Phrynobatrachus* sp. aff. *werneri* is here presented – this morpho-species is similar to *P. werneri* (Nieden, 1910) and has not been observed on other mountains or in other collections. It is similar to *P. werneri*, but is more gracile like *P. chukuchuku* Zimkus, 2009 and has a more pointed snout (Fig 2). *Phrynobatrachus* sp. aff. *werneri* varies in colouration, with green or yellow bands or a narrow-yellow stripe down the centre of the back. As it has only been observed exclusively in forest with larger trees in Anyafouma Forest and Abuh Forest, it is proposed that it could be threatened by forest loss. It has also never been recorded >500m from the forest edge. It is represented by only one voucher specimen, but has not been observed on Mount Oku since 2009 for further collections to be made to create an appropriate type series to describe it. Following the assessment criteria of the IUCN, the extremely limited range (EOO is negligible, AOO= 8km^2) and sustained decline of this species would qualify it as Critically Endangered A2(a,b) B2(b) (IUCN Standards and Petitions Subcommittee 2016).

The calculated proportion of threatened species (assuming data deficient and not evaluated species will have an equivalent distribution of conservation status to threatened species, Böhm et al. 2013) based on the recorded species (excluding *P.* sp. aff. *werneri*) was 44.2%. Of the 50 species, 19 amphibian species have already been assessed to be threatened with extinction at different intensities: seven Vulnerable; eight Endangered; four Critically Endangered (Table 1). Hypothesised threat assessments to all species predominately include: forest loss (23 out of 50 species); burning and overgrazing of grassland (four species); one case of overexploitation (Hairy Frogs, *Trichobatrachus robustus*); two cases of invasive species (*Xenopus longipes* Loumont & Kobel, 1991 and possibly *Phrynobatrachus njiomock* Zimkus & Gvoždík, 2013 threatened by exotic fish); and two possible cases of climate change being a threat (for the Mt Oku Subalpine Toad, *Wolterstorffina chirioi* Boistel & Amiet, 2001 and Spiny Puddle Frog, *Phrynobatrachus chukuchuku*). Threats that have not been appraised for these species include pollution and susceptibility to disease.

Of the newly described species so far un-assessed by the IUCN, it is proposed *P. njiomock* be given a classification of Critically Endangered (IUCN criteria: A2(a),B2(b)) due to its extremely limited range size (EOO=12 km^2, AOO=20 km^2) within and around Lake Oku. It has also not been observed since 2010 despite monthly monitoring at Lake Oku and the Oku summit. It is also proposed that both *P. jimzimkusi* Zimkus, Gvoždík & Gonwouo, 2013 and *P. steindachneri* Nieden, 1910 be given a classification of Critically Endangered A2(a), A4(a), B1(b), B2(b) due to their limited range sizes and recent, substantial declines (Hirschfeld et al. 2016). *Phrynobatrachus jimzimkusi* has been unobserved on Mt Oku since 2010, *P. steindachneri* since 2009. *Phrynobatrachus werneri*, while observed in agricultural areas, has also become rare, from high sample success rate of several individuals in 2006 and 2009 to no individuals or rare occurrences of singletons at those same localities from 2010 onward (Hirschfeld et al. 2016, TMD-B pers. obs.). It may thus be inferred that this species

Table 1. A working conservation checklist for the amphibians of Mt. Oku.

Taxon	Species authority	Global IUCN status (those inparentheses are inferred status)	Biogeography	Species Elevational Range (m)		Hypothesised threats	Sources – presence on Mt Oku
				Low	Peak		
Amphibians							
Gymnophiona							
Scolecomorphidae							
Crotaphatrema lamottei	Nussbaum, 1981	DD	MtO	2175	2398	FL	Nussbaum 1981; Doherty-Bone et al 2011a; Nyingchia and TMD-B pers. ob. 2012, 2013
Anura							
Arthroleptidae							
Arthroleptis adelphus	Perret, 1966	LC	CWA	900	1788	FL	VG pers. ob. 2005; TMD-B pers. ob. 2006
Arthroleptis palava	Blackburn, Gvoždík, Leaché, 2010	LC	CNMts	1400	2200	–	VG pers. ob. 2005, 2009; Blackburn et al. 2010; TMD-B pers. ob. 2006, 2009, 2012
Arthroleptis sp. aff. *poecilonotus*	–	(LC)	CWA	900	1788	–	Blackburn 2008a; TMD-B pers. ob. 2006, 2012; VG pers. ob. 2005, 2010
Arthroleptis cf. *perreti*	Blackburn, Gonwouo, Ernst, Rödel, 2009	VU	CNMts	1400	2200	–	VG pers. ob. 2005
Cardioglossa leucomystax	(Boulenger, 1903)	LC	CWA	900	1100	FL	VG pers. ob. 2005
Cardioglossa oreas	Amiet, 1972	EN	BamH	1900	2650	FL	Gartshore 1986; VG pers. ob. 2005, 2009; Blackburn 2008b; TMD-B pers. ob. 2006, 2010
Cardioglossa pulchra	Schiotz, 1963	EN	CNMts	900	1800	–	Gartshore 1986; TMD-B pers. ob. 2006; Blackburn 2008b
Cardioglossa schioetzi	Amiet, 1981	EN	CNMts	1640	1800	FL	Blackburn 2006
Leptopelis modestus	(Werner, 1898)	LC	CWA	900	2160	FL	TMD-B pers. ob. 2012
Leptopelis nordequatorialis	Perret, 1966	LC	CNMts	1000	2000	–	Gartshore 1986; TMD-B pers. ob. 2006, 2009, 2012
Leptopelis notatus	(Peters, 1875)	LC	CWA	900	1100	–	VG pers. ob. 2005, 2009
Astylosternus cf. *diadematus*	Werner, 1898	VU	CNMts	900	1350	–	VG pers. ob. 2005, 2010
Astylosternus montanus	Amiet, 1977	NT	CNMts	900	2030	–	TMD-B pers. ob. 2009

Taxon	Species authority	Global IUCN status (those inparentheses are inferred status)	Biogeography	Species Elevational Range (m)		Hypothesised threats	Sources – presence on Mt Oku
				Low	Peak		
Astylosternus ranoides	Amiet, 1977	EN	BamH	2200	2600	FL	Amiet 1977; TMD-B pers. ob. 2006, 2008, 2009, 2010, 2012
Astylosternus rheophilus	Amiet, 1977	VU	CNMts	1350	2500	FL	Amiet 1977; TMD-B pers. ob. 2006, 2008, 2009, 2010, 2012
Leptodactylodon axillaris	Amiet, 1971	CR	BamH	2300	2700	FL	TMD-B pers. ob. 2006
Leptodactylodon bicolor	Amiet, 1971	VU	CNMts	950	1788	FL	TMD-B pers. ob. 2006; Ndifon and TMD-B pers. ob. 2015
Leptodactylodon perreti	Amiet, 1971	EN	BamH	1500	2650	FL	Amiet 1980; TMD-B pers. ob. 2006, 2009, 2012
Trichobatrachus robustus	Boulenger, 1900	LC	CWA	900	1788	OV	TMD-B pers. ob. 2006, 2008; VG, pers. ob. 2010
Bufonidae							
Sclerophrys latifrons	(Boulenger, 1900)	LC	CWA	900	900	–	VG pers. ob. 2005
Sclerophrys maculatus	(Hallowell, 1855)	LC	SSA	900	1788	–	VG pers. ob. 2005; TMD-B pers. ob. 2006, 2008
Sclerophrys regularis	(Reuss, 1833)	LC	SSA	900	2500	–	TMD-B pers. ob. 2006, 2008
Sclerophrys superciliaris	(Boulenger, 1888)	LC	CWA	900	900	FL	VG pers. ob. 2005
Sclerophrys villiersi	(Angel, 1940)	EN	CNMts	1200	2500	FL, SV	VG, pers. ob. 2009
Werneria bambutensis	(Amiet 1972)	EN	BamH	1750	2600	FL, SV	Amiet 1972; VG pers. ob. 2005; TMD-B pers. ob. 2006
Wolterstorffina chirioi	Boistel & Amiet, 2001	CR	MtO	3000	3000	CL, SV	Boistel and Amiet 2001; Nyingchia and TMD-B pers. ob. 2012
Wolterstorffina mirei	(Perret, 1971)	EN	BamH	1800	2800	FL, SV	Boistel and Amiet 2001; Nyingchia and TMD-B pers. ob. 2008, 2009, 2012
Hyperoliidae							
Afrixalus "quadrivittatus"	Pickersgill, 2007	LC	SSA	900	1788	–	TMD-B pers. ob. 2006, 2008, 2012; Amiet 2009
Hyperolius ademetzi	Ahl, 1931	NT	CNMts	900	2220	–	VG pers. ob. 2005
Hyperolius igbettensis	Schiotz, 1963	LC	CWA	900	2010	–	TMD-B pers. ob. 2012
Hyperolius nitidulus	Peters, 1875	LC	CWA	900	2010	–	TMD-B pers. ob. 2012
Hyperolius riggenbachi	(Nieden, 1910)	VU	CNMts	1100	2010	–	Gartshore 1986; VG pers. ob. 2005, 2009, 2010; Khimal and TMD-B pers. ob. 2006, 2008, 2009, 2012

Taxon	Species authority	Global IUCN status (those inparentheses are inferred status)	Biogeography	Species Elevational Range (m)		Hypothesised threats	Sources – presence on Mt Oku
				Low	Peak		
Kassina maculosa	(Sternfeld, 1917)	LC	CWA	900	2600	–	Amiet 2007; VG pers. ob. 2005; TMD-B pers. ob. 2009
Petropedetidae							
Petropedetes sp. aff. *parkeri*		(DD)	CNMts	1090	1300	–-	VG pers. ob. 2005
Phrynobatrachidae							
Phrynobatrachus calcaratus	(Peters, 1863)	LC	CWA	900	1200	FL	VG pers. ob. 2005
Phrynobatrachus chukuchuku	Zimkus, 2009	CR	MtO	2230	2800	SV, CL	Zimkus 2009; TMD-B pers. ob. 2012
Phrynobatrachus cricogaster	Perret, 1957	VU	CNMts	900	1850	FL	VG pers. ob. 2005
Phrynobatrachus jimzimkusi	Zimkus, Gvoždík, Gonwouo, 2013	(CR)	CNMts	*c.*1300	*c.*2800	FL	Gartshore 1986; Zimkus and Gvoždík 2013; VG pers. ob. 2005, 2009; TMD-B pers. ob. 2006, 2008, 2009, 2010
Phrynobatrachus natalensis	(Smith, 1849)	LC	SSA	900	2200	–	TMD-B pers. ob. 2006
Phrynobatrachus njiomock	Zimkus & Gvoždík, 2013	(CR)	MtO	2200	2400	FL, IN	Gartshore 1986; Zimkus and Gvoždík 2013; Amiet 1978; VG pers. ob. 2005; Zimkus 2009; TMD-B pers. ob. 2006–2010
Phrynobatrachus schioetzi	Blackburn & Rödel, 2011	(VU)	CNMts	1500	2200	FL	Hořák and VG pers. ob. 2005; TMD-B pers. ob. 2006, 2008, 2009, 2012
Phrynobatrachus steindachneri	Nieden, 1910	VU (CR)	CNMts	1300	2460	FL	Amiet 1971; Gartshore 1986; Zimkus 2009; Zimkus and Gvoždík 2013; TMD-B pers. ob. 2006, 2009
Phrynobatrachus werneri	(Nieden, 1910)	LC (VU)	CNMts	1200	2200	–	Gartshore 1984 (from BMNH collection, not reported in 1986 paper); TMD-B pers. ob. 2006, 2009, 2012
Phrynobatrachus sp. aff. *werneri*	–	(CR)	MtO	*c.*2000	*c.*2800	FL	TMD-B pers. ob. 2006, 2008, 2009

Taxon	Species authority	Global IUCN status (those inparentheses are inferred status)	Biogeography	Species Elevational Range (m)		Hypothesised threats	Sources – presence on Mt Oku
				Low	Peak		
Pipidae							
Xenopus eysoole (previously referred to as *X. amieti*)	Evans, Carter, Greenbaum, Gvoždík, Kelley, McLaughlin, Pauwels, Portik, Stanley, Tinsley, Tobias & Blackburn, 2015	(NT)	BamH	1100	2175	–	Loumount and Kobel 1991; Gartshore 1986; Evans et al. 2015; Tropek and VG pers. ob. 2009; TMD-B pers. ob. 2006, 2008, 2009, 2012, 2015
Xenopus poweri (previously referred to as *X. laevis*)	Hewitt, 1927	LC	SSA	900	1788	–	Loumount and Kobel 1991; TMD-B pers. ob. 2006
Xenopus longipes	Loumount & Kobel, 1991	CR	MtO	2220	2220	IN, FL	Gartshore 1986; Loumount and Kobel 1991; Tinsely and Measey 2004; VG pers. ob. 2005; Blackburn et al. 2010; Doherty-Bone et al. 2013b; TMD-B, pers. ob. 2006–2015
Ptychadenidae							
Ptychadena cf. *mascareniensis* "D" (OTU 6; sensu Zimkus et al. 2017)	–	LC	SSA	900	*c.*2000	–	VG pers. ob. 2009; TMD-B 2006, 2009
Ptychadena cf. *oxyrhynchus*	(Smith, 1849)	LC	SSA	900	*c.*2000	–	VG pers. ob. 2010
Ptychadena taenioscelis	Laurent, 1954	LC	SSA	900	2029	–	TMD-B pers. ob. 2009

Abbreviations for IUCN Conservation Status: CR – Critically Endangered; EN – Endangered; VU – Vulnerable; NT – Near Threatened; LC – Least Concern; DD – Data Deficient.

Elevation range is provided for the species population living on Mt Oku.

Abbreviations for biogeography: SSA - widespread throughout sub-Saharan Africa; CWA - restricted to Central and West Africa countries, but still widespread; CNMts - restricted to the highlands of Cameroon and Nigeria; BamH restricted to the Bamenda Highlands, including Mt. Mbam, Mt. Lefo and Bamboutos Mts.; MtO restricted to Mt. Oku

Abbreviations for hypothesised threats: FL – forest loss and degradation; SV – threats to savanna species, including overgrazing and fire; CL – Climate Change; OV – Overexploitation; IN – Introduced Species, all inferred based on IUCN assessments and field observations of the authors. Threats from pollution and disease are unknown.

would be downgraded from Least Concern to Vulnerable A2(b),B1(b),B2(b) due to its decline at least on Oku and restricted range to the Highlands of Cameroon and Nigeria. If it has declined elsewhere across its range as on Oku and Manengouba, it might be downgraded further to Endangered. The newly described *Xenopus eysoole* Evans, Carter, Greenbaum, Gvoždík, Kelley, McLaughlin, Pauwels, Portik, Stanley, Tinsley, Tobias, Blackburn, 2015) show similar habitat affinities to its original synonym *X. amieti* Kobel, du Pasquier Fischberg & Gloor, 1980 in that it occurs almost exclusively in open areas, including in agriculture but restricted to elevations above 1100 m a.s.l. and thus vulnerable to climate change and possibly increases in use of agrochemicals (see below). It may thus be assessed to be Near Threatened by the IUCN criteria. These proposed changes to IUCN status discussed above would increase the proportion of threatened amphibian species on Mt Oku to 47.9%. If *P.* sp. aff. *werneri* is indeed a valid species, this would make the proportion of threatened species 48.9%.

Discussion

Intensive field work over the past 10 years was combined with published records on the amphibian species present on Mt Oku. One hundred years of surveys have yielded 50 species, of which nearly half have been recorded in the past 10 years. This has brought together knowledge to make improved research, monitoring and conservation planning possible. A significant proportion of these frogs were found to be threatened, and the intrinsic and extrinsic causes of this are discussed below.

Biogeography and Origin of Mount Oku's Amphibians

The amphibian fauna of Oku appear to share an affinity with nearby localities such as Mt Mbam, Mt Lefo and Bamboutos Mts, and sharing same restricted range and Bamenda Highland endemic species on Mt Tchabal Mbabo (Cameroon), Gotel Mountains (Cameroon – Nigeria) and Mambilla Plateau (Nigeria). For example, all firstly listed three peaks share species such as *Astylosternus ranoides* Amiet, 1977, *Cardioglossa oreas* Amiet, 1972 and the restricted range *Astylosternus rheophilus* Amiet, 1977. Oku and Bamboutos share *Werneria bambutensis* Amiet, 1972 and *Wolterstorffina mirei* Perret, 1971 (Perret 1972), while Oku and Tchabal Mbabo share very similar *Crotaphatrema* caecilian species, *C. lamottei* and *C. tchabalmbamboensis* Lawson, 2000 (Doherty-Bone et al. 2011a), and two lineages of the *Phrynobatrachus steindachneri* species complex (Zimkus and Gvoždík 2013). An adequate, robust appraisal (such as an analysis of similarity indices across the range) of the biogeography of Oku's amphibians is difficult due to: a) the absence of complete inventories at other localities around Cameroon and b) the turbulent taxonomy of Cameroon's amphibians over the last decades.

Understanding uniqueness and therefore potential recovery options (such as translocation) for Mt Oku's threatened amphibians requires an adequate appraisal of their evolutionary history. This is however not robustly possible at present due to: a) incomplete distribution and availability of genetic data; and b) an apparently low research effort on this topic. Evolutionary questions to address origins of some amphibian genera within the African continent have been investigated but few have addressed Mt Oku specifically. Exceptions include the discussion of speciation in *Cardioglossa* (Blackburn 2008) and hypothesis on the polyploid speciation and origin of *Xenopus* (Evans et al. 2015). The speciation of Oku's amphibian species is currently explained by the isolation of Oku from other mountains during Pleistocene climatic changes when habitats became fragmented (Amiet 2008). However, the relatively few Mt Oku endemic and numerous Bamenda Highland endemic species in relation to other northern mountains is consistent with phylogenetic evidence from certain avian species (Smith et al. 2000), suggesting the higher plateau of the "northern mountains" (Bamboutos, Oku, Mbam, Mambilla Plateau, Gotel Mts, Tchabal-Mbabo) had greater connectivity during cold and dry climatic phases. The phylogeny of the Cameroonian *Phrynobatrachus* and phylogeography of the *P. steindachneri* complex support hypotheses about pre-Pleistocene radiation and speciation events probably in connection to the orogeny and rich volcanic activities during the Late Tertiary (Zimkus and Gvoždík 2013).

Mount Oku Endemics

Endemic species of Mount Oku include the Lake Oku Clawed Frog, *Xenopus longipes* restricted to Lake Oku, the Mount Oku Subalpine Toad, *Wolterstorffina chirioi* restricted to the subalpine grasslands on the Mount Oku summit. The Spiny Puddle Frog, *Phrynobatrachus chukuchuku* also found only in the high elevation grasslands of the Oku summit. It is more abundant than *W. chirioi*, and has been found as far from the summit as the wetland at Kinkolong (2710 m a.s.l.). A tadpole barcoded with a matching haplotype has however been recorded at Abuh at a stream at the forest-grassland boundary (2,160 m a.s.l.) (Pfalzgraff et al. 2015), suggesting it may have a broader distribution but still restricted to montane grassland. It has recently been assessed to be Critically Endangered by the IUCN due to its restricted range and grazing pressure of its limited habitat (IUCN SSC Amphibian Specialist Group 2011). Here it is hypothesised that climate change may also threaten this species, as similarly hypothesised to threaten the sympatric *Wolterstorffina chirioi* (IUCN SSC Amphibian Specialist Group 2015a). The Puddle Frog, *Phrynobatrachus njiomock* was recently formally described (Zimkus and Gvoždík 2013) but was previously known as *P.* sp. 11 (sensu Amiet 1978) – this frog has also been referred to as *P.* cf. *steindachneri* (Doherty-Bone et al. 2008, 2013b) and resembles *P. steindachneri* and *P. jimzimkusi* which also occur on Mt Oku. This species is mostly restricted to the forest adjacent to Lake Oku, with some individuals found towards the summit.

The Mount Oku Caecilian, *Crotaphatrema lamottei* Nussbaum, 1981 – a very rare, limbless burrowing amphibian has only been recorded to Mt Oku. A similar species, *C. tchabalmbaboensis* occurs on Mt Tchabal Mbabo, Adamawa Region, ca.

230 km north-east. A recent study found these two species to have a very low genetic difference, suggesting they could be conspecific, possibly treated as different subspecies, but requires further examination of more specimens and molecular markers (Doherty-Bone et al. 2011a). It is possible that this caecilian is more widespread over the mountains (probably including the Nigerian Mambilla Plateau and Gotel Mts) but overlooked. The Oku community consider contact with this worm-like amphibian to be bad-juju (bringer of bad luck) and require a traditional medicine man to provide a cleansing potion on the event of digging up, touching or accidentally killing one (Doherty-Bone et al. 2011b). As it is still inconclusive whether or not this species is dependent on forest or if it is threatened by agriculture, it remains listed as Data Deficient by the IUCN.

The potential undescribed endemic puddle frog, *Phrynobatrachus* sp. aff. *werneri* has not been observed since 2009 in Anyafouma Forest, despite searches in 2012 to present. This makes taxonomic appraisals difficult as it is represented by only a single voucher specimen. So far it has not been observed on other mountains, including in the Bamenda Highlands. Careful appraisal of the taxonomy of this species is now needed in the absence of additional specimens, should no further individuals be observed. If individuals are observed, careful consideration should be put into collection of voucher specimens from what could be a recovering population of a Critically Endangered species (Minteer et al. 2014).

Bamenda Highlands Endemics

There are seven species that occur on Mt Oku which also occur on nearby mountains in the Bamenda Highlands, such as Mts Bamboutous, Lefo, Santa, and Mbam. These include Perret's Egg Frog, *Leptodactylodon perreti* Amiet, 1971 – The type specimen for this species was collected from Mt Mbam, with other populations found on Mt Oku. It can be found in most forests on Mt Oku, though it is difficult to find individuals, even when among a chorus. Their choruses have been heard from near the farm-forest boundary by Elak-Oku, to the woodland just below the Oku summit grassland. It has apparently never been observed outside of forest, and is probably a forest-dependent species. It has been listed as Endangered by the IUCN due to its restricted range and predicted vulnerability to forest loss. Another Egg Frog corresponding to *Leptodactylodon axillaris* has recently been found to occur on Mt Oku. This species was previously only recorded to Mt Bamboutos, and has thus been assessed to be Critically Endangered (IUCN SSC Amphibian Specialist Group 2013). The population corresponding to this species should be verified as to whether it is con-specific to those on Mt Bamboutos, given the potential for high elevation frogs to have cryptic species. Should con-specific status between the two mountains be confirmed, extinction of this species would be less imminent given the severe threats on Bamboutos.

The Bamboutos Small-tongue Toad, *Werneria bambutensis* type locality is Mt Bamboutos. There have been no individuals observed on Oku since 2006 (by TMD-

B) where it was rare: two were presented by local women in Elemighong who were out farming (but distance to forest was not clear), another captured in grassland near Anyafouma Forest, suggesting it is not entirely dependent on forest. The requirements of its full life-cycle are not clear however. Tadpoles were found by VG in a stream in a small forest patch at 2,100 m a.s.l. in the beginning of dry season (late November 2005). It is listed as Endangered by the IUCN because of its restricted range and threat from forest loss (Amiet 2004a).

The Mount Oku Long-fingered Frog, *Cardioglossa oreas* also occurs on Mt Bamboutos and Mt Lefo. Mt Manengouba has been cited as a locality for this species, but these reports have been attributed to its sister species *C. manengouba* Blackburn 2008 prior to the latter's description (Blackburn 2008b). It has been collected in a forest patch above Big Babanki (VG), high elevation grassland ~20 m from Anyafouma Forest boundary and at Lake Oku (TMD-B, VG) and other reports of an affinity with bamboo forest and streams (Gartshore 1986). It has been listed by the IUCN as Endangered, as it is thought to be dependent on forest (IUCN SSC Amphibian Specialist Group 2015b). Indeed *C. oreas* has never been found far (< 500 m) from the boundary of primary forest blocks. However, it seems that this species may survive even in small forest patches, which might serve for its dispersal across a fragmented landscape (VG, personal observation), and should be assessed further.

Other Bamenda Highland endemic species include the Mount Oku Wolterstorff Toad, *Wolterstorffina mirei* – Mt Oku is the type locality for this species, and is found also on Bamboutos (Perret 1972). These toads are quite rare on the mountain, found in both forest and higher savanna. The Central Night Frog, *Astylosternus ranoides* type locality is Mt Bamboutos. On Oku, it can consistently be observed around most streams in or near forest, particularly around Lake Oku. It is rarely found outside the forest and is likely a forest dependent species. It is one of the few montane endemic frog species to have not experienced a population crash as observed for other sympatric species on Mt Oku (Hirschfeld et al 2016).

Restricted-range mountain specialists

Restricted-range mountain specialists include Steindachner's Puddle Frog, *Phrynobatrachus steindachneri*, previously found in forests and forest openings throughout the Kilum-Ijim Forest, where it appeared to be the dominant leaf litter anuran together with similar, closely related *P. jimzimkusi* (Zimkus and Gvoždík 2013). *Phrynobatrachus steindachneri* is also recorded on mountains in western Adamawa Region, Cameroon, and eastern Nigeria, while *P. jimzimkusi* is distributed from Mt Oku southward to Mt Manengouba and westward to the Obudu Plateau in Nigeria (Zimkus and Gvoždík 2013). *Phrynobatrachus jimzimkusi* has been found in more deforested areas, such as in home-gardens in Elak-Oku, so could be more tolerant to forest loss. Both species have been previously lumped under the name *P. steindachneri*, which is currently listed as Vulnerable by the IUCN due to the threat from forest loss and degradation. As both species have sustained long term (since 2010) declines on Mt Oku (Hirschfeld et al. 2016; TMD-B pers. ob.), it is proposed they are more threatened than previously thought, and may require more severe conservation classification (see above).

Werner's Puddle Frog, *Phrynobatrachus werneri* was common in most habitats on Oku, including both primary montane forest and agricultural areas. It is restricted to the highlands of Cameroon and Nigeria, and is assessed to be Least Concern by the IUCN. As with other *Phrynobatrachus* in the highlands of Cameroon, it was once abundant (especiallly in Elemighong) but has undergone a dramatic and not easily explained decline in abundance (Hirschfeld et al. 2016), though a single specimen was found in Afua Swamp and another at the Mbi Crater in 2012 by TMD-B.

Other Cameroon highland species with broader ranges include the Cameroon Range Night Frog, *Astylosternus rheophilus* – found particularly in agricultural areas, but also in forest. This species is endemic to Cameroon, though may occur in Nigeria. It is found also on Mt Bamboutos, Mt Lefo, and on Tchabal Mbabo from where a separate subspecies was described (*A. r. tchabalensis* Amiet, 1977). It has been listed as Vulnerable by the IUCN as it could be threatened by habitat degradation, though it has a larger range than other similar *Astylosternus* species (Amiet 2004b).

Riggenbach's/Hieroglyphic Reed Frog, *Hyperolius riggenbachi* (Nieden, 1910)– This species has been observed as high as Elak-Oku (1960 m a.s.l.), and also collected at the Mbi Crater and Elemighong. It appears to thrive in agricultural settings and can be one of few species observed in most degraded areas. It has been listed as Vulnerable by the IUCN due to its restricted range, though it does not seem to be threatened by forest loss. Its resilience to agrochemicals and intensified land use is not known.

The Bamenda Reed Frog, *Hyperolius ademetzi* is endemic to the highlands of Cameroon and is found as far south as Mt Manengouba. This is the first report of this species occurring on Mt Oku, where it was observed by VG in the Kilum-Ijim Plantlife Sanctuary by Lake Oku. As it occurs mostly in grasslands, the IUCN has assessed it to be Near Threatened. Similarly, the Tree Frog, *Leptopelis nordequatorialis* is also found in open areas, including cultivated areas and swamps. It has been collected in Elemighong and Afua Swamp. It is restricted to the highlands of Cameroon and Nigeria, and has been assessed to be Least Concern by the IUCN. The extent of the resilience of these species to grazing, grassland burning, agrochemicals and intensification of land use is not understood.

A common pipid frog has been observed in the Oku Massif and had been previously referred to as *Xenopus amieti* (Loumont and Kobel 1991). However, frogs assigned to this species on Mt Oku have recently been assigned to a separate species, *Xenopus eysoole* (Evans et al. 2015). It is common throughout Oku and Bamenda Highlands. It is found in most disturbed habitats, strangely never in forest, but this could be explained by a preference for still waters and open areas, as opposed to the shaded, lotic habitats that dominate forests on Oku. The current range of *X. amieti* is now uncertain with regard to whether it is sympatric or allopatric to *X. eysoole*, with updated surveys needed to enable more accurate assessment of their conservation status.

Habitat and elevation affinities of Mount Oku's amphibians

Mt Oku's landscape has historically been altered by the demand for fuel wood, grazing of livestock, encroaching agriculture, extirpation of many larger animals, fire and possibly even climate change (Cheek et al. 2000). Understanding the habitat requirements of Oku's amphibians is therefore challenging due to "ghost of land use past" (*sensu* Harding et al. 1998), but critical for predicting their future population trends and planning appropriate action such as habitat protection. Habitat affinities are discussed rather than actual habitat requirements of Mt Oku's amphibians, as a consistent study on habitat requirements has yet to take place. Instead, data on which habitat each species has been observed is available (Gartshore 1986 and listed on the IUCN Red List: www.iucnredlist.org and references therein), but does not robustly predict consequences of habitat change or take elevation distribution into account. These consist mostly of anecdotal, expert opinions and/or surveys that suffer from design issues, such as pseudoreplication, confounding edge effects and the lack of adequate, clearly defined control sites (Gardner et al. 2007). Addressing these issues is challenging due to the physical terrain and size of the study area. Identifying a pristine control site that represents a large block of undisturbed forest is also a challenge as most of the Kilum-Ijim Forest has been degraded, including the Plantlife Sanctuary. The latter is perhaps the most appropriate candidate for a control site, along with the sacred forest by Elak-Oku, which local elders claim is among the least disturbed in the Kilum (Oku) forest.

Emerging evidence suggests that most of the endemic and Bamenda Highland endemic species rely on forest on Mt Oku (see above and Table 1) and two endemic species occur exclusively in high elevation grasslands, though whether they are dependent on grassland or the climatic envelope montane grassland occurs at is not known. All these habitats are threatened by livestock grazing, which appears to be increasing in forests, with high densities on the grasslands. Some endemic species have very specific habitat affinities, such as *Phrynobatrachus njiomock* being predominantly recorded in the forest around Lake Oku, and the Lake Oku Clawed Frog (*X. longipes*) being restricted to Lake Oku. Differentiating affinities for habitat from affinities for particular elevations is challenging for those species that dwell at high elevations above 2,000 m a.s.l.. Forest-dependent species that are adapted to mid-elevations will be difficult to identify as most forest has been lost at mid-elevations, and any such species would be either locally extinct, occur at low abundance or occur in fringe habitat that obscures its complete elevational range. Resolving these issues will require consistent sampling on nearby mountains with varying deforestation histories at set elevations (i.e. 1,000 m, 1,500 m, 2,000 m, etc.), at least for those species shared by other mountains.

Conservation of Mount Oku's Amphibians

As with the rest of the world and Cameroon (i.e. Beebee and Griffiths 2005; Amiet 2008), issues affecting amphibians are effectively the same affecting the wider environment on Mt Oku. The loss and degradation of forests, chemical pollution, invasive species (including pathogens) and climate change all threaten the unique biodiversity of Oku as much as it threatens its amphibians. Predicting the specific threats is however difficult due to the paucity of robust ecological data. Amphibians require freshwater breeding habitats, as well as terrestrial habitats to forage. These also require physical conditions to allow eco-physiological persistence, such as moisture (when on land), low salinity and conditions to avoid predation (freedom from fish or cover from birds) (as reviewed in Smith and Sutherland, 2014). It is therefore possible to pre-emptively hypothesise threats in absence of robust data that could be obtained at a later date. Certain amphibian populations have however received consistent, specific survey attention, such as the Lake Oku Clawed Frog (*Xenopus longipes*), leaf-litter forest anurans, farmland species and more recently anurans of the high grasslands of the Oku summit. Below we discuss other threats to amphibians on Mt Oku, notably disease, climate change, pollution and invasive species.

Forest degradation and loss

Mt Oku has been inhabited by people for hundreds of years and it has been estimated that widespread deforestation occurred through clearance for agriculture during the rise of the centralised agrarian societies that are now the fondoms (= kingdoms) (Cheek et al. 2000). During the colonial era, the remaining forest on the top of Mt Oku was protected as a forest reserve, but following independence the new government forestry department allowed the forest to decline. Despite renewed protection measures for the Kilum-Ijim forests (see below), the forest is likely to continue to degrade due to edge-effects, extirpation of seed-dispersing birds and mammals (e.g. Maisels et al. 2001), incursions by livestock, likely climate change, chronic over-use by the local community and infrastructure developments.

Forest management is by local community forest management institutes (FMIs), with the exception of the government controlled (Ministry of Forestry and Wildlife) Kilum-Ijim Plantlife Sanctuary which includes Lake Oku. Forests are accessed extensively by the communities for placing bee hives, collection of medicinal plants, hunting and passage to other villages. Firewood is frequently collected from the forest: the FMI's have declared that only wood from dead trees be collected from the forest, but in recent years live trees cut down have been increasingly observed, as has the killing of trees through debarking (Stewart 2009; TMD-B, pers. ob.).

More recently, a section of forest was cut down in 2012 to make way for an ambiguous government funded tourism development near (though not immediately adjacent to) Lake Oku. This has apparently set a precedent for more forest clearance for development in 2015 with the Cameroon Baptist Convention clearing another patch of forest adjacent to the aforementioned site, to create a new church and a series of dormitories (TMD-B, pers. ob.). Should they become successful in attracting customers (no plans exist for low impact ecotourism), this is likely to result in further demand for fuel wood and general disturbance of the forest. Roads passing through the forests have recently been improved and are likely to increase access to the forest to extract resources such as bushmeat and fuelwood.

Livestock grazing

Livestock are found to regularly make incursions into the forest (pers. ob.). Livestock grazing has historically been on the increase, especially in the Summit grasslands (Maisels et al. 2000; Doherty-Bone 2015a) but also on most high elevation grasslands, such as by Anyafouma and Abuh. These often co-occur with incursions into the forests. All these practises are novel to these ecosystems, though the extent to which grazing by locally extinct larger mammals is replaced by domestic livestock is unknown. Livestock overgrazing represents a threat to amphibians through altering habitat by removal of vegetation, compacting soils, causing erosion into aquatic habitats, and restricting recruitment of native trees. This is in combination with burning by pastoralists in an attempt to provide better quality browse for animals, which would directly kill amphibians and alter their habitats.

Exploitation

Amphibians in Cameroon are in some areas commonly harvested for human consumption (Gonwouo and Rödel 2008). In the highland areas at Babanki, larger species of frog such as Hairy Frogs (*Trichobatrachus robustus*) and tadpoles of *Astylosternus* and *Trichobatrachus* are often consumed (VG pers. ob.). Collection of amphibians for the commercial pet trade apparently also occurs from Cameroon (based on animals presented for sale in Western countries claimed to originate from Cameroon, Herrel and van der Meijden 2014). However, the authors have not observed animals being collected for trade, or heard about brokers organizing local people to collect animals on Mt Oku so far, unlike elsewhere. There have been isolated incidents when Western animal dealers have contacted Cameroonian field assistants about acquiring species such as *X. longipes*. It was not clear how these dealers obtained the contact details of these field assistants, but these appeared to be prospective inquiries rather than trade *per se*. This is still activity that professional herpetologists should be aware of when training local guides and sharing their contact details.

Chemical pollution

The role of chemical pollution is an old topic in amphibian conservation biology (e.g. Rouse et al. 1999), but Africa has received an especially low research effort (Schiesari et al. 2007). Industry and use of agrochemicals is growing in Cameroon. Mt. Oku is still isolated from the effects of factories and is generally not subjected to industrial agriculture, with the exception of a tea estate adjacent to the Mbi Crater at Ndawara. However, local farmers are increasingly using agrochemicals for their crops, such as glyphosate herbicides that are on sale in many shops in villages. Other sources of pollution on Mt Oku include an increasing incidence of plastic refuse, electrical waste (especially batteries) and the growing number of vehicles in the area. Garbage is often found around the one section of lake that is visited by tourists and locals alike. Three roads bisect the Kilum-Ijim forest (Figure 1) and traffic has been noticed to increase in recent years following improvements to these roads (TMD-B. pers. obs.). Other forms of pollution include elevated UV-radiation from ozone depletion, which impacts certain species of northern hemisphere amphibians in the laboratory (Blaunstein et al. 1994), but unknown for African amphibians, particularly those that occur at high elevations, such as on Oku. Responses of Mt Oku's amphibians to these multiple and growing sources of chemical pollution are unknown and warrant further investigation, which could include surveys on farms using particular chemicals, and toxicity experiments on target species to field-relevant agrochemical mixtures.

Climate change

Tropical mountains are predicted to undergo dramatic ecological changes as a result of global climate change, including changes to temperature and precipitation (Beniston et al. 1996). In Cameroon, temperatures have been increasing since at least 1960, with changes to rainfall predicted to increase incidents of drought (Molua 2006). This has the potential to impact the amphibian assemblage of Mt Oku. Firstly, there may be a shift in distribution based on elevation as temperatures change, with endemic species at higher elevations moving even higher, reducing their range size (as observed in Madagascar, Raxworthy et al. 2008). Another effect will be changes to hydrology associated with changes in precipitation. This could lead to potentially reduced availability of streams and ponds for breeding, affecting both breeding phenology and density of amphibians across habitats. Further consequences of this could include increased competition between species and increased transmission of disease. Other consequences of increased drought will be a decline in invertebrates, reducing the prey-base for amphibians (Donnelly and Crump 1998). High elevation endemic species hypothesised to be most threatened by climate change on Mt Oku are the Subalpine Toad, *Wolterstorffina chirioi* (Boistel and Amiet 2001), with the sympatric Spiny Puddle Frog, *Phrynobatrachus chukuchuku* possibly also threatened due to its restricted climatic-range. It is however likely there are many more amphibians on Mt Oku threatened by climate change.

Disease

Disease is a major issue in the conservation of amphibians worldwide (Dazak et al. 1999; Skerratt et al. 2007; Duffus 2009). The amphibian-killing chytrid fungus

(*Batrachochytrium dendrobatidis - Bd*) for example has caused declines of many amphibian species. Cameroon has one of the oldest records for this pathogen, recorded in a frog collected from eastern lowland Cameroon in 1933 (Soto-Azat et al. 2010). A 2006 survey of amphibians on Mt Oku found no evidence for the presence of this pathogen (Doherty-Bone et al. 2008) but subsequent sampling has found it to be present at a prevalence comparable to other regions of Africa (Doherty-Bone et al. 2013a; Hirschfeld et al. 2016). However, the role of *Bd* in amphibian population viability in Cameroon is still cryptic due to new findings that multiple strains of varying virulence occur within Africa (Farrer et al. 2011). While *Bd* is hypothesised to originate from Africa (Weldon et al. 2004), it has more recently been associated with declines of amphibians in the Highlands of Cameroon, including Mt Oku where it had not been detected prior to declines (Hirschfeld et al. 2016). Sequencing of strains of this fungus from localities in Cameroon is now a priority to understand if strains are from hyper-virulent or benign variety (one sample was said to be from the pan-global hypervirulent strain, but was not accompanied by peer-reviewed data in Hirschfeld et al. 2016). Using archived museum specimens to determine the long-term history of this pathogen on Mt Oku will test the endemic versus novel pathogen hypothesis in Cameroon (Soto-Azat et al. 2010; Doherty-Bone et al. 2013a). Research on the susceptibility of Cameroonian amphibians to infection by *Bd* could also take place, particularly the presence of skin peptides and microbiota that provide resistance to this fungal pathogen (Harris et al. 2006; Woodhams et al. 2007). This work will help determine the threat posed by *Batrachochytrium* pathogens to Cameroon's amphibians.

Amphibian chytrid fungus is not the only pathogen that can cause declines in amphibians, with other diseases emerging (Duffus 2009). An enigmatic disease has been observed in the Lake Oku Clawed Frog (*X. longipes*) since 2006, where many frogs have been found with lesions and necrotic limbs, but with the agent still unidentified (Blackburn et al. 2010a; Doherty-Bone et al. 2013b). Ranavirus has been found in one of these clinical specimens, but its presence needs to be verified and impacts on Cameroonian amphibians assessed. The role of disease for the conservation of Mt Oku's amphibians remains uncertain and requires monitoring to detect emergences and declines so conservation action can be planned. Biosecurity should be practised by field workers to prevent the spread of novel and unknown pathogens to naïve amphibian populations (Phillott et al. 2010).

Invasive alien species

Invasive alien species are organisms established by humans either intentionally or unintentionally outside of their natural range into a novel ecosystem that would not normally encounter that species. When they proliferate and spread without control, they can cause severe damage to native species, ecosystems and economies. Amphibians are particularly affected by invasive alien species, notably by introduced predators (especially fish) and pathogens (Kats and Ferrer 2003). On Mt Oku, the biggest threat from invasive alien species comes from aquaculture and the introduction of fish (possibly *Tilapia nilotica*) into Lake Oku (Gartshore 1986; Tinsley and Measey 2004). The latter has not yet happened but is still a looming threat to the endemic Lake Oku Clawed Frog, as stocking the lake is often discussed by the Oku community elders (TMD-B pers. ob.). Small-scale aquaculture already takes place in the form of numerous fish ponds around Mt Oku. The potential for fish to escape from ponds and invade Lake Oku have not been fully investigated, though all ponds are downhill from the lake, making passive escape following rainfall less imminent. However, other animals such as birds might spread organisms from these ponds to Lake Oku, especially fungal pathogens that can be carried by these animals (Garmyn et al. 2012). These ponds are often found to provide habitats for some anurans, especially *Xenopus eysoole*, but whether or not they breed in the presence of fish, or intensively stocked fish ponds is unknown. The domestic cat (*Felis domesticus*) is a particularly harmful invasive species in many regions (Lowe et al. 2000) and are beginning to become popular in Elak-Oku, owing to their value in controlling rodents in households. The potential of densities of cats to increase in the region and for them to establish populations is uncertain, but is potentially a new invasive alien species and should be monitored. Other invasive species that might threaten amphibians on Mt Oku are unknown. As with preventing new and unknown diseases, biosecurity should be practised by field workers (including tour guides) to prevent spread of new invasive alien species through inspecting, cleaning and drying equipment (Anderson et al 2015).

Past, present and future conservation action

Conservation interventions relevant to amphibians on Oku have been indirect or patchy at best. The Birdlife International-Cameroon government Kilum-Ijim Forest Project has had some success in encouraging an environmental ethic in the local communities (Abott et al. 2001; TMD-B and VG pers. ob.) and reducing (but not stopping) the rate of forest loss and degradation (GIS Unit Royal Botanic Gardens Kew, downloaded 2016). The current status quo is the main forest block, the Kilum-Ijim Forest divided into 11 community forest management institutes, with the Kilum-Ijim Plantlife Sanctuary a protected government reserve that includes Lake Oku. This latter protected area is however infrequently patrolled by the forest guards, who seem to have a conflicting agenda with members of the community through prohibition of access to one part of the lake's shore (TMD-B, pers. ob.). This hence occasionally affects local rights, tourism and perceptions of conservation by local people, who frequently ignore the access prohibition (TMD-B, pers. obs.). The Mbi Crater is also a government protected area, but again is infrequently patrolled, seems to have no management plan or mitigation against agrochemical run-off from the local tea estate. The forests around Big Babanki have no protection at all. The wetland habitat of Afua Swamp also has no protection, and the surrounding forest has been heavily degraded in the past decade (Khimal Peter, former Kilum-

Ijim Project technician, pers. comm.), mostly by cattle grazing, which also threatens rare plants (Maisels et al. 2000).

Improving the future prospects for Mt Oku's amphibians needs to build on these existing conservation interventions. This requires a concerted movement from sporadic surveys and preliminary appraisals of the human scale to taxonomic appraisals using modern methods, robust ecological and socio-economic study and active interventions (such as those listed in Smith and Sutherland 2014). Recent campaigns to collect better reference collections to enable molecular phylogenetic analysis to test taxonomic hypotheses have made significant advances (Zimkus 2009; Blackburn et al. 2010b; Doherty-Bone et al. 2011a; Zimkus and Gvoždík 2013). These have been coupled with engagement with the local communities raising awareness: public outreach meetings (Doherty-Bone 2011, 2015b), participation in traditional rituals to facilitate field surveys (Doherty-Bone et al. 2011b) and even naming of new species using indigenous languages (Zimkus 2009; Blackburn et al. 2010b; Zimkus and Gvoždík 2013; Evans et al. 2015). Work addressing threats directly also need to be focused into sustainable actions. For example, initial conservation action plans have been prepared for the habitats of three Critically Endangered amphibians: Lake Oku and the Oku Summit (Doherty-Bone, 2014, 2015). These action plans have been prepared in collaboration with local, regional, national and international representatives in Oku, as recommended in a newly accepted paradigm for environmental management in developing countries (Smith et al. 2009). These action plans have strong involvement with local stakeholders, particularly appropriate for the anurans restricted to the summit grasslands of Oku, where conflicts exist with livestock herders (Maisels et al. 2000). E*x-situ* colonies of captive *Xenopus longipes* are being developed (Michaels et al. 2015; Tapley et al. 2016) following recommendations of the species' 2004 IUCN assessment (Tinsley and Measey, 2004), though it should be noted that *ex-situ* conservation projects can have limited effectiveness, particularly without adequate planning and collaboration *in situ* (Tapley et al 2015). Small steps have been made in addressing the threats to the Lake Oku Clawed Frog: firstly acquiring ecological data on this species; training local researchers; engaging and informing the community on issues such as the risks of fish introductions; and advocating the general importance of amphibians and environment on Oku. The impact of these conservation action plans have still to be assessed. Despite this, much work is needed to reinforce the long term prospects for this particular species and habitat.

The amphibian fauna of Mt Oku is particularly rich but threatened, and despite over 40 years of field research, more species may yet be described, more sections of the mountain need to be surveyed and the biology and ecology of these species to be adequately studied. The status of the many endemic and restricted-range species remains uncertain, though it is likely most are under pressure from multiple-threats for a changing region, country and globe. These threats can be addressed through protection of forest and high-elevation savanna with careful control of agrochemicals and biosecurity, with roles for international, national, regional and local conservation professionals and stakeholders alike.

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Appendix

Table 1A. Museum accession numbers for newly recorded taxa to Mount Oku, Cameroon. Abbreviations for museums: BM – Natural History Museum London, UK; NHMUK – Natural History Museum London UK, (new tag code); NMP6V – National Museum, Prague, Czech Republic

Species	Accession #	Year	Locality
Arthroleptis adelphus	NMP6V 73369	2005	Bamo Forest
	photo voucher	2006	Elemighong
Arthroleptis sp. aff. *poecilonotus*	NMP6V 73343/1-2,5	2005	Mejung
	NMP6V 74668	2010	
	BM20052327,2499-500	2012	Elemighong
Arthroleptis cf. *perreti*	NMP6V 73367, -35	2005	Mendong Buo
	BM20051932	2013	Emfveh-mi Forest
Astylosternus cf. *diadematus*	NMP6V 73373/1-2,	2005	Kedjom-Keku
	73374, 74674	2009	Mejung
Asytlosternus montanus	BM2008.442	2009	Afua Swamp
Cardioglossa leucomystax	NMP6V 73398/1-5	2005	Bamo Forest
Leptodactylodon axillaris	photo voucher	2006	Anyafouma Forest
Leptodactylodon bicolor	photo voucher	2006	Elemighong
	photo voucher	2015	Lui-Oku
Leptopelis modestus	ZMB 79624 (tadpole)	2012	Abu Forest
Leptopelis notatus	NMP6V 73394, 74608	2005	Mejung
		2009	
Hyperolius ademetzi	NMP6V 73384	2005	Lake Oku
Hyperolius igbettensis	BM20051994	2012	Mbi Crater
Hyperolius nitidulus	BM20051996-97	2012	Mbi Crater
Petropedetes sp. aff. *parkeri*	NMP6V 73389/1-2, 73391/1-2	2005	Kedjom-Keku, Mejung
Phrynobatrachus calcaratus	NMP6V 73399/1-12	2005	Bamo Forest
Phrynobatrachus cricogaster	NMP6V 73393/1-6	2005	Bamo Forest
Phrynobatrachus natalensis	photo voucher	–	Chuaku
	NHMUK201368,90-91	–	Lake Kuk
Phrynobatrachus schioetzi	NMP6V 73438	2005	Kedjom Keku (Mendong Buo)
	BM2008.537	2008	Klilum-Ijim Plantlife Sanctuary
	BM2008.551	2009	Afua Swamp
Phrynobatrachus sp. aff. *werneri*	BM2008.542	2009	Anyafouma Forest
Ptychadena cf. *mascareniensis*	BM2008.477	2009	Kissotin
	NMP6V 74606/1-2	2009	Mejung
Ptychadena cf. *oxyrhynchus*	NMP6V 74667/1-3	2010	Mejung
Ptychadena taenioscelis	BM2008.476	2009	Afua Swamp
Sclerophrys latifrons	NMP6V 73395/1-2	2005	Bamo Forest
Sclerophrys maculata	NMP6V 73378, 73380	2005	Bamo Forest, Mejung
Sclerophrys regularis	photo voucher	2006	Elemighong
Sclerophrys superciliaris	photo voucher	2005	Bamo Forest
Sclerophrys villiersi	NMP6V 74647	2009	Bambui
Trichobatrachus robustus	photo voucher	2006	Elemighong
	–	2008	Mbam-Oku
	–	2010	Kedjom-Keku

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RESEARCH ARTICLE

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Herpetofauna of the Northwest Amazon forest in the state of Maranhão, Brazil, with remarks on the Gurupi Biological Reserve

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Abstract

Understanding the biodiversity of an area is the first step for establishing effective interventions for conservation, especially when it comes to herpetofauna, since 4.1% and 9.2%, respectively, of Brazilian amphibians and reptiles are endangered. The aim of this study is to identify the composition of the herpetofauna occurring in the Northwest Amazonian state of Maranhão, with a focus on the Gurupi Biological Reserve and surrounding areas. Samples were collected between May 2012 and October 2013 (18 months), through pitfall traps, time constrained active search, and opportunistic encounters, and these records were supplemented by specimens collected by third parties and by bibliographic records. A total of 131 species were recorded: 31 species of amphibians and 100 species of reptiles (six testudines, 30 lizards, two amphisbaenas, 60 snakes and two alligators), including some species new to the state of Maranhão and the northeast region of Brazil. This inventory contributes to the knowledge of the herpetofauna for the Belém Endemism Center, the most devastated region of the Brazilian Amazon, and considered poorly sampled.

Keywords

Amphibians, Belém Center of Endemism, inventory, new records, reptiles

Introduction

Conservation units in the Amazon (National Parks, ecological stations, extractive reserves, national forests, biological reserves, etc.) are of fundamental importance for the conservation of biodiversity in this biome (Peres 2005; Silva 2005).

With regard to formulating effective management plans, it is of fundamental importance for biodiversity conservation to know the composition of the fauna and flora. Faunal lists, especially in regions that represent sampling gaps and that refer to groups with a high percentage of endangered species should be emphasized (Peres 2005).

In this context, regarding the herpetofauna, there are in Brazil 1080 known species of amphibians and 773 species of reptiles of which 4.1% and 9.2% are endangered, respectively (Costa and Bérnils 2015; Segalla et al. 2016).

Biodiversity inventories enable us to identify the degree of regional endemism, new trigger points, new species and the actual conservation status of the species recorded, besides enriching regional and national scientific collections (Moura et al. 2014).

It is noteworthy that species lists are highly important for our understanding of the environmental conservation, since the occurrence of bioindicator species (either opportunistic or vulnerable) can be used to determine the effects of environmental disturbance in the area, and therefore, pave a way to efficient interventions and conservation policies (Brown and Freitas 2002).

The state of Maranhão is known for its rich biodiversity (Martins and Oliveira 2011), lack of researchers and extensive sampling gaps, especially regarding the herpetofauna in the Amazon biome of northwestern Maranhão.

Among the indicators of the need for wildlife studies in Maranhão are the articles of the last decades that describe new species in Amazonia, especially in the eastern Amazon (Caramaschi 2010; Vaz-Silva et al. 2015), as well as the few publications, concentrated mainly on snakes (Cunha and Nascimento 1993; Freitas et al. 2014), lizards (Avila-Pires and Vitt 1998; Avila-Pires 1995; Freitas et al. 2013) and anurans (Andrade et al. 2003; Rodrigues et al. 2003; Andrade et al. 2011; Barreto et al. 2011; Miranda et al. 2012; Miranda et al. 2013; Matavelli et al. 2013; Freitas et al. 2014b, c, d; Vaz-Silva et al. 2015).

This study aims to record the composition of amphibians and reptiles of the REBIO Gurupi and adjacent regions of the northwest Amazon of Maranhão state, a conservation unit of utmost importance, composing the largest and last block of continuous forests in the Belém Endemism Center (Martins and Oliveira 2011).

Materials and methods

Study Area: The Gurupi Biological Reserve (03°58'32"S 46°46'52"W) (Figure 1), was created by Law 95 614 1988 and occupies an original area of 341,650 hectares in the municipalities of Bom Jardim, Centro Novo do Maranhão and São João do Caru, in the state of Maranhão (IBAMA 2006). The climate is humid type B2, with moderate water deficit in the summer, average annual rainfall exceeding 2,000 and average temperature greater than 24°C, with the soil type being argisoil (Martins and Oliveira 2011). This work was performed and focused only in Gurupi and the adjacent rainforests of the State of Maranhão, northwest Amazon.

Sampling: Samples were collected between May 2012 and October 2013 (18 months), with pitfall traps, time constrained active search, and opportunistic encounters in the forested areas and access roads to REBIO (road killed individuals). They also included those collected by third parties and bibliographic records.

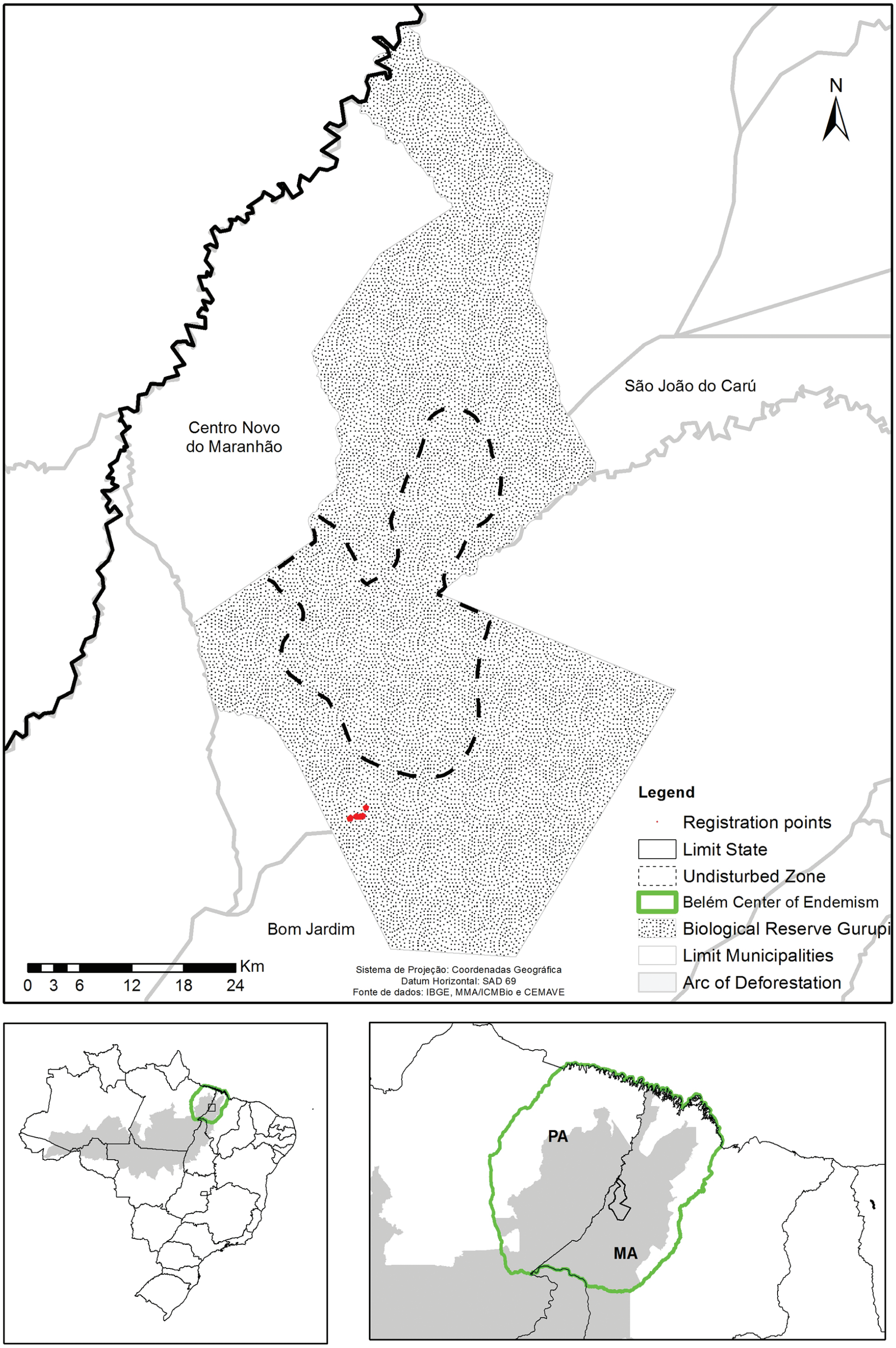
The pitfall traps were composed of seven lines of five buckets (60 L) connected by a plastic canvas with a length of 10m, totaling 70 meters of intercept lines (Line 1: 03°59'14"S, 46°47'53"W; Line 2: 03°59'11"S, 46°47'50"W; Line 3: 03°59'05 "S, 46°47'27"W; Line 4: 03°59'06"S, 46°47'25 "W; Line 5: 03°59'07"S, 46° 47'15"W; Line 6: 03°59'03"S, 46°47'04"W; Line 7: 03°58'32"S, 46°46'52"W), each line was about 3 km distant from one another. Traps were open around the clock for five days during each sampling campaign. These were concentrated in the rainy season, December 2012, January and May 2013, totaling an effort of 5400 hours/ bucket.

The time-constrained active search was conducted over three campaigns for three nights in December 2012, January and May 2013, by a team made up of five collectors, totaling an effort of 135 man hours according to the methodologies of Martins and Oliveira (1998), and Bernarde and Abe (2006). The search was concentrated in the area adjacent to aquatic environments near the southern tip of REBIO Gurupi (04°00'20"S, 46°46'41"W).

The collected amphibians were euthanized by applying lidocaine ointment (lidocaine) on the ventral region and the reptiles through overdosing with ether (Callefo, 2002; Franco and Salomão 2002). All specimens were fixed with 10% formalin and then preserved in 70% ethanol.

Voucher specimens were deposited in the collection of Herpetology and Paleontology of the Federal Rural University of Pernambuco CHP-UFRPE (tumble numbers in Appendix 1) and the University of São Paulo Museum of Zoology MZUSP (tumble numbers in Appendix 1). Important bibliographic databases added to this work were Cunha and Nascimento (1993) for snakes, Avila-Pires (1995) for lizards and Barreto et al. (2011) for amphibians and reptiles.

Data analysis: In order to evaluate the efficiency of the collection effort in the analyzed fragment, species rarefaction curves were constructed with 1000 randomizations generated based on the data matrix of the relationship between richness and abundance (ICE index) through the statistical program Ecosim version 7.0 (Gotelli and Enstminger 2003).



Sistema de Projeção: Coordenadas Geográfica
Datum Horizontal: SAD 69
Fonte de dados: IBGE, MMA/ICMBio e CEMAVE

Figure 1. Map with the location of the study area, in the Gurupi Biological Reserve, Maranhão, Brazil.

Results

A total of 131 species composing the local herpetofauna was recorded: 31 species of amphibians (seven families) and 100 species of reptiles, which included six testudines, 30 lizards, two amphisbaenians, 60 snakes and two crocodylians (Figure 3, 4).

Of the 131 species recorded for the northwest Amazon in Maranhão, 78 species (62%) were recorded in this study and 53 (38%) included results from previously published data (Cunha and Nascimento 1993; Avila-Pires 1995; Barreto et al. 2011).

Discussion

The Belém Endemism Center (EC) is the smallest among the eight EC in the Amazon region, and has the largest loss of forest cover, with about 70% of its total area already destroyed (Silva et al. 2005; Fearnside 2006).

The diversity of amphibians and reptiles of the Belém Endemism Center is very high, as in other endemic centers, especially those of the eastern Amazon region, such as the ECs Guyana, Xingu and Tapajós: 85 species of snakes further down the Amazon River, in Santarem, state of Pará at EC Tapajós (Frota et al. 2005); 62 species of amphibians and reptiles in the Biological Reserve Tapirape, also at Pará, in the EC Xingu (Bernardo et al. 2012); 71 species of amphibians in the National Forest of Carajás, also at the EC Xingu (Pinheiro et al. 2012); 175 species of amphibians and reptiles in the National Forest of Saracá-Taquera, in theEC Guiana at Pará (Morato et al. 2014.); 53 species of snakes for the National Forest of Caxiuanã (at the EC Xingu (Santos-Costa et al. 2015); 78 species of amphibians and reptiles in the northern part of the state of

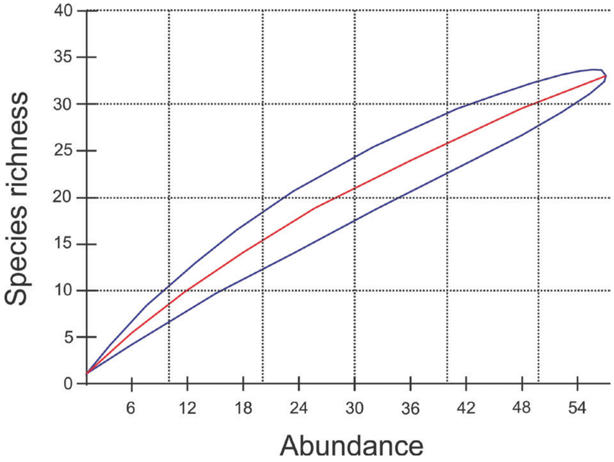


Figure 2. Thinning curves representing the accumulated richness of species of frogs and lizards at REBIO Gurupi. The richness was recorded by passive and active collection between December 2012 and May 2013. The center line is the average (randomizing 1000 times) and the lines on the side represent the associated standard deviation.

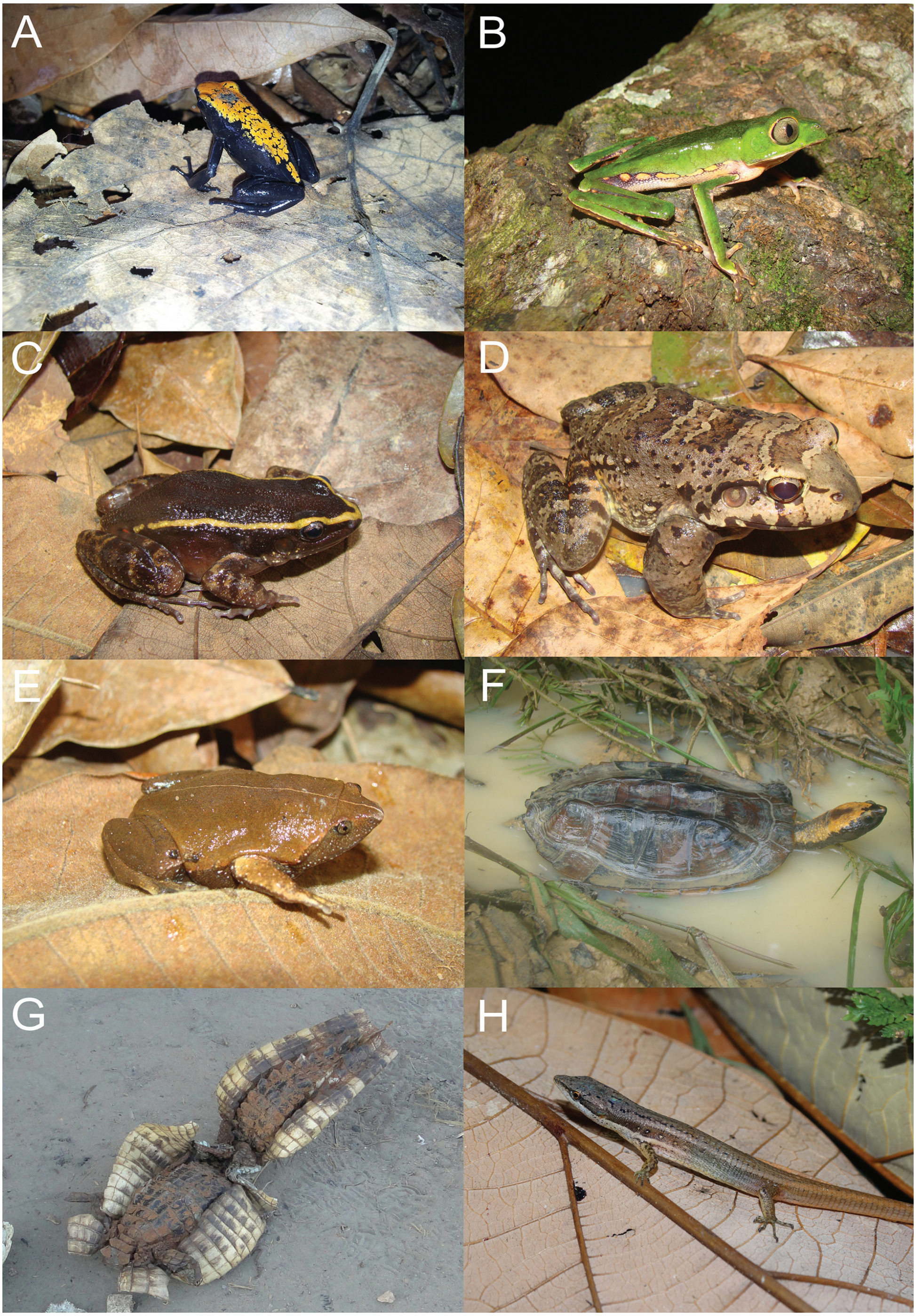


Figure 3. Some amphibians and reptiles recorded in the Gurupi Biological Reserve, Maranhão, Brazil. **A** *Adelphobates galactonotus* **B** *Phyllomedusa vaillanti* **C** *Lithodytes lineatus* **D** *Leptodactylus paraensis* **E** *Ctenophryne geayi* **F** *Platemys platicephala* **G** *Paleosuchus trigonatus* **H** *Cercosaura argulus*. Photo credit of *P. trigonatus* (**G**) belong to Eloisa Mendonça and M. A. de Freitas for others.

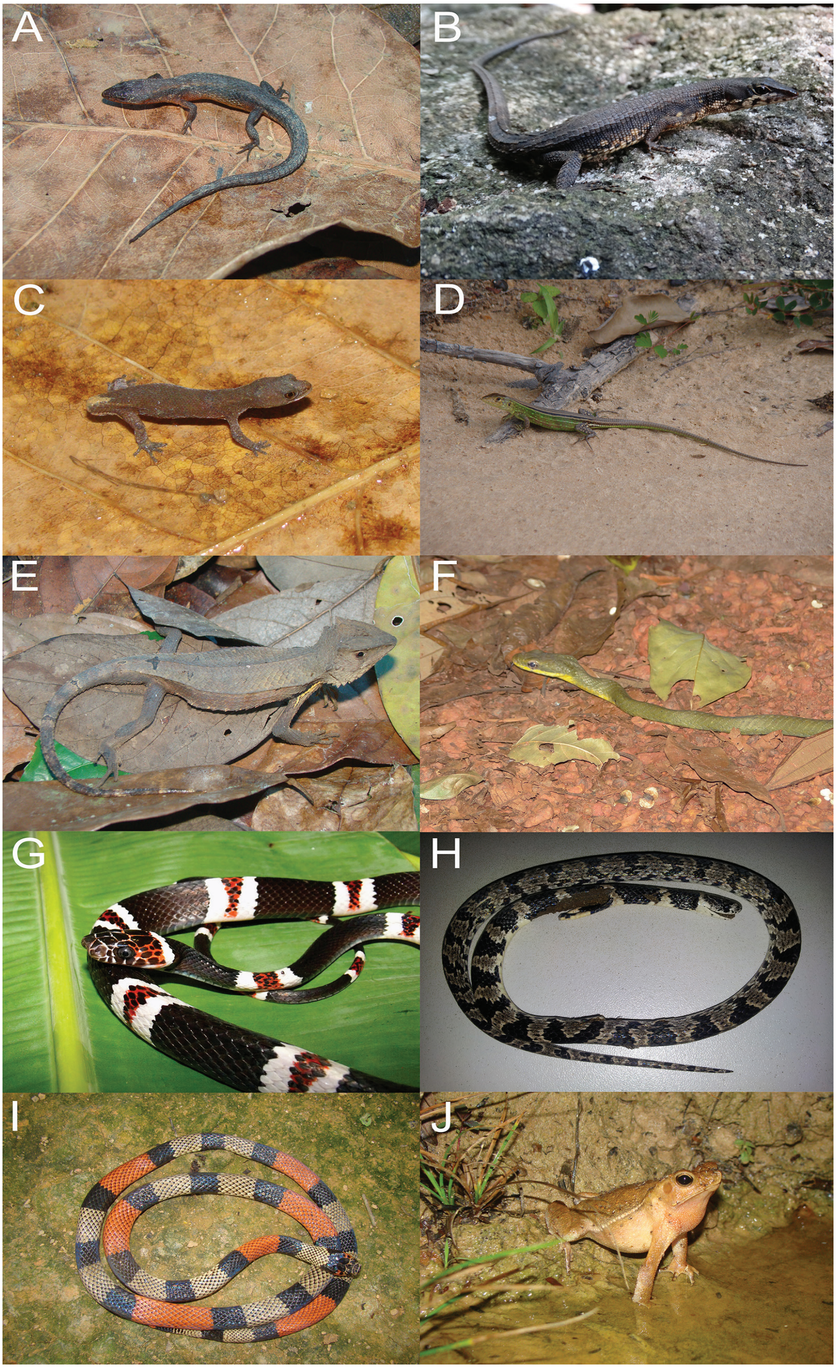


Figure 4. Some amphibians and reptiles recorded in the Gurupi Biological Reserve, Maranhão, Brazil. **A** *Leposoma percarinatum* **B** *Neusticurus bicarinatus* **C** *Coleodactylus septentrionalis* **D** *Cnemidophorus cryptus* **E** *Stenocercus dumerilii* **F** *Phrynonax poecilonotus* **G** *Rhinobothryum lentiginosum* **H** *Sibynomorphus mikanii septentrionalis* **I** *Micrurus spixii martiusi* **J** *Rhinella gildae*. Photo credits of *P. poecilonotus* (**F**) belong to Eloisa Mendonça, *R. lentiginosum* (**G**) to Saymon Albuquerque and M. A. de Freitas for others.

Table 1. Herpetofauna recorded between May 2012 and October 2013 in the Gurupi Biological Reserve, Maranhão, Brazil. (Type of record: EO = Opportunistic Encounter; BA = Constrained active search; Pitfalls = pitfall trap, DS = secondary data).

* First occurrence for the state of Maranhão and northeast Brazil.
** Species endemic to the Belém center of endemism.

FAMILY	SPECIES	TYPE OF RECORD
Bufonidae	*Rhinella gildae* Vaz-Silva, Maciel, Bastos & Pombal Jr, 2015**	EO - DS (Vaz-Silva et al. 2015)
	Rhinella marina (Linnaeus, 1758)	EO/Pitfalls/BA
Craugastoridae	*Pristimantis fenestratus* (Steindachner, 1864)	BA
Dendrobatidae	*Adelphobates galactonotus* (Steindachner, 1864)**	BA
Hylidae	*Dendropsophus marmoratus* (Laurenti, 1768)	EO
	Dendropsophus cf. minusculus	DS (Barreto et al. 2011)
	Dendropsophus nanus (Boulenger, 1889)	DS (Barreto et al. 2011)
	Dendropsophus minutus (Peters, 1872)	DS (Barreto et al. 2011)
	Dendropsophus rubicundulus (Reinhardt & Lütken, 1862)	DS (Barreto et al. 2011)
	Hypsiboas boans (Linnaeus, 1758)	EO
	Hypsiboas fasciatus (Günther, 1859 "1858")	DS (Barreto et al. 2011)
	Hypsiboas punctatus (Schneider, 1799)	BA
	Hypsiboas multifasciatus (Günther, 1859)	DS (Barreto et al. 2011)
	Osteocephalus taurinus Steindachner, 1862	BA
	Scinax fuscomarginatus (A. Lutz, 1925)	DS (Barreto et al. 2011)
	Scinax nebulosus (Spix, 1824)	DS (Barreto et al. 2011)
	Scinax ruber (Laurenti, 1768)	EO
	Sphaenorhynchus lacteus (Daudin, 1800)	DS (Benício et al. 2011)
	Trachycephalus typhonius (Linnaeus, 1758)	EO
	Phyllomedusa hypochondrialis (Daudin, 1800)	DS (Barreto et al. 2011)
	Phyllomedusa vaillantii Boulenger, 1882 *	BA (Freitas et al. 2014)
Leptodactylidae	*Adenomera andreae* (Müller, 1923)	BA/Pitfalls/EO
	Leptodactylus fuscus (Schneider, 1799)	EO
	Leptodactylus mystaceus (Spix, 1824)	BA/Pitfalls/EO
	Leptodactylus rhodomystax Boulenger, 1884	(Sá et al. 2014)
	Leptodactylus paraensis Heyer, 2005 ** -	EO
	Leptodactylus troglodytes A. Lutz, 1926	EO
	Leptodactylus gr *latrans*	DS (Barreto et al. 2011)
	Lithodytes lineatus (Schneider, 1799)*	(Freitas et al. 2014)
Leiuperidae	*Physalaemus cuvieri* Fitzinger, 1826	BA/Pitfalls
Microhylidae	*Ctenophryne geayi* Mocquard, 1904 *	(Freitas et al. 2014)
Chelidae	*Platemys platycephala* (Schneider, 1792) *	EO
Geoemydidae	*Rhinoclemmys punctularia* (Daudin, 1801)	EO
Kinosternidae	*Kinosternon scorpioides* (Linnaeus, 1766)	EO
Podocnemididae	*Podocnemis unifilis* Troschel, 1848	EO
Testudinidae	*Chelonoidis carbonarius* (Spix, 1824)	EO
	Chelonoidis denticulatus (Linnaeus, 1766)	EO
Alligatoridae	*Caiman crocodilus* (Linnaeus, 1758)	EO
	Paleosuchus trigonatus (Schneider, 1801) *	EO
Dactyloidae	*Dactyloa punctata* (Daudin, 1802)	BA
	Norops fuscoauratus (D'Orbigny, 1837)	BA/Pitfalls/EO
	Norops tandai (Ávila-Pires, 1995)	DS (Ávila-Pires 1995)

FAMILY	SPECIES	TYPE OF RECORD
Gekkonidae	*Hemidactylus mabouia* (Moreau de Jonnès, 1818)	EO
Gymnophthalmidae	*Micrablepharus maximiliani* (Reinhardt & Luetken, 1862)	EO
	Cercosaura argulus Peters, 1863	(Freitas et al. 2013)
	Cercosaura ocellata Wagler, 1830	DS (Ávila-Pires 1995)
	Colobosaura modesta (Reinhardt & Luetken, 1862)	DS (Ávila-Pires 1995)
	Arthrosaura reticulata (O'Shaughnessy, 1881)	DS (Ávila-Pires 1995)
	Leposoma percarinatum Muller, 1923 *	Pitfalls
	Neusticurus bicarinatus (Linnaeus, 1758)**	DS (Ávila-Pires 1995)
Iguanidae	*Iguana iguana* (Linnaeus, 1758)	EO
Leiosauridae	*Enyalius leechii* (Boulenger, 1885)	DS (Ávila-Pires 1995)
Mabuyidae	*Copeoglossum nigropunctatum* (Spix, 1825)	Pitfalls
Phyllodactylidae	*Thecadactylus rapicauda* (Houttuyn, 1782)	EO
Polychrotidae	*Polychrus acutirostris* Spix, 1825	DS (Ávila-Pires 1995)
	Polychrus marmoratus (Linnaeus, 1758)	EO
Sphaerodactylidae	*Coleodactylus septentrionalis* (Vanzolini, 1980)**	Pitfalls
	Gonatodes humeralis (Guichenot, 1855)	Pitfalls/BA
Teiidae	*Ameiva ameiva* (Linnaeus, 1758)	Pitfalls/EO/BA
	Cnemidophorus cryptus Cole & Dessauer, 1993 *	EO
	Kentropyx calcarata Spix, 1825	Pitfalls/BA
	Salvator merianae (Duméril & Bibron, 1839)	EO/BA
	Tupinambis teguixin (Linnaeus, 1758)	DS (Ávila-Pires 1995)
	Dracaena guianensis Daudin, 1801	DS (Ávila-Pires 1995)
Tropiduridae	*Plica umbra* (Linnaeus, 1758)	Pitfalls
	Stenocercus dumerilii (Steindachner, 1867)**	Pitfalls
	Tropidurus hispidus (Spix, 1825)	EO/BA
	Uracentron azureum (Linnaeus, 1758)	DS (Ávila-Pires 1995)
	Uranoscodon superciliosus (Linnaeus, 1758)	BA
Amphisbaenidae	*Amphisbaena alba* Linnaeus, 1758	DS (Barreto et al. 2011)
	Amphisbaena amazonica Vanzolini, 1951	EO
Typhlopidae	*Amerotyphlops brongersmianus* (Vanzolini, 1976)	Pitfalls
Aniliidae	*Anilius scytale* (Linnaeus, 1758)	EO
Boidae	*Boa constrictor* Linnaeus, 1758	EO
	Corallus hortulanus (Linnaeus, 1758)	EO
	Epicrates cenchria (Linnaeus, 1758)	EO
	Eunectes murinus (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
Colubridae	*Chironius carinatus* (Linnaeus, 1758)	EO
	Chironius exoletus (Linnaeus, 1758)	EO
	Chironius fuscus (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
	Chironius multiventris Schmidt & Walker, 1943	DS (Cunha and Nascimento 1993)
	Chironius scurrulus (Wagler in Spix, 1824)	DS (Cunha and Nascimento 1993)
	Drymarchon corais (Boie, 1827)	EO
	Drymoluber dichrous (Peters, 1863)	DS (Cunha and Nascimento 1993)
	Leptophis ahaetulla (Linnaeus, 1758)	EO
	Mastigodryas bifossatus (Raddi, 1820)	DS (Cunha and Nascimento 1993)

FAMILY	SPECIES	TYPE OF RECORD
Colubridae	*Mastigodryas boddaerti* (Sentzen, 1796)	EO
	Oxybelis aeneus (Wagler in Spix, 1824)	DS (Barreto et al. 2011)
	Oxybelis fulgidus (Daudin, 1803)	EO
	Phrynonax poecilonotus (Peters, 1867)*	EO
	Rhinobothryum lentiginosum (Scopoli, 1785) *	EO
	Spilotes pullatus (Linnaeus, 1758)	EO
	Tantilla melanocephala (Linnaeus, 1758)	Pitfalls
Dipsadidae	*Atractus alphonsehogei* Cunha & Nascimento, 1983	DS (Cunha and Nascimento 1983)
	Atractus schach (Boie, 1827)	DS (Cunha and Nascimento 1993)
	Atractus snethlageae Cunha & Nascimento, 1983	DS (Cunha and Nascimento 1993)
	Dipsas catesbyi (Sentzen, 1796)	EO
	Dipsas variegata (Duméril, Bibron & Duméril, 1854)	DS (Cunha and Nascimento 1993)
	Erythrolamprus aesculapii (Linnaeus, 1766)	DS (Cunha and Nascimento 1993)
	Erythrolamprus cobella (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
	Erythrolamprus miliaris chrysostomus (Griffin, 1916)	DS (Cunha and Nascimento 1993)
	Erythrolamprus oligolepis (Boulenger, 1905)	DS (Cunha and Nascimento 1993)
	Erythrolamprus poecilogyrus (Wied, 1825)	EO
	Sibon nebulatus (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
	Sibynomorphus mikanii septentrionalis Cunha, Nascimento & Hoge, 1980 **	EO (Freitas et al. 2014)
	Imantodes cenchoa (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
	Leptodeira annulata (Linnaeus, 1758)	EO/BA
	Helicops angulatus (Linnaeus, 1758)	DS (Barreto et al. 2011)
	Hydrops martii (Wagler in Spix, 1824)	DS (Cunha and Nascimento 1993)
	Pseudoeryx plicatilis (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
	Philodryas olfersii (Liechtenstein, 1823)	EO
	Philodryas viridissima (Linnaeus, 1758)	EO
	Clelia plumbea (Wied, 1820)	EO
	Oxyrhopus melanogenys Cunha & Nascimento, 1983	BA
	Oxyrhopus petolarius (Reuss, 1834)	EO
	Pseudoboa coronata Schneider, 1801	DS (Cunha and Nascimento 1993)
	Pseudoboa neuwiedii (Duméril, Bibron & Duméril, 1854)	EO
	Pseudoboa nigra (Duméril, Bibron & Duméril, 1854)	EO
	Siphlophis cervinus (Laurenti, 1768)	DS (Cunha and Nascimento 1993)
	Taeniophallus quadriocellatus Santos-Jr, Di-Bernardo & Lema, 2008	DS (Cunha and Nascimento 1993)

FAMILY	SPECIES	TYPE OF RECORD
Dipsadidae	*Xenodon merremii* (Wagler in Spix, 1824)	EO
	Xenodon severus (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
Elapidae	*Micrurus filiformis* (Günther, 1859)	DS (Cunha and Nascimento 1993)
	Micrurus hemprichii (Jan, 1858)	DS (Cunha and Nascimento 1993)
	Micrurus lemniscatus lemniscatus (Linnaeus, 1758)	DS (Cunha and Nascimento 1993)
	Micrurus spixii martiusi Schmidt, 1953 **	BA
	Micrurus surinamensis (Cuvier, 1817)	DS (Cunha and Nascimento 1993)
Viperidae	*Bothrops atrox* (Linnaeus, 1758)	BA/EO
	Bothrops brazili Hoge, 1954	DS (Cunha and Nascimento 1993)
	Bothrops taeniatus Wagler in Spix, 1824	DS (Cunha and Nascimento 1993)
	Lachesis muta (Linnaeus, 1766)	DS (Cunha and Nascimento 1993)

Maranhão (Barreto et al. 2011). It is noteworthy that the last of these studies includes species of open areas, which are present due to the strong influence of Cerrado vegetation in northern Maranhão, as seen in the inventory of Miranda et al. (2012), which lists 42 species of reptiles for the coastal region of Maranhão, which is characterized by open vegetation and the predominance of coastal dunes.

The species richness of REBIO Gurupi is second only to the richness sampled in the National Forest of Saracá-Taquera in northern Pará, located in the Guyana Endemism Center, more than 1000 km from REBIO Gurupi (Morato et al. 2014).

Regarding sampling efficiency, the collection effort and methods used were not effective, as not all niches were sampled. As a result, the curve implied 33 amphibian species and 78 reptile species at the site. As the curves did not reach an asymptote, we believe that site has potential for species that have not yet been recorded. This is borne out by the additional 58 species not found during the field work but recorded in the literature for the area (Cunha and Nascimento 1993; Avila-Pires 1995; Barreto et al. 2011) (Figure 2). Regarding collection methods, pitfall traps yielded only 33 species of the 78 found in the field, chiefly amphibians, frogs, and fossorial lizards.

Conclusion

Since only 38% of the recorded species included in this inventory were obtained from literature (Table 1), it is possible to ascertain that our sampling reflects a great share of the total herpetofauna from Northwestern Maranhão. With this richness, the Gurupi Biological Reserve stands out as a biodiversity hotspot in the Amazon, reinforcing the need for its protection, and its position as the most important restricted use conservation unit of the Belém Endemism Center.

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Appendix I

Specimens examined

Rhinella marina CHP-UFRPE 1810 – *Rhinella gildae* CHP-UFRPE 1848 *Leptodactylus paraensis* CHP-UFRPE 1811-1845 – *Leptodactylus petersii* MZUSP – 154062 – *Lithodytes lineatus* CHP-UFRPE 1815 – *Leptodactylus mystaceus* CHP-UFRPE 1838-44-1863-64-1878 – *Physalaemus cuvieri* CHP-UFRPE 1834-37 - *Pristimantes fenestratus* CHP-UFRPE 1862-2042, MZUSP 154061 - *Phyllomedusa vaillantii* CHP-UFRPE 1874-2621 - *Hypsiboas punctatus* CHP-UFRPE 1877 - *Ctenophryne geayi* CHP-UFRPE 1820-1859-1876 - *Scinax ruber* CHP-UFRPE 1816 - *Dentropsophus marmoratus* CHP-UFRPE 1818 - *Trachycephalus tyfonius* CHP-UFRPE 1824-25 – *Adenomera andreae* MZUSP 154063-69 *Cercosaura argulus* CHP-UFRPE 2622 - *Amphisbaena amazonica* CHP-UFRPE 2065 - *Plica umbra* CHP-UFRPE 1826-28-1873 - *Iguana iguana* CHP-UFRPE 1870 - *Micrablepharus maximiliani* CHP-UFRPE 1866-69 - *Norops fuscoauratus* CHP-UFRPE 1817-1865 - *Tropidurus hispidus* CHP-UFRPE 1846 - *Copeoglosum nigropunctatum* CHP-UFRPE 1832-33 - *Gonatodes humeralis* CHP-UFRPE 1821-23 - *Coleodactylus septentrionalis* CHP-UFRPE 1819 - *Uranoscodon superciliosus* CHP-UFRPE 1813 - *Polychrus marmoratus* CHP-UFRPE 1856 - *Kentropys calcarata* CHP-UFRPE 1830-31-MZUSP 104280 – *Leposoma percarinatum* MZUSP 104278 – *Stenocercus dumerilii* MZUSP 104279 – *Cnemidophorus cryptus* MZUSP 104281 – *Salvator merianae* MZUSP-MTR 23332 - *Platemys platicephala* CHP-UFRPE 1814 - *Kinosternum scorpioides* CHP-UFRPE 2126 - *Amerotyphlops brongersmianus* CHP-UFRPE 1850 - *Anilius scytale* CHP-UFRPE 1860 - *Boa constrictor* CHP-UFRPE 2439 - *Corallus hortulanus* CHP-UFRPE 2457 - *Spilotes pullatus* CHP-UFRPE 1873 - *Leptophis ahaetulla* CHP-UFRPE 2267 - *Tantila melanocephala* CHP-UFRPE 1829 - *Sibynomorpus mikani septentrionalis* CHP-UFRPE 1858-2234-38 - *Oxyrhopus pelotarius* CHP-UFRPE 2220 - *Oxyrhopus melanogenys* CHP-UFRPE 1829 - *Pseudoboa nigra* CHP-UFRPE 1852-53 - *Pseudoboa newiedii* CHP-UFRPE 1860 - *Erythrolamprus poecilogyrus* CHP-UFRPE 1875-1851 - *Leptodeira annulata* CHP-UFRPE 1847 - *Philodryas olfersii* CHP-UFRPE 1854 - *Dipsas catesbyi* CHP-UFRPE 1872 - *Micrurus spixii martiusi* CHP-UFRPE 1812 - *Bothrops atrox* CHP-UFRPE 1855.