

Why be original? Two new species of *Choeradoplana* resembling the type species of the genus in their external aspects (Platyhelminthes, Continenticola)

Giuly Gouvêa Iturralde¹, Ana Leal-Zanchet¹

¹ Instituto de Pesquisas de Planárias and Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos, 93022-750 São Leopoldo, Rio Grande do Sul, Brazil

Corresponding author: Ana Maria Leal-Zanchet (zanchet@unisinos.br)

Academic editor: Tom Artois | Received 10 September 2018 | Accepted 31 October 2018 | Published 7 January 2019

<http://zoobank.org/AA071B67-392E-4B82-ADEC-CA05E6ED7770>

Citation: Iturralde GG, Leal-Zanchet AM (2019) Why be original? Two new species of *Choeradoplana* resembling the type species of the genus in their external aspects (Platyhelminthes, Continenticola). ZooKeys 813: 1–19. <https://doi.org/10.3897/zookeys.813.29565>

Abstract

The genus *Choeradoplana* Graff, 1896 encompasses 16 species, most of them found in Brazil. Herein two new species of this genus are described from remnants of *Araucaria* moist forests, located in the states of Paraná and Santa Catarina, south Brazil. Both species resemble the type-species of the genus, *C. iheringi*, showing brownish dorsal surface covered by dark-brown flecks. However, regarding their anatomy, the new species differ from *C. iheringi* and other congeners by a long and horizontal disposed permanent papilla. In such aspects, both species resemble *C. benyai*, but differ from this species, as well as from each other, in details of the prostatic vesicle, ejaculatory duct, and penis papilla.

Keywords

Araucaria Forest, Neotropical region, taxonomy, Tricladida

Introduction

The genus *Choeradoplana*, proposed by Graff (1896), has a Neotropical distribution, with most species recorded from Brazil. Its representatives show a cephalic region with a glandulo-muscular organ and longitudinal cutaneous musculature with a portion sunk into the mesenchyme (Graff 1899, Ogren and Kawakatsu 1990). The genus was reviewed by

Froehlich (1955). Carbayo and Leal-Zanchet (2003) and Carbayo and Froehlich (2012) suggested the inclusion of other features in its diagnosis. Currently, the genus encompasses 16 species, six of them occurring in areas of ombrophilous *Araucaria* forest (Graff 1899, Ogren and Kawakatsu 1990, Leal-Zanchet and Souza 2003, Carbayo and Froehlich 2012, Negrete and Brusa 2012, Lemos et al. 2014, Álvarez-Presas et al. 2017).

The *Araucaria* forest is a phytophysiognomy of the Atlantic Forest, which harbours a high species richness of land flatworms (Sluys 1998, Leal-Zanchet and Carbayo 2000, Castro and Leal-Zanchet 2005, Antunes et al. 2008, Leal-Zanchet and Baptista 2009; Baptista et al. 2010, Leal-Zanchet et al. 2011, Amaral et al. 2014). A recent inventory of land flatworms in areas of *Araucaria* moist forest located in the states of Paraná and Santa Catarina, in south Brazil, indicated the occurrence of brownish specimens with dark-brown flecks over the dorsal surface, similar to the type-species of the genus, *Choradoplana iheringi* Graff, 1899. Anatomical and histological analyses indicated that they belong to two different species that are herein described.

Materials and methods

Flatworms were sampled in two protected areas located in the Iguassu River Drainage Basin, in south Brazil, namely the *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, and 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. Specimens were collected by visual search during the night, when they are more active.

Just after sampling, colour pattern, body shape and dimensions of live specimens were recorded. They were then euthanised using boiling water and fixed in neutral formalin 10%. After fixation, specimens were 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 characteristics. 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:

cmc	common muscle coat;	di	dorsal insertion of pharynx;
cov	common glandular ovovitelline duct;	dm	dorsal cutaneous musculature;
cs	creeping sole	e	eyes;
de	dorsal epidermis;	ej	ejaculatory duct;
		fc	female canal;

gc	glandular cushions;	pp	pharyngeal pouch;
go	gonopore;	pv	prostatic vesicle;
i	intestine;	r	retractor muscle;
im	internal musculature;	rg	rhabditogen glands;
m	mouth;	sc	secretory cells;
mm	mesenchymal muscles;	sg	shell glands;
mn	“muscle net”;	slm	sunken longitudinal cutaneous muscles;
n	nerve plate;	sv	spermiducal vesicle;
nlm	normal longitudinal cutaneous muscles;	t	testes;
om	outer musculature;	v	vitelline follicles;
ov	ovovitelline ducts;	ve	ventral epidermis;
p	penis papilla;	vi	ventral insertion of pharynx.

Taxonomy

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Genus *Choeradoplana* Graff, 1896

***Choeradoplana longivesicula* sp. n.**

<http://zoobank.org/FC60603B-DBA9-4B55-BB17-FF9E0B337C61>

Figures 1–11

Type-material. Holotype: MZUSP PL.2143: leg. JAL Braccini, 6 June 2015, General Carneiro (*Araucaria* Natural Heritage Private Reserve), PR, Brazil – anterior tip: transverse sections on 24 slides; anterior region at the level of the ovaries: sagittal sections on 27 slides; pre-pharyngeal region: transverse sections on 8 slides; pharynx: sagittal sections on 31 slides; copulatory apparatus: sagittal sections on 25 slides. **Paratype:** MZU PL.00292: leg. I Rossi, 6 June 2015, General Carneiro (*Araucaria* Natural Heritage Private Reserve), state of Parana, Brazil – anterior tip: transverse sections on 27 slides; anterior region at the level of the ovaries: sagittal sections on 24 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx: sagittal sections on 22 slides; copulatory apparatus: sagittal sections on 19 slides.

Diagnosis. A species of *Choeradoplana* with dorsal surface covered by irregular small dark-brown flecks and thin median light stripe; pharynx bell-shaped; sperm ducts opening laterally into proximal wall of prostatic vesicle; prostatic vesicle tubular and unpaired, narrowing to open through tip of penis papilla as an ejaculatory duct; penis papilla cylindrical and almost symmetrical, filling entire common atrium; atrium oval-elongate and unfolded, without differentiation between male and female regions.

Description. External features. Body elongate with parallel margins (Figs 1, 2), elliptical in cross section; anterior end expanded, posterior end slight pointed. Cephalic region (ca. 3 mm long) with two glandular cushions separated by a median longitudinal slit in the ventral surface. Maximum length 67 mm when crawling; 50 mm after fixation (Table 1). Mouth at median third of body; gonopore at posterior third of body (Table 1).

Live specimens with dorsal surface covered by dark-brown pigmentation constituted by irregular, small flecks. Yellowish ground colour visible on cephalic region, on body margins, as well as on thin median stripe occurring along the body except for cephalic region (Figs 1, 2). Ventral surface whitish before and after fixation. After fixation, dorsal pigmentation fades.

Eyes absent on anterior tip (first 1.5 mm of body, corresponding to 3% of body length). Afterwards, eyes initially monolobate and uniserial. Eyes become trilobate and pluriserial after 3 mm and sparser towards posterior tip. No clear halos around eyes. Pigment cups between 20 µm and 30 µm in diameter.

Sensory organs, epidermis and body musculature. Sensory pits, as simple invaginations (15–20 µm deep), absent on anterior tip, occurring in a single row between 3% and 10% of body length. Three types of glands discharge through whole epidermis of pre-pharyngeal region: rhabditogen glands with xanthophil rhammites (ventrally with smaller rhabdites) and cyanophil glands with amorphous secretion, as well as few erythrophil glands with fine granular secretion (Figs 3, 4). Creeping sole occupies 82% of body width. Glandular margin absent. Glands discharging through anterior tip of body with similar arrangement as in other species of the genus.

Table 1. Measurements, in mm, of specimens of *Choeradoplana longivesicula* 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. 2143	Paratype MZU PL.00292
Length at rest	34	47
Width at rest	6	4
Maximum length in extension	62	67
Maximum width in extension	3	3
Length*	52	49
Width*	4	4
DM*	33(63)	30 (61)
DG*	39(75)	35 (71)
DMG*	6	5
DPVP*	1.8	1.6
Creeping sole %	86	82
Ovaries	18(35)	16 (32)
Anteriormost testes	17(33)	15 (30)
Posteriormost testes	28(54)	26 (53)
Length of prostatic vesicle	2.5	2.3
Length of penis papilla	2.1	1.9
Length of atrium	2.4	2.3
Female canal	0.8	0.7
Common glandular ovovitelline duct	0.1	0.1

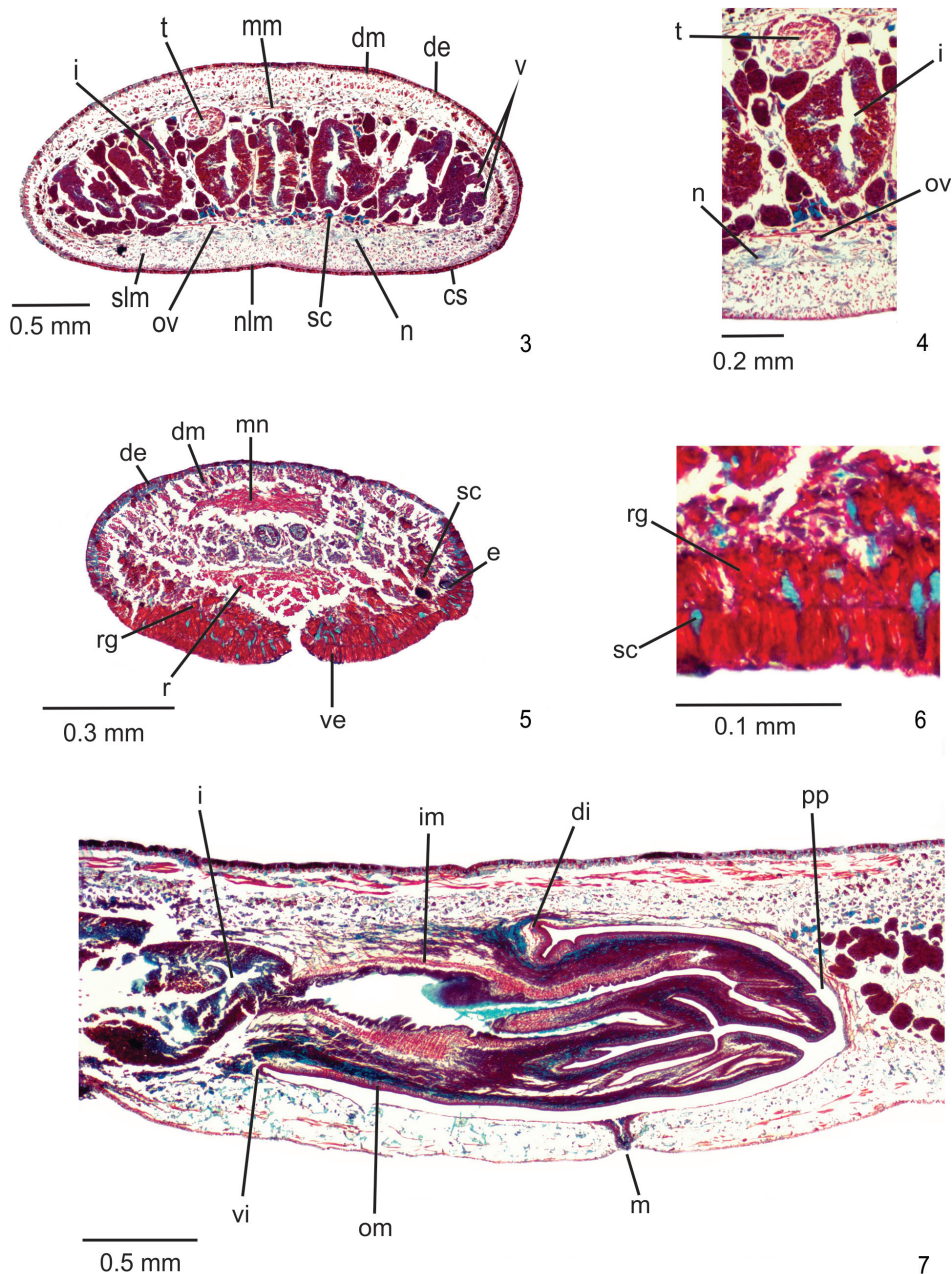


1



2

Figures 1, 2. *Choeradoplana longivesicula* sp. n., paratype MZU PL. 00292, **1, 2** dorsal view, with part of whitish ventral surface visible in **1**. Arrows indicate the anterior tip.



Figures 3–7. *Choeradoplana longivesicula* sp. n., paratype MZU PL. 00292 **3, 4** pre-pharyngeal region, transverse sections **5, 6** anterior region of body, transverse sections **7** pharynx, sagittal section, with anterior tip to the left.

Cutaneous musculature with usual three layers (circular, oblique, and longitudinal layers), showing part of ventral longitudinal layer, as well as some muscle bundles of dorsal longitudinal layer, imbedded in mesenchyme (Fig. 3, Table 2). Longitudinal layer between four and eight times thicker than other two cutaneous layers in pre-pharyngeal

Table 2. Body height and cutaneous musculature in the median region of a transverse section of the pre-pharyngeal, in micrometres, and ratio of the thickness of cutaneous musculature to the height of the body (mc:h index) of specimens of *Choeradoplana longivesicula* sp. n.

Measurement	Holotype MZUSP PL.2143	Paratype MZU PL.00292
Dorsal circular	3	4
Dorsal oblique	25	24
Dorsal longitudinal	100	108
Dorsal imbedded	20	18
Dorsal total	148	154
Ventral circular	2	3
Ventral oblique	15	13
Ventral longitudinal	40	38
Ventral imbedded	70	67
Ventral total	127	121
Body height	1421	1413
Mc:h(%)	19	19

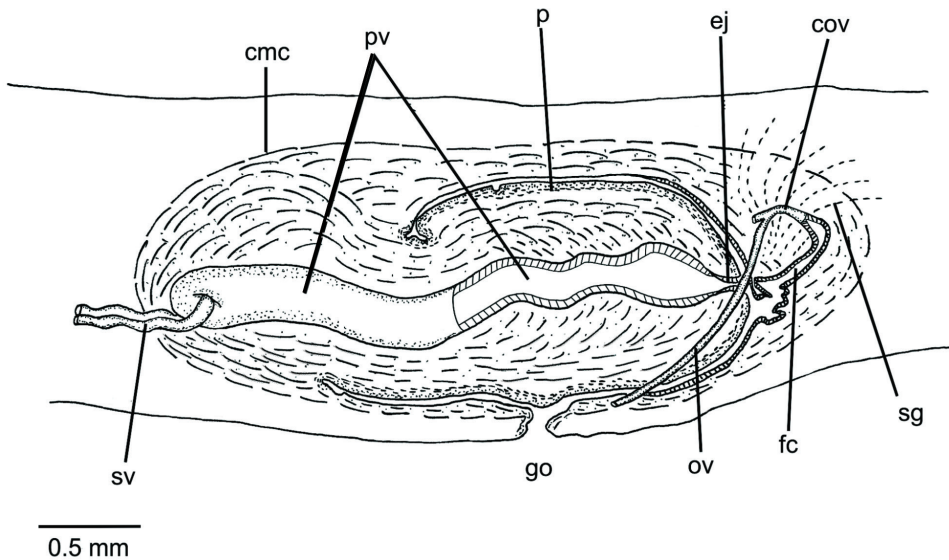
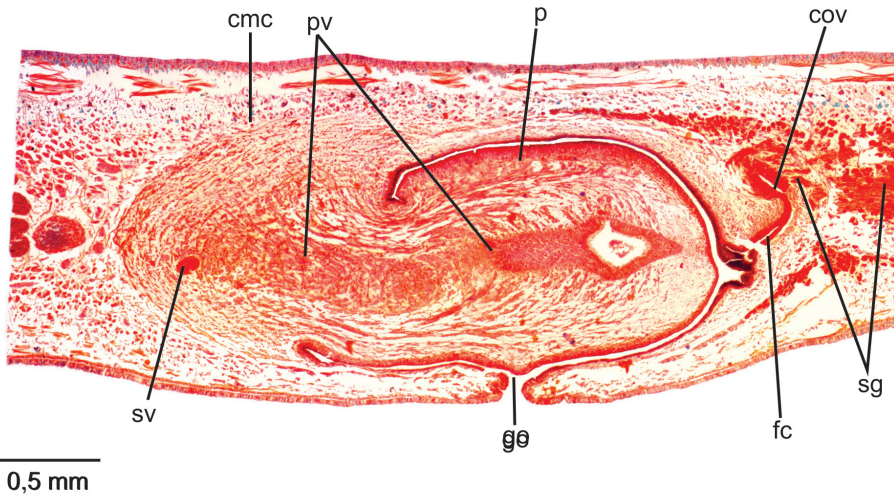
region (Table 2). Cutaneous musculature as thick paramedially as medially. Ventral musculature slightly thinner than dorsal in pre-pharyngeal region. Ratio between cutaneous musculature and body height (mc:h) ca. 19% (Table 2). In cephalic region, cutaneous musculature with similar arrangement as in other species of the genus (Fig. 5).

Mesenchymal musculature (Fig. 3) weakly developed, mainly composed of three layers: (1) dorsal subcutaneous, located mainly close to cutaneous musculature, with decussate fibres (2–3 fibres thick), (2) supra-intestinal transverse (3–5 fibres thick) and (3) sub-intestinal transverse (4–6 fibres thick). In cephalic region, mesenchymal musculature with similar arrangement as in other species of the genus (Fig. 5).

Digestive System. Pharynx bell-shaped, ca. 6% of body length, occupies ca. 90% of pharyngeal pouch. Mouth slightly posterior to dorsal insertion next to end of median third of pharyngeal pouch (Fig. 7). Oesophagus absent.

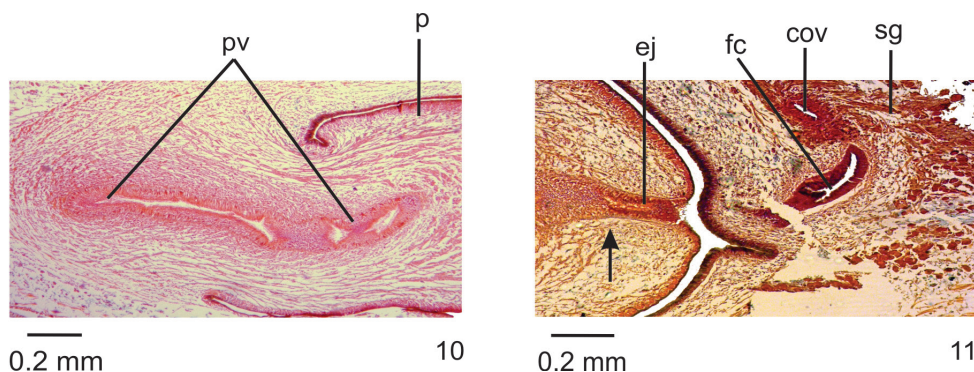
Reproductive organs. Testes in one or two irregular rows on either side of body, located beneath dorsal transverse mesenchymal muscles, between intestinal branches (Fig. 3), begin slightly anteriorly to ovaries, in anterior third of body, and extend to next to root of the pharynx (Table 1). Sperm ducts dorsal to ovovitelline ducts, under or among fibres of sub-intestinal transverse mesenchymal musculature, in pre-pharyngeal region (Figure 3). They form spermiducal vesicles posteriorly to pharynx. Sperm ducts enter common muscle coat, ascend slightly and open laterally into proximal wall of prostatic vesicle. Intrabulbar prostatic vesicle, tubular and unpaired, traverses both penis bulb and papilla (Fig. 10), narrowing to open through tip of papilla as an ejaculatory duct (Fig. 11). Penis papilla, cylindrical and almost symmetrical, filling entire common atrium. Dorsal insertion of penis papilla slightly shifted posteriorly. Common atrium oval-elongate and unfolded, without differentiation between male and female atria (Figs 8, 9).

Prostatic vesicle lined with high pseudostratified epithelium with few ciliated cells, receiving numerous openings of glands with ill-stained, coarse granular secretion, sometimes containing an erythrophil core. In addition, sparse openings of other two types of glands occur throughout the epithelium of prostatic vesicle: cells with xanthophil, coarse granular secretion, and cell with amorphous, cyanophil secretion.



Figures 8, 9. *Choeradoplana longivesicula* sp. n., holotype **8** copulatory apparatus, sagittal section **9** sagittal composite reconstruction of copulatory apparatus. Anterior tip to the left.

Ejaculatory duct lined with columnar, densely ciliated epithelium. Both penis papilla and atrium lined with non-ciliated columnar epithelium, becoming cuboidal towards tip of papilla, showing a xanthophil apical region. Numerous glands of two types open through epithelial lining of penis papilla and atrium: one with xanthophil, coarse granular secretion and the other with amorphous, cyanophil secretion. In addition, a third type, with fine granular, erythrophil secretion, opens through epithelial lining of penis papilla. Muscularis of penis papilla (30–60 μ m) composed of subepithelial layer with circular fibres followed by some longitudinal fibres; that of atrium composed of longitudinal and circular interwoven fibres (10–15 μ m).



Figures 10, 11. *Choeradoplana longivesicula* sp. n., holotype, sagittal sections **10** prostatic vesicle **11** ejaculatory duct and female canal. Anterior tip to the left. Arrow indicates the transition from the prostatic vesicle to the ejaculatory duct.

Vitelline follicles situated between intestinal branches, well developed. Ovaries ovoid, ca. twice longer than wide, measuring 0.2 mm in its antero-posterior axis. They are located dorsally to the ventral nerve plate, in anterior third of body. Ovo-vitelline ducts emerge laterally from median third of ovaries, then run posteriorly immediately dorsal to the nerve plate. Behind the gonopore, the ovovitelline ducts ascend posteriorly and medially inclined, and unite, dorsally to the female canal, forming the common glandular ovovitelline duct. Female canal relatively long and C shaped. This canal opens into bottom of posterior part of atrium, where a constriction occurs (Figs 8, 9).

Female canal lined with erythrophil, pseudostratified epithelium. Three types of glands open through the epithelium of female canal: abundant cells with finely granular, erythrophil secretion, cells with coarse granular, xanthophil secretion, and scarce cells with amorphous, cyanophil secretion. Muscularis of female canal composed of longitudinal and circular interwoven fibres (20–30 μ m)

Gonopore canal almost vertical at the sagittal plane. Common muscle coat highly developed, especially at penis bulb (Figs 8, 9), with interwoven oblique, circular and longitudinal fibres.

Etymology. The name is composed of the Latin adjective *longus* (long) and the Latin *vesicula*, alluding to the elongate prostatic vesicle.

Distribution. known only from the type-locality, General Carneiro, Paraná, Brazil.

***Choeradoplana cyanoatria* sp. n.**

<http://zoobank.org/424F7AF0-33EE-4ED0-9500-27EB77503752>

Figures 12–18

Type-material. Holotype: MZUSP PL.2144: leg. JAL Braccini, 2 June 2015, Três Barras (National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 17 slides; anterior region at the level of the ovaries: sagittal sections on 16



Figure 12. *Choeradoplana cyanoatria* sp. n., holotype, dorsal view. Arrow indicates the anterior tip.

slides; pre-pharyngeal region: transverse sections on 7 slides; pharynx and copulatory apparatus: sagittal sections on 25 slides.

Diagnosis. A species of *Choeradoplana* with dorsal surface covered by irregular small dark-brown flecks; pharynx bell-shaped; sperm ducts opening subterminally into prostatic vesicle; prostatic vesicle oval-elongate and folded, becoming funnel-shaped proximally and forming an elongate duct inside penis papilla; penis papilla, conical, long and almost symmetrical, with dorsal insertion shifted posteriorly, filling the whole atrium.

Description. External features. Body elongate with parallel margins (Fig. 12), sub-cylindrical in cross section; anterior end expanded, posterior end slight pointed. Cephalic region (ca. 3 mm long) with two glandular cushions and a median slit in the ventral surface. Maximum length 20 mm when resting; 50 mm after fixation (Table 3). Mouth at median third of body; gonopore at posterior third of body (Table 3).

Live specimens with dorsal surface covered by irregular, small dark-brown flecks over all body length including cephalic region (Fig. 12). Yellowish ground colour visible on cephalic region, on body margins, as well as on thin, inconspicuous median stripe occurring along the anterior body half except for cephalic region. Ventral surface pale yellow. After fixation, dorsal pigmentation remains brownish; ventral surface becomes whitish with darker body margins.

Eyes absent on cephalic region (first 1.2 mm of body, corresponding to 2.4% of body length). After that, eyes initially monolobate and uniserial. Eyes become trilobate and pluriserial after 3 mm, becoming sparser towards posterior tip. No clear halos around eyes. Diameter of pigment cups between 24 μ m and 32 μ m in diameter.

Table 3. Measurements, in mm, of the holotype of *Choeradoplana cyanoatria* 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.

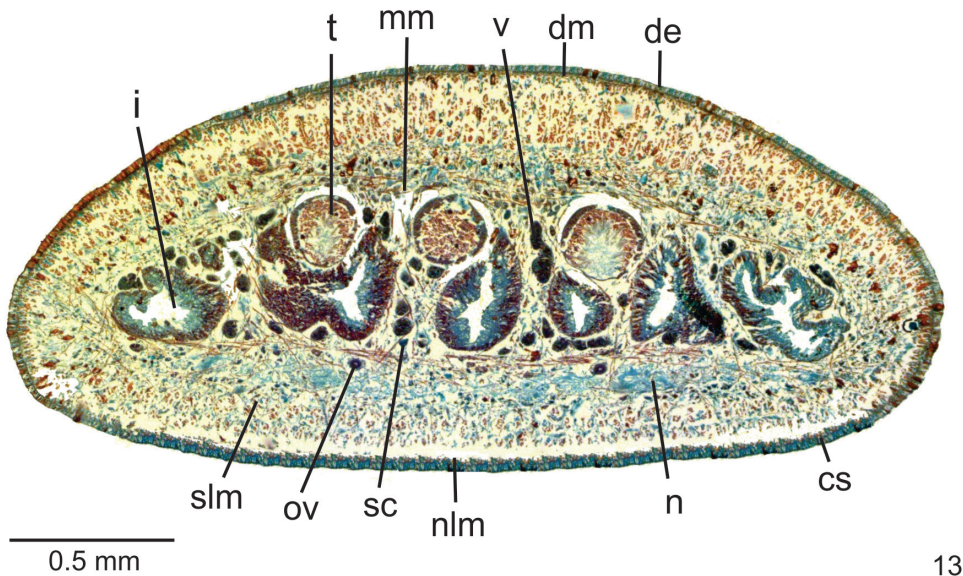
Measurement	HolotypeMZUSP PL.2144
Length at rest	20
Width at rest	4
Maximum length in extension	28
Maximum width in extension	3
Length*	50
Width*	4
DM*	33(66)
DG*	40(80)
DMG*	7
DPVP*	1.2
Creeping sole %	89
Ovaries	8 (16)
Anteriormost testes	9 (18)
Posteriormost testes	29 (58)
Length of prostatic vesicle	0.6
Length of penis papilla	1.3
Length of the atrium	1.6
Female canal	0.4
Common glandular ovovitelline duct	0.1

Sensory organs, epidermis and body musculature. Sensory pits, as simple invaginations (15–18 μ m deep), absent on anterior tip, occurring in a single row between 3% and 10% of body length.

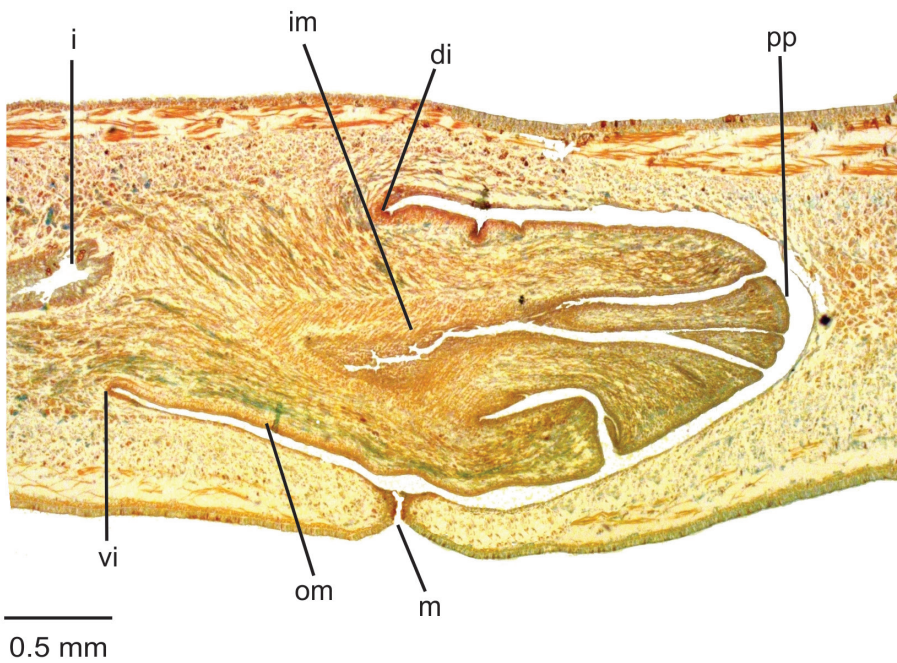
Three types of glands discharge through whole epidermis of pre-pharyngeal region: rhabditogen glands with xanthophil rhammites (ventrally with smaller rhabdites) and cyanophil glands with amorphous secretion, as well as few xanthophil glands with coarse granular secretion (Fig. 13). Creeping sole occupies 89% of body width. Glandular margin absent. Glands discharging through anterior tip of body with similar arrangement as in other species of the genus.

Cutaneous musculature with usual three layers (circular, oblique, and longitudinal layers), with part of ventral longitudinal layer, as well as few muscle bundles of dorsal longitudinal layer, imbedded in mesenchyme (Fig. 13, Table 4). Longitudinal layer between five and eight times thicker than other two cutaneous layers in pre-pharyngeal region (Table 4). Cutaneous musculature as high paramedially as medially. Ventral musculature thinner than dorsal in pre-pharyngeal region. Mc:h 22% (Table 4). In cephalic region, cutaneous musculature with similar arrangement as in other species of the genus.

Mesenchymal musculature (Fig. 13) weakly developed, mainly composed of three layers: (1) dorsal subcutaneous, located mainly close to cutaneous musculature, with decussate fibres (2 fibres thick), (2) supra-intestinal transverse (2–4 fibres thick) and (3) sub-intestinal transverse (5–7 fibres thick). In cephalic region, mesenchymal musculature with similar arrangement as in other species of the genus.



13



14

Figures 13, 14. *Choeradoplana cyanoatria* sp. n., holotype **13** pre-pharyngeal region, transverse section **14** pharynx, sagittal section, with anterior tip to the left.

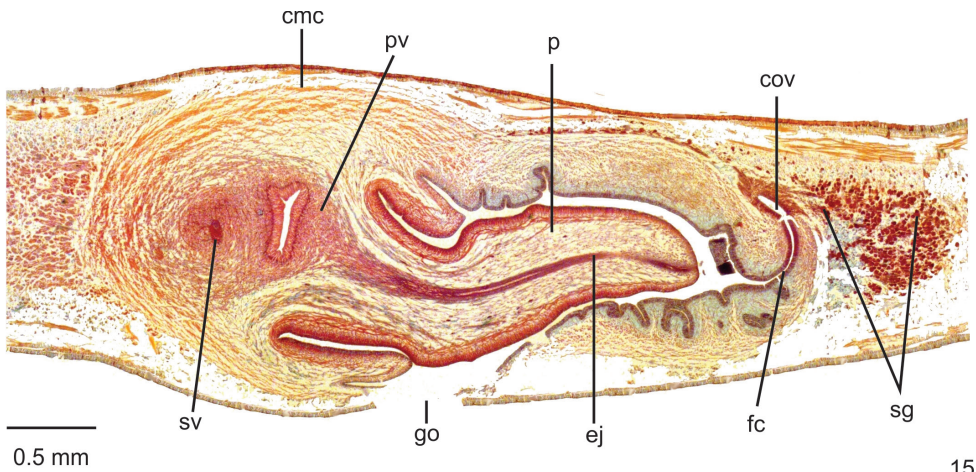
Digestive system. Pharynx bell-shaped, as long as 7% of body length, occupies almost entire pharyngeal pouch. Mouth almost at the same transversal level as dorsal insertion in the beginning of median third of pharyngeal pouch (Fig. 14). Oesophagus absent.

Table 4. Body height and cutaneous musculature in the median region of a transverse section of the pre-pharyngeal, in micrometres, and ratio of the thickness of cutaneous musculature to the height of the body (mc:h index) of the holotype of *Choeradoplana cyanoatria*.

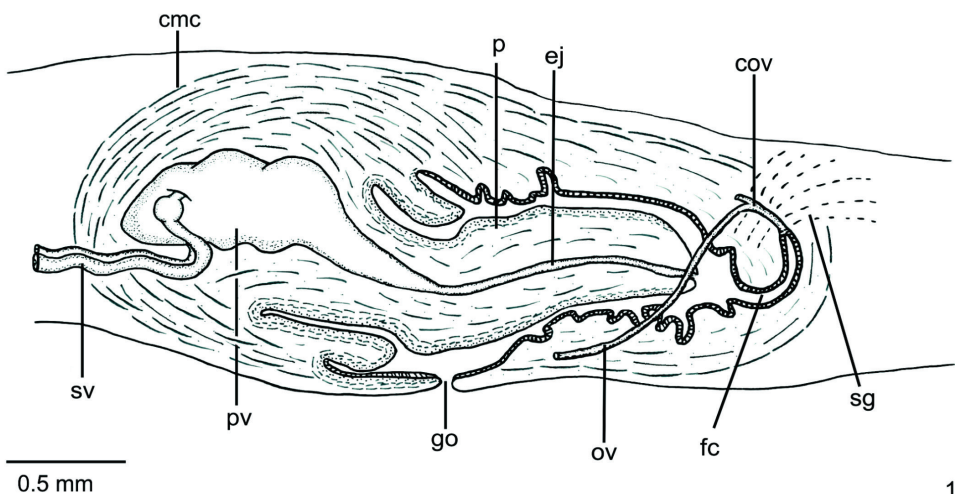
Measurement	Holotype MZUSP PL.2144
Dorsal circular	4
Dorsal oblique	8
Dorsal longitudinal	33
Dorsal imbedded	27
Dorsal total	72
Ventral circular	2
Ventral oblique	15
Ventral longitudinal	130
Ventral imbedded	5
Ventral total	152
Body height	1025
Mc:h(%)	22

Reproductive organs. Testes in two or three irregular rows on either side of body, located beneath dorsal transverse mesenchymal muscles, between intestinal branches (Fig. 13). They begin slightly anteriorly to ovaries, in anterior sixth of body, to just the root of the pharynx (Table 3). Sperm ducts dorsal to ovovitelline ducts, medially displaced, under or among fibres of sub-intestinal transverse mesenchymal musculature, in pre-pharyngeal region (Fig. 13). They form spermiducal vesicles posteriorly to pharynx. Sperm ducts enter common muscle coat, recurve, and open subterminally into prostatic vesicle. Intrabulbar prostatic vesicle, oval-elongate and folded, becoming funnel-shaped both proximally and distally (Fig. 17). Inside penis papilla, prostatic vesicle narrows and forms an elongate duct that opens through tip of the papilla. Penis papilla, conical, long and almost symmetrical, filling the whole atrium. The dorsal insertion of the penis papilla is posteriorly shifted (Figs 15, 16). Folded atrium without anatomical or histological differentiation between male and female regions. Close to papilla insertions, longitudinal folds represent part of papilla wall.

Prostatic vesicle and proximal third of ejaculatory duct receive abundant openings of cells with coarse granular, erythrophil secretion, besides sparse amorphous, cyanophil secretion, besides a third type of gland containing heavy cyanophil granules. Distal two thirds of ejaculatory duct receives openings from numerous glands with amorphous, cyanophil secretion. Muscularis of ejaculatory duct thin (5µm) composed of longitudinal fibres. Abundant glands with densely distributed, coarse granular, xanthophil secretion and numerous glands with amorphous, cyanophil secretion open through epithelial lining of penis papilla, besides sparse erythrophil glands through lining of penis papilla. Numerous glands with amorphous, cyanophil secretion and scattered glands with erythrophil, fine granular secretion open through epithelial lining of the atrium, which is cyanophil. Muscularis of penis papilla (40–80µm) composed of subepithelial layer with circular fibres followed by layer with longitudinal fibres, both layers well developed. Posteriorly to the gonopore, necks of cyanophil glands concentrate subepithelially; subepithelial muscle fibres of atrium scattered among these cell necks (Fig. 18).



15

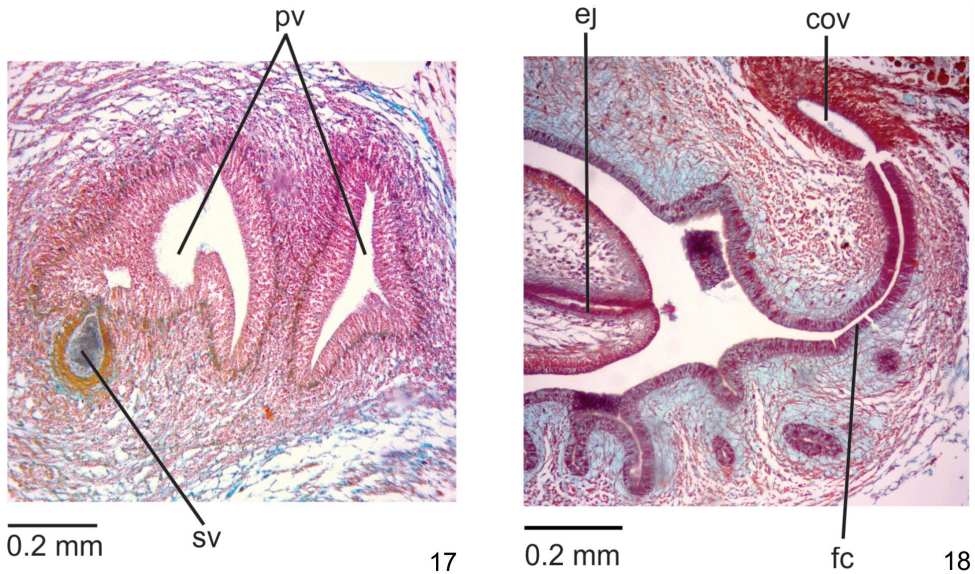


16

Figures 15, 16. *Choeradoplana cyanoatria* sp. n., holotype **15** copulatory apparatus, sagittal section **16** sagittal composite reconstruction of copulatory apparatus. Anterior tip to the left.

Vitelline follicles, situated between intestinal branches, well developed. Ovaries ovoid, 1.5 times longer than wide, measuring 0.3 mm in its antero-posterior axis. They are located dorsally to the ventral nerve plate, in anterior sixth of body. Ovitelline ducts emerge laterally from median third of ovaries, and run posteriorly immediately above nerve plate. Behind gonopore, the ovitelline ducts ascend posteriorly and medially inclined, uniting to form a common glandular ovitelline duct. This duct is situated dorsally to the relatively long, C shaped female canal, which opens into the atrium (Figs 15, 16).

Shell glands of two types: with coarse granular, erythrophil secretion, as well as with coarse granular, xanthophil secretion, the cells bodies of which occur among cell



Figures 17, 18. *Choeradoplana cyanoatria* sp. n., holotype, sagittal sections **17** prostatic vesicle **18** ejaculatory duct, and female canal. Anterior tip to the left.

bodies of cyanophil glands. Towards female canal, the lining epithelium becomes pseudostratified and erythrophil. Two types of glands open through the epithelium of the female canal: erythrophil glands with finely granular secretion and cyanophil glands with amorphous secretion both sparsely distributed. Muscularis of female canal (20–40µm) composed of interwoven circular and longitudinal fibres.

Gonopore canal vertical at the sagittal plane. Common muscle coat highly developed, especially at penis bulb (Figs 15, 16), with interwoven oblique, circular and longitudinal fibres.

Etymology. The name is a composite of the Latin adjective *cyano* (blue) and the Latin *atria*, referring to the abundant cyanophil secretion opening through the atrium.

Distribution. Known only from the type-locality, Três Barras, Santa Catarina, Brazil.

Notes on ecology and distribution

Choeradoplana longivesicula was recorded only in its type-locality, the *Araucaria* Natural Heritage Private Reserve, state of Parana, in a site showing an initial stage of regeneration with poorly developed understorey (Rossi and Leal-Zanchet 2017, Amaral et al. 2018). *Choeradoplana cyanoatria* occurred only in its type-locality, the Três Barras National Forest, state of Santa Catarina, located ca. 150 km east from the type-locality of *C. longivesicula*, in an area of *Araucaria* moist forest. Both species showed low abundance during night samplings.

Discussion

Both new species described herein match the diagnostic features of the genus *Choeradoplana*, namely a cephalic region that is curved backwards, a cephalic glandulo-muscular organ, and a cutaneous longitudinal musculature with a portion internal to the subcutaneous nerve plexus throughout the body, among others (Ogren and Kawakatsu 1990, Carbayo and Froehlich 2012, Carbayo et al. 2013).

Regarding external features, both new species resemble the type-species, *C. iheringi*, as well as *C. banga* Carbayo & Froehlich, 2012, *C. bocaina* Carbayo & Froehlich, 2012, *C. benyai* Lemos & Leal-Zanchet, 2014, *C. agua* Carbayo et al. 2017, *C. pucupucu* Carbayo et al. 2017 and *C. abaiba* Carbayo et al. 2017. All these species show a brownish dorsal pigmentation usually consisting of irregular, small dark-brown flecks (Carbayo and Froehlich 2012, Lemos et al. 2014, Carbayo et al. 2017).

With respect to the anatomy of the copulatory organs, by presenting a long, permanent penis papilla, the new species are easily differentiated from the species with an eversible penis papilla, namely *C. abaiba*, *C. agua*, *C. albonigra* (Riester 1938), *C. banga* Carbayo & Froehlich, 2012, *C. bocaina*, *C. gladismariae* Carbayo & Froehlich, 2012, *C. iheringi*, *C. langi* (Graff 1894), and *C. pucupucu* (Graff 1899, Riester 1938, Carbayo and Froehlich 2012, Carbayo et al. 2017). The presence of a long penis papilla horizontally disposed and occupying the whole length of the atrium distinguishes both new species from *C. bilix* Marcus, 1951, *C. catua* Froehlich, 1955, *C. marthae* Froehlich, 1955, and *C. crassiphalla* Negrete & Brusa, 2012. These four species have a short penis papilla that is obliquely disposed in the male atrium (Marcus 1951, Froehlich 1955, Negrete and Brusa 2012). *Choeradoplana longivesicula* and *C. cyanoatria* also differ from *C. minima* Lemos and Leal-Zanchet, 2014, which shows an inverted penis (Lemos et al. 2014).

Both species share with *C. benyai* a long penis papilla with horizontal orientation. However, by having a cylindrical and long prostatic vesicle that traverses the penis papilla. *Choeradoplana longivesicula* differs from *C. benyai* that shows a globose prostatic vesicle with folded wall restricted to the penis bulb (Lemos et al. 2014). In addition, a long ejaculatory duct traverses the penis papilla of *C. benyai*, whereas in *C. longivesicula* the prostatic vesicle opens into the tip of the penis papilla through a constriction, as if it were a short ejaculatory duct. Regarding shape of the prostatic vesicle and ejaculatory duct, *C. cyanoatria* is quite similar to *C. benyai* and can be easily differentiated from *C. longivesicula*. The elongate conical penis papilla of *C. cyanoatria* distinguishes it from *C. benyai*, which has a cylindrical and relatively longer penis papilla (Lemos et al. 2014). *Choeradoplana longivesicula* and *C. cyanoatria* show a long atrium with a continuous muscle coat and without anatomical or histological distinction between male and female regions, thus differentiating them both from *C. benyai*. The latter shows male and female atria with independent muscle coats and different gland types opening into these regions (Lemos et al. 2014).

Acknowledgements

We acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil, Nr. 306853/2015-9 and CNPq/PELD Site 9, Nr. 403817/20129) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for research grants and fellowships in support of this study. We thank Prof. Dr. C.A. Sanquetta and his research group in the Universidade Federal do Paraná, especially Dr. A.L. Rodrigues, for their help in the planning and organization of the field trips. We acknowledge I Rossi and JAL Braccini for their help in sampling flatworms and E Benya for an English review of the text. I Rossi is also acknowledged for the photos in the figures 1, 2, and 12. Dr Fernando Carbayo and Dr Hugh Jones are thanked for their suggestions on an early draft of the manuscript.

References

- Amaral SV, Rossi I, Iturralde GG, Leal-Zanchet AM (2014) Land flatworms (Platyhelminthes: Tricladida) in remnants of deciduous forest in the northeast region of southern Brazil. *Biota Neotropica* 14(1): 1–6. <https://doi.org/10.1590/S1676-06020140045>
- Amaral SV, Ribeiro GG, Valiati VH, Leal-Zanchet AM (2018) Body doubles: an integrative taxonomic approach reveals new sibling species of land planarians). *Invertebrate Systematics* 32: 533–550. <https://doi.org/10.1071/IS17046>
- Antunes MB, Marques DIL, Leal-Zanchet AM (2008) Composição das comunidades de planárias terrestres (Platyhelminthes, Tricladida, Terricola) em duas áreas de floresta estacional decidual do sul do Brasil. *Neotropical Biology Conservation* 31: 34–38.
- Baptista VA, Oliveira SM, Leal-Zanchet AM (2010) Inventário de planárias terrestres (Platyhelminthes, Tricladida) em remanescente de Floresta Estacional Decidual do sul do Brasil. *Biota Neotropica* 10(2): 247–252. <https://doi.org/10.1590/S1676-06032010000200027>
- Carbayo F, Álvarez-Presas M, Olivares CT, Marques FPL, Froehlich EM, Riutort M (2013) Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: proposal of taxonomic actions. *Zoologica Scripta* 42(5): 508–528. <https://doi.org/10.1111/zsc.12019>
- Carbayo F, Froehlich EM (2012) Three new Brazilian species of the land planarian *Choeradoplana* (Platyhelminthes: Tricladida: Geoplaninae) and an emendation of the genus. *Journal of Natural History* 46(19–20): 1153–1177. <https://doi.org/10.1080/00222933.2012.657699>
- Carbayo F, Leal-Zanchet AM (2003) Two new genera of geoplaninid land planarians (Platyhelminthes: Tricladida: Terricola) of Brazil in the light of cephalic specializations. *Invertebrate Systematics* 17: 449–468. <https://doi.org/10.1071/IT01035>
- Carbayo F, Leal-Zanchet AM, Vieira EM (2002) Flatworms (Platyhelminthes, Tricladida, Terricola) diversity versus man-induced disturbance in ombrophilous rainfor-

- est from Southern Brazil. *Biodiversity and Conservation* 11: 1091–1104. <https://doi.org/10.1023/A:1015865005604>
- Carbayo F, Silva MS, Riutort M, Álvarez-Presas M (2017) Rolling into the deep of the land planarian genus *Choeradoplana* (Tricladida, Continenticola, Geoplanidae) taxonomy. *Organisms Diversity & Evolution* 18: 1–24. <https://doi.org/10.1007/s13127-017-0352-4>
- Castro RA, Leal-Zanchet AM (2005) Composição de comunidades de planárias terrestres (Platyhelminthes) em área de floresta estacional e de campo na região central do Rio Grande do Sul, Brasil. *Acta Biologica Leopoldensia* 27: 147–150.
- Froehlich CG (1955) Sobre morfologia e taxonomia das Geoplanidae. *Boletim da Faculdade de Filosofia Ciências e Letras da Universidade de São Paulo, série Zoologia* 19: 195–279. <https://doi.org/10.11606/issn.2526-3382.bffclzoologia.1954.120092>
- Graff, LV (1896) Über das System und die geographische Verbreitung der Landplanarien. *Verhandlungen der deutschen zoologischen Gesellschaft* 6: 61–75.
- Graff LV (1899) Monographie der Turbellarien: II. Tricladida Terricola. (landplanarien), Verlag von Wilhelm Engelmann, Leipzig.
- Leal-Zanchet AM, Baptista VA (2009) Planárias terrestres (Platyhelminthes, Tricladida) em remanescentes de Floresta com Araucária. In: Fonseca CR, Souza AF, Leal-Zanchet AM, Dutra T, Backes A, Ganade G (Eds) *Floresta com Araucária: Ecologia, Conservação e Desenvolvimento Sustentável*. Holos, Ribeirão Preto, 199–207.
- Leal-Zanchet AM, Baptista V, Campos LM, Raffo JF (2011) Spatial and temporal patterns of land flatworms assemblages in Brazilian Araucaria forests. *Invertebrate Biology* 130: 25–33. <https://doi.org/10.1111/j.1744-7410.2010.00215.x>
- Leal-Zanchet AM, Carbayo F (2000) Fauna de planárias terrestres (Platyhelminthes, Tricladida, Terricola) da Floresta Nacional de São Francisco de Paula, RS, Brasil: Uma análise preliminar. *Acta Biologica Leopoldensia* 22: 19–25.
- Leal-Zanchet AM, Souza SA (2003) Redescricao de *Choeradoplana iheringi* Graff (Platyhelminthes, Tricladida, Terricola). *Revista Brasileira de Zoologia* 20: 523–530. <https://doi.org/10.1590/S0101-81752003000300026>
- Lemos SV, Cauduro GP, Valiati VH, Leal-Zanchet AM (2014) Phylogenetic relationships within the flatworm genus *Choeradoplana* Graff (Platyhelminthes: Tricladida) inferred from molecular data with the description of two new sympatric species from *Araucaria* moist forests. *Invertebrate Systematics* 28: 605–627. <https://doi.org/10.1071/IS14003>
- Marcus E (1951) Turbellaria brasileiros. *Boletim da Faculdade de Filosofia Ciências e Letras da Universidade de São Paulo, série Zoologia* 16(9): 5–215. <https://doi.org/10.11606/issn.2526-4877.bsffclzoologia.1951.125221>
- Negrete L, Brusa F (2012) *Choeradoplana crassiphalla* sp. nov. (Platyhelminthes: Tricladida: Geoplanidae): a new species of land planarian from the Atlantic Forest of Argentina. *Studies on Neotropical Fauna and Environment* 47: 227–237. <https://doi.org/10.1080/01650521.2012.735071>
- Ogren RE, Kawakatsu M (1990) Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) Part I: Geoplaninae. *Bulletin of the Fuji Women's College* 28(2): 79–166.
- Romeis B (1989) *Mikroskopische Technik*. Urban und Schwarzenberg, München.

- Riester A (1938) Beiträge zur Geoplaniden-Fauna Brasiliens. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 441: 1–88.
- Rossi I, Amaral S, Ribeiro GG, Cauduro GP, Fick I, Valiati VH, Leal-Zanchet AM (2015) Two new Geoplaninae species (Platyhelminthes: Continenticola) from Southern Brazil based on an integrative taxonomic approach. Journal of Natural History 50(13–14): 1–29. <https://doi.org/10.1080/00222933.2015.1084057>
- Rossi I, Leal-Zanchet AM (2017) Three new species of *Cratera* Carbayo et al., 2013 from Araucaria forests with a key to species of the genus (Platyhelminthes, Continenticola). ZooKeys 643: 1–32. <https://doi.org/10.3897/zookeys.643.11093>
- Sluys R (1998) Land planarians (Platyhelminthes, Tricladida, Terricola) in biodiversity and conservation studies. Pedobiologia 42: 490–494.

The first stygobiont species of Coleoptera from Portugal, with a molecular phylogeny of the *Siettitia* group of genera (Dytiscidae, Hydroporinae, Hydroporini, Siettitiina)

Ignacio Ribera¹, Ana Sofia P.S. Reboleira²

1 Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra), Barcelona, Spain **2** Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 København Ø, Denmark

Corresponding author: Ignacio Ribera (ignacio.ribera@ibe.upf-csic.es)

Academic editor: M. Michat | Received 14 September 2018 | Accepted 12 November 2018 | Published 7 January 2019

<http://zoobank.org/05B3292B-ECF0-41AA-9EC5-F3FF0BAAE35D>

Citation: Ribera I, Reboleira ASPS (2019) The first stygobiont species of Coleoptera from Portugal, with a molecular phylogeny of the *Siettitia* group of genera (Dytiscidae, Hydroporinae, Hydroporini, Siettitiina). ZooKeys 813: 21–38. <https://doi.org/10.3897/zookeys.813.29765>

Abstract

Iberoporus pluto **sp. n.**, the first stygobiont beetle from Portugal (Dytiscidae, Hydroporinae), is described from a single female from the cave Soprador do Carvalho (Coimbra). The species is highly troglomorphic, depigmented, blind, and with elongated appendages not adapted for swimming. A molecular phylogeny based on a combination of three mitochondrial and two nuclear genes showed the new species to be sister to *I. cermenius* Castro & Delgado, 2001 from Córdoba (south of Spain), within the subtribe Siettitiina of the tribe Hydroporini. Both species are included in a clade with *Siettitia avenionensis* Guignot, 1925 (south of France) and *Rhithrodytes agnus* Foster, 1992 and *R. argaensis* Fery & Bilton, 1996 (north of Portugal), in turn sister to the rest of species of genus *Rhithrodytes* Bameul, 1989, in what is here considered the *Siettitia* group of genera. We resolve the paraphyly of *Rhithrodytes* by transferring the two Portuguese species to *Iberoporus* Castro & Delgado, 2001, *I. agnus* (Foster, 1992), **comb. n.** and *I. argaensis* (Fery & Bilton, 1996), **comb. n.**

Keywords

Diving beetles, groundwater, new species, stygofauna, troglomorphy

Introduction

The knowledge of the subterranean fauna from Portugal has significantly increased over the last decade, with the description of a high number of obligate subterranean species (tripling their number) and the establishment of new biogeographic patterns (Reboleira 2012). A high number of these species are stygobiont (i.e., confined to groundwater), mostly from wells in the north of the country, where evapotranspiration is higher (Reboleira et al. 2011, 2013). They include 62 species of crustaceans, mostly asellids, syncarids and amphipods, and one species of annelid (Reboleira et al. 2013).

In this work we describe the first stygobiont species of Coleoptera from Portugal, a diving beetle of the subtribe Siettitiina (Dytiscidae, Hydroporinae, Hydroporini; type genus: *Siettitia* Abeille de Perrin, 1904). Siettitiina includes the only known European genera of Dytiscidae which have stygobiont members: *Siettitia*, with two species in France, *Iberoporus* Castro & Delgado, 2001, with one species in south Spain, *Etruscodytes* Mazza et al., 2013, with one Italian species, and *Graptodytes* Seidlitz, 1887, with the Moroccan *G. eremitus* Ribera & Faille, 2010 among several epigean members (Ribera and Faille 2010, Nilsson and Hájek 2018a). The subtribe also includes some North American stygobiont species, with an uncertain phylogenetic position (Miller et al. 2013, Kanda et al. 2016, Miller and Bergsten 2016, Nilsson and Hájek 2018b). The new species is known from a single female found in a well-studied cave in central Portugal. Despite multiple visits to the same cave no additional specimens have been found, so we describe here the species on the basis of its morphological singularity and of the molecular data that places it unambiguously among the west Mediterranean species of Siettitiina.

Material and methods

Taxon sampling, DNA extraction and sequencing

For the phylogenetic placement of the new species we used the datasets of Ribera and Faille (2010) and Abellán et al. (2013), with the inclusion of additional sequences (mostly nuclear genes) and taxa (Table 1). Most notably is the inclusion of *Siettitia avenionensis* Guignot, 1925, the second oldest described stygobiont water beetle worldwide. Partial sequences of the genes COI and 18S were obtained from a larva preserved in 70% ethanol, collected in 1989 (Table 1). Other attempts to extract and sequence different larvae from the same locality collected in 1984 and 1992 (Ph. Richoux leg.) proved unsuccessful. Extractions of single specimens were non-destructive, using a standard phenol-chloroform method or the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). Vouchers and DNA samples are kept in the collections of the Museo Nacional de Ciencias Naturales, Madrid (MNCN), the Institute of Evolutionary Biology, Barcelona (IBE) and the Natural History Museum of Denmark (NHMD).

Table 1. Material used in the molecular phylogeny of the *Siettitia* group of genera, with locality, collector, and EMBL accession numbers. Newly obtained sequences are in bold typeface. Nomenclature follows Nilsson and Hájek (2018a).

<i>N</i>	Species	Voucher	Locality, date, and collector	COI-5'	COI-3'	16S+	18S	H3
1	<i>Gnaptodytes aequalis</i>	NHM-IR206	Morocco: Debdou, Meson forestiere; 6.4.1999, I Ribera, P Aguilera, C Hernando, A Millán	LS999725	HM588264	AY250910	AJ850509	EF670184
2	<i>G. atlantis</i>	MNCN-AI921	Morocco: Lac Afenourir, Azrou; 29.4.2000, I Ribera	LS999726	HM588265	HM588602	LS999692	LS999771
3	<i>G. bilineatus</i>	MNCN-AI608	Sweden: Västerbotten prov., Åmsele, Vindelälven; 18.9.2005, AN Nilsson	LS999727	HM588267	HM588603	LS999693	LS999772
4	<i>G. castilianus</i>	MNCN-AI1316	Spain: Navarra, Pitillas; pond in crossroad; 21.7.2004, I Ribera, A Cieslak	HF947943	HM588268	HM588604	LS999694	LS999773
5	<i>G. delectus</i>	MNCN-AI1092	Tenerife (Spain): Chamorga, Bco. Roque Bermejo; 20.7.2006, A Castro	LS999728	HM588269	HM588605	LS999695	LS999774
6	<i>G. eremitus</i>	IBE-AF33	Morocco: Tiqqi, cave Doussoulile; 28.7.2008, JM Bichain et al.	LS999729	HM588271	HM588606	LS999696	LS999775
7	<i>G. flavipes</i>	NHM-IR40	Spain: Huelva, Almonte, poblado forestal; 26.7.1998, I Ribera	–	HM588273	AY250914	AJ318730	EF056561
8	<i>G. fractus</i>	MNCN-AI627	Spain: Córdoba, Sa. de Córdoba, Arroyo de los Arenales; 16.4.2005, A Castro	LS451100	HM588274	HM588608	LS453474	LS453168
9	<i>G. granularis</i>	MNCN-AI609	Sweden: Västerbotten prov., Åmsele, Vindelälven; 18.9.2005, AN Nilsson	LS999730	HM588278	HM588611	LS999697	LS999776
10	<i>G. ignotus</i>	NHM-IR531	Spain: Girona, Estany de Capmany, 3.2001, P Aguilera	LS999731	HM588287	AY250915	AJ850510	EF670185
11	<i>G. kuchtae</i>	MNCN-AI177	Mallorca (Spain): Ternelles, Torrent de Ternelles; 14.10.2004, I Ribera, A Cieslak	LS999732	HM588288	HM588614	LS999698	LS999777
12	<i>G. laeticulus</i>	MNCN-HI16	Algeria: Algeria, Ain Damous; 24.8.2006, S Bouzid	–	HM588300	HM588621	LS999699	LS999778
13	<i>G. pictus</i>	MNCN-AI660	Poland: Zachodniopomorsky, Dygowo; pond; 16.8.2004, I Ribera, A Cieslak	LS999733	HM588290	HM588615	LS999700	LS999779
14	<i>G. pietrii</i>	MNCN-DM37	Tunisia: Rd. Beja-Teboursouk, NW Teboursouk; 23.10.2001, I Ribera, A Cieslak	LS999734	HM588292	HM588616	LS999701	LS999780
15	<i>G. sedilloti sedilloti</i>	NHM-IR585	Cyprus; 3.2001, K Miller	LS451098	HM588294	HM588619	LS453473	LS453167
16	<i>G. sedilloti phrygius</i>	MNCN-AI111	Chios (Greece): Marmaro marsh; 19.4.2004, GN Foster	LS999735	HM588293	HM588618	LS999702	LS999781
17	<i>G. siculus</i>	MNCN-AH162	Sicily (Italy): Parco dei Nebrodi, Stream Trail Lago Urio; 13.6.2007, P Abellán, F Picazo	LS999736	HM588295	HM588620	LS999703	LS999782
18	<i>G. varius</i>	MNCN-AH160	Sicily (Italy): Parco dei Nebrodi, Stream Trail Lago Urio; 13.6.2007, P Abellán, F Picazo	LS999737	HM588297	HM588622	LS999704	LS999783
19	<i>G. veterator veterator</i>	MNCN-AH161	Sicily (Italy): Parco dei Nebrodi, Stream Trail Lago Urio; 13.6.2007, P , F Picazo	LS451095	HM588304	HM588625	LS453472	LS453105
20	<i>G. veterator behmingi</i>	MNCN-AI774	Turkey: Düzce, Rd. to Kartalkaya from Çaydurt; 23.4.2006, I Ribera	LS999738	HM588303	HM588624	LS999705	LS999784
21	<i>Iberoporus cermenius</i>	NHM-IR276	Spain: Cordoba, Priego de Cordoba; 29.4.2000, A Castro	LS451107	AY250958	AY250918	AJ850511	EF670186
22	<i>I. pluto</i> sp. n.	IBE-AN151	Portugal: Soprador do Carvalho; 24.10.2014, ASPS Reboleira	LS999739	LS999756	LS999763	LS999706	LS999785
23	<i>Metaporus meridionalis</i>	NHM-IR34	Spain: Albacete, Robledo, Ojos de Villaverde; 7.9.1997, I Ribera	–	HM588307	AY250919	AJ318739	EF670187
24	<i>Porhydrus genei</i>	IBE-RA86	Algeria: Garaet Ain Nechma, nr Ben-Azzouz (Skikda); 29.6.2009, S Bouzid	LS999740	HF931320	HF931543	LS999707	LS999786

N	Species	Voucher	Locality, date, and collector	COI-5'	COI-3'	16S+	18S	H3
25	<i>P. lineatus</i>	NHM-IR24	England (UK): Sommerset Levels, Chilton Trinity; 4.7.1998, I Ribera	LS999741	AY250973	AY250933	AJ318743	EF670188
26	<i>P. obliquesignatus</i>	IBE-RA147	Italy: Piano Grande, Piano di Castelluccio; 20.7.2009, M Toledo	LS999742	HF931305	LS999764	LS999708	LS999787
27	<i>P. vicinus</i>	MNCN-AH113	Portugal: Cercal, ephemeral pond btw. Cercal and Vilanova; 24.1.2008, I Ribera	LS999743	HF931132	HF931350	LS999709	LS999788
28	<i>Rhithrodytes agnus</i>	MNCN-AI1007	Portugal: Viana do Castelo, N Ponte de Lima, W Labruja; 28.5.2006, H Fery	LS999744	HF931143	HF931362	LS999710	LS999789
29	<i>R. argaensis</i>	MNCN-AI179	Portugal: Serra de Arga, Pools on summit; 9.5.2005, DT Bilton	HF948005	HF931183	HF931405	LS999711	LS999790
30	<i>R. bimaculatus</i>	IBE-RA727	Spain: Huesca, Aragüés del Puerto; 23.7.2011, I Esteban	LS999745	LS999757	LS999765	LS999712	LS999791
31	<i>R. crux</i>	MNCN-AI302	Italy: Alessandria, stream; 2.5 km S Praglia; 18.10.2002, I Ribera, A Cieslak	LS451084	HF931187	HF931410	LS453475	LS453108
32	<i>R. numidicus</i>	MNCN-DM34	Tunisia: Rd. Tabarka-Aïn-Draham, stream Aïn-Draham; 23.10.2001, I Ribera, A Cieslak	–	LS999758	LS999766	LS999713	LS999792
33	<i>R. sexguttatus</i>	NHM-IR183	Corsica (France): Porto-Vecchio: l'Ospedale; 18.9.1999, I Ribera, A Cieslak	–	AY250975	AY250936	AJ850513	EF670190
34	<i>Siettitia avenionensis</i>	MNCN-AI897	France: Barbestane; 22.2.1992, J Dalmon	–	LS999759	–	LS999714	–
35	<i>Stictometes abellani</i>	IBE-PA312	Spain: Ciudad Real, PN Cabañeros; 7.7.2008, A Millán and col.	LS451083	HF931298	HF931530	LS453469	LS453169
36	<i>S. azruensis</i>	NHM-IR661	Morocco: Moyen Atlas, nr. Azrou, Col du Zad; 16.4.2001, Pellecchia, Pizzetti	LS999746	AY250979	AY250940	LS999715	LS999793
37	<i>S. canariensis</i>	IBE-AF114	Gran Canaria (Spain): Barranco Güigüi grande; 1.4.2008, J Hájek, K Kaliková	LS999747	HF931113	HF931330	LS999716	LS999794
38	<i>S. epipleuricus</i>	MNCN-AH73	Portugal: Serra de São Mamede, Portalegre: r. Caia; 25.7.1998, I Ribera	LS999748	LS999760	LS999767	LS999717	–
39	<i>S. escheri</i>	MNCN-AH107	Morocco: Asilah, rd. N1, stream ca.; 4 km S Asilah; 27.3.2008, I Ribera, P Aguilera, C Hernando	LS999749	HF931130	HF931349	LS999718	LS999795
40	<i>S. formosus</i>	MNCN-AH108	Morocco: Asilah, rd. N1, stream ca.; 4 km S Asilah; 27.3.2008, I Ribera, P Aguilera, C Hernando	LS999750	HF931131	LS999768	LS999719	LS999796
41	<i>S. lepidus</i>	MNCN-AI632	Spain: Córdoba, Sierra Morena, cta. Villaviciosa; 16.4.2005, A Castro	LS999751	LS999761	LS999769	LS999720	LS999797
42	<i>S. occidentalis</i>	NHM-IR529	Portugal: Algarve; 2001, P Aguilera	–	AY250980	AY250942	–	LS999798
43	<i>S. optatus</i>	MNCN-AI1089	Spain: Jaén, Sierra de Cazorla, cta. Del Tranco; 3.8.2006, A Castro	LS999752	LS999762	LS999770	LS999721	LS999799
44	<i>S. optatus</i>	NHM-MsC	Corsica (France): Porto-Vecchio: l'Ospedale; 18.9.1999, I Ribera, A Cieslak	–	AY250981	AY250943	AJ850514	EF670192
45	<i>S. rebecca</i>	MNCN-AH72	Portugal: Serra Estrela, Sabugueiro, r. above village; 12.5.2005, I Ribera	LS999753	FR851207	FR851208	LS999722	LS999800
46	<i>S. rufulus</i>	MNCN-AI1299	Sardinia (Italy): Road from Óschiri to Mount Limbara; 17.10.2006, GN Foster	LS999754	HF931179	HF931400	LS999723	LS999801
47	<i>S. samai</i>	IBE-AF142	Algeria: Oued Bagrat; 24.3.2006, S Bouzid	LS999755	HF931119	HF931336	LS999724	LS999802

Examples of most species of Palaearctic *Siettitiina* were included, including all stygobiont or interstitial species with the exception of *Graptodytes aurasius* Jeannel, 1907 (Algeria), *Siettitia balsetensis* Abeille de Perrin, 1904 (France) and *Etruscodytes nethuns*

Mazza et al., 2013 (Italy). Trees were rooted in the split between *Graptodytes*+*Metaporus* Guignot, 1945 and the rest of Siettitiina, based on previous phylogenetic results (Ribera et al. 2008, Abellán et al. 2013).

Fragments of five genes in five sequencing reactions were sequenced, three mitochondrial (1) 5' end of cytochrome c oxidase subunit 1 (COI-5, "barcode" fragment of Hebert et al. 2003); (2) 3' end of cytochrome c oxidase subunit 1 (COI-3); (3) 5' end of 16S RNA plus the Leucine tRNA plus 5' end of NADH dehydrogenase subunit I (16S); and two nuclear fragments (4) an internal fragment of the small ribosomal unit, 18S RNA (18S) and (5) an internal fragment of Histone 3 (H3). Details on primers used are provided in Table 2. Sequences were assembled and edited with Geneious v6.0.6 (Kearse et al. 2012); new sequences (111) have been submitted to the EMBL database with accession numbers LS999692-LS999802 (Table 1).

Phylogenetic analyses

Edited sequences were aligned using the online version of MAFFT 7 with the G-INS-I algorithm (Katoh and Toh 2008).

BEAST 1.8 (Drummond and Rambaut 2007) was used for Bayesian phylogenetic analyses, using a molecular-clock approach for estimating divergence times. We applied a partition by genes with uncorrelated lognormal relaxed clocks to estimate substitution rates and a Yule speciation process as the tree prior, using GTR+I+G and HKY+I+G evolutionary models. We calibrated the tree using rates estimated in Andújar et al. (2012) for a genus of Carabidae (*Carabus* Linnaeus, 1758), in the same suborder Adephaga (rate of 0.0113 [95% confidence interval 0.0081 – 0.0147] substitutions per site per million years (subst/s/Ma) for COI-5; 0.0145 [0.01 – 0.0198] subst/s/Ma for COI-3 and 0.0016 [0.001 – 0.0022] subst/s/Ma for 16S+tRNA). Analyses were run for 100 million generations, assessing that convergence was correct and estimating

Table 2. Primers used in the amplifying and sequencing reactions.

Gene	Primer	Sequence	Reference
COL-3'	Jerry (5')	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)
	Pat (3')	TCCAATGCACTAATCTGCCATATTA	
	Chy (5')	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	Ribera et al. (2010)
	Tom (3')	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	
COI-5'	Uni LepF1b	TAATACGACTCACTATAGGGATTCAACCAATCATAAAGATATTGGAAC	Hebert et al. (2004)
	Uni LepR1	ATTAACCCCTCACTAAAGTAAACTTCTGGATGTCCAAAAAATCA	
16S+trnL+nad1	16SaR (5')	CGCCTGTTTAAACAAAAACAT	Simon et al. (1994)
	ND1 (3')	GGTCCCTTACGAATTTGAATATATCCT	
	16Sb	CCGGTCTGAACCTCAGATCATGT	
18S	18S 5'	GACAACCTGGTTGATCCTGCCAGT(1)	Shull et al. (2001)
	18S b5.0	TAACCGCAACAACCTTTAAT(1)	
H3	H3aF (5')	ATGGCTCGTACCAAGCAGACRCG	Colgan et al. (1998)
	H3aR (3')	ATATCCTTTRGGCATRATRTGTAC	

the burn-in fraction with Tracer v1.6 (Drummond and Rambaut 2007). We also used a fast Maximum Likelihood (ML) heuristic algorithm in RAXML-HPC2 (Stamatakis 2006) in the CIPRES Science Gateway (Miller et al. 2010), using the same partition scheme as in BEAST with a GTR+G evolutionary model independently estimated for each partition and assessing node support with 100 pseudoreplicates with a rapid bootstrapping algorithm (Stamatakis et al. 2008).

Results

The two BEAST analyses (GTR and HKY evolutionary models) resulted in identical topologies and very similar branch lengths, although convergence for GTR evolutionary models was poor for some genes (nad1, 18S), so we present here only the results of the HKY models (Fig. 1). The topology was also almost identical to that obtained with RAXML (Fig. 1).

We obtained a well-supported, well-resolved phylogeny of Siettitiina (Fig. 1). In agreement with previous results we recovered three clades, *Graptodytes*+*Metaporus*, *Stictonectes* Brinck, 1943 + *Porhydrus* Guignot, 1945, and the *Siettitia* group of genera as here defined, including *Siettitia*, *Rhithrodytes*, and *Iberoporus* (plus most likely *Etruscodytes*, see Discussion). The new species was placed as sister to *Iberoporus cermenius* Castro & Delgado, 2001 with strong bootstrap support (BS = 73%), although in the Bayesian analyses the support was lower (posterior probability, pp = 0.73). Both species were in turn sister to *Rhithrodytes argaensis* Bilton & Fery, 1996 plus *R. agnus* Foster, 1992 in a very well supported clade (BS = 94; pp = 0.97), and then to *Siettitia* (Fig. 1). All other sampled species of *Rhithrodytes* were placed as sister to this clade, rendering the genus paraphyletic. In order to preserve the monophyly of *Rhithrodytes* we thus transfer the two species to the genus *Iberoporus*, *Iberoporus agnus* (Foster, 1992) comb. n. and *Iberoporus argaensis* (Bilton & Fery, 1996), comb. n.

According to our calibration, the separation between the new species and *Iberoporus cermenius* was dated at ca. 10 Ma (95% HPD 13.4–6.9 Ma), with a similar age for the split from *I. agnus* + *I. argaensis* (11.4 Ma [15.0–8.3]), during the Tortonian (Fig. 1).

Taxonomy

Iberoporus pluto sp. n.

<http://zoobank.org/3F0A115A-F9F0-4AE5-95BC-E4E918FA04BB>

Figures 2–4, 6

Type locality. Portugal, Penela, Gruta Soprador do Carvalho (39°59'N, 8°23'W) (Fig. 6).

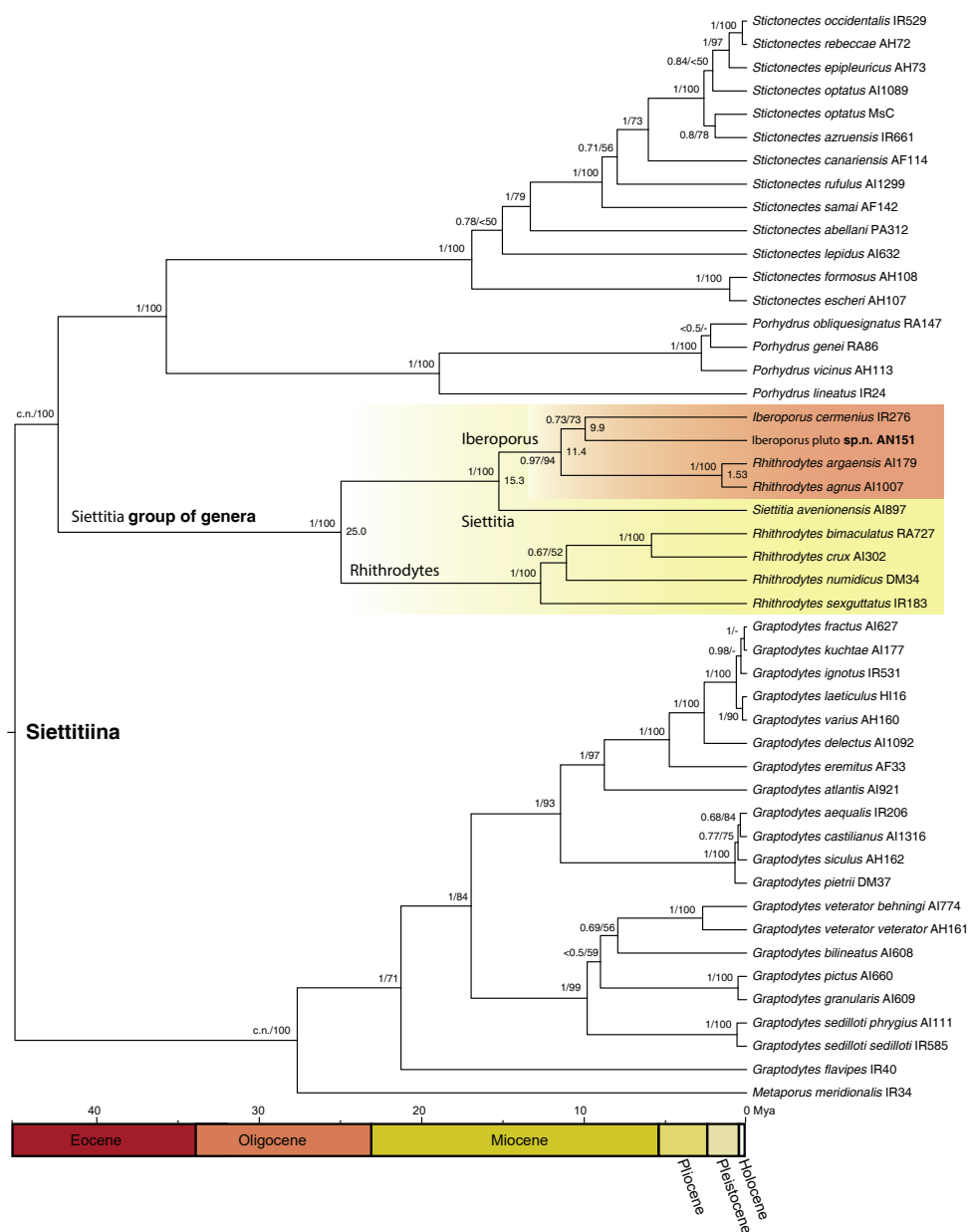


Figure 1. Phylogeny of the *Siettitia* group of genera, obtained with Bayesian methods. Numbers in nodes, Bayesian posterior probabilities/maximum likelihood bootstrap support (obtained in RAxML); c.n., constrained node in the Bayesian analysis. See Table 1 for details on the specimens.

Type material. **Holotype** female (NHMD) Portugal, Penela, Gruta Soprador do Carvalho, ASPS Reboleira leg., 24.X.2014, with red holotype label and DNA voucher label “IBE-AN151”.

Diagnosis. A blind and depigmented species of *Iberoporus*, larger and wider than the other subterranean species of the genus, with a cordiform pronotum without lateral stria, less prominent constriction between pronotum and elytra and with a more transverse pronotum. Appendages longer and more slender, especially antennae and pro- and mesotibiae. Male unknown.

Description. Body length 2.8 mm, maximum width 1.1 mm. Habitus: Body elongate, strongly parallel-sided (including pronotum and head) (Fig. 2), flattened in lateral view (Fig. 3a); in dorsal view lateral outline with a slight discontinuity between posterior angles of pronotum and base of elytra. Body and appendages uniformly pale orange (cuticle appears translucent after DNA extraction due to digestion of soft tissue).

Head (Fig. 2): Wide, anterior margin almost perfectly semicircular, deeply encased in pronotum, with two lateral dark scars in place of eyes; surface smooth, with very sparse small shallow punctures, surface weakly micro-reticulated, stronger on margins, glabrous. Antennae with ovoid pedicel, distal antennomeres conical, more elongate.

Pronotum (Figs 2, 3): Cordiform, margins sinuated, anterior part slightly wider than head, posterior part narrower than head and base of elytra; anterior margin more or less straight (except angles), angles strongly acute; posterior margin sinuated, angles acute; sides without rim, anterior margin with transverse depression with irregular row of large punctures; posterior margin with some sparse large punctures very loosely forming a row. Pronotum without sublateral stria on each side, with only a slight depression and very irregular row of larger punctures. Surface smooth, with fine shallow punctures denser on disk, with very fine microreticulation, stronger near margins, cells not contiguous; centre of disc with small longitudinal rectangular mark. Pronotum with long lateral sensorial setae (Fig. 3b).

Elytra (Figs 2, 3): almost parallel-sided on basal 2/3, apical third regularly acuminate. Sides of elytra with weak rim, not visible from above. In lateral view margin of elytra almost straight, only very weakly ascending to humeral angle in anterior quarter; epipleuron not visible until shoulders. Surface with same structure as on pronotum, with very sparse larger punctures; larger punctures forming very loose and irregular lines on elytra; more distinct near to suture and on disk. With long sensorial setae on margins (Fig. 3b). Without traces of hind wings.

Ventral surface (Fig. 4): Uniformly pale, colour similar to dorsal surface. Prosternal process lanceolate, apex acuminate; not reaching anteromedial metaventral process. Epipleuron becoming narrower short before mid-length, without oblique carina near shoulder. Metepisternum more or less triangular in shape. Metacoxal lines obsolete; joint hind margin of metacoxal processes incised; lobes of processes rounded.

Legs (Figs 2–4): long and slender, especially posterior legs. Metafemora very thin, not enlarged, regularly curved; without natatorial setae.

Etymology. From “Πλούτων” (Ploutōn), the ruler of the underworld in the Greek mythology. Name in apposition.

Notes on the habitat. Soprador do Carvalho is a cave with approximately 4 km of horizontal development (Fig. 7). It is the largest cave of the so-called Dueça Speleologi-



Figure 2. Habitus of *Iberoporus pluto* sp. n., dorsal view (holotype, after DNA extraction). Scale bar: 1 mm.

cal System, located in the north-eastern part of the Sicó karst area in central Portugal (Neves et al. 2005). The subterranean stream feeds the spring of the Dueça River, a contributor to the Mondego River. The substrate of the river is mostly composed of

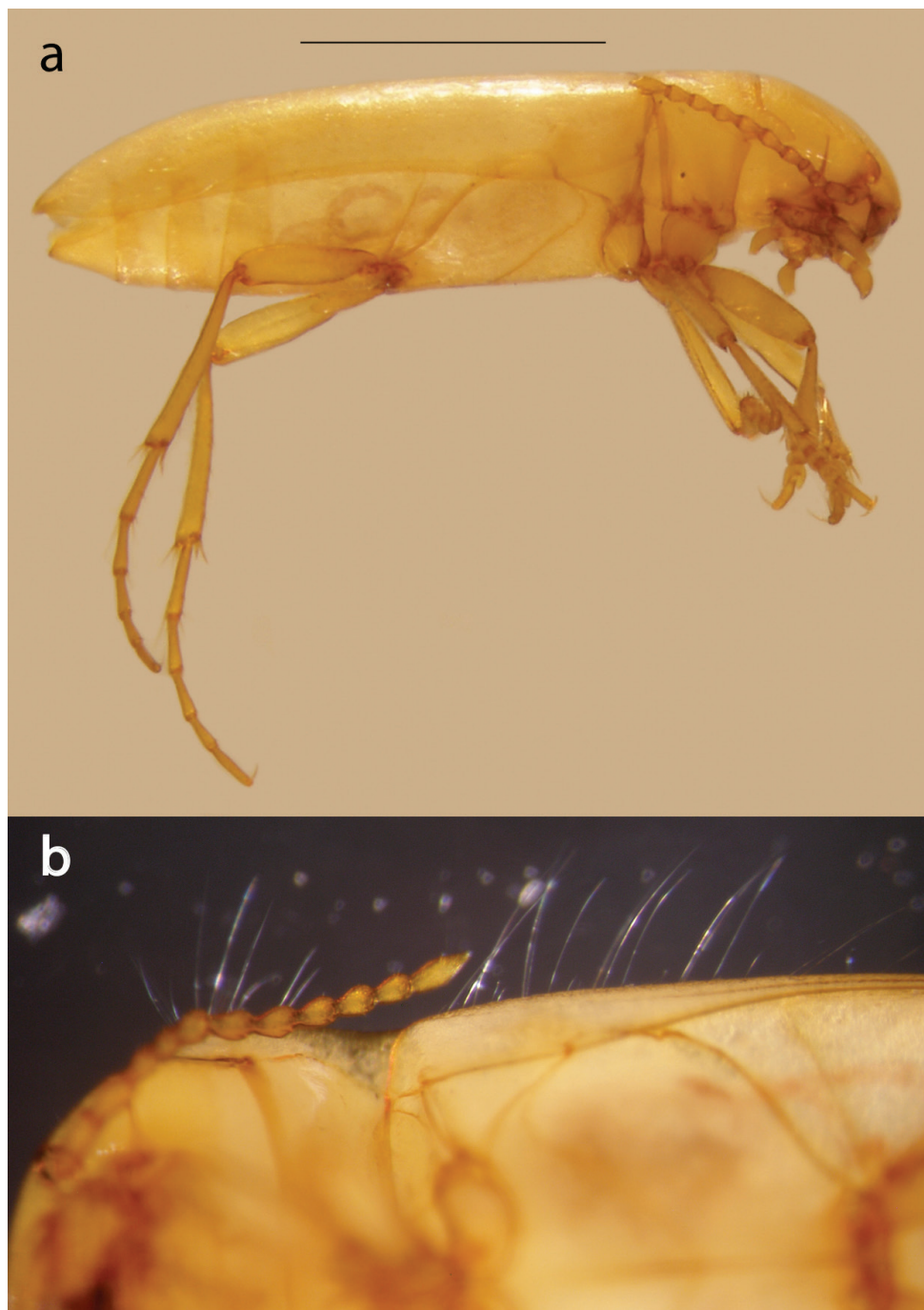


Figure 3. *Iberoporus pluto* sp. n., holotype. **a** Lateral view (scale bar, 1 mm) **b** Detail of the sensory setae of pronotum and elytra (both previous to DNA extraction).



Figure 4. *Iberoporus pluto* sp. n., holotype, ventral view (previous to DNA extraction).



Figure 5. Habitus of the species of *Iberoporus*. **a** *I. cermenius* (modified from Millán et al. 2014) **b** *I. agnus* comb. n. **c** *I. argaensis* comb. n. (both modified from Fery 2016).

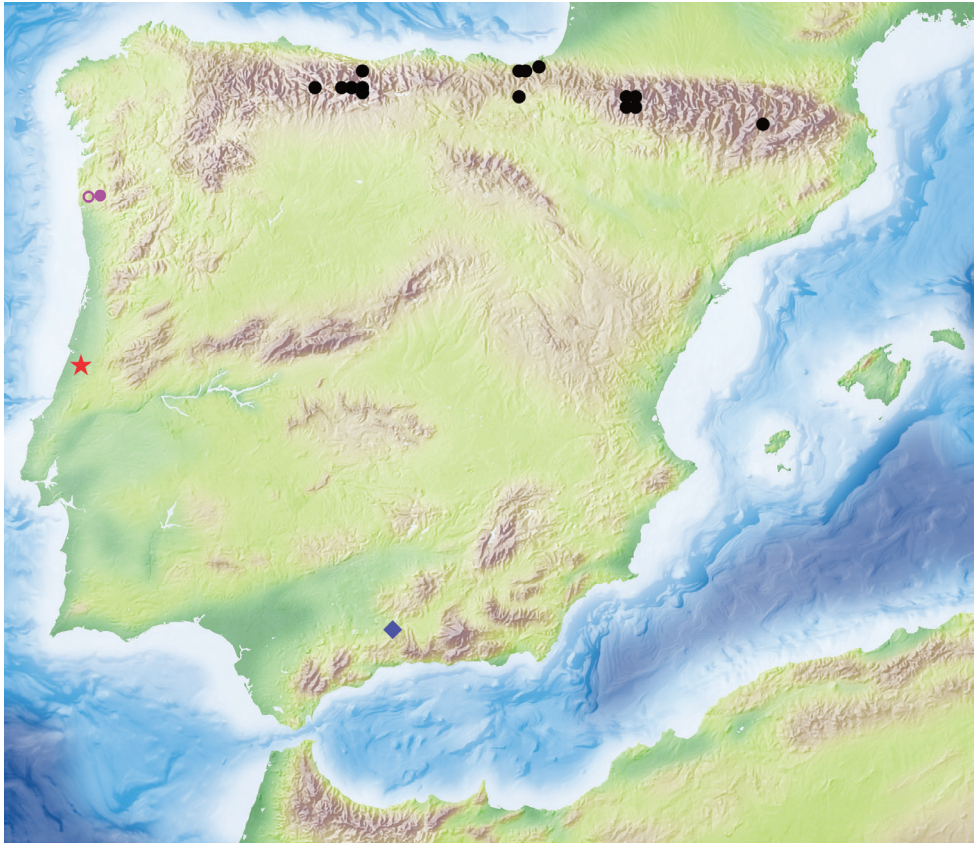


Figure 6. Distribution map of the Iberian species of *Rhithrodytes* and *Iberoporus*. Key: red star, *I. pluto* sp. n.; blue diamond, *I. cermenius*; filled purple circle, *I. argaensis* comb. n.; empty purple circle, *I. agnus* comb. n.; black circles, *R. bimaculatus* (data from Millán et al. 2014).

clasts and gravel, with large clay deposits on the margins. The specimen was found in the bottom of a clay pound connected to the margin of the subterranean stream. Other invertebrate stygobionts are found in this stream, such as a new species of the asellid genus *Proasellus* and of the amphipod genus *Pseudoniphargus*, and unidentified copepods (Reboleira 2012). In the terrestrial compartment of the cave, several cave-adapted species are known: the pseudoscorpion *Occidenchthonius duecensis* Zaragoza & Reboleira, 2018; the millipede *Scutogona minor* Enghoff & Reboleira, 2013; the woodlice *Trichoniscoides sicoensis* Reboleira & Taiti, 2015 (which has an amphibian behaviour and can be collected inside the stream totally submerged) and *Porcellio cavernicolus* Vandel, 1946; and the dipluran *Podocampa* cf. *fragiloides* Silvestri, 1932 (Enghoff and Reboleira 2013, Reboleira et al. 2015, Zaragoza and Reboleira 2018). Over recent years, the cave is being explored for tourism. This may represent a major threat, as tourists constantly trample the bottom of the subterranean stream where the new species was found.

Remarks. *Iberoporus pluto* sp. n. is most similar in its external morphology to *I. cermenius*. Both share a similar shape of the head, a cordiform pronotum without



Figure 7. Soprador do Carvalho Cave, type locality of *Iberoporus pluto* sp. n.

lateral stria, and similar general appearance (Figs 2, 5a). In the absence of males of *I. pluto* sp. n. (and in addition to the genetic differences), both species can be easily separated by the body shape, larger and wider in *I. pluto* sp. n., and with a less prominent constriction between pronotum and elytra (clearly visible in *I. cermenius*) and with a more transverse pronotum. The appendages of *I. pluto* sp. n. are also longer and more slender, especially the antennae and the pro- and mesotibiae (Figs 2, 5a). *Iberoporus cermenius* has also well-defined parasutural rows on the elytra formed by large punctures, which are absent in *I. pluto* sp. n.

Discussion

We obtained for the first time a phylogeny of Siettitiina including a species of its type genus, *Siettitia*. Despite the incomplete data, there is strong support for the existence of a clade including *Siettitia*, *Iberoporus*, and *Rhithrodytes*, what we call the *Siettitia* group of genera. Our results also clearly demonstrate the parayphyly of *Rhithrodytes*, and the need to transfer two of the species to maintain its monophyly. The relationships between *Rhithrodytes* and the other three European stygobiont genera of Siettitiina (*Siettitia*, *Iberoporus*, and *Etruscodytes*), although widely recognised, had not been clearly established. Originally, the genus *Rhithrodytes* was erected for a group of species

of *Graptodytes* (the group IV of Zimmermann 1919, or the group “crux” of Guignot 1947) with a curved apex of the median lobe of the aedeagus, a lateral stria running the whole length of the pronotum (Bameul 1989) and (as recognised later), a transverse carina in the epipleura (Fery 2013). With the exception of the epipleural carina, the rest of the characters are shared with the subterranean genus *Siettitia*, which has been for long recognised to be closely related to some of the species included in *Rhithrodytes* (e.g., *R. bimaculatus* (Dufour, 1852); Régimbart 1905, Zimmermann 1932) (Table 3).

Subsequent to the description of *Rhithrodytes* two genera were described each for a single European stygobiont species: *Iberoporus* and *Etruscodytes*. *Iberoporus cermenius* shares the structure of the male genitalia with *Rhithrodytes* and *Siettitia*, but it is in particular very similar to that of *I. agnus* and *I. argaensis*. These two species (formerly in *Rhithrodytes*) have a more straight median lobe and a different shape of the apex of the parameres (Bilton and Fery 1996, Fery 2016).

The body shape of *I. agnus* and *I. argaensis* has also some similarities to the species of *Iberoporus*, parallel-sided and elongated (Figs 5b, c; see figs 12–19 in Fery 2016). *Iberoporus cermenius* shares with *Siettitia* the structure of the metacoxal processes, something that could be related to the subterranean habitat and a poor swimming ability (Castro and Delgado 2001).

Etruscodytes, described from a male and a female, also shares with *Rhithrodytes* and *Siettitia* the general structure of the aedeagus (note that the tip of the aedeagus in the figure of Mazza et al. 2013 seems to be broken) and the long lateral striae of the pronotum (Table 3), but nevertheless was described in a separate genus due to some morphological peculiarities (Mazza et al. 2013). Thus, according to the description by Mazza et al. (2013), the species would have (1) head wide and “subsquare” (regularly rounded in *Siettitia* and *Rhithrodytes*; although more similar to that of *Iberoporus*); (2) presence of short and flattened setae on pronotum and elytra; (3) prosternal process contacting anteromedial process of metaventricle (also in *Rhithrodytes*, not in *Siettitia* and *Iberoporus*, Table 3); (4) anteromedial process of metaventricle rounded (pointed in *Siettitia* according to Mazza et al. 2013); (5) ventrites II and III not fused (fused in *Siettitia* and *I. cermenius*, not in *I. pluto* sp. n. or *Rhithrodytes*); (6) elytra not completely fused (fused in *Siettitia*, not in *Iberoporus* and *Rhithrodytes*). Some of these characters seem to be clear autapomorphies related to the subterranean life (fusion of elytra or ventrites, particularly shaped setae, lack of lateral striae on the pronotum, lack of carina on the epipleuron), and others are of uncertain interpretation. Thus, the structure of the prosternal process is sometimes difficult to appreciate, but there do not seem to be fundamental differences between the species (note that in fig. 7 in Mazza et al. 2013 the prosternal process seems to fit below the anteromedial process of the metaventricle, which is likely an artefact), being the differences consequence of the different position of the mesocoxa (contiguous or not) and ultimately the width of the body, which in turn may depend on the habitat and ecology of the species. More data, especially molecular sequences of *Etruscodytes* and *Siettitia*, and the likely discovery of other subterranean taxa would contribute to the understanding of the evolution of this western Mediterranean lineage.

Table 3. Summary comparison of some character states among the taxa of the *Siettitia* group of genera (character states of *Etruscodytes* obtained from Mazza et al. 2013).

Character and character state	<i>Siettitia</i>	<i>Etruscodytes</i>	<i>Iberoporus cermenius</i> , <i>I. pluto</i> sp. n.	<i>Iberoporus agnus</i> , <i>I. argaensis</i>	<i>Rhithrodytes</i> sensu novo
sublateral pronotal stria	long	long	absent	long	long
subhumeral epipleural carina	absent	absent?	absent	present	present
pigmentation of elytra	weak	weak	weak	strong	generally strong
eyes	absent	absent	absent	present	present
body shape, general	parallel	parallel	parallel	oval-parallel	generally oval
constriction at bases of pronotum and elytra	absent	absent	present	absent	absent
contact between prosternal process and anteromedial metaventral process	absent	present	absent	present	present
ventrites II and III	fused	not fused	fused in <i>I. cermenius</i>	not fused	not fused
elytra	fused	partly fused?	not fused	not fused	not fused

Acknowledgements

We thank all collectors listed in Table 1 for their material, in particular P Richoux (Lyon) for providing specimens of *Siettitia avenionensis*; A Cardoso and A Villastrigo (IBE) for laboratory work; and H Fery (Berlin) for multiple comments and improvements of the manuscript. Molecular work was partly funded by projects CGL2013-48950-C2-1-P and CGL2016-76705-P (AEI/FEDER, UE) to IR. ASR is supported by a research grant (15471) from VILLUM FONDEN. The holotype was collected under permits of the Instituto de Conservação da Natureza e das Florestas.

References

- Abellán P, Sánchez-Fernández D, Picazo F, Millán A, Lobo JM, Ribera I (2013) Preserving the evolutionary history of freshwater biota in Iberian National Parks. *Biological Conservation* 162: 116–126. <https://doi.org/10.1016/j.biocon.2013.04.001>
- Andújar C, Serrano J, Gómez-Zurita J (2012) Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): Assessment of methodological decisions on rate and node age estimation. *BMC Evolutionary Biology* 12: 40. <https://doi.org/10.1186/1471-2148-12-40>
- Bameul F (1989) Description de *Rhithrodytes*, nouveau genre d'Hydrophilinae d'Europe et d'Afrique du Nord: analyse phylogénétique et biogéographie (Coleoptera: Dytiscidae). *Annales de la Société Entomologique de France* N.S. 25: 481–503.
- Bilton DT, Fery H (1996) Revisional notes on *Rhithrodytes* Bameul 1989, with the description of a new subspecies and the introduction of *Rhithrodytes dorsoplagiatus* (Fairmaire) as a valid species (Coleoptera, Dytiscidae). *Linzer Biologische Beiträge* 28: 917–931.
- Castro A, Delgado JA (2001) *Iberoporus cermenius*, a new genus and species of subterranean water beetle (Coleoptera : Dytiscidae) from Spain. *Aquatic Insects* 23: 33–43. <https://doi.org/10.1076/aqin.23.1.33.4931>

- Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* 46: 419–437. <https://doi.org/10.1071/ZO98048>
- Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214. <https://doi.org/10.1186/1471-2148-7-214>
- Enghoff H, Reboleira ASPS (2013) A new cave-dwelling millipede of the genus *Scutogona* from central Portugal (Diplopoda, Chordeumatida, Chamaesomatidae). *Zootaxa* 3736(2): 175–186. <http://dx.doi.org/10.11646/zootaxa.3736.2.5>
- Fery H (2013) *Rhithrodytes*—are we all blind? *Latissimus* 34: 13–15.
- Fery H (2016) Notes on *Rhithrodytes* Bameul, 1989, with the description of *R. minimus* nov. sp. from Algeria (Coleoptera, Dytiscidae: Hydroporini, Sietitiina). *Linzer Biologische Beiträge* 48: 431–450.
- Guignot F (1947) Coléoptères hydrocanthares. *Faune de France* 48: 1–287.
- Hebert PD, Ratnasingham S, De Waard JR (2003) Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* 270: S96–S99. <https://doi.org/10.1098/rsbl.2003.0025>
- Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America* 101(41): 14812–14817. <https://doi.org/10.1073/pnas.0406166101>
- Kanda K, Gómez RA, Van Driesche, RV, Miller KB, Maddison DR (2016) Phylogenetic placement of the Pacific Northwest subterranean endemic diving beetle *Stygoporus oregonensis* Larson & LaBonte (Dytiscidae, Hydroporinae). *ZooKeys* 632: 75–91. <https://doi.org/10.3897/zookeys.632.9866>
- Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298. <https://doi.org/10.1093/bib/bbn013>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Mazza G, Cianferoni F, Rocchi S (2013) *Etruscodytes nethuns* n. gen., n. sp.: the first phreatic water beetle from Italy (Coleoptera: Dytiscidae: Hydroporinae). *Italian Journal of Zoology* 80(2): 233–241.
- Millán A, Sánchez-Fernández D, Abellán P, Picazo F, Carbonell JA, Lobo JM, Ribera I (2014) *Atlas de los coleópteros acuáticos de España peninsular*. Madrid: Ministerio de Agricultura, Alimentación y Medio Ambiente, 819 pp.
- Miller KB, Bergsten J (2016) *Diving beetles of the world: Systematics and biology of the Dytiscidae*. Johns Hopkins University Press, Baltimore, 320 pp.
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE) IEEE, New Orleans*, 1–8. <https://doi.org/10.1109/GCE.2010.5676129>

- Miller KB, Jean A, Alarie Y, Hardy N, Gibson R (2013) Phylogenetic placement of North American subterranean diving beetles (Insecta: Coleoptera: Dytiscidae). *Arthropod Systematics & Phylogeny* 71: 75–90.
- Neves J, Pessoa M, Redinha N (2005) O sistema espeleológico do Dueça. *Espeleo Divulgação* 6: 14–18.
- Nilsson AN, Hájek J (2018a) Catalogue of Palaearctic Dytiscidae (Coleoptera), 88 pp. Internet Version 31.I.2018. <http://www.waterbeetles.eu> [Accessed 11 September 2018]
- Nilsson AN, Hájek J (2018b) A world catalogue of the family Dytiscidae (Coleoptera, Adephaga), 304 pp. Internet version 31.I.2018. Available from: <http://www.waterbeetles.eu> [Accessed 11 September 2018]
- Régimbart M (1905) Note sur le *Siettitia balsetensis*. *Bulletin de la Société Entomologique de France* 1905: 252.
- Reboleira ASPS (2012) Biodiversity and conservation of subterranean fauna of Portuguese karst. Ph.D. thesis, University of Aveiro, 333 pp. <http://hdl.handle.net/10773/10865>
- Reboleira ASPS, Borges P, Gonçalves F, Serrano ARM, Oromí P (2011) The subterranean fauna of a biodiversity hotspot region - Portugal: an overview and its conservation. *International Journal of Speleology* 40(1): 23–37. <http://dx.doi.org/10.5038/1827-806X.40.1.4>
- Reboleira ASPS, Gonçalves F, Oromí P (2013) Literature survey, bibliographic analysis and a taxonomic catalogue of subterranean fauna from Portugal. *Subterranean Biology* 10: 51–60. <http://dx.doi.org/10.3897/subtbiol.10.4025>
- Reboleira ASPS, Gonçalves F, Oromí P, Taiti S (2015) The cavernicolous Oniscidea (Crustacea: Isopoda) of Portugal. *European Journal of Taxonomy* 161: 1–61. <http://dx.doi.org/10.5852/ejt.2015.161>
- Ribera I, Fresneda J, Bucur R, Izquierdo A, Vogler AP, Salgado JM, Cieslak A (2010) Ancient origin of a Western Mediterranean radiation of subterranean beetles. *BMC Evolutionary Biology* 10: 29. <https://doi.org/10.1186/1471-2148-10-29>
- Ribera I, Faille A (2010) A new microphthalmic stygobitic *Graptodytes* Seidlitz from Morocco, with a molecular phylogeny of the genus (Coleoptera, Dytiscidae). *Zootaxa* 2641: 1–14. <https://doi.org/10.11646/zootaxa.2351.1.1>
- Ribera I, Vogler AP, Balke M (2008) Phylogeny and diversification of diving beetles (Coleoptera: Dytiscidae). *Cladistics* 24: 563–590. <https://doi.org/10.1111/j.1096-0031.2007.00192.x>
- Seidlitz G (1887) Bestimmungs-Tabelle der Dytiscidae und Gyrinidae des europäischen Faunengebietes. *Verhandlungen des Naturforschenden Vereines in Brünn* 25(1886): 3–136.
- Shull VL, Vogler AP, Baker MD, Maddison DR, Hammond PM (2001) Sequence alignment of 18S ribosomal RNA and the basal relationships of Adephagan beetles: evidence for monophyly of aquatic families and the placement of Trachypachidae. *Systematic Biology* 50: 945–969. <https://doi.org/10.1080/106351501753462894>
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America* 87: 651–701. <https://doi.org/10.1093/aesa/87.6.651>

- Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* 57: 758–771. <https://doi.org/10.1080/10635150802429642>
- Zaragoza JA, Reboleira ASPS (2018) Five new hypogean *Occidenchthonius* (Pseudoscorpiones, Chthoniidae) from Portugal. *Journal of Arachnology* 46(1): 81–103. <https://doi.org/10.1636/JoA-S-17-031.1>
- Zimmermann A (1919) Die Schwimmkäfer des Deutschen Entomologischen Museums in Berlin-Dahlem. *Archiv für Naturgeschichte* 83A(12) (1917): 68–249.
- Zimmermann A (1932) Monographie der paläarktischen Dytisciden. III. Hydroporinae (3. Teil). *Koleopterologische Rundschau* 18: 69–111.

Without a body of evidence and peer review, taxonomic changes in Liolaemidae and Tropiduridae (Squamata) must be rejected

Jaime Troncoso-Palacios¹, Margarita Ruiz De Gamboa², Roberto Langstroth³,
Juan Carlos Ortiz⁴, Antonieta Labra⁵

1 Programa de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, Independencia 1027, Casilla 70005, Santiago, Chile **2** Centro de Investigación en Medio Ambiente (CENIMA), Universidad Arturo Prat, Casilla 121, Iquique, Chile **3** Colección Boliviana de Fauna, Calle 27 Cota Cota, La Paz, Bolivia **4** Departamento de Zoología, Facultad de Ciencias naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile **5** Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, Norway

Corresponding author: Jaime Troncoso-Palacios (jtroncosopalacios@gmail.com)

Academic editor: J. Penner | Received 17 August 2018 | Accepted 5 December 2018 | Published 7 January 2019

<http://zoobank.org/C404C299-10C8-41A9-85C6-31BC36A2009E>

Citation: Troncoso-Palacios J, Ruiz De Gamboa M, Langstroth R, Ortiz JC, Labra A (2019) Without a body of evidence and peer review, taxonomic changes in Liolaemidae and Tropiduridae (Squamata) must be rejected. ZooKeys 813: 39–54. <https://doi.org/10.3897/zookeys.813.29164>

Abstract

In his recent self-published book "Reptiles en Chile", Diego Demangel Miranda presented 13 taxonomic changes for liolaemid and tropidurid lizards. While these could be considered validly published according to the International Code of Zoological Nomenclature, we show that these taxonomic propositions lack the necessary scientific rigor in terms of replicability, specimen work, lack of peer review and that they do not follow best practices accepted by the herpetological community. Therefore, we hereby invalidate all 13 taxonomic changes proposed in this book, leaving the taxonomy unaffected. Finally, we call attention to the potentially negative consequences of using these taxonomic changes in conservation and environmental impact studies as incorrect decisions might be taken in relation to the species involved.

Keywords

Taxonomy, best practices, International Code of Zoological Nomenclature, synonymy, *Liolaemus*, *Microlophus*, *Phymaturus*

Introduction

Field guides are a common source of information for the general public interested in the identification of plants and animals in a region. However, popular guide-books are not intended to be vehicles for taxonomic decisions and should follow the taxonomy supported by the most recent peer-reviewed scientific studies. A reliable taxonomy, the science of describing, naming, and/or synonymizing taxa (Engelhorn and Seberg 2006), requires multiple lines of evidence (e.g., de Queiroz 1998) supported by the appropriate comparisons of voucher specimens (Ceríaco et al. 2016). Furthermore, any such study needs to be submitted to the opinions and comments of other experts; thus, taxonomic studies should be published only after peer review (Kaiser et al. 2013; Schutze et al. 2017). Whereas the International Code of Zoological Nomenclature (ICZN 1999; hereafter the Code) regulates the nomenclatural acts, it does not have the aim of regulating the methods by which taxonomic decisions should be generated and how any resulting change should become part of the scientific record (Kaiser 2013). This situation allows publication of taxonomic decisions in a non-scientific manner, with insufficient or unsuitable methodology, with lack of evidence, or via self-publication without peer review. In many cases, these unscientific changes are numerous and have affected several taxonomic groups, as has been carefully critiqued (Jäch 2007a, b; Wallach et al. 2009; Kaiser 2013, 2014a; Reynolds et al. 2014; Rhodin et al. 2015). The most controversial cases in herpetology are the names created by Raymond Hoser, who self-publishes the *Australasian Journal of Herpetology* (see Kaiser et al. 2013). Hoser's publications (e.g., Hoser 2012a, b) may include the basic requirements for valid nomenclature, such as availability of the publication, names for proposed taxa, holotype for species descriptions and diagnoses for proposed taxa (Wüster et al. 2001). However, these are provided in unacceptable ways, without proper evidence or peer review and sometimes with substantial plagiarism and ethical breaches (Kaiser et al. 2013; Denzer et al. 2015; Rhodin et al. 2015). While one case regarding Hoser's self-published names is pending a ruling by the International Commission on Zoological Nomenclature (e.g., Kaiser 2014b; Rhodin et al. 2015), nine herpetologists, supported by 100 other herpetologists as well as several large herpetological societies and journals, have determined that unscientific taxonomic decisions in herpetology without a body of evidence and without peer review should be unacceptable for the purposes of herpetological taxonomy (Kaiser et al. 2013).

Recently, Diego Demangel Miranda published the book "Reptiles en Chile" (Demangel Miranda 2016a), which is an exceptional source of high-quality photographs of living Chilean non-avian reptiles, especially relevant for species without previously published photographs. However, the book includes several taxonomic changes in the Liolaemidae and Tropiduridae along with several changes in the geographic distribution for various species. Here, we examine whether these taxonomic changes fulfill the currently recognized best practices in herpetology (Kaiser et al. 2013).

Features of "Reptiles en Chile" by Demangel Miranda (2016a)

The 619-page book starts with 20 pages of general overview (cover page, acknowledgments, prologue, preface, and presentation). Thereafter, it provides generalities about reptiles, as well as some information of their characteristics, evolution, and species concepts (pp. 21–39). It then provides a list of Chilean reptile species accepted by the author (pp. 40–44), and guidelines to facilitate the identification of the Chilean reptile groups in the field (pp. 45–50). Thereafter (pp. 51–59), the book gives data on biogeography and species conservation, followed by a glossary (pp. 60–63), and an explanation (pp. 64–65) of how to use the book according to the information provided for each species.

The bulk of the book (pp. 66–583) provides accounts of each species, including (for almost all species) three pages of photos of live individuals attributed to the species (each indicating the locality). Each species account begins with a caption indicating the species name, authority, and, in some cases, a list of “formal synonyms or other scientific names used in the last 30 years” (our translation, p. 64). However, the synonymies do not indicate the authority or year. Each species account has one page with text, including the etymology of the name, type locality, diagnosis, morphological features, distribution, natural history notes, some specific references, as well as a distribution map and usually three small photos. In total, this section has 143 pages of text (i.e., the text occupies a column, with maps and species photos occupying another column) and 375 pages of photos. Remarkably, the species accounts include three species names proposed by the author but following an unusual presentation for taxonomic descriptions. While standard taxonomic publication introduces the first use of the name and then presents the diagnostic traits and description of the holotype and other types in consecutive pages (e.g., Quinteros et al. 2014), Demangel Miranda (2016a) provided the first use of the name and the diagnostic features on consecutive pages of the book, but placed the brief holotype descriptions at the end of the book in a section entitled “Taxonomic notes” (pp. 584–597, Fig. 1).

The taxonomic notes section includes the changes proposed in the book: one for Tropiduridae (one synonymy) and 12 for Liolaemidae (nine synonymies and three proposed species). On pages 596–597, the author lists the type specimens for the three proposed species and provides a brief description of the holotypes. These two pages of descriptions also include three small photographs displaying the three holotypes in life as well as a photo of two live individuals (placed on the same rock), stating that these are individuals of two of the described species. Finally, the literature cited in Demangel Miranda’s book is presented on pp. 598–612, followed by the name index (pp. 614–618).

Although Demangel Miranda (2016a) included several taxonomic decisions, there is no section describing the methods he used to arrive at these decisions. Such a section is fundamental in any scientific study, including, for example, other recent descriptions of Chilean *Liolaemus* (e.g., Núñez et al. 2000; Valladares 2004; Esquerré et al. 2013; Quinteros et al. 2014). While there are some statements in Demangel Miranda

PROPOSED NAMES IN DEMANGEL MIRANDA (2016a)			
First use of the names followed by one page with diagnostic traits and general features	Photographs of live individuals attributed to each proposed species (no vouchers)	UNRELATED TEXT	Brief description of holotypes
Liolaemus igneus pp. 266–267	pp. 268–269		Liolaemus igneus p. 596
Liolaemus tacora pp. 476–477	pp. 478–479		Liolaemus tacora p. 596
Liolaemus tolhuaca pp. 484–485	pp. 486–487		Liolaemus tolhuaca p. 597

Figure 1. Outline of the non-standard presentation of the three species proposed by Demangel Miranda (2016a).

(2016a) which indicate that the author performed “scale counts,” he did not describe how these were done. Beyond the five specimens used for the descriptions of his three proposed species, Demangel Miranda (2016a) stated that he examined the holotype of *L. lopezi* Ibarra-Vidal, 2005 (p. 586), one specimen of *L. molinai* Valladares et al., 2002 (p. 590), two specimens of *L. frassinettii* Núñez, 2007 (p. 590), one specimen of *L. carlosgarini* Esquerré et al., 2013 (p. 592), and two syntypes of *L. melanopleurus* (Philippi, 1960) (p. 592). These 12 specimens are not listed in an appendix of material examined, contrary to standard practice in taxonomy (e.g., Lobo and Espinoza 2004; Abdala and Quinteros 2008; Breitman et al. 2011b; Avila et al. 2012). Furthermore, Demangel Miranda (2016a) also stated that he examined the type series of *L. velosoi* Ortiz, 1987, and *Microlophus tarapacensis* (Donoso-Barros, 1966) at the MZUC (Museo de Zoología de la Universidad de Concepción), but he did not provide catalog numbers. He also indicated that he reviewed MZUC specimens labeled as *L. brattstroemi* Donoso-Barros, 1961, indicating that these were not the types, and that he instead assigned these to *L. cyanogaster* (Duméril & Bibron, 1837), but he, again, did this without providing catalog numbers or data to support this claim. This lack of examined material is a serious problem of the proposed taxonomic changes included in this book.

Copies of Demangel Miranda's book were first available on 30 June 2016 (2000 copies). We remark that some copies were sold before the launching (pers. obs.). The book was published by Fauna Nativa Ediciones and, to our knowledge, this is the first and sole book published by this company. Moreover, Fauna Nativa Ediciones is the publishing arm of Fauna Nativa Consultores SPA (<http://www.faunanativa.cl>), which is a company owned by Diego Demangel Miranda dedicated to wildlife evaluation services in regard to environmental impact studies. No editor is mentioned by Demangel Miranda (2016a), but the verso of the title page indicates "Texts", "Photographs" and "Photographic edition" by Diego Demangel and "Style revision", "General design", "Graphical design", and "Layout" are credited to other people. Thus, it can be concluded that Diego Demangel Miranda was solely responsible of the final review of the text (i.e., acted as editor) and that he took all decisions in relation to the taxonomic changes made.

Taxonomic changes proposed in Demangel Miranda (2016a)

Demangel Miranda (2016a) started the book indicating that "... [this] is not a conventional scientific work" (p. 17), which may cause some uncertainties for the reader. Then, in the first paragraph of the section where the taxonomic changes are presented (pp. 584–597), Demangel Miranda stated that "...it was not possible to review all the literature regarding the different species and therefore many readers may be disappointed with the text in their hands..." (our translations, p. 584) which also may cause some uncertainties. The 13 taxonomic changes made by Demangel Miranda (2016a) in *Liolaemidae* and *Tropiduridae* are summarized in Table 1.

The main failure of the taxonomic changes proposed by Demangel Miranda (2016a) is that these were not published via peer review. As demonstrated above, he was both his own editor and the owner of the publisher which produced the book. Although Demangel Miranda (2016a, p. 6) stated that Juan Carlos Torres-Mura, a Chilean zoologist, reviewed "some sections of the book", this cannot be considered a peer review because Torres-Mura reviewed only "part of the text" and it is unclear which sections. Moreover, in a scientific publication the author does not choose the reviewers (while some journals allow suggesting reviewers, which is different than choosing) and because Demangel Miranda himself is credited as responsible for all texts (editor), this procedure cannot be considered to fulfill the objectives of an appropriate peer review (see Voight and Hoogenboom 2012). As recommended by Kaiser et al. (2013), peer review should involve at least two independent reviewers and an editor who can objectively be considered experts in the field of the manuscript under review. This lack of peer review is a strong argument to indicate that the taxonomic acts in Demangel Miranda (2016a) should not be accepted by the herpetological community.

In the following paragraphs, we provide additional information to support our conclusions that Demangel Miranda (2016a) also failed to meet the accepted best practice in herpetological taxonomy. There is no "body of evidence" to support his

Table 1. Summary of the taxonomic changes proposed by Demangel Miranda (2016a). Details of recommendation are explained in the text. *Liolaemus* subgenera are according to Abdala and Quinteros (2014).

Family	Genus / Subgenus (if correspond)	Taxon	Demangel Miranda (2016a) proposed	Recommendation
Liolaemidae	<i>Liolaemus</i> / <i>Liolaemus</i> (sensu stricto)	<i>Liolaemus brattstroemi</i> Donoso-Barros, 1961	Synonym of <i>Liolaemus pictus</i> (Duméril & Bibron, 1837)	Not acceptable
		<i>Liolaemus chungara</i> Quinteros, Valladares, Semham, Acosta, Barrionuevo & Abdala, 2014	Synonym of <i>Liolaemus alticolor</i> Barbour, 1909	
		<i>Liolaemus kuhlmanni</i> Müller & Hellmich, 1933	Synonym of <i>Liolaemus zapallarensis</i> Müller & Hellmich, 1933	
		<i>Liolaemus velosoi</i> Ortiz, 1987	Synonym of <i>Liolaemus platei</i> Werner, 1898	
	<i>Liolaemus</i> / <i>Eulaemus</i>	<i>Liolaemus lopezi</i> Ibarra-Vidal, 2005	Synonym of <i>Liolaemus ornatus</i> Koslowsky, 1898	
		<i>Liolaemus morandae</i> Breitman, Parra, Pérez & Sites, 2011	Synonym of <i>Liolaemus lineomaculatus</i> Boulenger, 1885	
		<i>Liolaemus scolaroi</i> Pincheira-Donoso & Núñez, 2005	Synonym of <i>Liolaemus zuliyae</i> Cei & Scolaro, 1996	
	<i>Liolaemus</i>	<i>Liolaemus igneus</i>	Proposed species	
		<i>Liolaemus tacora</i>	Proposed species	
		<i>Liolaemus tolhuaca</i>	Proposed species	
Tropiduridae	<i>Phymaturus</i>	<i>Phymaturus aguedae</i> Troncoso-Palacios & Esquerré, 2014	Synonym of <i>Phymaturus darwini</i> Núñez, Veloso, Espejo, Veloso, Cortés & Araya, 2010	
		<i>Phymaturus damasense</i> Troncoso-Palacios & Lobo, 2012	Synonym of <i>Phymaturus maulense</i> , Núñez, Veloso, Espejo, Veloso, Cortés & Araya, 2010	
	<i>Microlophus</i>	<i>Microlophus yanezi</i> (Ortiz, 1980)	Synonym of <i>Microlophus theresioides</i> (Donoso-Barros, 1966)	

synonymies and proposed species, which could have been done by using the available literature and by appropriate data analysis, as is required by best practices (Kaiser et al. 2013).

Synonymies

One general problem is that Demangel Miranda (2016a) did not refer to material examined for the junior or senior synonyms proposed, apart from the holotype of *L. lopezi* (all other specimens listed are not involved in the taxonomic changes). This omission is problematic as the examination and listing of specimens are key aspects of correct

taxonomic practice (see Dubois 2017a), which allows others to build knowledge based on the new data. Moreover, the lack of a section describing how the synonymies were developed or the proposed species were described (i.e., lack of materials and methods) makes the conclusions reached by Demangel Miranda (2016a) a non-replicable result.

Liolaemus brattstroemi

Demangel Miranda (2016a, pp. 394, 590–591) declared *Liolaemus brattstroemi* Donoso-Barros, 1961 to be a junior synonym of *L. pictus* (Duméril & Bibron, 1837). However, he provided no comparative data for *L. pictus* (neither from reviewed vouchers nor references) to support this claim. The author only supported this proposed synonymy by a visit to the type locality of *L. brattstroemi*, where he found lizards that he considered assignable only to *L. pictus*, without indication of how many individuals were analyzed to reach this conclusion or provide a reliable data analysis.

Liolaemus chungara

Demangel Miranda (2016a, pp. 142, 591) included *Liolaemus chungara* Quinteros et al., 2014, as a junior synonym of *L. alticolor* Barbour, 1909. Demangel Miranda (2016a, p. 591) proposed this synonymy based on the presence and absence of precloacal pores in the males he found during a field trip to the type locality of *L. chungara*. However, there is no indication of how many males were sampled and no information on the examined voucher specimens (neither of *L. chungara* nor of *L. alticolor*) was provided. In addition, the accuracy of the determination of the precloacal pores in the field remains unclear, considering that an appropriate observation of these types of pores requires the use of magnifying lenses, whose use was not indicated by Demangel Miranda (2016a). Moreover, it was not indicated how he concluded that all the observed males were of the same species or how he concluded that this supposed variation is “a relatively common feature” in *L. alticolor*.

Liolaemus lopezi

Demangel Miranda (2016a, pp. 374, 585–586) included *Liolaemus lopezi* Ibarra-Vidal, 2005, as a junior synonym of *L. ornatus* Koslowsky, 1898. In contrast to the procedure used to propose the other synonymies in his book, in the case of *L. lopezi* Demangel Miranda examined the holotype of this species (Museo Regional de Concepción, CHMHNC 1099); however, he did not review vouchers of *L. ornatus*.

He cited Pincheira-Donoso and Núñez (2005) as his only source of data for *L. ornatus* morphological variation (p. 585) and despite being unsure if these data really belong to *L. ornatus* (p. 586), he still proposed the synonymy.

Liolaemus kuhlmanni

Demangel Miranda (2016a, pp. 512, 592) included *Liolaemus kuhlmanni* Müller & Hellmich, 1933, as a junior synonym of *L. zapallarensis* Müller & Hellmich, 1933, as previously proposed by Pincheira-Donoso and Núñez (2005) but rejected by Lobo et al. (2010a). Demangel Miranda (2016a) proposed this synonymy without providing information on the specimens analyzed (i.e., vouchers reviewed) or any other supporting evidence (e.g., data of compared scale count ranges or morphological measures, statistical analysis, molecular data). While referring to Lobo et al. (2010a) in an unrelated paragraph (e.g., p. 590), Demangel Miranda (2016a) did not mention that the same study had rejected the prior synonymy of *L. kuhlmanni* under *L. zapallarensis*. This procedure clearly did not fulfill “the third line of evidence” that a reliable taxonomic study needs to follow (Kaiser et al. 2013, p. 18), because there is an important omission of a key published scientific study that must have been included in the “body of knowledge” on *L. kuhlmanni*.

Liolaemus morandae

Demangel Miranda (2016a, pp. 306, 586) included *Liolaemus morandae* Breitman et al., 2011 as a junior synonym of *L. lineomaculatus* Boulenger, 1885. Breitman et al. (2011b) split *L. lineomaculatus* into three species: *L. morandae*, *L. avilae*, and *L. lineomaculatus* (with allopatric distributions from north to south, respectively), based on a principal component analysis (PCA), a multivariate analyses (MPMANOVA), and a multilocus phylogeny with examination of 36 specimens of these species. Demangel Miranda (2016a, p. 586) proposed the synonymy based on comparisons of live individuals that he found during field trips to Aysén and Magallanes Regions, Chile, but without inclusion of animals from the type locality of *L. morandae*. He claimed that “the diagnosis provided by Breitman et al. (2011b) does not allow a proper separation between *L. morandae* and *L. lineomaculatus*” (Demangel Miranda 2016a, p. 586), without mention *L. avilae*. Moreover, he pointed out that an integrative taxonomic study should be performed to evaluate if it is appropriate to split *L. lineomaculatus* into species or subspecies, without acknowledging the study already published by Breitman et al. (2011b). While best practices indicate the need for “rigorous” taxonomic analyses (Kaiser et al. 2013, p. 8), Demangel Miranda (2016a) failed in regard to this synonymy due to his total lack of evidence to refute the results of Breitman et al. (2011b), which is indeed an integrative taxonomy study in *Liolaemus*.

Liolaemus scolaroï

Demangel Miranda (2016a, pp. 516, 585) included *Liolaemus scolaroï* Pincheira-Donoso & Núñez, 2005 as a junior synonym of *L. zullyae* Cei & Scolaro, 1996. Demangel Miranda (2016a) based his synonymy on a field trip to the type locality of *L. scolaroï*,

during which he claims to have examined live individuals, but without providing supporting data, analysis, and results. Moreover, Demangel Miranda (2016a) did not refer to the previous publications (Breitman et al. 2011a, 2014) that had already suggested this possible synonymy.

Liolaemus velosoi

Demangel Miranda (2016a, pp. 398, 593) included *Liolaemus velosoi* Ortiz, 1987, as a junior synonym of *L. platei* Werner, 1898. Although Demangel Miranda (2016a) stated that he reviewed the *L. velosoi* type series, he did not provide catalog numbers and did not mention examining any voucher specimens of *L. platei*. Demangel Miranda (2016a) did not provide any data for the scale counts considered diagnostic for these species (Ortiz 1987; Pincheira-Donoso and Núñez 2005) or other type of evidence apart from the photos in which he stated that the color variation overlaps between species. The mtDNA phylogeny of Troncoso-Palacios et al. (2015) showed a deep genetic divergence between these species, but this was not taken in account by Demangel Miranda (2016a) as part of the body of knowledge for this species.

Phymaturus aguedae* and *P. damasense

Demangel Miranda (2016a, pp. 530, 534, 593) included *Phymaturus aguedae* Troncoso-Palacios & Esquerré, 2014, as a junior synonym of *P. darwini* Núñez et al., 2010, and included *P. damasense* Troncoso-Palacios & Lobo, 2012 as a junior synonym of *P. maulense* Núñez et al., 2010. Demangel Miranda (2016a) based both synonymies on live individuals (no vouchers were listed) that he observed in field trips, but in both cases he did not list all the visited localities and he only provided the same ambiguous sentence for both synonymies: “[I] have carried out multiple field trips to the localities where [these *Phymaturus*] are known”. He also indicated that “an inter-population analysis was performed” without indicating the methodology, data or results of this analysis. Additionally, he stated that he compared scale counts and scale sizes (i.e., preocular and canthal) on live individuals, without indication of how many individuals were examined. It should be indicated that scales in *Phymaturus* are very small and taxonomic studies performed in this genus have declared the use of magnification lenses for proper observation (e.g., Lobo et al. 2010b), which suggest that field observations of scales made by Demangel Miranda are neither rigorous, appropriate, nor reliable.

Microlophus yanezi

Demangel Miranda (2016a, pp. 560, 595) included *Microlophus yanezi* (Ortiz, 1980) as a junior synonym of *M. theresioides* (Donoso-Barros, 1966). Ortiz (1980) described

M. yanezi and distinguished it from *M. theresioides* based on the average counts of mid-body scales and average counts of scales in the fourth toe lamellae. However, Demangel Miranda (2016a) only compared the ranges of these scale counts based on Ortiz (1980), without vouchers or another data source, and without mention of the average differences, or additional evidence to refute Ortiz's (1980) conclusions. Moreover, without evidence as in the case of *L. morandae*, Demangel Miranda (2016a) attempted to undermine a previous published scientific study.

Species names proposed by Demangel Miranda (2016a)

Demangel Miranda (2016a) proposed three names: *Liolaemus igneus* (p. 266), *L. tacora* (p. 478) and *L. tolhuaca* (p. 486), with small sample sizes ($n = 3$, $n = 1$ and $n = 1$, respectively) and through an odd presentation that does not follow the standard taxonomic descriptions (see Fig. 1). The holotypes were placed in the Museo Nacional de Historia Natural de Chile. All the proposed names lack a hypothesis of group membership apart of from being assigned to the genus *Liolaemus*, a very diverse genus (257 species; Abdala and Quinteros 2014) which includes two well-supported subgenera, each composed of several groups (see Lobo et al. 2010a; Abdala and Quinteros 2014). However, none of the species proposed by Demangel Miranda (2016a) were assigned to either of these subgenera. This omission is a failure to fulfill the first step of the best practices in herpetology when species are described (Kaiser et al. 2013, p. 8).

Moreover, Demangel Miranda (2016a) did not provide analyses and appropriate methodology to support his species hypotheses. For example, the “diagnostic features” were based solely on some color patterns, and shape and size of some scales. When the author attempts to utilize the size of the specimens as diagnostic feature, he only used ambiguous comparative expressions such as “bigger than” or “smaller than”. Although *Liolaemus* comprises some 257 species, Demangel Miranda (2016a) precariously compared his three proposed species with only five *Liolaemus* species (without indication of reviewed vouchers): he only compared *L. igneus* and *L. tacora* against *L. jamesi* and with each other; he compared *L. tolhuaca* against only four other *Liolaemus* (*L. buergeri*, *L. lonquimayensis*, *L. scorialis* and *L. zabalai*). This is worsened by the fact that Demangel Miranda (2016a) failed to provide evidence of the supposed species as cohesive populations, which is necessary according to the best practices (Kaiser et al. 2013, p. 18). For example, *L. igneus* is based on three specimens collected at three different localities (p. 596), without evidence to support that these three specimens conformed a recognizable cohesive population assignable to a species. Moreover, *L. tacora* and *L. tolhuaca* are based only on one specimen each (pp. 597–98), but despite this, he indicated variation for some features (e.g., scale count ranges, differences between males and females) without reference to any paratypes or other material examined or reference (i.e., the source of the variability of his data is unknown). Remarkably, for *L. igneus* and *L. tacora*, Demangel Miranda (2016a, p. 267 and p. 477, respectively) included a “species bibliography” listing “Abdala et al. (2008)”, “Quinteros et al. (2008)”, and

“Quinteros and Abdala (2011)”, but there are neither indications of the aim of these references in the context of the characterizations nor their inclusion in the book bibliography. We can only speculate that these may refer to studies describing Argentine *Liolaemus* species. These facts allow us to conclude that Demangel Miranda (2016a) did not fulfill the second step of the best practices, to test the taxonomic hypothesis through a “rigorous, honest, and appropriate methodology” (Kaiser et al. 2013, p. 8).

As previously pointed out, the major problem with the three species names proposed by Demangel Miranda (2016a) and all his other taxonomic decisions is that they were not published via peer review, which is in opposition to the third step of the best practices proposed by Kaiser et al. (2013, p. 8).

Finally, Demangel Miranda (2016a) does not denote an effort to maintain taxonomic stability in *Liolaemus*, *Phymaturus* and *Microlophus*, as he proposed 13 taxonomic decisions for two squamate families, with very few reviewed vouchers and without reliable analyses, and rather represented his own vision in an authoritarian way, which is not in agreement with the appropriate practices in taxonomy (Kaiser et al. 2013, Schutze et al. 2017).

Final remarks

In addition to the taxonomic instability produced by the propositions already discussed, the taxonomic and distributional changes performed in the book may potentially have major negative consequences affecting society at large. The use of this type of unreliable taxonomy by agencies or institutions dealing with biodiversity and conservation problems can lead to incorrect decisions with potential negative consequences such as the loss of biological resources (Wilson 1985; Pilon and Chase 2007; Georges et al. 2011; Kaiser et al. 2013; Thomson et al. 2018). Although the last updated reptile list for Chile (Ruiz de Gamboa 2016) did not consider Demangel Miranda’s (2016a) taxonomic changes because of the “lack of scientific rigor” in it, Demangel Miranda (2016a) has already begun to be used by the Chilean government agencies that deal with conservation and biodiversity. For example, “Vertebrados en Peligro de la Región Metropolitana de Santiago, Chile” [Endangered Vertebrates from the Metropolitan Region of Santiago, Chile] by Carrasco-Lagos et al. (2016) and the background record for conservation purposes of *Hydrophis platurus* (Tala, 2016), both published by the Chilean Ministry of the Environment, cite Demangel Miranda (2016a). Demangel Miranda (2016a) also is cited in environmental documents involved in the approval of the proposal of a power generation plant submitted to the Chilean Environmental Impact Assessment System (file N° 52, http://seia.sea.gob.cl/expediente/expedientesEvaluacion.php?modo=ficha&id_expediente=2131347751). The problem is that while Demangel Miranda (2016a) compiled useful information, at the same time the publication changes several important aspects for numerous Chilean lizard species without scientific support (e.g., distributional ranges and taxonomy). Remarkably, the three names proposed and two of the synonymies (*L. kuhlmanni* and *P. damasense*) by

Demangel Miranda (2016a) have been also followed by the widely used “Reptile Database” (Uetz et al. 2018, accessed in December 3, 2018), without indication of why these two synonymies are followed whereas the other are not.

The book also generates confusion about what is a taxonomic synonym. The listing of names as synonyms by Demangel Miranda (2016a) does not follow the accepted practice (e.g., names do not include any author citations or year), which could lead to incorrect interpretations. For example, he recognizes *L. islugensis* and *L. cf. pantherinus* as full species, but at the same time, includes each in the synonymy of the other (pp. 274, 378).

Later in 2016, Demangel Miranda launched a second field guide entitled “Reptiles del Centro Sur de Chile” (Demangel Miranda 2016b). This new book is a simplified version of Demangel Miranda (2016a) that covers only the species of central and southern Chile and does not include species descriptions or new synonymies. The guide reflects the taxonomic and distributional changes of Demangel Miranda (2016a), hence, we recommend to not follow it as taxonomically reliable source.

Finally, concerning the Code, Article 8.1 requires that for a study to be considered as published for the purposes of zoological nomenclature “it must be issued for the purpose of providing a public and permanent scientific record”. The problem here is that the Code does not define “scientific record”. Demangel Miranda (2016a) himself declares that “the book is not a conventional scientific publication” (p. 17), as is evidenced by the lack of several sections and procedures typically used in scientific publications such materials and methods, analyses of the characters and publication via peer review. For us, it is clear that he did not intend the book to be a scientific review of Liolaemidae and *Microlophus*, but rather the purpose was the diffusion of knowledge of the Chilean reptile for a wide range of Chilean readers. Some authors have stated that the names proposed in publications that are not intended to be “scientific record” should be considered as not valid nomenclatural act (e.g., Busack et al. 2016), a detailed clarification of this issue is provided by Schleip (2014); if this strict interpretation of the Art. 8.1 is applied to Demangel Miranda (2016a), the three names proposed could be considered as not valid, but we think that the sole publication of these names without fulfilling the best practices (Kaiser et al. 2013) should be enough to avoid their use. Additionally, we suggest that Art. 13.1 of the Code, needs to be improved because this requires that the description must include the characters “purported to differentiate the taxon” but not that these achieve this goal, which in our opinion is insufficient as requirement and have been also noted as unclear by Dubois (2017b).

Acknowledgements

We thank all our colleagues who encouraged us to write this review. JTP thanks Mario Penna for his support, and MRGA thanks Beca Conicyt Doctorado Nacional 2012, 21120577. We thank Hinrich Kaiser, Andrés Sebastián Quinteros, Richard Etheridge and Scott A. Thomson for all their suggestions and constructive criticism that allowed us to significantly improve this manuscript.

References

- Abdala CS, Quinteros AS (2008) Una nueva especie de *Liolaemus* (Iguania, Liolaemidae) endémica de la Sierra de Fiambalá, Catamarca, Argentina. Cuadernos de Herpetología 22: 35–47.
- Abdala CS, Quinteros AS (2014) Los últimos 30 años de estudios de la familia de lagartijas más diversa de Argentina. Actualización taxonómica y sistemática de Liolaemidae. Cuadernos de Herpetología 28: 55–82.
- Abdala CS, Quinteros AS, Espinoza RE (2008) Two new species of *Liolaemus* (Iguania: Liolaemidae) from the Puna of Northwestern Argentina. Herpetologica 64: 458–471. <https://doi.org/10.1655/08-022R1.1>
- Avila LJ, Pérez CHF, Medina CD, Sites Jr JW, Morando M (2012) A new species of lizard of the *Liolaemus elongatus* clade (Reptilia: Iguania: Liolaemini) from Curi Leuvu River Valley, northern Patagonia, Neuquén, Argentina. Zootaxa 3325: 37–52.
- Breitman MF, Avila LJ, Sites Jr JW, Morando M (2011a) Lizards from the end of the world: Phylogenetic relationships of the *Liolaemus lineomaculatus* section (Squamata: Iguania: Liolaemini). Molecular Phylogenetics and Evolution 59: 364–376. <https://doi.org/10.1016/j.ympev.2011.02.008>
- Breitman MF, Parra M, Pérez CHF, Sites Jr JW (2011b) Two new species of lizards from the *Liolaemus lineomaculatus* section (Squamata: Iguania: Liolaemidae) from southern Patagonia. Zootaxa 3120: 1–28. <https://doi.org/10.5281/zenodo.208130>
- Breitman MF, Minoli I, Avila LJ, Medina CD, Sites Jr JW, Morando M (2014) Lagartijas de la provincia de Santa Cruz (Argentina): Distribución geográfica, diversidad genética y estado de conservación. Cuadernos de Herpetología 28: 83–110.
- Busack SD, Salvador A, Bauer AM, Kaiser H (2016) *Darevskia* and *Iberolacerta* (Reptilia, Lacertidae): Arribas, 1997 or 1999? The correct dating of two nomenclatural acts affecting Palearctic lizards, and validation of the name *Caucasilacerta* Harris, Arnold & Thomas, 1998. Bionomina 10: 61–73. <https://doi.org/10.11646/bionomina.10.1.4>
- Carrasco-Lagos P, Moreno RA, Tala C, Ibarra-Vidal H, Duarte M (2016) Vertebrados en peligro de la Región Metropolitana de Santiago, Chile. Seremi del Medio Ambiente Región Metropolitana de Santiago, Universidad Santo Tomás, Ministerio del Medio Ambiente, Exp Ediciones al Conocimiento, Instituto de Ecología y Biodiversidad (IEB-Chile), Minera Florida, Santiago, 52 pp.
- Ceríaco LMP, Gutiérrez EE, Dubois A (2016) Photography-based taxonomy is inadequate, unnecessary, and potentially harmful for biological sciences. Zootaxa 4196: 435–445. <https://doi.org/10.11646/zootaxa.4196.3.9>
- de Queiroz K (1998) The general lineage concept of species, species criteria, and the process of speciation. In: Howard DJ, Berlocher SH (Eds) Endless Forms: Species and Speciation. Oxford University Press, New York, 57–75.
- Demangel Miranda D (2016a) Reptiles en Chile. Fauna Nativa Ediciones, Santiago, 619 pp.
- Demangel Miranda D (2016b) Guía de Campo – Reptiles del centro sur de Chile. Corporación Chilena de la Madera, Concepción, Chile, 187 pp.
- Denzer W, Manthey U, Wagner P, Böhme W (2015) A critical review of Hoser's writings on *Draconinae*, *Amphibolurinae*, *Laudakia* and *Uromastycinae* (Squamata: Agamidae). Bonn zoological Bulletin 64: 117–138.

- Dubois A (2017a) The need for reference specimens in zoological taxonomy and nomenclature. *Bionomina* 12: 4–38. <https://doi.org/10.11646/bionomina.12.1.2>
- Dubois A (2017b) Diagnoses in zoological taxonomy and nomenclature. *Bionomina* 12: 63–85. <https://doi.org/10.11646/bionomina.12.1.8>
- Engelhoff H, Seberg O (2006) A taxonomy of taxonomy and taxonomists. *The Systematist* 27: 13–15.
- Esquerré D, Núñez H, Scolaro JA (2013) *Liolaemus carlosgarini* and *Liolaemus riodamas* (Squamata: Liolaemidae), two new species of lizards lacking precloacal pores, from Andean areas of central Chile. *Zootaxa* 3619: 428–452. <https://doi.org/10.11646/zootaxa.3619.4.2>
- Georges A, Spencer RJ, Welsh M, Shaffer HB, Walsh R, Zhang X (2011) Application of the precautionary principle to taxa of uncertain status: the case of the Bellinger River turtle. *Endangered Species Research* 14: 127–134. <https://doi.org/10.3354/esr00350>
- Hoser RT (2012a) Three new species of *Stegonotus* from New Guinea (Serpentes: Colubridae). *Australasian Journal of Herpetology* 12: 18–22.
- Hoser RT (2012b) A review of the taxonomy of the living crocodiles including the description of three new tribes, a new genus, and two new species. *Australasian Journal of Herpetology* 14: 9–16.
- ICZN (1999) International Code of Zoological Nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature, London. <http://www.nhm.ac.uk/hosted-sites/iczn/code>
- Jäch MA (2007a) Vandalism in taxonomy. *Koleopterol. Rundschau* 77: 38.
- Jäch MA (2007b) Vandalism in taxonomy (continued). *Koleopterol. Rundschau* 77: 146.
- Kaiser H (2013) The Taxon Filter, a novel mechanism designed to facilitate the relationship between taxonomy and nomenclature, vis-à-vis the utility of the Code's Article 81 (the Commission's plenary power). *Bulletin of Zoological Nomenclature* 70: 293–302. <https://doi.org/10.21805/bzn.v70i4.a17>
- Kaiser H (2014a) Best practices in herpetological taxonomy: Errata and addenda. *Herpetological Review* 45: 257–268.
- Kaiser H (2014b) Comment on *Spracklandus* Hoser, 2009 (Reptilia, Serpentes, Elapidae): request for confirmation of the availability of the generic name and for the nomenclatural validation of the journal in which it was published. *Bulletin of Zoological Nomenclature* 71: 30–35.
- Kaiser H, Crother BJ, Kelly CMR, Luiselli L, O'Shea M, Ota H, Passos P, Schleich WD, Wüster W (2013) Best practices: in the 21st century, taxonomic decisions in herpetology are acceptable only when supported by a body of evidence and published via peer-review. *Herpetological Review* 44: 8–23.
- Lobo F, Espinosa RE (2004) Two new *Liolaemus* from the Puna region of Argentina and Chile: Further resolution of purported reproductive bimodality in *Liolaemus alticolor* (Iguania: Liolaemidae). *Copeia* 2004: 850–867. <https://doi.org/10.1643/CH-03-241R1>
- Lobo F, Espinoza RE, Quinteros AS (2010a) A critical review and systematic discussion of recent classification proposals for liolaemid lizards. *Zootaxa* 2549: 1–30.
- Lobo F, Abdala C, Valdecantos S (2010b) Taxonomic studies of the genus *Phymaturus* (Iguania: Liolaemidae): Description of four new species. *South American Journal of Herpetology* 5: 102–126. <https://doi.org/10.2994/057.005.0205>

- Núñez H, Navarro J, Veloso A (2000) *Liolaemus foxi*, una nueva especie de lagarto para el Norte de Chile (Squamata: Reptilia: Sauria). Boletín del Museo Nacional de Historia Natural, Chile 49: 117–130.
- Ortiz JC (1980) Estudio comparativo de algunas poblaciones de *Tropidurus* de la costa chilena. Anales del Museo de Historia Natural de Valparaíso 13: 267–279.
- Ortiz JC (1987) Une nouvelle espèce de *Liolaemus* (Sauria, Iguanidae) du Chili. Bulletin du Museum National d'Histoire Naturelle, Paris, Section A, Zoologie, Biologie et Ecologie Animales 9: 265–270.
- Pillon Y, Chase MW (2007) Taxonomic exaggeration and its effects on orchid conservation. Conservation Biology 21: 263–265. <https://doi.org/10.1111/j.1523-1739.2006.00573.x>
- Pincheira-Donoso D, Núñez H (2005) Las especies chilenas del género *Liolaemus* Wiegmann, 1834 (Iguania: Tropiduridae: Liolaeminae). Taxonomía, sistemática y evolución. Publicación Ocasional del Museo Nacional de Historia Natural, Chile 59: 1–486.
- Quinteros AS, Abdala CS (2011) A new species of *Liolaemus* of the *Liolaemus montanus* section (Iguania: Liolaemidae) from northwestern Argentina. Zootaxa 2789: 35–48.
- Quinteros AS, Valladares P, Semham R, Acosta JL, Barrionuevo S, Abdala CS (2014) A New Species of *Liolaemus* (Iguania: Liolaemidae) of the *alticolor-bibronii* Group from Northern Chile. South American Journal of Herpetology 9: 20–29. <https://doi.org/10.2994/SAJH-D-13-00040.1>
- Reynolds GR, Niemiller ML, Revell LJ (2014) Toward a tree-of-life for the boas and pythons: multilocus species-level phylogeny with unprecedented taxon sampling. Molecular Phylogenetics and Evolution 71: 201–213. <https://doi.org/10.1016/j.ympev.2013.11.011>
- Rhodin AGJ, Kaiser H, van Dijk PP, Wüster W, O'Shea M, Archer M, Auliya M, Boitani L, Bour R, Clausnitzer V, Contreras-MacBeath T, Crother BI, Daza JM, Driscoll CA, Flores-Villela O, Frazier J, Fritz U, Gardner AL, Gascon C, Georges A, Glaw F, Grazziotin FG, Groves CP, Haszprunar G, Havaš P, Hero J-M, Hoffmann M, Hoogmoed MS, Horne BD, Iverson JB, Jäch M, Jenkins CL, Jenkins RKB, Kiester AR, Keogh JS, Lacher Jr TR, Lovich JE, Luiselli L, Mahler DL, Mallon D, Mast R, McDiarmid RW, Measey J, Mittermeier RA, Molur S, Mosbrugger V, Murphy RW, Naish D, Niekisch M, Ota H, Parham JF, Parr MJ, Pilcher NJ, Pine RH, Rylands AB, Sanderson JG, Savage JM, Schleip W, Scrocchi GJ, Shaffer HB, Smith EN, Sprackland R, Stuart SN, Vetter H, Vitt LJ, Waller T, Webb G, Wilson EO, Zaher H, Thomson S (2015) Comment on “*Spracklandus* Hoser, 2009 (Reptilia, Serpentes, Elapidae): request for confirmation of the availability of the generic name and for the nomenclatural validation of the journal in which it was published. Bulletin of Zoological Nomenclature 72: 65–78.
- Ruiz de Gamboa M (2016) Lista actualizada de los reptiles de Chile. Boletín Chileno de Herpetología 3: 7–12.
- Schleip WD (2014) Two new species of *Leiopython* Hübner, 1879 (Pythonidae: Serpentes): non-compliance with the International Code of Zoological Nomenclature leads to unavailable names in zoological nomenclature. Journal of Herpetology 48: 272–275. <https://doi.org/10.1670/13-157>
- Schutze MK, Bourtzis K, Cameron SL, Clarke AR, de Meyer M, Hee AKW, Hendrichs J, Krosch MN, Mwatawala M (2017) Integrative taxonomy versus taxonomic authority without peer-review: The case of the Oriental fruit fly, *Bactrocera dorsalis* (Tephritidae). Systematic Entomology 42: 609–620. <https://doi.org/10.1111/syen.12250>

- Tala C (2016) *Hydrophis platurus*. Ministerio del Medio Ambiente, Chile. http://www.mma.gob.cl/clasificacionespecies/fichas13proceso/fichas-inicio/Pelamis_platurus_INICIO_13RCE.pdf
- Thomson SA, Pyle RL, Ah Yong ST, Alonso-Zarazaga M, Ammirati J, Araya JF, Ascher JS, Audisio TL, Azevedo-Santos VM, Bailly N, Baker WJ, Balke M, Barclay MVL, Barrett RL, Benine RC, Bickelstaff JRM, Bouchard P, Bour R, Bourgoin T, Boyko CB, Breure ASH, Brothers DJ, Byng JW, Campbell D, Ceriaco LMP, Cernák I, Cerretti P, Chang C, Cho S, Copus JM, Costello MJ, Cseh A, Csuzdi C, Culham A, D'Ellia G, et al. (2018) Taxonomy based on science is necessary for global conservation. *PLoS Biology* 16: e2005075. <https://doi.org/10.1371/journal.pbio.2005075>
- Troncoso-Palacios J, Schulte JA II, Marambio-Alfaro Y, Hiriart D (2015) Phenotypic variation, phylogenetic position and new distributional records for the poorly known *Liolaemus silvai* Ortiz, 1989 (Iguania: Iguanidae: Liolaemini). *South American Journal of Herpetology* 10: 71–81. <https://doi.org/10.2994/SAJH-D-14-00007.1>
- Uetz P, Freed P, Hošek J (2018) The Reptile Database. <http://www.reptile-database.org> [Accessed on: 2018–12–3]
- Valladares JF (2004) Nueve especie de lagarto del género *Liolaemus* (Reptilia: Liolaemidae) del norte de Chile, Previamente confundido con *Liolaemus* (= *Phrynosaura*) *reichei*. *Cuadernos de Herpetología* 18: 43–53.
- Voight ML, Hoogenboom BJ (2012) Publishing your work in a Journal: understanding the peer review process. *The International Journal of Sports Physical Therapy* 7: 452–460.
- Wallach V, Wüster W, Broadley DG (2009) In praise of subgenera: taxonomic status of cobras of the genus *Naja* Laurenti (Serpentes: Elapidae). *Zootaxa* 2236: 26–36. <https://doi.org/10.5281/zenodo.190422>
- Wilson EO (1985) The biodiversity crisis: A challenge to science. *Issues in Science and Technology* 2: 20–29.
- Wüster W, Bush B, Keogh JS, O'Shea M, Shine R (2001) Taxonomic contributions in the “amateur” literature: comments on recent descriptions of new genera and species by Raymond Hoser. *Litteratura Serpentina* 21: 67–79, 86–91.

A new species of forest snake of the genus *Rhadinaea* from Tropical Montane Rainforest in the Sierra Madre del Sur of Oaxaca, Mexico (Squamata, Dipsadidae)

Vicente Mata-Silva¹, Arturo Rocha², Aurelio Ramírez-Bautista³,
Christian Berriozabal-Islas³, Larry David Wilson⁴

1 Department of Biological Sciences, The University of Texas at El Paso, Texas 79968-0500, USA **2** Department of Biological Sciences, El Paso Community College, Texas 79968-0500, USA **3** Centro de Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca-Tulancingo Km 4.5, Colonia Carboneras, C. P. 42184, Mineral de la Reforma, Hidalgo, Mexico **4** Centro Zamorano de Biodiversidad, Escuela Agrícola Panamericana Zamorano, Departamento de Francisco Morazán, Honduras; 16010 SW 207th Avenue, Miami, Florida 33187-1056, USA

Corresponding author: Vicente Mata-Silva (vmata@utep.edu)

Academic editor: R. Jadin | Received 9 September 2018 | Accepted 10 December 2018 | Published 7 January 2019

<http://zoobank.org/418B781C-1AEE-45CC-ADF0-7B1778FE2179>

Citation: Mata-Silva V, Rocha A, Ramírez-Bautista A, Berriozabal-Islas C, Wilson LD (2019) A new species of forest snake of the genus *Rhadinaea* from Tropical Montane Rainforest in the Sierra Madre del Sur of Oaxaca, Mexico (Squamata, Dipsadidae). ZooKeys 813: 55–65. <https://doi.org/10.3897/zookeys.813.29617>

Abstract

Content of the dipsadid genus *Rhadinaea* has changed considerably since Myers' 1974 revision. Three species groups are recognized currently in the genus. Our fieldwork in Oaxaca in June 2018 produced a single specimen of *Rhadinaea* considered to represent a new taxon. This new species is described from converted Premontane Wet Forest in the municipality of Santa Catarina Juquila in the Sierra Madre del Sur of southern Oaxaca, Mexico. It is most closely related to *Rhadinaea laureata*, from which it can be distinguished easily by color pattern and scutellation, and represents a species group distinct from the other three occupying the genus.

Keywords

Morphology, new serpent species, Santa Catarina Juquila municipality, Southern Mexico, taxonomy

Resúmen

La conformación del género *Rhadinaea* ha cambiado considerablemente desde la revisión de Myers en 1974. Tres grupos de especies son reconocidos actualmente en el género. Nuestro trabajo de campo en Oaxaca en junio de 2018 produjo un espécimen de *Rhadinaea* que consideramos que representa un nuevo taxón. Describimos esta nueva especie de bosque húmedo premontano en el Municipio de Santa Catarina Juquila en la Sierra Madre del Sur en el sur de Oaxaca, México. Esta especie está más estrechamente relacionada con *Rhadinaea laureata*, de la cual se puede distinguir fácilmente por medio del patrón de color y escutelación, y representa un grupo distinto a los tres que forman parte del género.

Palabras claves

Morfología, nueva especie de serpiente, municipalidad de Santa Catarina Juquila, el sur de México, taxonomía

Introduction

The dipsadid genus *Rhadinaea* was once considered one of the largest genera of snakes in the Western Hemisphere. Myers' (1974) well-known and well-regarded revision of this genus dealt with 45 species arranged into eight species groups. In the ensuing years, however, additional systematic work of a morphometric nature has reduced the content of *Rhadinaea*, largely by attrition, owing to allocation of a number of species placed in this genus by Myers (1974) to other genera. Myers (2011) resurrected the genus *Rhadinella* to contain the species he had placed earlier in the *Rhadinaea godmani* group. The eleven species he included in this group in 1974 had been augmented to 15 by his 2011 paper and now comprises a total of 19 species, including 18 listed at the Reptile Database (<http://reptile-database.reptarium.cz/>; accessed 15 June 2018) and one other species, *Rhadinella stadelmani*, which was resurrected by Mendelson and Kizirian (1995), but not placed in the genus *Rhadinella* by Myers (2011), as noted by García-Vázquez et al. (2018). Savage and Crother (1989) placed the *Rhadinaea lateristriga* group of Myers (1974), with seven species, along with the species of *Pliocercus* in the genus *Urotheca*. Other authors (Myers and Cadle 1994; Smith et al. 1995; McCranie 2011), however, rejected Savage and Crother's conclusions in part, preferring to continue to recognize *Pliocercus* as a genus distinct from *Urotheca*. Finally, Myers and Cadle (1994) placed the *Rhadinaea brevirostris* group of Myers (1974), with six species, in the resurrected genus *Taeniophallus*. As a result of the changes engendered in this systematic work over the last 44 years, *Rhadinaea* has been restructured to include only 21 species, arranged in five species groups (the *flavilata* group, containing *R. flavilata* and *R. laureata*; the *decorata* group, with *R. bogertorum*, *R. cuneata*, *R. decorata*, *R. forbesi*, *R. gaigeae*, *R. hesperia*, *R. macdougalli*, *R. marcellae*, *R. montana*, *R. myersi*, *R. nuchalis* [described by García-Vázquez et al. 2018], *R. omiltemana*, and *R. quinquelineata*; the *taeniata* group, with *R. fulvivittis* and *R. taeniata*; the *calligaster* group, with only *R. calligaster*; and the *vermiculaticeps* group, with *R. pulveriventris*, *R. sargenti*, and *R. vermiculaticeps*).

Two of the five species groups (*calligaster* and *vermiculaticeps* groups) currently recognized in *Rhadinaea* are extralimital to Mexico, occurring from Costa Rica to Colombia (Pérez-Higareda et al. 2002; Savage 2002; Köhler 2008; Ray 2017) and do not ap-

pear to contain taxa that are closely related to the one described herein. Another of the five groups (the *flavilata* group) comprises two species, one of which is distributed in the southeastern United States (*R. flavilata*) and the other in the Sierra Madre Occidental and the Trans-Mexican Volcanic Belt of western and central Mexico (*R. laureata*).

Apart from *R. laureata* mentioned in the previous paragraph, there are 15 other species distributed in Mexico (Heimes 2016; García-Vázquez et al. 2018), which are arranged in two species groups recognized by Myers (1974). These two groups are the *taeniata* group, with three species, and the *decorata* group, with 13 species. The distinctions among the three species groups of *Rhadinaea* represented in Mexico (*decorata*, *laureata*, and *taeniata* groups) are treated below in the Discussion section of this paper.

In general, species of the genus *Rhadinaea* are semifossorial, secretive, and infrequently encountered in the field, which means that significantly large sample sizes are difficult to impossible to accrue to assist in systematic studies and for other purposes. In June of 2018, however, while conducting general herpetofaunal surveys in the Sierra Madre del Sur of southern Oaxaca, Mexico, a single specimen of a *Rhadinaea* was encountered that we consider representing a species new to science, which we place in a new species group within this genus, and is described below.

Materials and methods

We conducted fieldwork in the area around the type locality of the new species described herein in early June, 2018, at moderate elevations in disturbed premontane wet forest now used for the cultivation of coffee. The holotype was preserved in full-strength ethyl alcohol to allow for molecular study. It was deposited in the collection of the Centro de Investigaciones Biológicas at the Universidad Autónoma del Estado de Hidalgo, Mexico.

We examined and measured the holotype with a stereomicroscope and precision digital calipers to the nearest 0.1 mm. The format for the description generally follows that of Batista et al. (2016). The ventral scales were counted using the methodology of Dowling (1951). We used a slash mark (/) to delineate characters that differ from the left and right sides of the holotype. We used the following abbreviations for morphological measurements:

SVL	(snout–vent length),	HL	(head length),
TL	(tail length),	HW	(head width), and
TOL	(total length),	ED	(eye diameter).

The sex was determined by the presence of partially everted hemipenes. The color pattern in life was described based on the color catalogue of Köhler (2012).

Systematic account

Rhadinaea eduardoi sp. n.

<http://zoobank.org/9D5AA496-27F7-4657-B1E9-59FA9901F81B>

Figures 1–5, Table 1

Common name. English: Eduardo's forest snake. Spanish: Hojarasquera de Eduardo

Holotype. CIB-5457 (original field number VMS-2029), a subadult male from Mexico, Oaxaca, municipality of Santa Catarina Juquila, El Obispo, 1,320 m (UTM 681141.99, 1789988.05 [= 16.183573, -97.305614, datum WGS 84]), collected by Eduardo Mata-Silva on 6 June 2018 at 1800 hrs (Fig. 1).

Diagnosis. A snake of the genus *Rhadinaea* that can be distinguished from all congeners by the following combination of morphological features: supralabials 7, with 3rd and 4th entering orbit; 120 ventrals; 71 subcaudals; one subpreocular (lower preocular); 17 dorsal scales throughout body; a head pattern lacking postorbital pale markings but having a pale line extending from the lower rear quadrant of the eye to the ultimate supralabial and slightly beyond, and a midbody dorsal color pattern of a lateral series of black dots in the lower apex of the scales of row V and a middorsal line confined to the middorsal scale row consisting of a series of disjunct spots on the posterior apex of otherwise dark brown scales.

Description of holotype (Figs 2–4). Subadult male, as evidenced by size of the body and partially everted hemipenes; SVL 196 mm; TL 90 mm; TOL 286 mm, TL 31.5% of TOL; head slightly wider than body; HL 8.29 mm; HW 5.73 mm; ED 1.79 mm, about 21.6% of HL; rostral 1.86 mm long and 0.92 mm wide; internasal on right 1.15 mm long and 1.04 mm wide, on left 1.04 × 0.88, contacting anterior and posterior nasals, rostral, and one/both prefrontals; one large preocular subtended by a small subpreocular (lower preocular); two subequal postoculars; temporals 1 + 2, separating supralabials vi and vii from parietals; 7 supralabials, 1st in contact with rostral, anterior and posterior nasals, and 2nd supralabial, 2nd in contact with posterior nasal (narrowly), 1st supralabial, loreal, 3rd supralabial, 3rd in contact with loreal (narrowly), subpreocular, orbit, and 4th supralabial, 4th in contact with 3rd supralabial, orbit, lower postocular, and 5th supralabial, 5th supralabial in contact with 4th supralabial, lower postocular, and 6th supralabial; 6th supralabial in contact with 5th supralabial, lower postocular (narrowly), anterior temporal, lower posterior temporal, and 7th supralabial, 7th supralabial in contact with 6th supralabial, lower posterior temporal, and two posttemporal scales; 8/9 infralabials, 1st pair in medial contact, four in contact with anterior chinshields, 5th the largest; mental 1.29 mm long and 0.90 mm wide, separated from anterior chinshields by medial contact of 1st pair of infralabials; anterior and posterior chinshields more or less subequal in size; four preventrals between posterior chinshields and 1st ventral; smooth dorsal scales arranged in 17 longitudinal rows throughout the body, with no apical pits; 120 ventrals, cloacal scute divided; and 71 paired subcaudals between cloacal scute and terminal spine.

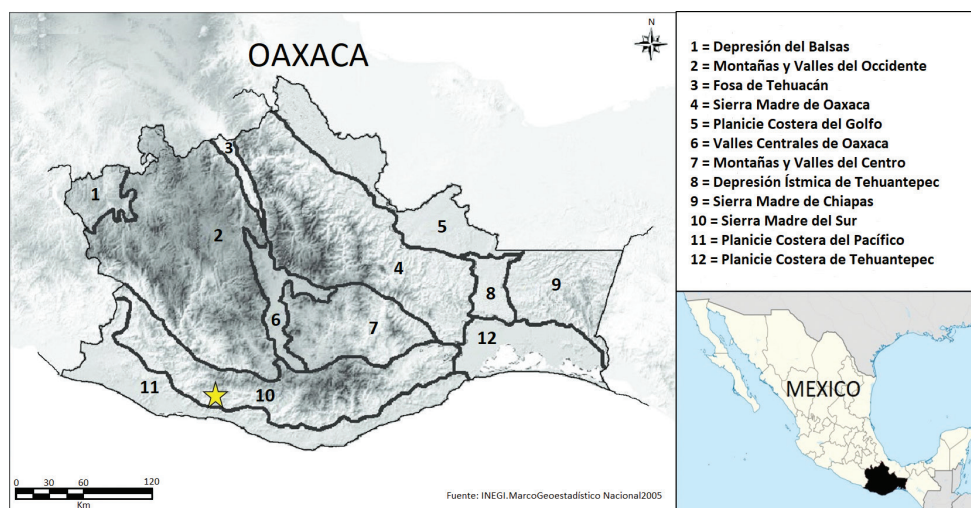


Figure 1. Map depicting the site (star) where *Rhadinaea eduardoi* was found.



Figure 2. Head and anterior portion of body of holotype of *Rhadinaea eduardoi*.

Coloration in life of holotype (Figs 2, 3a, b). The dorsum and lateral portion of the head above the pale lip line is Cinnamon-Rufous (color 31). A white line begins posterior to the posteroventral quadrant of the eye and extends posteriorly to disappear on the neck. A white dash outlined above by black is present on the side of the neck about three scales posterior to the parietal scales and is separated from the pale line on the body. The region of the dorsum of the body above scale row five is Hazel (color 26). A disjunct black stripe is present on the middorsal scale row, consisting of a series of black dots, each of which markings occupies the posterior portion of each dorsal

Table 1. Distinguishing features of three species groups of *Rhadinaea* represented in Mexico (based on data in Myers 1974), compared to a new species group erected for the new species described herein.

<i>Rhadinaea</i> species groups in Mexico				
Distinguishing Features	<i>decorata</i>	<i>flavilata</i>	<i>taeniata</i>	<i>eduardoi</i>
Number of supralabials	Normally 8–9, sometimes 7	Normally 7	8	7
Number of infralabials	10	9	10	8–9
Subpreocular	Normally present	Absent	Usually present	Present
Head pattern	Conspicuous pale postocular marking extending from, or lying a short distance behind, upper rear edge of eye	Vague, sometimes scarcely discernible pale line outlined below by often diffuse, broken black line	Conspicuous pale stripe behind (and usually in front of) eye confluent with pale dorsolateral stripe	Conspicuous pale postocular marking extending from the lower rear edge of eye
Dark stripes	Variable, but with narrow dark line on rows 4 or 5	Diffuse or absent	Distinct, well-defined middorsal and lateral stripes	Diffuse, poorly defined middorsal and lateral stripes
Anal ridges in males	Usually present, but sometimes absent	Usually present	Usually present	Absent
Ventrals in males	110–175	112–167	140–180	120
Ventrals in females	114–186	118–176	157–197	–
Subcaudals in males	56–137	68–97	83–121	71
Subcaudals in females	60–120	59–92	78–114	–



Figure 3. Holotype of *Rhadinaea eduardoi* in life.



Figure 4. Dorsal and ventral views of the preserved holotype of *Rhadinaea eduardoi*.

scale. The middorsal and lateral portions of the color pattern are separated by a narrow Pale Buff (color 1) line on scale row five that is bordered below by a black spot on the ventral apex of each scale. The lateral region of the dorsum is Cinnamon-Rufous (color 31) that becomes pale and flecked. The chin and anterior portion of the venter is dark gray flecked with black, grading to cream for the remainder of the venter. The lateral portion of each ventral on the anterior portion of the body bears a black spot.

Coloration in preservative (Fig. 4). The dorsum of the head is dark brown. The lateral portion of the snout is very dark brown. The postocular and temporal region of the head is dark brown. The first four supralabials are dark gray, heavily mottled with very dark brown. The pale line on the upper portion of the postocular supralabials is white, bounded above by a black border and below by a broken black mottled border. A pair of longitudinally-arranged white spots bordered above by a black border are located just posterior to the temporal scales on the right side. These posttemporal spots are in line with a black-bordered white stripe and separated from this stripe's point of origin by a single dorsal scale. The white supralabial stripe gradually disappears posterior to the head. The underside of the head is gray, heavily speckled with small black dots. This coloration continues onto the anterior portion of the venter, with the speckling decreasing in intensity. A black spot is present at the lateral apex of the ventral scales on the anterior venter. The dark speckling and spotting fades between ventrals, with the remainder of the venter and the ventral portion of the tail a pale yellow. The middorsal region of the body is dark brown. The middorsal row consists of a series of black dots positioned on the posterior apex of each middorsal scale. The dark brown scales of the middorsal and lateral regions of the body are separated by a pale line on the dorsal portion of scale row iv underlain by a black line on the ventral portion of the same scale row.

Etymology. This species is named in honor of Eduardo Mata-Silva, collector of the holotype. Eduardo is the younger brother of the senior author of this paper, is a resident of Río Grande, Oaxaca, and is a highly valued member of our field crew working in Oaxaca. He also outshines the rest of the crew when it comes to finding snakes, as evidenced by his discovery of the holotype of the snake described herein.

Habitat and natural history observations. *Rhadinaea eduardoi* is resident in an area of converted Premontane Wet Forest, which presently supports a plantation of



Figure 5. Habitat where holotype of *Rhadinaea eduardoi* was found.

shade-grown coffee (Fig. 5). The holotype was found active at 1800 hrs on leaf litter approximately 10 m from a stream after a very light rain. Other herpetofaunal species encountered at this site were the anurans *Craugastor pygmaeus*, *Ptychohyala leonhardschultzei*, and *Exerodonta sumichrasti*, and the lizards *Norops* sp. and *Holcosus undulatus*.

Distribution. This species is known only from the type locality in the Sierra Madre del Sur of southern Oaxaca, Mexico (Fig. 1).

Conservation status. *Rhadinaea eduardoi* joins the extensive cadre of conservation category priority one species in Mexico designated by Johnson et al. (2017). This species is placed in this category due to its high EVS value and its restriction to a single physiographic region in Mexico. Its EVS can be calculated as $6 + 8 + 2 = 16$, placing it in the middle of the high vulnerability category. This score is based on a contributory score of 6 for geographic distribution, because it is known only from the type locality, 8 for ecological distribution, because it is known only from a single forest formation, and 2 for human persecution, because it is semifossorial, non-venomous, and generally escapes human notice. It is restricted furthermore to the Sierra Madre del Sur. The EVS of 16 for this species matches that for *Rhadinaea bogertorum* (Johnson et al. 2017), another species of *Rhadinaea* endemic to the state of Oaxaca (Mata-Silva et al. 2015). Using reasoning similar to that employed by Batista et al. (2016), we opine that *Rhadinaea eduardoi* can be placed in the Critically Endangered category, according to IUCN criteria B1ab(iii)+2ab(iii).

Discussion

As noted in the introduction, Myers (1974) recognized eight species groups within the genus *Rhadinaea*, which has now been reduced to five, given that three of these groups have been allocated to other genera by subsequent authors (see introduction). Two of these five species groups, the *calligaster* and *vermaculiceps* groups, are restricted in distribution to Lower Central America and have members remotely related to the members of the other three species groups, the center of distribution of which is in Mexico. The largest of these three groups is the *decorata* group, most species of which are restricted in distribution to Mexico, with one species (*R. decorata*) currently considered to range from Mexico to Colombia (Myers 1974). Another group of two species, the *laureata* group, contains one species found in Mexico and another distributed in the southeastern United States. The third group, the *taeniata* group, consists of three species, all restricted in distribution to Mexico. The principal distinctions among these three species groups are indicated in Table 1.

The new species described herein does not appear to belong to any of the three species groups of *Rhadinaea* represented in Mexico. It is distinguished easily from the members of the *decorata* group in having seven supralabials instead of eight or nine and eight or nine infralabials instead of 10. It differs from the members of the *taeniata* group in the same features (seven versus eight supralabials and eight or nine versus 10 infralabials), as well as having many fewer ventrals and subcaudals (120 and 71 versus 140–197 and 78–121, respectively). Although it agrees with the members of the *flavilata* group in having 7 supralabials, *R. eduardoi* has a subpreocular (lower preocular) and a strikingly different dorsal color pattern. In our opinion, *Rhadinaea eduardoi* should be placed in a species group of its own (Table 1). Nonetheless, *R. eduardoi* appears to share its closest relationship with the members of the *flavilata* group, because of the presence of seven supralabials, with the 3rd and 4th entering the orbit. Logically, *R. laureata* would appear to be the most likely candidate as the closest known relative of *R. eduardoi*, but these two species are rather unlike one another in color pattern of the head and body, as well as in the much higher ventral numbers in male *R. laureata* (150–167, as opposed to 120 in the male holotype of *R. eduardoi*) and the much higher subcaudals in male *R. laureata* (86–97, as opposed to 71 in the male holotype of *R. eduardoi*). The color pattern of *R. laureata* consists of “a broad (three to five scale rows), gray dorsal stripe on a golden brown body, and little or no indication of lateral striping. There is a pale line through the top of the eye and another pale line that crosses the neck immediately behind the head; the last line may be confluent at its lower end with a line on the supralabials” (Myers 1974: 55). In addition, the distributional range of *R. laureata* is relatively remote from that of *R. eduardoi*, in the Trans-Mexican Volcanic Belt and the Sierra Madre Occidental, as opposed to the Sierra Madre del Sur.

Mexico is a country of significant herpetofaunal endemism, reported by Johnson et al. (2017) at 61.1% (on the basis of 789 endemic species of a total of 1,292 species). Mata-Silva et al. (2015) reported the state of Oaxaca to be the most speciose state in Mexico, with a total herpetofauna of 442 species. Of these 442 species, 164 are country endemics and 93 are state endemics. Combining these two figures indicates a total

proportion of endemism in Oaxaca of 58.1% (257/442). The physiographic region in which *Rhadinaea eduardoi* was discovered is the Sierra Madre del Sur (SMS), which Mata-Silva et al. (2015) reported as the second most speciose physiographic region in terms of herpetofauna of the 12 regions they recognized in the state. The number of species these authors reported in the SMS is 154, 34.8% of the 442 species known from the state at that time. This figure is second only to the number for the Sierra Madre de Oaxaca (216 species or 48.9% of the total of 442 species). The SMS regional herpetofauna was reported by Mata-Silva et al. (2015) to consist of 42 anurans, six salamanders, one caecilian, 46 lizards, 53 snakes, and six turtles. Of the 154 SMS species, 58 are country endemics and 25 are state endemics. The combined percentage of endemism for the SMS is 53.9% (83/154). Of the 53 species of snakes in the SMS, 18 are country endemics and three are state endemics; the combined percentage of snake endemism is 39.6% (21/53). The three state endemic SMS snake species are *Tantilla oaxacae*, *T. triseriata*, and *Micrurus ephippifer*. Thus, *Rhadinaea eduardoi* is an addition to this list, as well as to the overall list of endemic species in Mexico. *Rhadinaea eduardoi* also joins two other species of *Rhadinaea*, *R. fulvivittis* and *R. myersi*, resident in the SMS of Oaxaca that are endemic to Mexico (Mata-Silva et al. 2015).

Acknowledgments

We are especially grateful to the Bolán-Mata family and to V. Mata-Pérez for their assistance and hospitality. The collecting permit (SGPA/DGVS/004007/18) was issued by SEMARNAT to ARB, with extension to VMS, EMS, AR, and LDW. We also thank Jonathan A Campbell and Robert C Jadin for reviewing and improving the manuscript.

References

- Batista A, Mebert K, Lotzkat S, Wilson LD (2016) A new species of centipede snake of the genus *Tantilla* (Squamata: Colubridae) from an isolated premontane forest in eastern Panama. *Mesoamerican Herpetology* 3: 949–960.
- Dowling HG (1951) A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97–99.
- García-Vázquez UO, Pavón-Vázquez CJ, Blancas-Hernández JC, Blancas-Calva E, Centenero-Alcalá E (2018) A new rare species of the *Rhadinaea decorata* group from the Sierra Madre del Sur de Guerrero, Mexico (Squamata, Colubridae). *ZooKeys* 780: 137–154. <https://doi.org/10.3897/zookeys.780.25593>
- Heimes P (2016) *Herpetofauna Mexicana Volume I: Sankes of Mexico*. Edition Chimaira, Frankfurt am Main, 572 pp.
- Johnson JD, Wilson LD, Mata-Silva V, García-Padilla E, DeSantis DL (2017) The endemic herpetofauna of Mexico: organisms of global significance in severe peril. *Mesoamerican Herpetology* 4: 543–620.

- Köhler G (2008) Reptiles of Central America (2nd edn). Herpeton, Offenbach, 400 pp.
- Köhler G (2012) Color Catalogue for Field Biologists. Herpeton, Offenbach, 49 pp.
- Mata-Silva V, Johnson JD, Wilson LD, García Padilla E (2015) The herpetofauna of Oaxaca, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 2: 5–62.
- McCranie JR (2011) The Snakes of Honduras. *Contributions to Herpetology*, vol 26, Society for the Study of Amphibians and Reptiles, Ithaca, New York, 724 pp.
- Mendelson JR, Kizirian DA (1995) Geographic variation in *Rhadinaea hempsteadae* (Serpentes: Colubridae) with the description of a new species from Chiapas, Mexico. *Herpetologica* 51: 301–313.
- Myers CW (1974) The systematics of *Rhadinaea* (Colubridae), a genus of New World snakes. *Bulletin of the American Museum of Natural History* 153: 1–262.
- Myers CW (2011) A new genus and new tribe for *Enicognathus melanauchen* Jan, 1863, a neglected South American snake (Colubridae: Xenodontinae), with taxonomic notes on some Dipsadinae. *American Museum Novitates* 3(715): 1–33.
- Myers CW, Cadle JE (1994) A new genus for South American snakes related to *Rhadinaea obtusa* Cope (Colubridae) for the “*Rhadinaea*” *brevirostris* group. *American Museum Novitates* 3(102): 1–33.
- Pérez-Higareda G, López-Luna MA, Chiszar D, Smith HM (2002) Additions to and Notes on the Herpetofauna of Veracruz, Mexico. *Bulletin of Chicago Herpetological Society* 37: 67–68.
- Ray JM (2017) Snakes of Panama: A Field Guide to all Species. Team Snake Panama, CreateSpace Independent Publishing Platform, 213 pp.
- Savage JM (2002) The Amphibians and Reptiles of Costa Rica: A Herpetofauna between Two Continents, between Two Seas. The University of Chicago Press, Chicago, 954 pp.
- Savage JM, Crother BI (1989) The status of *Pliocercus* and *Urotheca* (Serpentes: Colubridae), with a review of included species of coral snake mimics. *Zoological Journal of the Linnean Society* 95: 335–362. <https://doi.org/10.1111/j.1096-3642.1989.tb02315.x>
- Smith HM, Wallach V, Chiszar D (1995) Observations on the snake of the genus *Pliocercus*, I. *Bulletin of the Maryland Herpetological Society* 31: 204–214.

Three new species of the genus *Zodarion* (Araneae, Zodariidae) from China

Bao-Shi Zhang^{1,2}, Feng Zhang³

1 College of Life Sciences, Hebei Normal University, Shijiazhuang, Hebei 050024, China **2** Department of Biochemistry, Baoding University, Baoding, Hebei 071051, China **3** The Key Laboratory of Invertebrate Systematics and Application, College of Life Sciences, Hebei University, Baoding, Hebei 071002, China

Corresponding author: Feng Zhang (dudu06042001@163.com)

Academic editor: S. Li | Received 13 September 2018 | Accepted 7 December 2018 | Published 7 January 2019

<http://zoobank.org/75FB5405-5829-4012-8148-EDA92ED5E082>

Citation: Bao-Shi Z, Feng Z (2019) Three new species of the genus *Zodarion* (Araneae, Zodariidae) from China. ZooKeys 813: 67–87. <https://doi.org/10.3897/zookeys.813.29683>

Abstract

Three new species of the genus *Zodarion* Walckenaer, 1826, from China, are described as *Zodarion apertum* sp. n. (♂♀, from Xinjiang), *Z. planum* sp. n. (♂, from Shaanxi), and *Z. ovatum* sp. n. (♂♀, from Yunnan).

Keywords

Asia, *italicum* group, *lutipes* group, taxonomy, zodariides

Introduction

The genus *Zodarion* was established by Walckenaer in 1826, with *Enyo nitida* Audouin, 1826 as its type species. Jocqué (1991) synonymized *Argus* Walckenaer, 1842, *Clotho* Walckenaer, 1837, *Enyo* Savigny & Audouin, 1825, *Lucia* C. L. Koch, 1837, and *Metargus* F. O. Pickard-Cambridge, 1902 with *Zodarion*. Marusik and Koponen (2001) transferred seven Central Asian and Chinese *Zodarion* species to *Zodariellum*. However, Jocqué and Henrard (2015) rejected the conclusions of Marusik and Koponen (2001) and transferred sixteen species (including two Chinese species) from *Zodariellum* to *Zodarion*.

Presently, *Zodarion* is the largest genus of the subfamily Zodariinae, and includes 160 species. The genus has been recorded in European, Asian, and North African countries (Jocqué 1991; Bosmans 1994, 1997, 2009; Marusik and Koponen 2001;

Pekár et al. 2011; Bosmans et al. 2014; Jocqué and Henrard 2015; Li and Lin 2016; WSC 2018), and each species has a limited distribution. From Asia, 34 species are known. 24 species are only known from females and 14 from males (World Spider Catalog 2018). Species of *Zodarion* are rare in China, with only three documented species: *Zodarion chaoyangense* Zhu & Zhu, 1983, from Liaoning and Hebei provinces; *Z. furcum* Zhu, 1988 from Hebei Province; and *Z. hunanense* Yin, 2012 from Hunan Province (Li and Lin 2016). During the examination of spider collections from China, we recognized three new zodariid species which are described here as *Zodarion apertum* sp. n., *Z. planum* sp. n., and *Z. ovatum* sp. n.

Materials and methods

All specimens have been preserved in 75% ethanol and were examined, illustrated, and measured under a Tech XTL-II stereomicroscope equipped with an Abbe drawing tube. Photographs were taken with a Leica M205A stereomicroscope fitted with a Leica DFC550 camera and LAS software (ver. 4.6). Carapace length was measured medially from the anterior margin to the rear margin of the carapace. Eye size was measured as the maximum diameter of the lens in dorsal or frontal view. The measurements of legs are shown as total length (femur, patella, tibia, metatarsus, tarsus). Only one paratype was measured. Epigynes were cleared in a warm solution of potassium hydroxide, and then transferred to 75% ethanol for drawing. All measurements are given in millimeters. All specimens studied are deposited in the Museum of Hebei University (MHBUS), Baoding, China.

The following abbreviations are used in the text and figures legends:

ALE	anterior lateral eyes;
AME	anterior median eyes;
MOQ	median ocular quadrangle;
PLE	posterior lateral eyes;
PME	posterior median eyes;
Z	Zodariidae.

Taxonomy

Family Zodariidae Thorell, 1881

Genus *Zodarion* Thorell, 1887

Type species. *Enyo nitida* Audouin, 1826.

Most *Zodarion* species are active at night and hide during the day in self-made retreats. Jocqué (1991) provided a generic diagnosis: the number of spinnerets reduced

to two in males and six in females, the dense cover of flattened incised hairs on the tegument, and no more than one dorsal femoral spine. Bosmans (1994, 1997, 2009) revised the European *Zodariion* and classified them into 12 groups. Large AME are widely distributed among the taxa in the femoral organ clade, though species of the *Z. thoni*-group have small AME, which is one of the diagnostic characters of *Zodariellum*. *Z. apertum* and *Z. planum* are close to Central Asian spiders considered by Marusik and Koponen (2001) in *Zodariellum*, all these species have similar RTA and bulb. Therefore, the genus *Zodariion* needs to be carefully examined and revised in the future.

***Zodariion apertum* sp. n.**

<http://zoobank.org/029D1AF1-EEBA-410B-91F1-A92C12B7E6E3>

Figures 1–3

Type material. **Holotype** ♂ (Z-Xinjiang-200905-11), Luntai County (41°22'N, 84°11'E), Xinjiang Uygur Autonomous Region, China, 27 May 2009, Dong Sun leg. **Paratype:** 1 ♀ (Z-Xinjiang-200905-12), same data as holotype.

Diagnosis. The male of *Z. apertum* sp. n. is very similar to that of *Z. mongolicum* (Marusik & Koponen, 2001) in having a fold on the apical tegular apophysis, a relatively wider bulb, and the wide and flat apical part of the retrolateral tibial apophysis enlarged. However, it can be distinguished from *Z. mongolicum* by the posteriorly downward direction of the embolic base (down-retrolaterally in *Z. mongolicum*), the retrolateral tibial apophysis with acinal apical margin in ventral view (flat in *Z. mongolicum*), and the posterior tip of the conductor being at the 6-o'clock-position (5-o'clock-position in *Z. mongolicum*) (Figs 2A–C, 3A–C). The female of this new species resembles that of *Z. zebra* Charitonov, 1946, *Z. spasskyi* Charitonov, 1946, and *Z. proszynskii* Nenilin & Fet, 1985 in having a straight margin of the pocket and closer apices of the two spermathecae, though the spiracles of the spermathecae are smaller than in the latter three species (Figs 2D, E, 3D, E).

Etymology. The specific name is from the Latin *apertum*, in reference to the uncovered terminal of the retrolateral tibial apophysis; adjective.

Description. Male (holotype): total length 3.65; carapace 1.79 long, 1.37 wide; opisthosoma 2.02 long, 1.33 wide. Carapace (Fig. 1A) declining, yellow-brown, furnished with black net-like stripes, flat thorax, and smooth tegument. Longitudinal foveae black. Clypeus 0.16 high, yellow brown. Anterior eye row slightly procurved, posterior eye row strongly procurved in dorsal view. Ocular area black. Eye sizes and interdistances: AME 0.18, ALE 0.12, PME 0.08, PLE 0.10, AME–AME 0.08, AME–ALE 0.01, ALE–ALE 0.55, AME–PME 0.07, PME–PME 0.24, PME–PLE 0.04, PLE–PLE 0.52, ALE–PLE 0.03. MOQ 0.32 long, anterior width 0.41, posterior width 0.40. Mouthparts (Fig. 1B): chelicerae yellow-brown, with two anterior and one posterior teeth on margins of fang furrows, terminal part with row of black hairs; endites yellowish, apices paler and provided with dense black scopula; labium triangular, 0.23 long, 0.29 wide, yellow-brown, apices paler. Sternum (Fig. 1B) 0.90 long, 1.04 wide, yellowish, lateral



Figure 1. *Zodarion apertum* sp. n., habitus (**A, C** dorsal view **B, D** ventral view) **A, B** Male holotype **C, D** Female paratype.

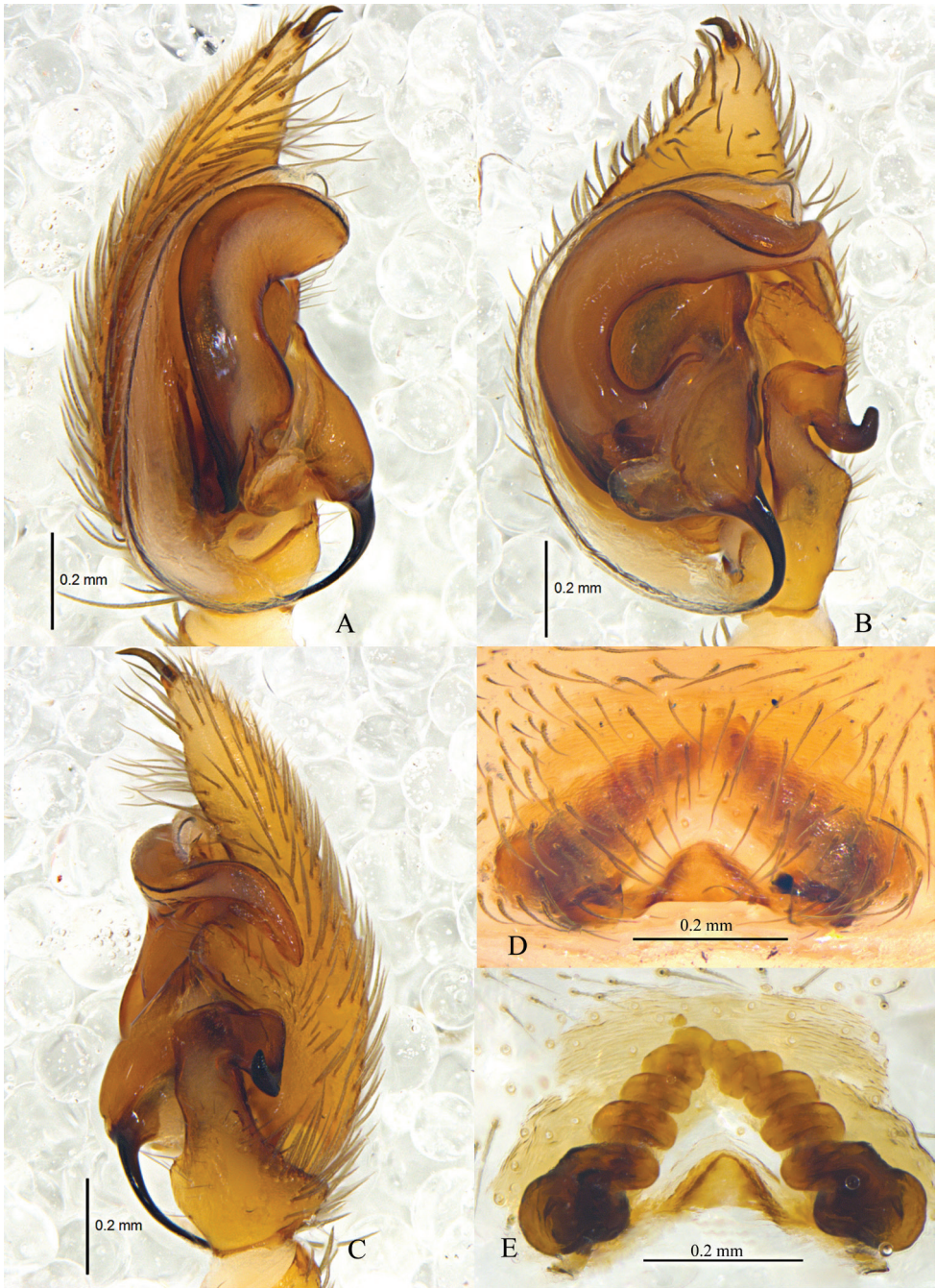


Figure 2. *Zodarium apertum* sp. n., male holotype left palp (A–C) and female paratype (D, E) A Prolateral view B Ventral view C Retrolateral view D Epigynum, ventral view E Vulva, dorsal view.

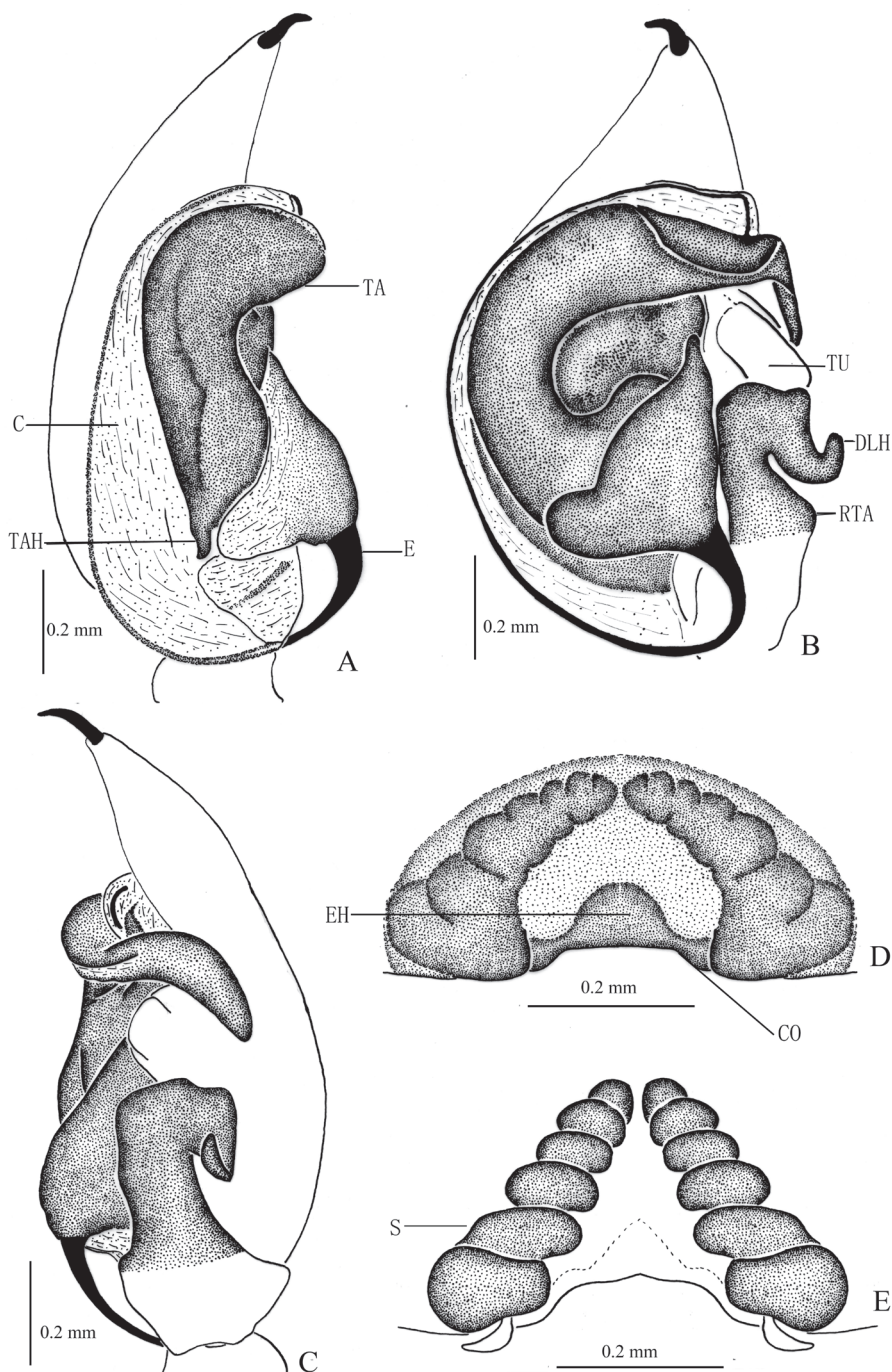


Figure 3. *Zodarion apertum* sp. n., male holotype (A–C) and female paratype (D, E) A–C Left male palp (A prolateral view B ventral view C retrolateral view) D Epigynum, ventral view E Vulva, dorsal view. Abbreviations: C, conductor; DLH, dorsolateral hook; E, embolus; EH, epigynal hood; RTA, retrolateral tibial apophysis; S, spermatheca; TA, tegular apophysis; TAH, tegular apophysis hook; TU, tutaculum.

margin dark, provided with sparse black setae, its lateral margin with inter- and intra-coxal triangles. Legs (Fig. 1A, B) yellow-brown. Leg measurements: leg I 5.39 (1.05 + 0.51 + 1.47 + 1.66 + 0.70), II 4.47 (0.90 + 0.46 + 1.54 + 1.00 + 0.57), III 5.85 (1.68 + 0.48 + 1.53 + 1.52 + 0.64), IV 6.24 (1.57 + 0.58 + 1.31 + 1.70 + 1.08). Opisthosoma covered with pale short hairs. Dorsum of opisthosoma gray, covered with large irregular black patches; venter gray, with small black patches. Spinnerets gray (Fig. 1A, B).

Palp (Figs 2A–C, 3A–C). Coxae of palps white, other sections yellow; length to width ratio of femur 3.0, length to width ratio of patella 1.2; retrolateral tibial apophysis enlarged, about 3 times the tibial length, apical part wide and flat, apical margin acinal, with thin hook-shaped dorsolateral terminal; cymbium with terminal spine, tutaculum obvious; tegular apophysis wide and strong, apical part with a fold, retrolaterally with long and beak shaped extension, tegular apophysis hook S-shaped in prolateral view, extends to basal embolus; membranous conductor long, lamellate and running almost along whole course of the embolus; base of embolus almost triangular.

Female. Total length 4.65: carapace 1.73 long, 1.12 wide; opisthosoma 2.96 long, 1.92 wide. Carapace yellow-brown. Clypeal height 0.21. Eye diameters: AME 0.19, ALE 0.11, PME 0.08, PLE 0.08. Eye sizes and interdistances: AME–AME 0.07, AME–ALE 0.02, ALE–ALE 0.51, AME–PME 0.08, PME–PME 0.22, PME–PLE 0.04, PLE–PLE 0.48, ALE–PLE 0.04. MOQ 0.32 long, front width 0.36, back width 0.33. Mouthparts (Fig. 1D): labium 0.23 long, 0.31 wide. Sternum 0.83 long, 0.97 wide. Leg measurements: I 4.78 (1.28 + 0.55 + 1.06 + 1.30 + 0.59), II 4.58 (1.30 + 0.42 + 0.93 + 1.23 + 0.70), III 4.73 (1.24 + 0.45 + 1.02 + 1.28 + 0.74), IV 6.14 (1.62 + 0.53 + 1.39 + 1.62 + 0.98). Dorsum of opisthosoma black; venter white. Other colors as in male (Fig. 1C, D).

Epigyne (Figs 2D, E, 3D, E). Posterior part with central hood, which is bathing-cap-shaped, 0.6 long, 0.13 wide, posteromedian margin straight, copulatory openings situated in posterior part of epigyne; long and spiraled spermathecae visible through integument, apices of two spermathecae converging and touching each other, base of two spermathecae distant (about 4 times the spermathecal diameter), brown spermathecae with 5 coils.

Distribution. China (Xinjiang).

Zodarion planum sp. n.

<http://zoobank.org/CAB3D10A-A662-4AEC-8829-35D9157EFDB3>

Figures 4, 5

Type material. **Holotype** ♂ (Z-Shaanxi-198606-22), Baoji City (34°22'N, 107°09'E), Shaanxi Province, China, 22 June 1986, Mingsheng Zhu leg.

Diagnosis. The male of *Z. planum* is very similar to those of *Z. sytchevskajae* Nenilin & Fet, 1985, *Z. chaoyangense* Zhu & Zhu, 1983, and *Z. furcum* Zhu, 1988, as all have dorsolateral processes extending from the middle part of the retrolateral tibial apophysis, though *Z. planum* can be distinguished from the others by the wide and fluent margins of the dorsolateral process (obviously curving in the other three species) (Figs 4C–E, 5A–C).

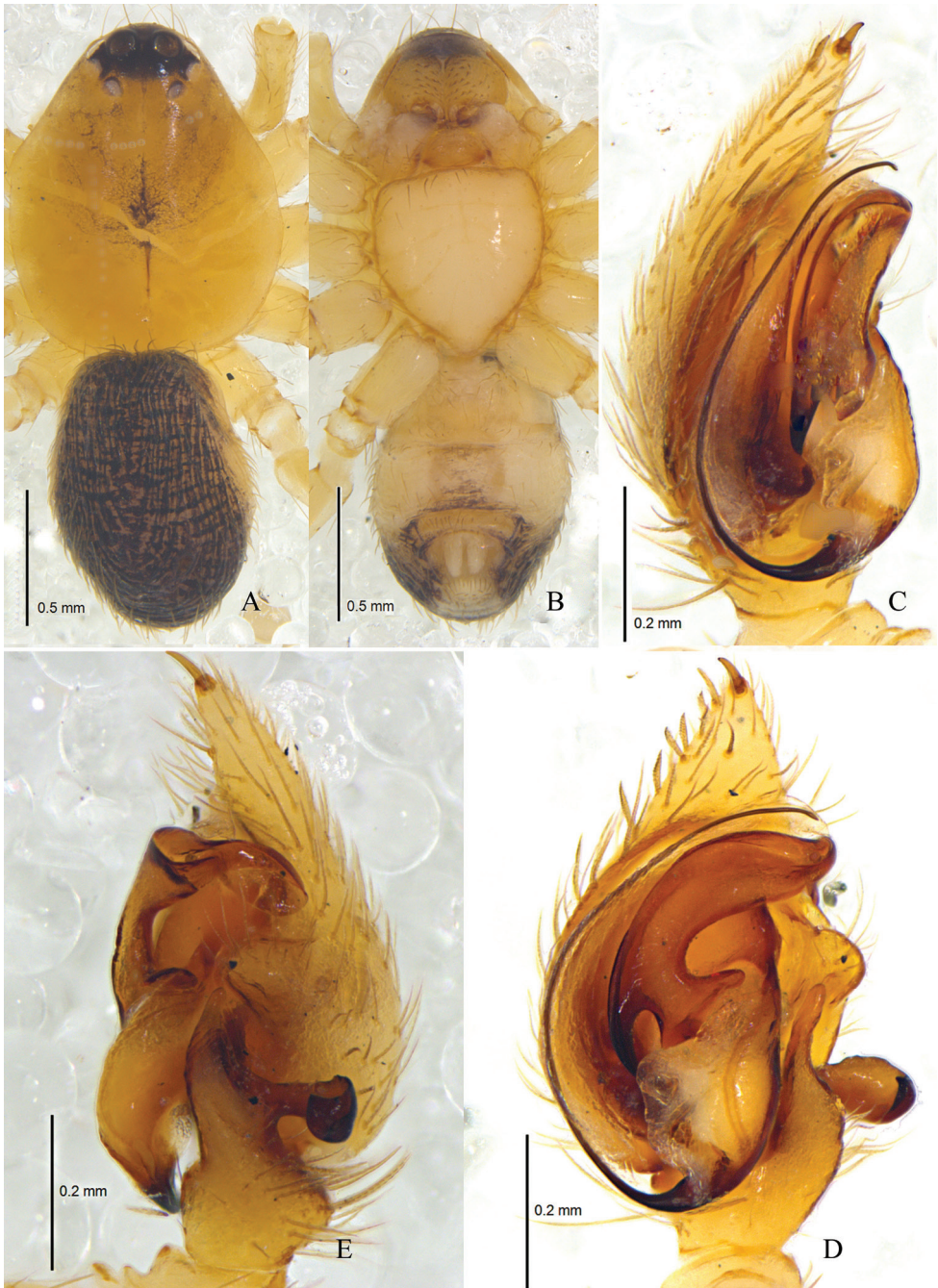


Figure 4. *Zodarion planum* sp. n., male holotype (**A–E**) **A, B** Habitus (**A** dorsal view **B** ventral view) **C–E** Left male palp (**C** prolateral view **D** ventral view **E** retrolateral view).

Etymology. The specific name is from the Latin *planum*, in reference to the dorso-lateral hook-shaped process of the retrolateral tibial apophysis; adjective.

Description. Male (holotype): total length 2.22; carapace 1.20 long, 1.00 wide; opisthosoma 1.02 long, 0.74 wide. Carapace (Fig. 4A) declining, longer than wide, yellow-brown, furnished with inconspicuous black netlike stripes. Clypeus 0.16 high, yellow-brown. Anterior eye row slightly procurved, posterior eye row strongly procurved in dorsal view. Ocular area black. Eye sizes and interdistances: AME 0.13, ALE 0.08, PME 0.07, PLE 0.07, AME–AME 0.04, AME–ALE 0.06, ALE–ALE 0.42, AME–PME 0.08, PME–PME 0.16, PME–PLE 0.02, PLE–PLE 0.42, ALE–PLE 0.02. MOQ 0.26 long, anterior width 0.28, posterior width 0.29. Mouthparts (Fig. 4B): chelicerae yellow-brown, with two anterior and one posterior teeth on margins of fang furrows, terminal part with row of black scopulae, fangs short; endites yellowish, apices paler and provided with dense black scopula; labium triangular, 0.16 long, 0.25 wide, yellow-brown. Sternum (Fig. 4B) 0.70 long, 0.64 wide, white, lateral margin dark, provided with sparse black setae, its lateral margin with inter- and intra-coxal triangles. Legs (Fig. 4A, B) yellow-brown; femora with dorsal spines. Leg measurements: leg I 5.32 (0.98 + 0.51 + 1.47 + 1.66 + 0.70), II 2.78 (0.78 + 0.38 + 0.65 + 0.59 + 0.38), III 4.41 (0.92 + 0.38 + 1.54 + 1.00 + 0.57), IV 5.79 (1.12 + 0.58 + 1.31 + 1.70 + 1.08). Opisthosoma (Fig. 4A, B) covered with black short setae. Dorsum of opisthosoma black; venter white, median part with wide dark gray band. Spinnerets (Fig. 4B) white, laterally with blackish patches.

Palp (Figs 4C, D, 5A–C). Coxae of palps white, other sections yellow; length to width ratio of femur 2.7, length to width ratio of patella 1.2; retrolateral tibial apophysis about 2.5 times the tibial length, thin apex finger-shaped, dorsolateral hook-shaped apophysis long and flat; cymbium with terminal spine, tutaculum obvious; tegular apophysis of moderate size, retrolaterally with flat and wide extension, tegular apophysis hook nearly straight in prolateral view; membranous conductor long, lamellate, and running almost along whole course of embolus; basal embolus almost triangular.

Female unknown.

Distribution. China (Shaanxi).

Remarks. The two new species *Z. apertum* sp. n. and *Z. planum* sp. n., together with most known East Asian and Central Asian species of the *Zodarion* (i.e. *Z. asiaticum* Tyschchenko, 1970, *Z. bekuzini* Nenilin, 1985, *Z. chaoyangense* Zhu & Zhu, 1983, *Z. continentale* Andreeva & Tyschchenko, 1968, *Z. furcum* Zhu, 1988, *Z. mongolicum* (Marusik & Koponen, 2001), *Z. nenilini* Eskov, 1995 (also distributed in European area and Urals of Russia), *Z. proszynskii* Nenilin & Fet, 1985, *Z. schmidtii* (Marusik & Koponen, 2001), *Z. spasskyi* Charitonov, 1946, *Z. hunanense* Yin, 2012, *Z. sytchevskajae* Nenilin & Fet, 1985, *Z. volgouralensis* (Ponomarev, 2007) (also distributed in Astrakhan of Russia), and *Z. zebra* Charitonov, 1946) appear to comprise an undescribed group with the following common characters: the long and thin embolus rising at the prolateral or basal part of the tegulum; tegular apophysis wide and strong, with a downwardly-directed hook; a modified apical portion of the retrolateral tibial

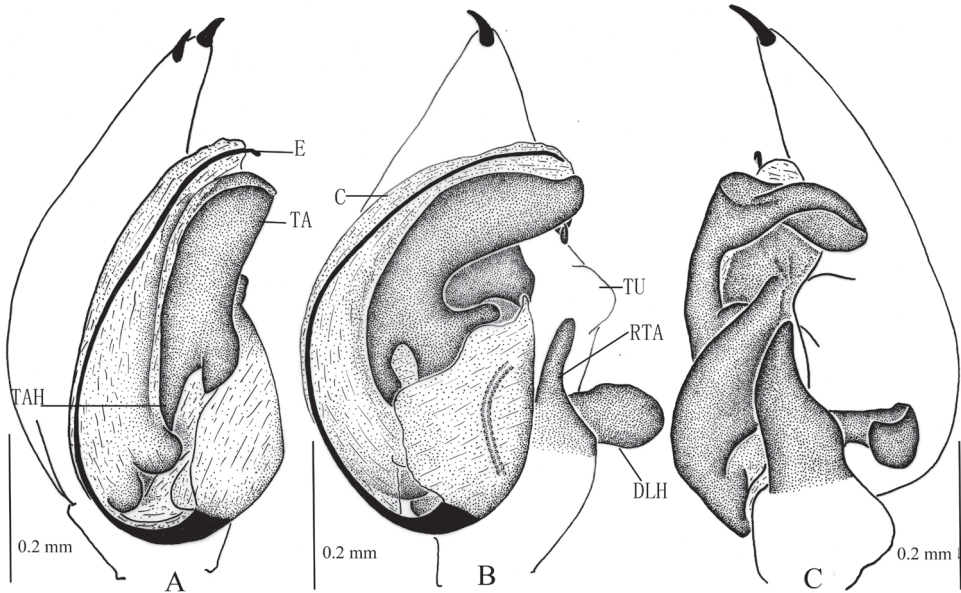


Figure 5. *Zodarion planum* sp. n. **A–C** Left male palp of male holotype (**A** prolateral view **B** ventral view **C** retrolateral view). Abbreviations: C, conductor; DLH, dorsolateral hook; E, embolus; RTA, retrolateral tibial apophysis; TA, tegular apophysis; TAH, tegular apophysis hook; TU, tutaculum.

apophysis turning dorsally; cymbium with tutaculum; epigyne with incised postero-median margin and median hood; apical parts of spiraled spermathecae converging. They are different from the *lutipes* group (Bosmans 2009) in having a long conductor, the tutaculum of the cymbium, and the converging apex of the spermathecae.

Zodarion hunanense was described based only on a female specimen from Hunan province of China. The possibility exists that *Z. planum* sp. n. is conspecific with *Z. hunanense*.

***Zodarion ovatum* sp. n.**

<http://zoobank.org/4C6933CB-C3BA-43C1-A952-05BED360B6B2>

Figures 6–8

Type material. Holotype ♂ (Z-Yunnan-200505-18), Yuanmou County (25°51'N, 101°45'E), Yunnan Province, China, 26 May 2005, collector unknown. **Paratypes:** 71 ♂ (Z-Yunnan-200505-19 - Z-Yunnan-200505-89) and 33 ♀ (Z-Yunnan-200505-90 - Yunnan-200505-122), same data as holotype.

Diagnosis. The males of *Z. ovatum* sp. n. are similar to those of *Zodarion nitidum* (Audouin, 1826), *Z. christae* Bosmans, 2009, *Z. deltshevi* Bosmans, 2009, and *Z. samos* Bosmans, 2009 because of the flagelliform embolus rising at the basal part of



Figure 6. *Zodarium ovatum* sp. n., male holotype (**A, B**) and female paratype (**C, D**) Habitus (**A, C** dorsal view **B, D** ventral view).

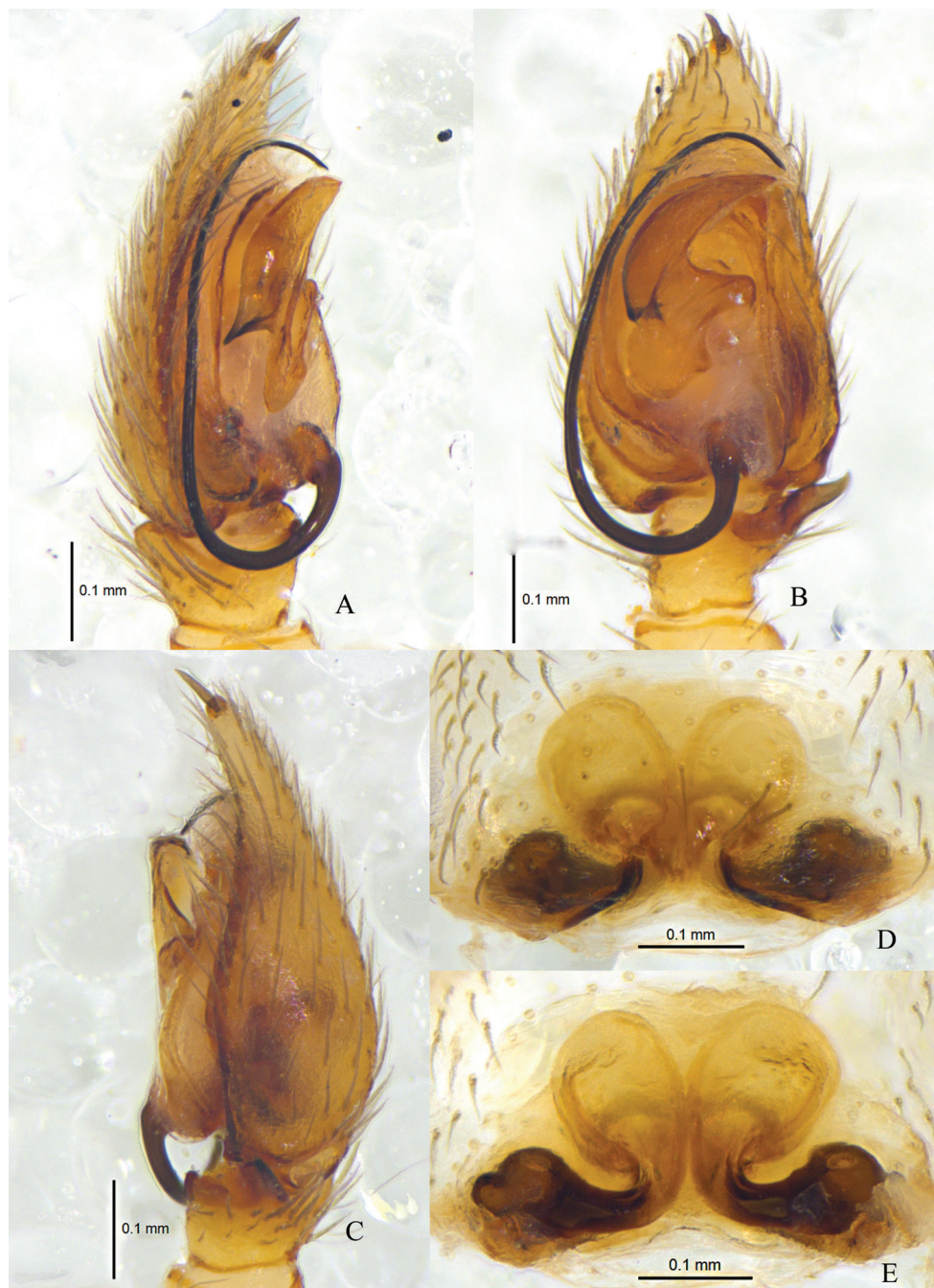


Figure 7. *Zodarion ovatum* sp. n., male holotype left palp (**A–C**) and female paratype (**D,E**) **A** Prolateral view **B** Ventral view **C** Retrolateral view **D** Epigynum, ventral view **E** Vulva, dorsal view.

the tegulum, the small tegular apophysis and the retrolateral tibial apophysis terminally pointed in ventral view, though it can be distinguished from the others by the small oval base of the embolus (triangular in the other three species), and the lack of a gland in the base of the cymbium (present in the other three species). The females of *Z. ovatum* sp. n. are similar to female *Z. soror* (Simon, 1873) in having swollen copulatory ducts, and also to *Z. ludibundum* Simon, 1914 and *Z. nigriceps* (Simon, 1873) by the oblique lateral margins of epigyne, but the copulatory ducts of *Z. ovatum* sp. n. are longitudinally arranged (Figs 7A–E, 8A–E) rather than oblique in the three other species.

Etymology. The specific name is from the Latin *ovatum*, in reference to the oval shape of the swollen copulatory ducts; adjective.

Description. Male (holotype): total length 1.85; carapace 0.99 long, 0.68 wide; opisthosoma 0.88 long, 0.64 wide. Carapace (Fig. 6A) longer than wide, yellow-brown, median part with faint black patch in front of black fovea, thorax flat, tegument smooth and shiny. Clypeus 0.09 high, yellow-brown. Anterior eye row slightly procurved, posterior eye row strongly procurved in dorsal view. Ocular area black. Eye sizes and interdistances: AME 0.11, ALE 0.03, PME 0.04, PLE 0.04, AME–AME 0.05, AME–ALE 0.03, ALE–ALE 0.33, AME–PME 0.07, PME–PME 0.16, PME–PLE 0.03, PLE–PLE 0.33, ALE–PLE 0.01. MOQ 0.21 long, anterior width 0.24, posterior width 0.25. Mouthparts (Fig. 6B): chelicerae yellow-brown, with two anterior and one posterior teeth on margins of fang furrows, terminal part with row of black scopulae, fangs short; endites yellow, apices paler and provided with dense black scopula; labium triangular, 0.11 long, 0.18 wide, yellow-brown, median part with semi-orbicular brown band. Sternum (Fig. 6B) 0.52 long, 0.55 wide, white, lateral margin dark, provided with sparse black setae, its lateral margin with inter- and intra-coxal triangles. Legs (Fig. 6A, B) brown. Leg measurements: leg I 2.83 (0.75 + 0.26 + 0.59 + 0.78 + 0.45), II 2.54 (0.62 + 0.24 + 0.45 + 0.79 + 0.44), III 2.62 (0.71 + 0.20 + 0.46 + 0.80 + 0.45), IV 3.56 (0.79 + 0.31 + 0.80 + 1.01 + 0.65). Opisthosoma (Fig. 6A, B) oval, covered with black short setae. Dorsum of opisthosoma gray, covered by many dark patches; venter white. Spinnerets (Fig. 6B) pale yellow.

Palp (Figs 7A–C, 8A–C). Palps yellow brown; length to width ratio of femur 2.5, length to width ratio of patella 1.3; retrolateral tibial apophysis as long as tibia, thin and slightly curved in ventral view, but wide in retrolateral view, without dorsolateral process; tegular apophysis large and complex, tip turning gradually tapering, hook of tegular apophysis pointed posteriorly in prolateral view; membranous conductor short; base of embolus small and oval, connected to tegulum via white membrane.

Female. One of the specimens (Z-Laos-11–25) measured: total length 2.65; carapace 1.20 long, 0.84 wide; opisthosoma 1.48 long, 1.05 wide. Carapace yellow-brown. Clypeal height 0.09. Eye sizes and interdistances: AME 0.13, ALE 0.09, PME 0.05, PLE 0.08, AME–AME 0.04, AME–ALE 0.03, ALE–ALE 0.41, AME–PME 0.06, PME–PME 0.21, PME–PLE 0.02, PLE–PLE 0.38, ALE–PLE 0.02. MOQ 0.60 long, front width 0.70, back width 0.26. Mouthparts (Figure 1D): labium 0.17 long, 0.21

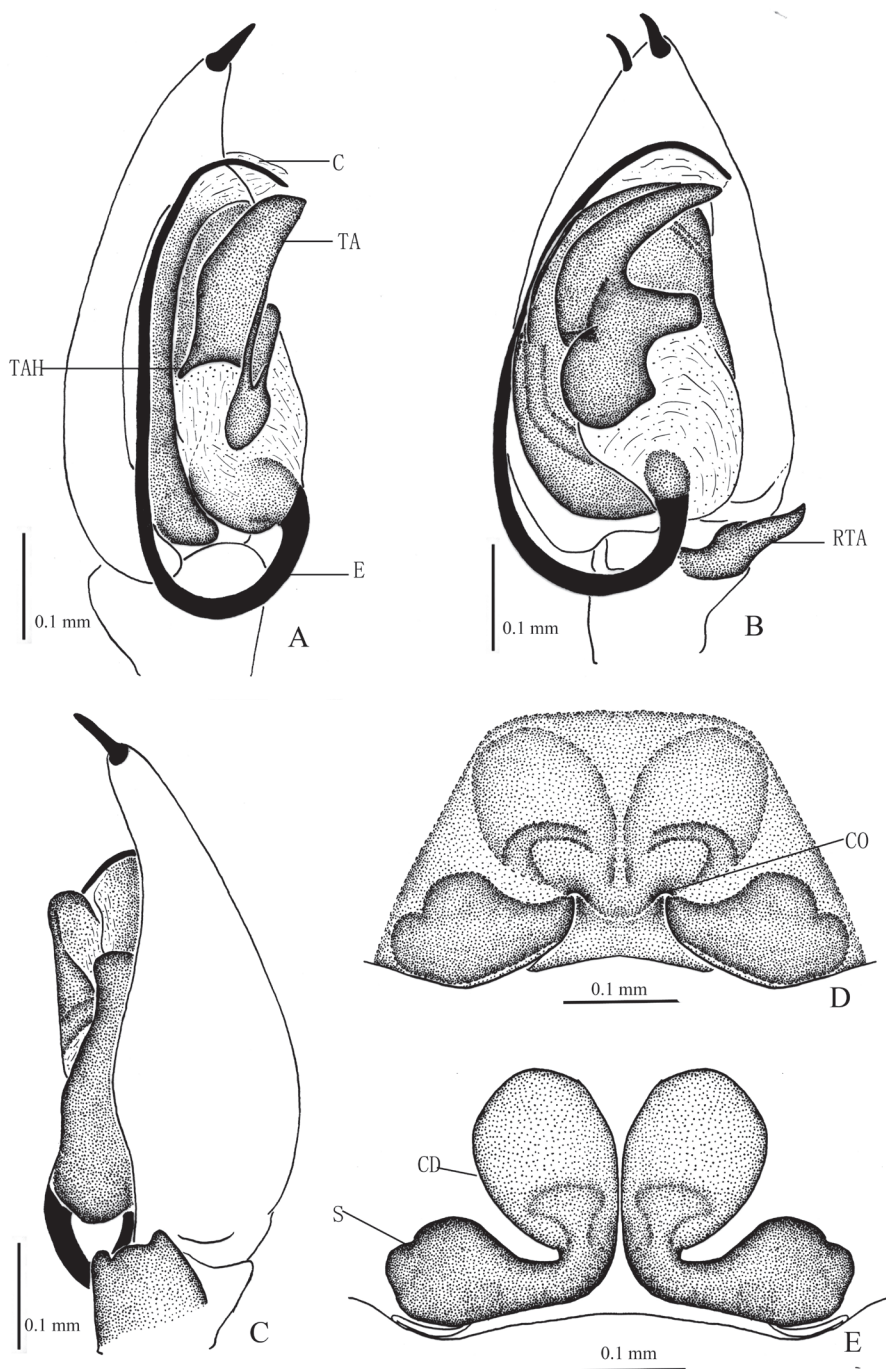


Figure 8. *Zodarion ovatum* sp. n., male holotype (**A–C**) and female paratype (**D, E**) **A–C** Left male palp (**A** prolateral view **B** ventral view **C** retrolateral view) **D** Epigynum, ventral view **E** Vulva, dorsal view. Abbreviations: C, conductor; CD, copulatory ducts; CO, copulatory opening; E, embolus; RTA, retrolateral tibial apophysis; S, spermatheca; TA, tegular apophysis; TAH, tegular apophysis hook.

wide. Sternum 0.83 long, 0.97 wide. Leg measurements: I 2.66 (0.71 + 0.22 + 0.59 + 0.50 + 0.64), II 2.37 (0.81 + 0.17 + 0.47 + 0.64 + 0.28), III 2.54 (0.72 + 0.23 + 0.34 + 0.79 + 0.46), IV 3.79 (1.07 + 0.27 + 0.63 + 1.18 + 0.64). Dorsum of opisthosoma black; venter gray-brown. Other coloration as in male (Fig. 6C, D).

Epigyne with two oblique chitinous sutures, copulatory openings situated almost in the central part of epigyne; anterior part of copulatory ducts swollen, visible through integument; spermathecae small, situated posteriorly and well separated (Figs 7D, E, 8D, E).

Distribution. China (Yunnan).

Remarks. The males of species *Z. ovatum* sp. n. belong to the *lutipes* group with their long embolus rising at the posterior part of the tegulum; tibial apophysis short, robust and without lateral process. The females of *Z. ovatum* sp. n. resemble the species of the *italicum* group (Bosmans 1997) with their parallel or converging chitinous sutures on the epigyne and swollen copulatory ducts.

Zodarion chaoyangense Zhu & Zhu, 1983

Figures 9, 10

Zodarion chaoyangensis Zhu and Zhu 1983: 137, fig. a–h; Song et al. 1999: 431, fig. 257P, Q, 258A; Song et al. 2001: 327, fig. 210A–D; Jocqué and Henrard 2015: 21.

Zodariellum chaoyangense: Marusik and Koponen 2001: 41.

Material examined. 1♂ and 1♀, Zanhuan County (37°65'N, 114°35'E), Hebei Province, China, time and collector unknown.

Description. See Zhu and Zhu (1983).

Distribution. China (Liaoning, Hebei).

Zodarion furcum Zhu, 1988

Figures 11, 12

Zodarion furcum Zhu 1988: 354, fig. 5–9; Song et al. 1999: 431, fig. 257R, S, 258B; Song et al. 2001: 328, fig. 211A–E; Jocqué and Henrard 2015: 21.

Zodariellum furcum Marusik and Koponen 2001: 41.

Material examined. 1♂ and 1♀, Shijiazhuang City (38°15'N, 114°12'E), Hebei Province, China, 17 May 1985, Mingsheng Zhu leg.

Description. See Zhu (1988).

Distribution. China (Hebei).



Figure 9. *Zodarion chaoyangense* Zhu & Zhu, 1983, male holotype (**A, B**) and female paratype (**C, D**) Habitus (**A, C** dorsal view **B, D** ventral view).

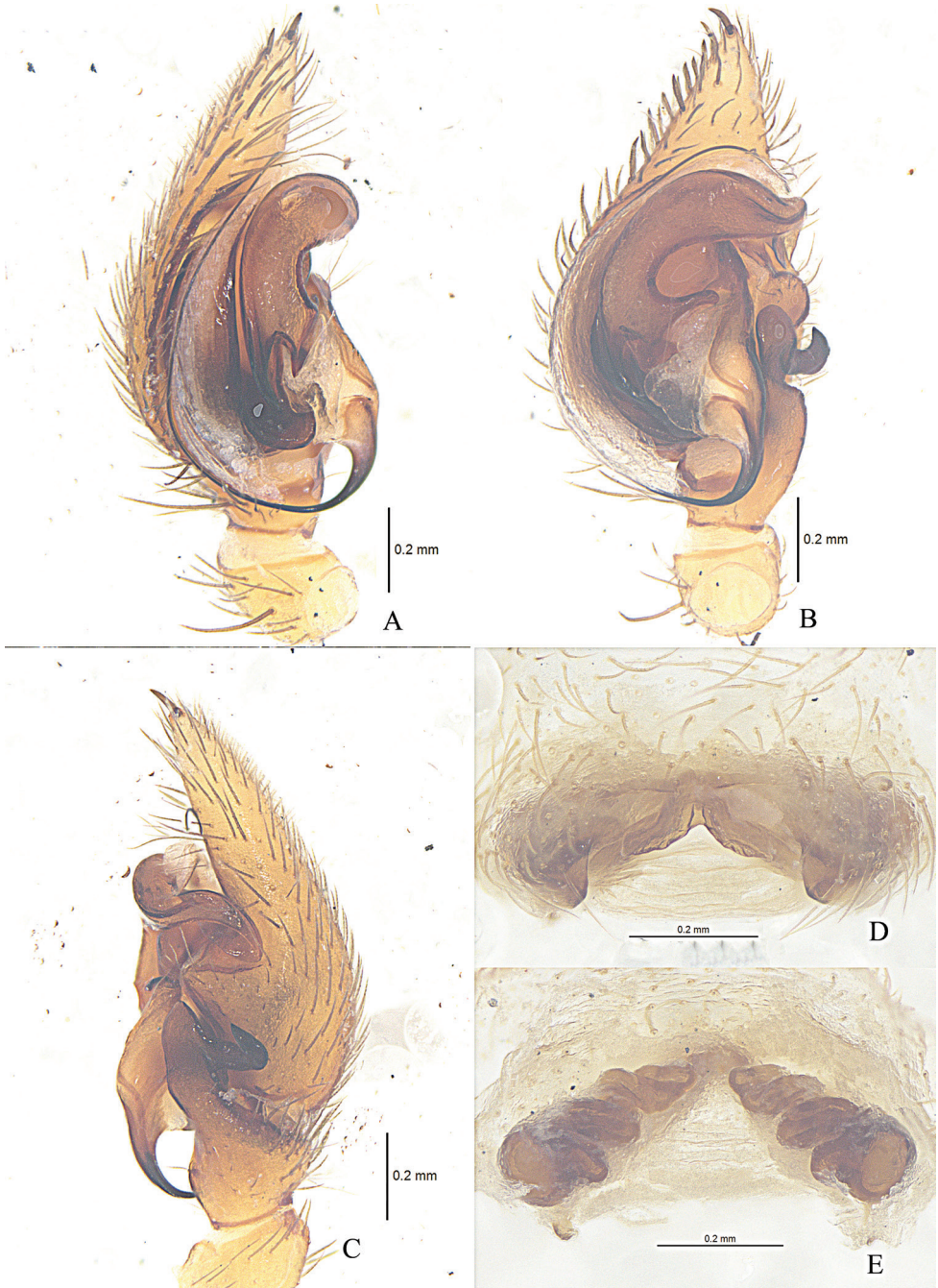


Figure 10. *Zodarion chaoyangense* Zhu & Zhu, 1983, male holotype left palp (**A–C**) and female paratype (**D, E**) **A** Prolateral view **B** Ventral view **C** Retrolateral view **D** Epigynum, ventral view **E** Vulva, dorsal view

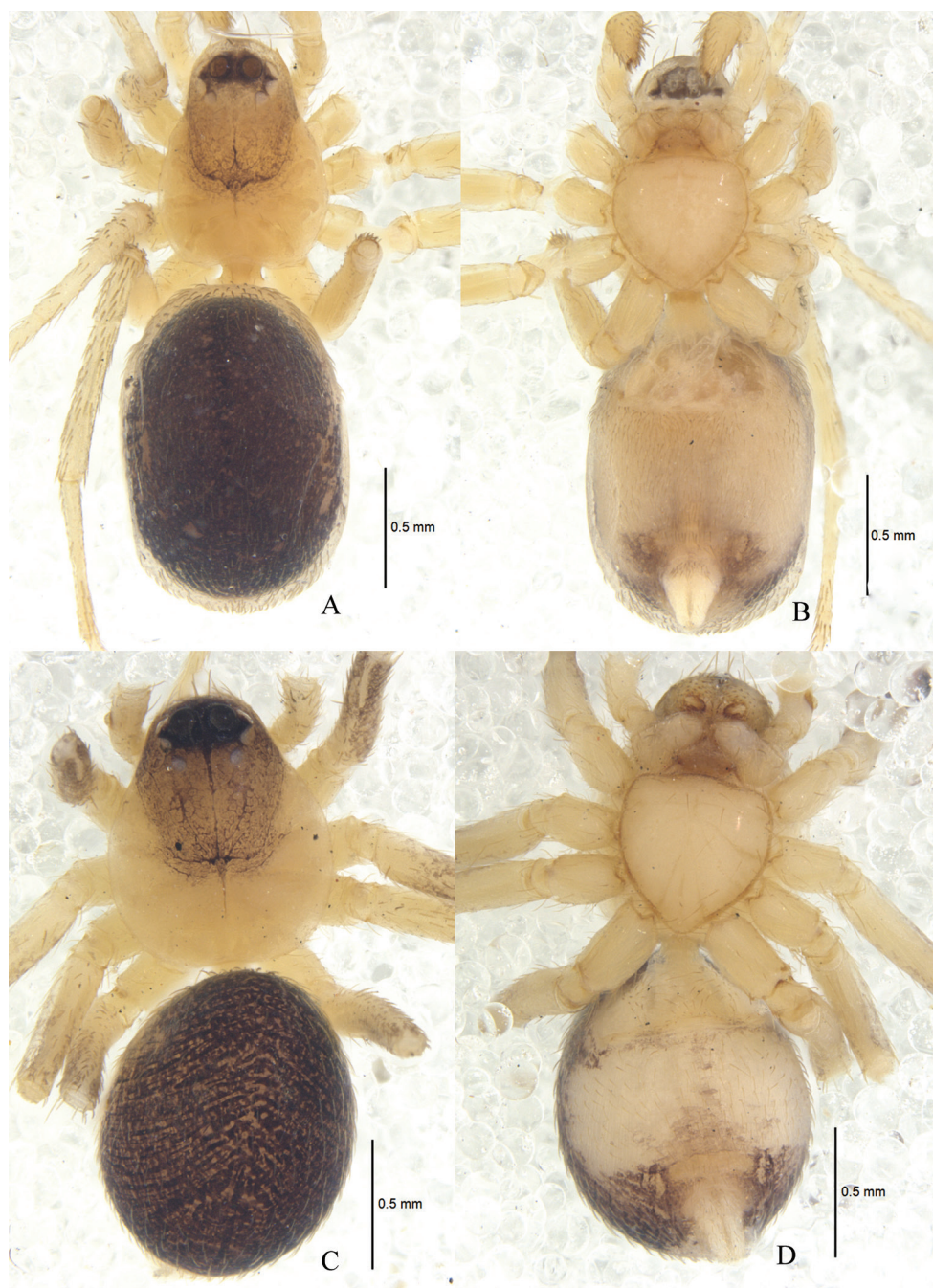


Figure 11. *Zodarion furcum* Zhu, 1988, male holotype (**A, B**) and female paratype (**C, D**) Habitus (**A, C** dorsal view **B, D** ventral view).

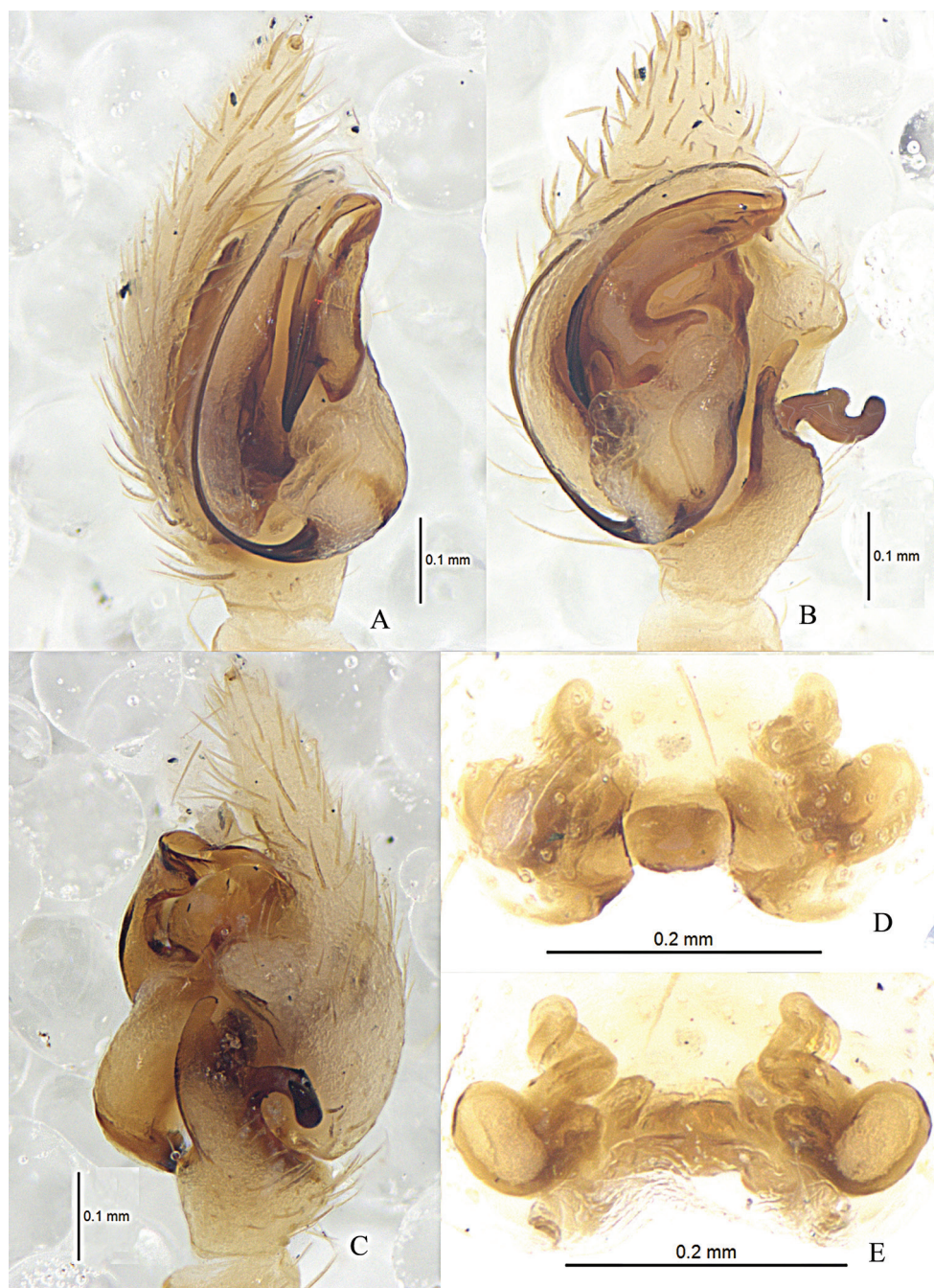


Figure 12. *Zodarion furcum* Zhu, 1988, male holotype left palp (**A–C**) and female paratype (**D, E**) **A** Prolateral view **B** Ventral view **C** Retrolateral view **D** Epigynum, ventral view **E** Vulva, dorsal view.

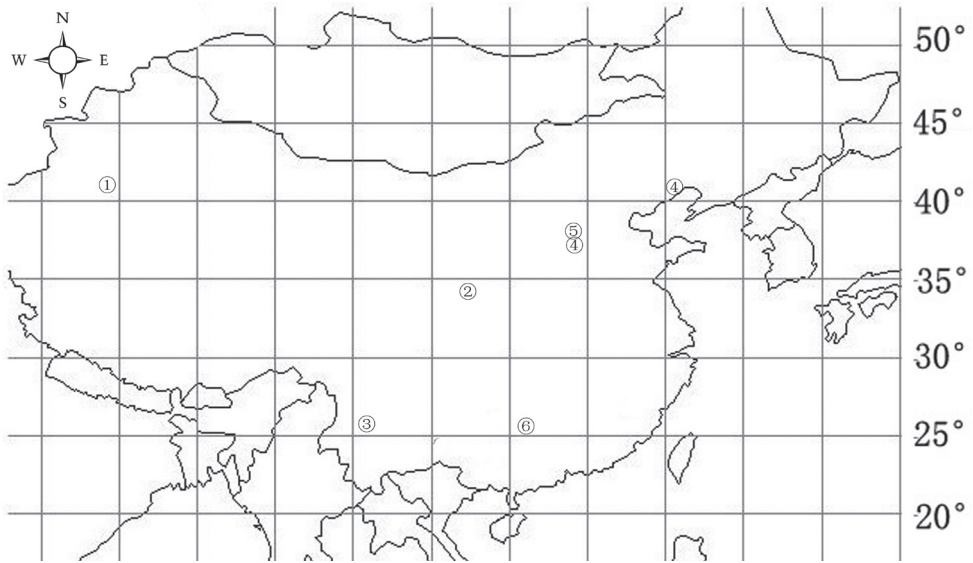


Figure 13. Records of *Zodarion* species in China. ① *Zodarion apertum* sp. n. ② *Zodarion planum* sp. n. ③ *Zodarion ovatum* sp. n. ④ *Zodarion chaoyangense* Zhu & Zhu, 1983 ⑤ *Zodarion furcum* Zhu, 1988 ⑥ *Zodarion hunanense* Yin, 2012.

Zodarion hunanense Yin, 2012

Zodarion hunanensis Yin in Yin et al. 2012: 1148, fig. 611a–e.

Description and figures. See Yin (2012).

Distribution. China (Hunan).

Acknowledgements

We are grateful to Dr Shuqiang Li, Dr Yuri Marusik and Zhisheng Zhang for valuable suggestions, and to the linguistic editor and Professor Malcolm Lyon (University of Manchester, UK) for improving the English. We are grateful to Mingsheng Zhu and Dong Sun for the collection of specimens. This study was supported by the National Natural Science Foundation of China (No. 31471969) and the Natural Science Foundation of Hebei Province (No. C2017104011).

References

- Bosmans R (1994) Revision of the genus *Zodarion* Walckenaer, 1833 in the Iberian Peninsula and Balearic Islands (Araneae, Zodariidae). Eos 69: 115–142.

- Bosmans R (1997) Revision of the genus *Zodarion* Walckenaer, 1833, part II. Western and Central Europe, including Italy (Araneae: Zodariidae). Bulletin of the British Arachnological Society 10(8): 265–294.
- Bosmans R (2009) Revision of the genus *Zodarion* Walckenaer, 1833, part III. South East Europe and Turkey (Araneae: Zodariidae). Contributions to Natural History 12: 211–295.
- Bosmans R, Özkütük RS, Varli SV, Kunt KB (2014) Description of a new *Zodarion* Walckenaer, 1826 from Turkey (Zodariidae; Araneae). Turkish Journal of Zoology 38: 99–101. <https://doi.org/10.3906/zoo-1303-11>
- Jocqué R (1991) A generic revision of the spider family Zodariidae (Araneae). Bulletin of the American Museum of Natural History 201: 1–160. <http://hdl.handle.net/2246/894>
- Jocqué R, Henrard A (2015) Revalidation of *Acanthinozodium* Denis, 1966 with description of three new species and discovery of a remarkable male palpal character (Araneae, Zodariidae). European Journal of Taxonomy 114: 1–23. <https://doi.org/10.5852/ejt.2015.114>
- Li SQ, Lin YC (2016) Species Catalogue of China. Vol. 2. Animals, Invertebrates (I) Arachnida: Araneae. Beijing, Science Press, 549 pp.
- Marusik YM, Koponen S (2001) Spiders of the family Zodariidae from Mongolia (Arachnida: Araneae). Reichenbachia 34: 39–48.
- Peckár S, Cardoso P, Barriga JC, Carvalho JC (2011) Update to the zodariid spider fauna of the Iberian peninsula and Madeira (Araneae: Zodariidae). Zootaxa 2814: 19–32. <https://doi.org/10.11646/zootaxa.2814.1.2>
- Song DX, Zhu MS, Chen J (2001) The Fauna of Hebei, China: Araneae. Hebei University of Science and Technology Publishing House Shijiazhuang, 510 pp.
- Song DX, Zhu MS, Chen J (1999) The Spiders of China. Hebei University of Science and Technology Publishing House Shijiazhuang, 640 pp.
- World Spider Catalog (2018) World Spider Catalog, version 19.0. Natural History Museum Bern. <http://wsc.nmbe.ch> [Accessed on: 2018-1-17]
- Yin CM, Peng XJ, Yan HM, Bao YH, Xu X, Tang G, Zhou QS, Liu P (2012) Fauna Hunan: Araneae in Hunan, China. Hunan Science and Technology Press, Changsha, 1590 pp.
- Zhu CD, Zhu SF (1983) A new species of spider of the genus *Zodarium* (Araneae: Zodariidae). Journal of the Bethune Medical University 9(supplement): 137–138.
- Zhu MS (1988) A new spider of the genus *Zodarium* from China (Araneae: Zodariidae). Acta Zootaxonomica Sinica 13: 353–355.

Completely engaged three-dimensional mandibular gear-like structures in the adult horned beetles: reconsideration of bark-carving behaviors (Coleoptera, Scarabaeidae, Dynastinae)

Wataru Ichiishi¹, Shinpei Shimada¹, Takashi Motobayashi¹, Hiroaki Abe¹

¹ Department of Biological Production, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai-cho 3-5-3, Fuchu, Tokyo 183-8509, Japan

Corresponding author: Hiroaki Abe (wfem@cc.tuat.ac.jp)

Academic editor: Andrey Frolov | Received 22 August 2018 | Accepted 18 December 2018 | Published 7 January 2019

<http://zoobank.org/84A4ADAA-4301-4890-BC45-04985BAFD8A2>

Citation: Ichiishi W, Shimada S, Motobayashi T, Abe H (2019) Completely engaged three-dimensional mandibular gear-like structures in the adult horned beetles: reconsideration of bark-carving behaviors (Coleoptera, Scarabaeidae, Dynastinae). ZooKeys 813: 89–110. <https://doi.org/10.3897/zookeys.813.29236>

Abstract

Adult horned beetles (Coleoptera, Scarabaeidae, Dynastinae) such as *Trypoxylus dichotomus* (Linnaeus, 1771) exhibit bark-carving behaviors to feed on tree sap, in part by using small projections of the clypeus. However, in the present experiments, adult horned beetles (*T. dichotomus* and *Dynastes hercules* (Linnaeus, 1758)) used their mandibles and not the projections of the clypeus to carve bark. Our findings show the presence of completely engaged mandibular interlocking, gear-like surface structures in molar areas that guide mandible opening and closure, and lead to completely synchronous movements of adult horned beetle mandibles. Three-dimensional shapes of these mandibular gear-like structures are complex and remained in contact after the death of a beetle. Moreover, adult horned beetles often performed bark-carving behaviors using only the mandible of one side, suggesting that the primary role of the mandibular gear-like structure is to prevent breakage of the mandible by transmitting load from one mandible to the other. Among the 22 Dynastinae and 16 other beetle species examined (not Dynastinae), the gear-like structure was found in all the Dynastinae species and in no other species.

Keywords

beetle, bark-carving behavior, gear-like structure, horned beetle, mandible

Introduction

Horned beetles (Coleoptera, Scarabaeidae, Dynastinae), including those of the genera *Dynastes* (Linnaeus, 1758), *Megasoma* (Linnaeus, 1758), *Chalcosoma* (Linnaeus, 1758), *Eupatorus* (Hope, 1831), and *Trypoxylus* (Linnaeus, 1771), are mostly large insects. Adults are typically sexually dimorphic and males have long horns extending from the head and thorax, whereas females have no horns. The largest *Dynastes hercules* (Linnaeus, 1758) male adults measure more than 160 mm from the tip of the thoracic horn to the end of the abdomen (Imamori 2011) (Suppl. material 1). Adults feed on the sap of wounded trees, whereas horned beetle larvae feed on rotten wood and wood litter. Horned beetles are imported to Japan from foreign countries as popular insect pets, and numerous books describe corresponding characteristics and breeding methods (Unno 2006, McMonigle 2012). Therefore, the life cycle, behavior, and breeding methods of horned beetles are well known in Japan. However, little is known about the mouthparts of horned beetles.

The horned beetle species *Trypoxylus dichotomus* (Linnaeus, 1771) is very common in Japan (Suppl. material 1), and numerous individuals are present in Satoyama, which is an area between mountain foothills and arable flat land. In Satoyama, many *Quercus acutissima* and *Q. serrata* are planted for use as bed logs in traditional mushroom cultivation. *Trypoxylus dichotomus* larvae feed on the rotten wood and wood litter from these trees. Adults of this species emerge in the summer and feed on the sap from tree wounds that are thought to be made by boring insects such as the larvae of cossid moths (Yoshimoto and Nishida 2007). Adult *T. dichotomus* feed extensively on sap and bark-carving behaviors have been observed on *Fraxinus griffithii* trees (Hongo 2006), and these involved repeated head-scooping movements. Because the surfaces of *F. griffithii* trees are soft, adult *T. dichotomus* likely carve soft tree bark to obtain sap. In addition, previous real-time macroscopic video observations suggested that adult *T. dichotomus* carves bark using the projection of the clypeus, which is a chisel-like feature on the front of the head (Hongo 2006) (Fig. 1).

Under rearing conditions, when the small plastic food cup becomes empty, beetles exhibit bark-carving behavior to obtain more sap (in this case, jelly). These activities have been observed in nature, as telecast by Nippon Hoso Kyokai (NHK) in their 5 August 2007 telecast “Darwin has come! The king of beetles! Hercules”. In their televised animation, adult *D. hercules* reportedly start by biting the bark with the mandibles to soften the tree and then use the spatula-like projection of the clypeus to carve the softened bark.

Mouthpart forms vary greatly between insect species and are related to the diet but are classified into two basic types: one adapted for biting and chewing solid food (mandibulate) and the other adapted for sucking fluids (suctorial or haustellate) (Chapman 2013). Beetle mouthparts are much more uniform and are mostly of the biting-chewing types. Mandibles, or jaws, are shaped according to the food consumed by the insect (Nel and De Villiers 1988, Nel and Scholtz 1990, Sanderson and Jackson 2001). Many adults of lepidopteran species develop a proboscis and have mandibles that are either nonfunctional, vestigial, or absent (Grimaldi and Engel 2005). In some enlarged jaw forms, such as those of adult male stag beetles (Lucanidae), huge mandibles do not contribute to feed-

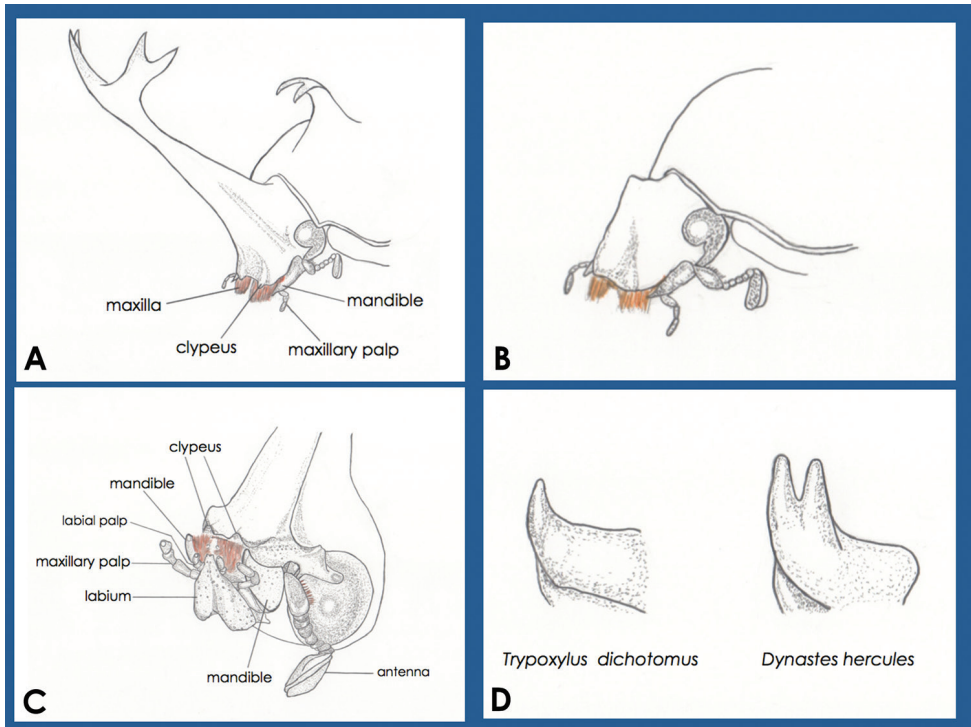


Figure 1. Diagrams of the mouthparts of adult horned beetles. **A** from an oblique anterior side of a *Trypoxylus dichotomus* male **B** from an oblique anterior side of a *T. dichotomus* female; names of mouthparts were omitted **C** View from an oblique ventral side of *T. dichotomus* male **D** diagram of two types of adult mandible tips; left, the tip of the left mandible of a *T. dichotomus* male from the left side; this tip does not branch off; right, the tip of a left mandible of a *Dynastes hercules* male from the left side; this tip branches off and is forked.

ing (Snodgrass 1993, Goyens et al. 2014, Goyens et al. 2016). The biting type, as found in grasshoppers and caterpillars, is the more typical, and the mandibles comprise single tooth-like pieces and move in a transverse plane (Sanderson and Jackson 2001, Clissold 2007). Moreover, as in mammalian anatomy, the more distal part of the mandible has a cutting function and is referred to as the incisor region, whereas the proximal part often has a grinding function and is referred to as the molar region (Chapman 1995). In recent studies of the mouthparts of insects, X-ray micro-CT analysis was used to investigate the internal morphology of insect heads without dissection or damage (Li et al. 2011, Beutel et al. 2014, Weihmann et al. 2015). In contrast, because the mandibles of horned beetles are small and inconspicuous, they have never been studied in detail.

Mouthparts of adult *T. dichotomus* horned beetles are illustrated in Fig. 1 and shows the presence of small and obscure mandibles on both sides of the head. The maxillae of adult horned beetles have orange brush shapes. In adult horned beetles, these maxillae are more outstanding than the mandibles (McMonigle 2012). Our observations indicate that projections of the clypeus in adult *T. dichotomus* or *D. hercules* are not sharp, and that intense bark carving may injure mouthparts such as the labrum.

Herein, we used video footage to study the relationship between bark-carving behaviors and structures of mouthparts of horned beetles, particularly those of *T. dichotomus* and *D. hercules*. In field experiments, angles and directions of video footage are restricted from dorsal or lateral sides because adult *T. dichotomus* and *D. hercules* hold on to the tree. Moreover, these beetles bury their head into the wounds of trees, precluding detailed filming of the mouthparts during bark-carving. Because *T. dichotomus* behaviors were analyzed in a seminal study (Hongo 2006) and *D. hercules* behaviors were telecast by NHK, we filmed bark-carving behaviors under controlled laboratory conditions using adult *T. dichotomus* and *D. hercules*, which are easy to breed in sufficient numbers for experiments.

Our observations indicated that *T. dichotomus* and *D. hercules* use their mandibles like a chisel to carve bark and do not use the projection of the clypeus (Suppl. materials 1–3). Moreover, *T. dichotomus* and *D. hercules* did not bite the bark by opening and closing of their mandibles. Furthermore, we observed completely synchronous movements of both mandibles. In subsequent analyses of the insect head, mandible movements were simultaneous and their asymmetrical hind regions resembled a gear structure. Approximately 1,700 species of horned beetles have been identified globally (Kohiyama 2014) and, although horn shapes and lengths in male adults vary between species, mouthpart structures are very similar. Thus, to determine whether gear-like structures of mandibles are common to these adult horned beetles, we examined 22 Dynastinae and 16 other beetle species (not Dynastinae). Our studies show that mandible gear-like structures are present only in the examined adult Dynastinae and are not present in 16 other beetle species.

Materials and methods

Insects

Among twenty-two horned beetle species three were maintained at the Tokyo University of Agriculture and Technology: *Trypoxylus dichotomus* (adult male length, 40–80 mm; adult female length, 40–60 mm (Bouchard et al. 2014)), *Dynastes hercules* (♂ 50–170 mm; ♀ 40–60 mm (Bouchard et al. 2014)), and *Endebius gideon* (Linnaeus, 1767) (body length, 35–75 mm (Bouchard et al. 2014)). The other horned beetle species *Chalcosoma atlas* (Linnaeus, 1758) (60–130 mm (Bouchard et al. 2014)), were purchased from Fujikon Co., Ltd. (<http://www.fujikon.net/>), Lumberjack Co., Ltd. (<http://www.lumber-j.com/>) and various insect-dealing internet sites (for example <http://64auc.sakura.ne.jp/>): *Chalcosoma chiron* (Oliver, 1789) (50–130 mm (Kohiyama 2014)), *Megasoma elephas* (Fabricius, 1775) (58–130 mm (Kohiyama 2014)), *Megasoma actaeon* (Linnaeus, 1758) (50–135 mm (Kohiyama 2014)), *Dynastes tityus* (Linnaeus, 1763) (40–60 mm (Bouchard et al. 2014)), *Dynastes neptunus* (Quensel, 1817) (50–150 mm (Kohiyama 2014)), *Dynastes satanas* (Moser, 1909) (55–115 mm (Kohiyama 2014)), *Eupatorus birmanicus* (Arrow, 1908) (40–50 mm (Kohiyama 2014)),

Haploscapanes Barbarossa (Fabricius, 1775) (26–56 mm (Kohiyama 2014)), *Allomyrina pfeifferi* (Redtenbacher, 1867) (27–40 mm (Kohiyama 2014)), *Augosoma centaurus* (Fabricius, 1775) (33–80 mm (Kohiyama 2014)), *Oryctes rhinoceros* (Linnaeus, 1758) (30–45 mm (Unno 2006)), *Eophileurus chinensis* (Faldermann, 1835) (18–25 mm (Kohiyama 2014)), *Golofa aegeon* (Hope, 1837) (58 mm (Imamori 2011)), *Golofa pizarro* (Hope, 1837) (25–40 mm (Unno 2006)), *Endebius florensis* (Lansberge, 1879) (77 mm (Imamori 2011)), *Eupatorus gracilicornis* (50–90 mm (Kohiyama 2014)), *Cyclocephala complanata* (Burmeister, 1847) (16 mm), and *Hexodon latissimus* (Olivier, 1789) (28 mm). The body weight of an adult male *D. hercules* was 22 g and adult female weighed 18 g, whereas the *T. dichotomus* adult male weighed only 8 g (Suppl. material 1).

Dynastes hercules are naturally found in the Neotropical region of southern Mexico to Bolivia and Trinidad, Guadeloupe, and Dominica in the West Indies (Ratcliffe and Cave 2015). *Dynastes hercules* are among the largest beetles and are the most recognizable insect species in the world. Adults fly mostly at night, especially during the first two hours after sunset (Bouchard et al. 2014). *Trypoxylus dichotomus* are the largest and best-known beetles in Japan. Adult males of this species use their long, forked horns in battles with rival males. Males are active all summer long, whereas females die soon after laying their eggs (Bouchard et al. 2014).

All larvae of horned beetles were fed on breeding fermentation mats (DEBURO Pro) that were purchased from Fujikon Co., Ltd. (<http://www.fujikon.net/>). All adults were fed on breeding jelly (DORCUS JELLY) containing animal protein, trehalose, collagen, and banana flavor (Fujikon Co., Ltd).

Synchronous movements and gear-like structures were characterized and compared between adult beetles and adults of various other insect species. Experiments were performed with five males and five females of *T. dichotomus* and *D. hercules*, and comparisons were made with single adult males of all other horned beetle species. Sixteen adult specimens of other beetle species (not Dynastinae) were collected from the field or purchased from insect-dealers. Sexes of these non-horned beetles were not recorded. Mouthparts of insect specimens were softened by immersion in water all day prior to observations of the synchronous movements and the gear-like structures of mandibles.

Observations of the mandible muscles

To observe the adductor and abductor muscles of the mandible, adult *T. dichotomus* specimens were fixed and preserved in 90% alcohol. Heads were dissected carefully using a file (Bkong YWE-B pencil type router; Yanase Corp., Hyogo, Japan) and tweezers. Mandible muscles were observed using binocular microscopy (Stemi 2000-C; Carl Zeiss Microscopy Co., Ltd.). We compared apparent volume of photographed adductor and abductor muscles. Ratios of adductor and abductor muscles in *T. dichotomus* biting type mandibles were compared with those of adult *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae).

Measurements of mandible strengths and resistance to breakage

Initially, horned heads were separated from dead male *T. dichotomus* specimens and were soaked in tap water overnight to soften the hardened articulations, and the labium and maxillae were then removed carefully using a file and tweezers. The average length from the tip of the horn to bottom of the head was 29.3 mm (Suppl. material 4). Twenty male heads with intact gear-like structures between right and left mandibles were used for measurements of right and left mandible strengths in break resistance analyses. Similarly, ten male heads with broken gear-like structures were used in tests of strengths according to breakage resistance. Head specimens were then fixed by sandwiching the horns between two plastic erasers (to prevent slipping) using a sponge and a small vice as shown in Fig. 2A. A paper container with a wire was attached to grip the tip of the mandible (Fig. 2B) and metal nuts of 4.5 g were used as weights. Load was applied to the mandibles at a right angle to the rotary direction of movement (Suppl. material 5). The container wire was then hung on the tip of a single mandible and the metal nuts were added stepwise to the container until the mandible was dislocated and fell from the mouth. Gross weights of nuts and the container were then recorded. Statistical analyses were performed using a generalized linear model (GLM) with R (Version 3.2.2). For multiple comparisons, Tukey-Kramer tests were used to identify differences between groups of mandibles.

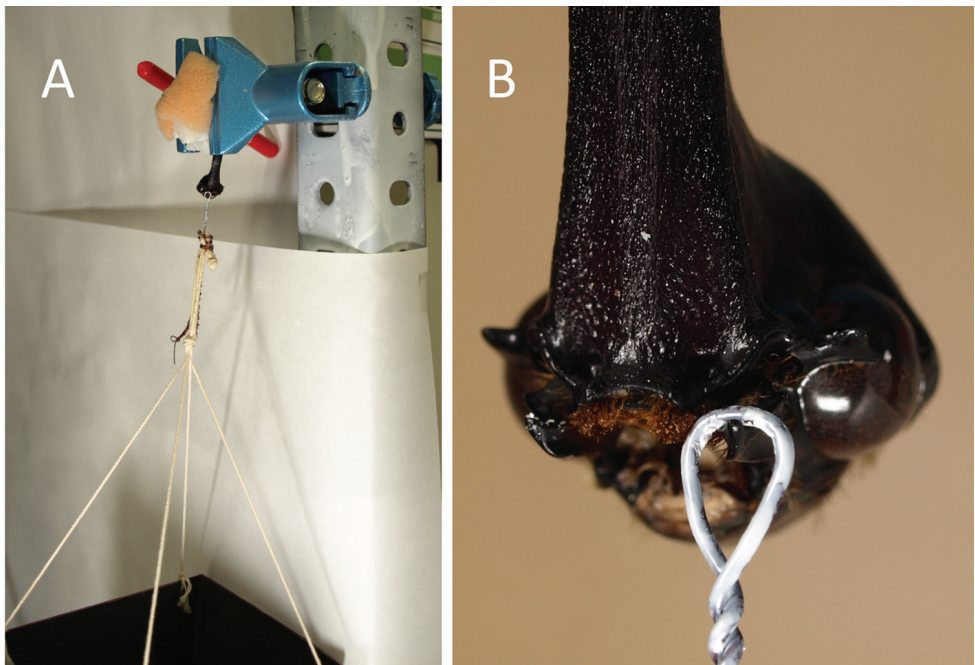


Figure 2. Measurements of mandible strengths of break resistance. **A** *T. dichotomus* horns were fixed in a small vice **B** wire was hung on the tip of the mandible.

Paraffin surface scratching tests with horned beetle heads

If horned beetles use mandibles to carve bark, the tips of the mandibles must be in contact with the tree surface earlier than the projections of the clypeus when the beetle takes the posture for bark carving. Therefore, we determined whether the tips of the mandibles or the projections of the clypeus touch the tree surface first. To this end, paraffin (solidification point about 51–53 °C) was melted, and a small piece of black crayon was melted and mixed in the plastic container to color the paraffin (Sterile No. 2 Square Schale; Eiken Kizai Co., Ltd. Tokyo, Japan), and was then cooled until it hardened. Initially, we determined whether tips of mandibles or the projections of clypeus made pits in the paraffin surface first. But pits on the paraffin were harder to see than scratches on the paraffin. Thus, heads of horned beetles of several genera were manually held at various forward angles (from 0° to 60°) between the horn and paraffin and were used to scratch the paraffin surface. Heads were also placed in the back position and the scars were photographed (Fig. 3).

Video recording and mandible gear-like structures filing procedures

Videos of bark-carving behaviors and actions of mandibles were recorded using a Sony Handycam HDR-PJ590V digital HD video recorder (Sony Corp., Tokyo, Japan). Perches comprised rotten wood and plates of jelly, which were made from logs that were used for cultivation of mushrooms, and were purchased from Fujikon Co., Ltd. We assumed that the bark-carving behavior observed in laboratory conditions on

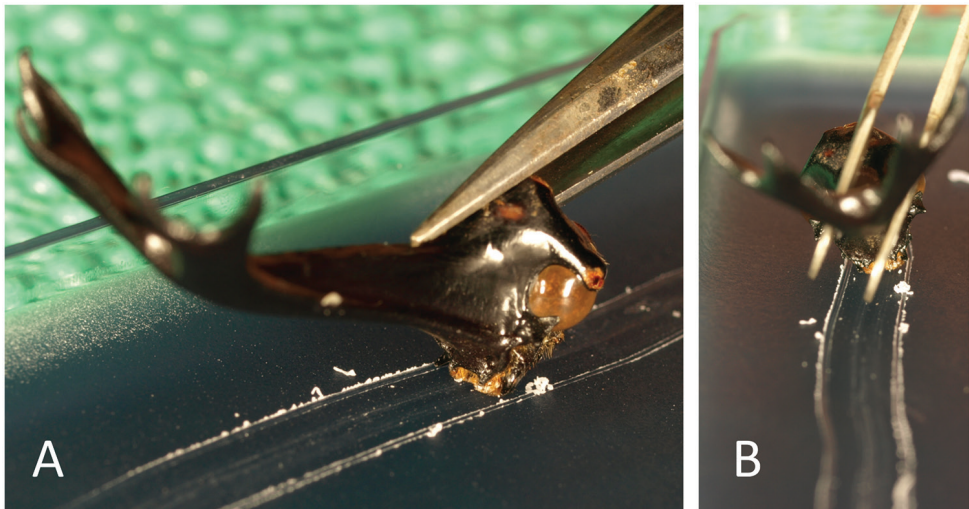


Figure 3. Scarring of flat paraffin surfaces; *T. dichotomus* heads were placed in the back position to show the widths of scars. **A** lateral view **B** frontal view. Only scratches from *T. dichotomus* are shown.

wood logs is similar to that observed on tree barks in the wild. Videos of bark-carving behaviors were recorded using adult *D. hercules* and *T. dichotomus*. The carving behavior of an adult male *T. dichotomus* was also recorded in a plastic green insect cage. To obtain specimens with broken gear-like structures between mandibles but no damage to articulation, gear regions of mandibles were disabled using a file (Bkong YWE-B pencil type router; Yanase Corp., Hyogo, Japan). The router bit was made from a nail and acrylic resin was used to bind mandibles. All materials were purchased from Fujikon Co., Ltd. (<http://www.fujikon.net/>) as an insect specimen kit.

Results

Bark-carving behaviors and mandibles

Mandibles of adult horned beetles are present on both sides of the head (Figs 1, 4A–C). Two types of mandible tips have been described previously (Rowland and Miller 2012); those in *Chalcosoma*, *Eupatorus*, and *Trypoxylus* do not branch off, are sharp, and turn upwards, and those of *Dynastes* and *Megasoma* species are shaped like forks and turn upwards (Fig. 1 and Suppl. material 6). No sexual differences in mandible forms were identified. Because mandible tips fail to make contact with each other, the mandibles of *T. dichotomus* and *D. hercules* do not have biting and chewing functions. The videos recorded in this study indicate that *T. dichotomus* and *D. hercules* use their mandibles to carve bark and do not use the projection of the clypeus. Use of the projection of the clypeus to carve bark or xylem would obscure the orange hair of the maxilla and labrum under the wood. However, in the images of adult male (from 20–40 s in Suppl. material 2 and 50 s to 1 min and at 30 s in Suppl. material 3), female *D. hercules* carving wood (from 1 s to 1 min and 10 s in Suppl. material 7) and adult male *T. dichotomus* beetles carving the green plastic cage (from 1 min and 28 s to 1 min and 35 s in Suppl. material 7), the orange hairs are visible. Moreover, during bark-carving, mandibles were fully opened (Suppl. materials 2, 3, 7) to about 30 degrees from the closed position (Fig. 5A–C), and microscope observations show that the tips of the mandibles were projected further forward than the projection of the clypeus. To confirm these observations, we scratched a flat paraffin surface using the heads of various horned beetle genera at various forward-position angles. In all cases, mandibles, rather than projections of the clypeus, scratched the paraffin. We showed only the scars from paraffin surface scratching experiments with *T. dichotomus* in Fig. 3.

Gear-like structures on mandibles

In further experiments, both mandibles of adult horned beetles moved in complete synchrony. Specifically, manual opening and closing of the right mandible of living adult *T. dichotomus* and *D. hercules* beetles were accompanied by synchronous opening

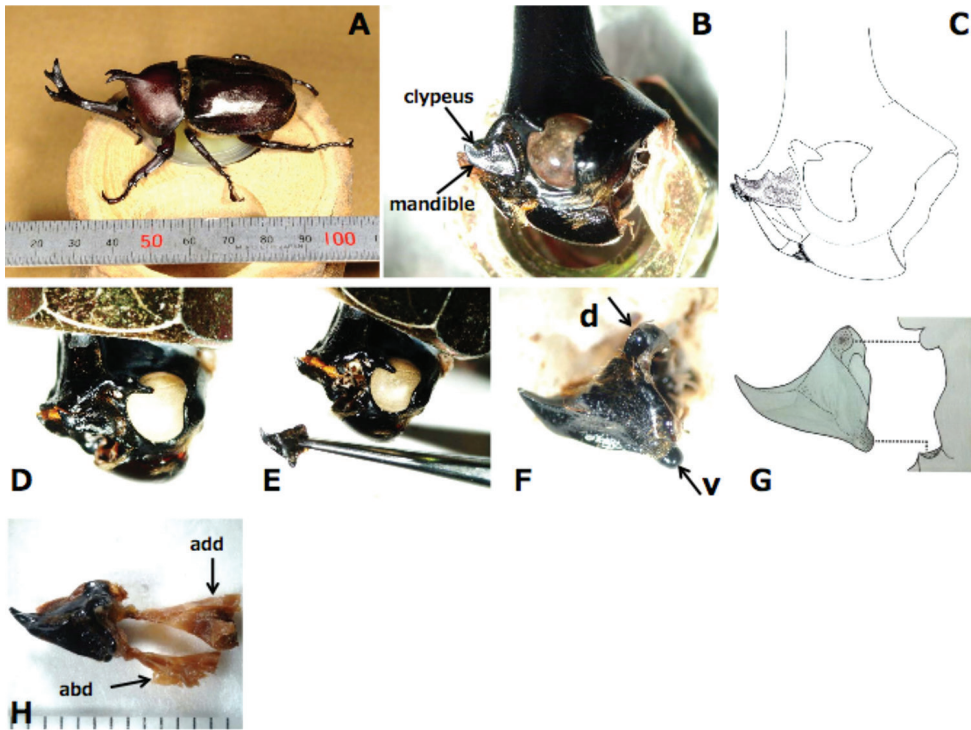


Figure 4. Mandibles of *T. dichotomus* adults. **A** A male adult viewed from the left side with a ruler **B** lateral view of the head from the left side; the clypeus and mandible are indicated by arrows. Antenna and maxillary palp parts were removed to expose the mandible **C** diagram of the head; the mandible is indicated by a stipple **D** a head before removing the mandible **E** a head after removing the mandible **F** the left side mandible was dissected from the cranium **G** diagram showing the double articulation of the mandible; the right side diagram is of the cranium **H** a left side mandible of *T. dichotomus* with attached muscles. Abbreviations: add = adductor muscle; abd = abductor muscle; d = dorsal articulation (socket); v = ventral articulation (condyle). Scale bar: 1 mm.

and closing of the left mandible. Similarly, movements to the left and right were precisely synchronous in both mandibles, and simultaneous mandible movements were also observed in dead insects (Suppl. material 8). These observations suggest that the mandibles are mechanically connected.

To investigate mechanical connections between left and right mandibles, we dissected the heads of *T. dichotomus* and *D. hercules* and observed mandible structures under a stereomicroscope. Mandibles of adult *T. dichotomus* and *D. hercules* are single, heavily sclerotized pieces with a dicondylic articulation. The ventral condyle of the mandible is a ball-like structure that fits into a socket like the acetabulum of the cranium, and the dorsal condyle of the cranium fits into an acetabulum of the mandible (Fig. 4D–G). This dicondylic articulation is typical for biting-chewing mouthparts. Because two points of the articulation are central to the axis of the mandibular movement, *T. dichotomus* and *D. hercules* mandibles can only move in a single plane. No sexual differences in

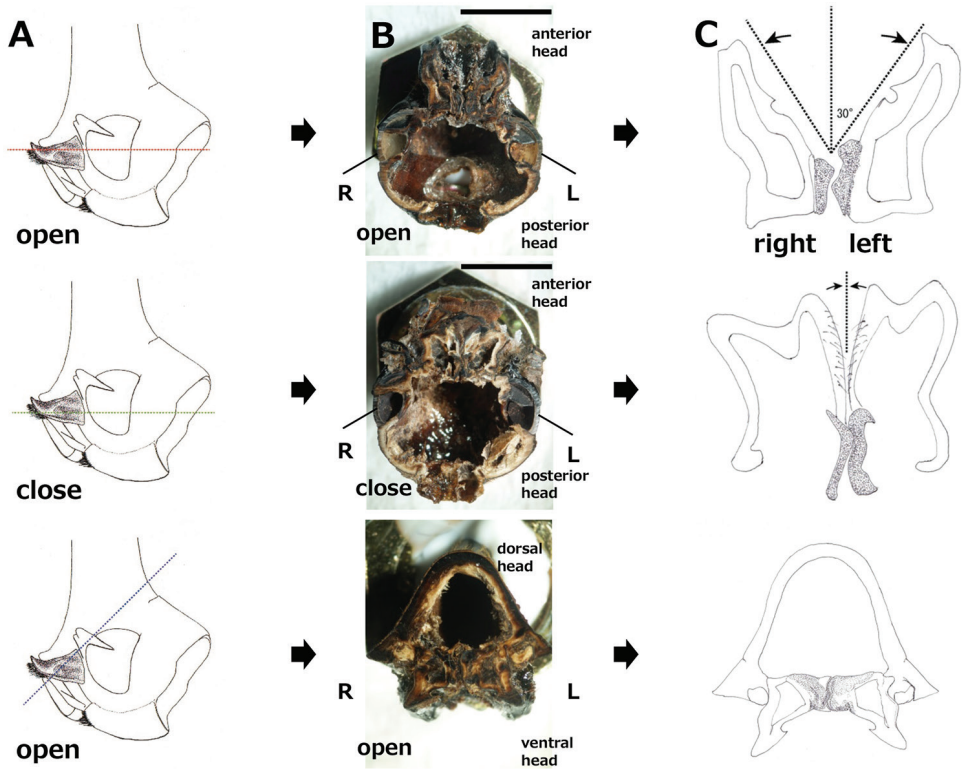


Figure 5. Engagement of gear-like structures on mandibles of *D. hercules* during opening and closing; engaged regions of mandibles were exposed using a file. **A** Lateral view of the head from the left side; dotted lines indicate the position of the transverse section **B** interior view of the head from the ventral side **C** diagram of the engagement region; gear-like structures are emphasized by stipple. Abbreviations: R = right compound eye; L = left compound eye. Scale bar: 7 mm.

mandible forms were identified. Mandibular muscles differed from those of typical biting-type insects. In particular, the adductor muscle of biting insects is generally very large and the abductor muscle is small (Chapman 2013, Snodgrass 1993), whereas in *T. dichotomus*, the adductor muscle is relatively small and the abductor muscle is larger than in other biting-type insects (Fig. 4H). Adductor and abductor muscles of mandibles are compared between *T. dichotomus* and typical biting type insect *L. migratoria* in Suppl. material 9. Furthermore, the posterior regions of mandibles that could not be observed from the outside were asymmetrical, and the gear-like structure was hard and engaged between the mandibles. Artificial devices that transmit force include V-belts and gears. The general definition of a gear is “a toothed machine part that engages successively with another toothed part to transmit motion or to change speed or direction.” Although the mandibles of *T. dichotomus* and *D. hercules* do not rotate 360°, their structure fulfills this definition, as observed in the mouth cavity after stripping the labium and maxillae. Initially, we suspected that these posterior region structures were molar cusps. However, adults of *T. dichotomus* and *D. hercules* could

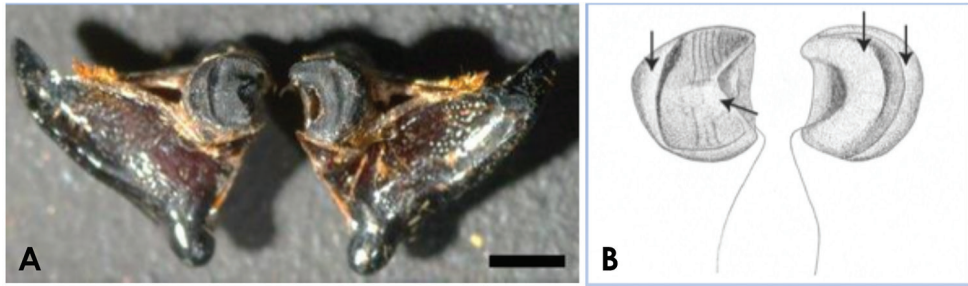


Figure 6. Gear-like structures on mandibles of adult horned beetles. To show the mandibular gear-like structure, the head was split along the anterior midline, and the mandibles were laid out and viewed from the inside. **A** Mandibles of a *T. dichotomus* male adult **B** diagram of structures in Fig. 6A; gear-like structures are shown as stipple. Arrows indicate the tip of each tooth. Scale bar: 2 mm.

not separate engaged mandible and these gear-like structures remained in contact after death (Suppl. material 8). In contrast, no gear structures of mandibles were observed in larvae of *T. dichotomus* (Suppl. material 10) and *D. hercules* (Suppl. material 11), and posterior regions of mandibles in these specimens were separable, indicating that the gear-like structures appear at the adult stage.

The three-dimensional shape of the mandible gear-like structure is very complicated (Fig. 6A, B). Because the gear-like structure with the engagement of mandibles was difficult to represent in two dimensions, we solidified the mandibles of *D. hercules* by filling with acrylic resin and then filed the solidified mandibles to expose horizontal and ventral sections. As shown in Fig. 6, left and right mandibles have two-gear teeth each, and the engagement point moved with opening and closing of mandibles (Suppl. material 8).

In further analyses, we observed gear-like structures of mandibles in adults of 20 other species of horned beetle, suggesting that the gear-like structure of mandibles is common to all adult horned beetles (Suppl. material 6).

Many insects have asymmetric mandibles during the larval or adult stages (Snodgrass 1993). Thus, we examined the posterior regions of the mandibles from various other insect species, but found neither synchronous movements nor mandible gear-like structures (Suppl. material 6).

Measurements of mandible break-resistance strengths

To investigate the roles of the mandible gear-like structures, we measured the break-resistance strengths of mandibles from dead adult *T. dichotomus* under load. Preliminarily, we noticed that considerable force was necessary to dislocate single sides of intact and engaged mandibles from the cranium using tweezers. Moreover, although two articulations remained in the mandibles, these could be dislocated easily. However, there was a possibility that initial loading may damage the remaining mandibles and its articulations. Therefore to exclude this possibility, we used one intact specimen for only one side

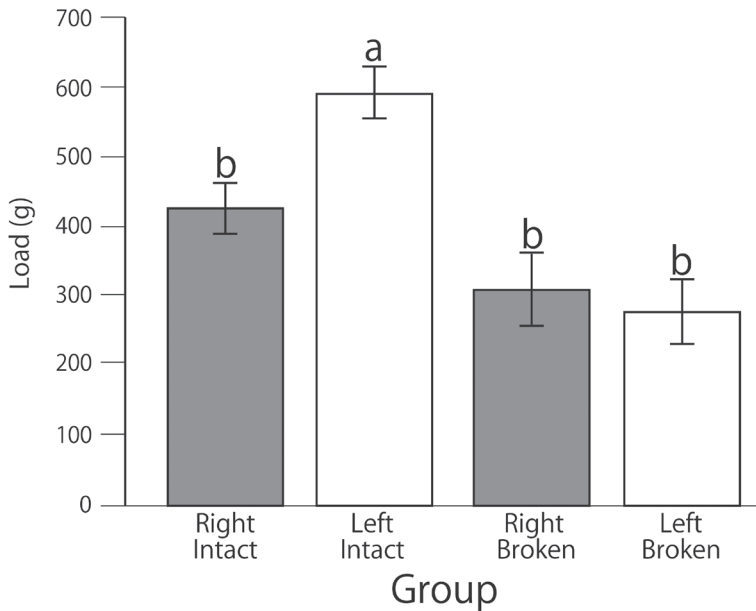


Figure 7. Load strengths of mandibles from a *T. dichotomus* adult male. Abbreviations: Right = right mandible; Left = left mandible. Intact = right and left mandibles in the intact state; Broken = the gear-like structure was broken before the measurement. Vertical bars indicate standard errors of the mean (SE). Significant differences between mandible break-resistance strengths are indicated by different letters (a, b; Tukey, $p < 0.05$).

mandible dislocation experiments (Fig. 7, Right, Intact and Left, Intact groups). Additionally, to obtain the specimen in which the gear-like connection between mandibles was broken without damage to articulation, we filed the gear-like structure region (Suppl. material 12) and performed further load-dislocation experiments. As shown in Fig. 7 (Left, Intact), a load of 600 g was required to dislocate the left side mandible from the cranium. On the other hand, a load of about 400 g was required to dislocate the right side mandible. Thus, we found that the break-resistance strengths of right and left mandibles differed in the intact state (Fig. 7). Disengaged mandibles were dislocated under smaller loads than those of required to dislocate engaged mandibles (Fig. 7, Suppl. material 4). Although no significant differences were observed between “Right”, “Intact”, and “Broken” mandibles, disengaged right mandibles (Broken) were dislocated under smaller load than those required to dislocate engaged mandibles (Intact). In contrast, there were significant differences between “Left”, “Intact”, and “Broken” mandibles. Moreover, the difference in strength between right and left mandibles was not observed after filing the gear region (Fig. 7, Right, Broken and Left, Broken groups). Finally, unlike man-made single gear structures that do not prevent movement up and down along the axis of rotation, the complicated gear-like structure of horned beetle mandibles prevents such movements during the use of only one mandible side (Fig. 5C).

Discussion

Adult *T. dichotomus* beetles exhibit bark-carving behaviors, and it is widely believed that small projections of the clypeus are involved in the process (Hongo 2006, Hongo 2012, Yagihashi et al. 2014). Our experiments and observations show that adults *T. dichotomus* beetles use their mandibles rather than the projection of the clypeus during bark-carving. We also demonstrate that adults *D. hercules* beetles use their mandibles to carve bark (Suppl. materials 2, 3, 7) and have completely engaged mandibular gear-like structures (Fig. 3, Suppl. material 8). These gears operate in two directions (open and close) and produce completely synchronous movements (Suppl. material 8).

Because mandible articulations of adult *T. dichotomus* and *D. hercules* beetles are placed under a considerable load during bark carving, this gear-like structure may primarily prevent the breakage of the mandibles. Accordingly, adult *D. hercules* exhibited bark-carving behaviors using only the mandible of one side, suggesting that the gear-like structure transmits the load from one mandible to the other, thus reducing the load on the mandible in use.

Although synchronous movements of both mandibles may enhance break resistance strength, this is likely an insufficient explanation for the evolutionary conservation of moving functions in adult horned beetles. In addition to carving, mandibles of *T. dichotomus* and *D. hercules* are likely to facilitate sucking of sap as indicated by the narrow mouth cavity. After closing both mandibles, the inside regions with orange hair form a thick sandwich with projections from the labium into the mouth cavity (Suppl. material 13, Fig. 8). Moths and butterflies possess a proboscis (sucking tube) adapted to sucking fluid (nectar and sap). In the case of *T. dichotomus* and *D. hercules*, which do not have tubes, the beetles close their mandibles to create a narrow straw-like opening through which they suck the sap, which flows easily up the narrow mouth cavity and then down the alimentary canal. In accordance with the present observations of mandibular engagement, this function does not require skillful or independent movements of horned beetle mandibles. Moreover, following injury of muscle or nervous connections of one mandible, synchronous movements of both mandibles would be preserved by the gear-like structure. In addition, it was difficult to remove the labium due to its hardness and highly sclerotized state. We suggest that it protects the mouth-parts, especially the maxillae.

Among very few studies of mandible movements in insects, investigations of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) showed that the mandibles of the two sides predominantly move together due to the synchronized activities of the adductor muscles shown in analyses of the sensory inputs required to maintain and change mandibular activities (Chapman 1995, Seath 1977a, Seath 1977b, Rast and Brauning 2001). In addition, *Odontomachus* ants (Hymenoptera: Formicidae) reportedly move both mandibles synchronously, and the required motor neuron circuits also allow independent mandible movements under certain conditions. *Odontomachus* ants move and control mandibular forces and speeds skillfully to perform various functions, including food con-

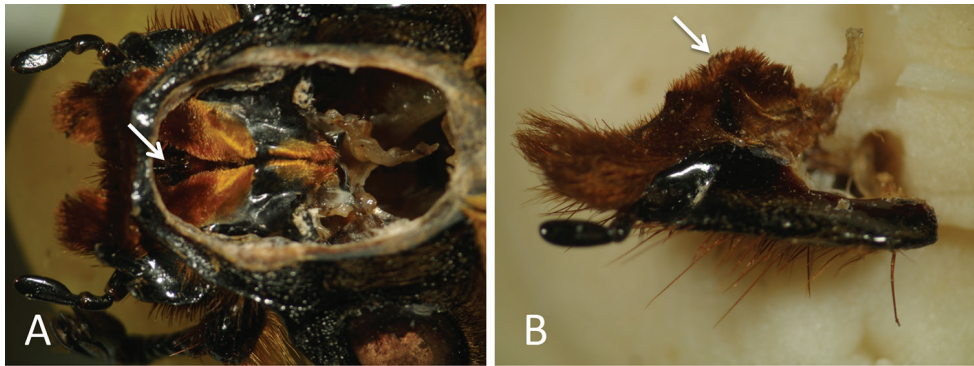


Figure 8. Mandibles and labium of *D. hercules*; engaged regions of mandibles were exposed using a file. **A** Interior view of the male head from the dorsal side; arrows indicate projections from the labium into the mouth cavity **B** lateral view of labium from the left side; arrows indicate projections into the mouth cavity.

sumption, brooding, and grooming (Just and Gronenberg 1999). It is thought that all ants use their mandibles skillfully (Holldobler and Wilson 1990). Hence, ant mandibles have evolved independence of left and right to enable skillful movements. In contrast, adult horned beetles do not move their mandibles skillfully and have small heads relative to their bodies. Horned beetle mandibles also have small adductor muscle quantities, suggesting that biting functions have been abandoned in favor of space in the head cavity. This may be advantageous for horned beetles, as they can bury their small heads into small wound to exude sap. Hence, mandibles of horned beetles may have evolved to have strength for dislocating load rather than skills by adopting a gear-like structure.

Currently, approximately 1,700 species of horned beetles have been identified in forests globally and most of these are in Southeast Asia and South America (Kohiyama 2014). Although horned beetle species vary in shapes and lengths of their adult horns, the mouthparts bear close resemblance to each other. Even between different species, the heads of the adult female horned beetles closely resemble each other. Moreover, the fundamental structural features of mandible gear-like structures were common in the present 22 species. Horned beetles have evolved into many species, but the fundamental structural features of mandibular gear-like structures are likely conserved in adults, because they are the most suitable form for bark carving.

Functional gears are found rarely in animals, and in the single (Hemiptera: Issidae) previous report, nymphs of the planthopper *Issus coleoptratus* had tiny rows of cuticular gear teeth (15–30 μm high) around curved medial surfaces of their two hindleg trochantera (Burrows and Sutton 2013). These gears of right and left trochantera are engaged during preparation for jumping and ensure that both hindlegs move at identical angular velocities to propel the body without yaw rotation. However, these gears occasionally fail to engage at the start of the propulsive phase of jumping due to separation before the jump. *Issus* gears also have only one direction of powered rotation and are lost during the final molt into adulthood. In contrast, the gear-like structures of horned beetles are comparatively large and heavily sclerotized, remaining completely engaged in two directions (open and close) throughout adulthood.

Multiple insect species are considered pests of living and dead or dying trees, and most bark beetles, such as *Pseudohylesinus nebulosus* and *Dendroctonus ponderosae* (Curculionidae), excavate egg galleries in fresh phloem. The locust borer *Megacyllene robiniae* (Coleoptera: Cerambycidae) is a phloem wood insect that attacks living trees (Knight and Heikkinen 1980), and both adults and larvae bite the wood with their mandibles. In contrast, adult horned beetles carve living trees or wood with their mandibles. In the present Suppl. materials 2, 3, 7, the bark is hard and the xylem is soft like rotten wood that has been used for cultivation of mushrooms, and the adult *T. dichotomus* and *D. hercules* carved xylem more easily than bark. In agreement, photographs of adult horned beetles show that their heads are buried in more fibrous stem plants such as sugar cane (Unno 2006). These photographs indicate that adult horned beetles loosen the stem using the same action as that for bark-carving. Therefore, we propose the general use of the terms “tree-carving” or “plant-carving” to denote these carving behaviors of adult horned beetles.

Acknowledgments

We are grateful to two anonymous reviewers for their helpful comments. We also thank Enago (www.enago.jp) for the English language review.

References

- Beutel RG, Friedrich F, Ge SQ, Yang XK (2014) *Insect Morphology and Phylogeny*. Walter de Gruyter GmbH, Berlin, Germany, 516 pp.
- Bouchard P, Bousquet Y, Carlton C, Chamorro ML, Escalona HE, Evans AV, Konstantinov A, Leschen RAB, Tirant SL, Lingafelter SW (2014) *The book of beetles*. The University of Chicago Press, Chicago, IL, USA, 656 pp. <https://doi.org/10.7208/chicago/9780226082899.001.0001>
- Burrows M, Sutton G (2013) Interacting gears synchronize propulsive leg movements in a jumping insect. *Science* 341: 1254–1256. <https://doi.org/10.1126/science.1240284>
- Chapman RF (1995) *Mechanics of Food Handling by Chewing Insects*. In: Chapman RF, de Boer G (Eds) *Regulatory Mechanisms in Insect Feeding*. Chapman & Hall, New York, 3–31. https://doi.org/10.1007/978-1-4615-1775-7_1
- Chapman RF (2013) In: Shimpson SJ, Douglas AE (Eds) *The Insect: Structure and Function* (5th ed). Cambridge University Press, Cambridge, 929 pp.
- Clissold F (2007) The biomechanics of chewing and plant fracture: Mechanisms and implications. *Advances in Insect Physiology* 34: 317–372. [https://doi.org/10.1016/S0065-2806\(07\)34006-X](https://doi.org/10.1016/S0065-2806(07)34006-X)
- Goyens J, Dirckx J, Dierick M, Hoorebeke LV, Aerts P (2014) Biomechanical determinants of bite force dimorphism in *Cyclommatus metallifer* stag beetles. *Journal of Experimental Biology* 217: 1065–1071. <https://doi.org/10.1242/jeb.091744>
- Goyens J, Dirckx J, Aerts P (2016) Jaw morphology and fighting forces in stag beetles. *Journal of Experimental Biology* 219: 2955–2961. <https://doi.org/10.1242/jeb.141614>

- Grimaldi D, Engel MS (2005) Evolution of Insects. Cambridge University Press, New York, 755 pp.
- Holldobler B, Wilson EO (1990) The Ants, The Belknap Press of Harvard University Press, Cambridge, 733 pp. <https://doi.org/10.1007/978-3-662-10306-7>
- Hongo Y (2006) Bark-carving behavior of the Japanese horned beetle *Trypoxylus dichotomus septentrionalis* (Coleoptera: Scarabaeidae). Journal of Ethology 24: 201–204. <https://doi.org/10.1007/s10164-006-0202-x>
- Hongo Y (2012) The Latest Science of Horned Beetles and Stag Beetles. Media factory, Tokyo, 191 pp. [In Japanese]
- Imamori M (2011) The Museum of Rhinoceros Beetles. Alice-kan, Tokyo, 232 pp. [In Japanese]
- Just S, Gronenberg J (1999) The control of mandible movements in the ant *Odontomachus*. Journal of Insect Physiology 45: 231–240. [https://doi.org/10.1016/S0022-1910\(98\)00118-8](https://doi.org/10.1016/S0022-1910(98)00118-8)
- Knight FB, Heikkinen HJ (1980) Principles of Forest Entomology (5th ed). McGraw-Hill Book, Inc. New York, 461 pp.
- Kohiyama K (2014) Rhinoceros Beetles: Micro Presence 4. Shuppan-Geijutsu-sha Co., Inc., 144 pp. [In Japanese]
- Li D, Zhang K, Zhu P, Wu Z, Zhou H (2011) 3D configuration of mandibles and controlling muscles in rove beetles based on micro-CT technique. Analytical and Bioanalytical Chemistry 401: 817–825. <https://doi.org/10.1007/s00216-011-5088-y>
- McMonigle O (2012) The Ultimate Guide to Breeding Beetles. Coachwhip Publications Landisville, PA, USA, 208 pp.
- Nel A, De Villiers WM (1988) Mouthpart structure in adult scarab beetles (Coleoptera: Scarabaeoidea). Entomologia Generalis 13: 95–114. <https://doi.org/10.1127/entom.gen/13/1988/95>
- Nel A, Scholtz CH (1990) Comparative morphology of the mouthparts of adult Scarabaeoidea (Coleoptera). Entomology Memoir- Republic of South Africa, Department of Agricultural Development, 80–84.
- Rast GF, Brauning P (2001) Insect mouthpart motor patterns: central circuits modified for highly derived appendages?. Neuroscience 108: 167–176. [https://doi.org/10.1016/S0306-4522\(01\)00406-7](https://doi.org/10.1016/S0306-4522(01)00406-7)
- Ratcliffe BC, Cave RD (2015) The dynastine scarab beetles of the West Indies (Coleoptera: Scarabaeidae: Dynastinae). Bulletin of the University of Nebraska State Museum 28: 1–346.
- Rowland JM, Miller KB (2012) Phylogeny and systematics of the giant rhinoceros beetles (Scarabaeidae: Dynastini). Insect Mundi: 0263.
- Sanderson ED, Jackson CF (2001) Super Review “ENTOMOLOGY” Insect Life & Behavior. Research & Education Association, Piscataway, NJ, USA, 390 pp.
- Seath I (1977a) Sensory feedback in control of mouthpart movements in the desert locust *Schistocerca gregaria*. Physiological Entomology 2: 147–156. <https://doi.org/10.1111/j.1365-3032.1977.tb00093.x>
- Seath I (1977b) The effects of increasing mandibular load on electrical activity in the mandibular closer muscles during feeding in the desert locust, *Schistocerca gregaria*. Physiological Entomology 2: 237–240. <https://doi.org/10.1111/j.1365-3032.1977.tb00109.x>
- Snodgrass RE (1993) Principles of Insect Morphology. Cornell University Press, Ithaca, 667 pp.

- Unno K (2006) Beetles (4th ed). Co. DATA HOUSE Inc., Shinjuku, Tokyo, Japan, 208 pp. [In Japanese]
- Weihmann T, Kleinteich T, Gorb SN, Wipfler B (2015) Functional morphology of the mandibular apparatus in the cockroach *Periplaneta americana* (Blattodea: Blattidae) – a model species for omnivore insects. *Arthropod Systematics and Phylogeny* 73: 477–488.
- Yagihashi T, Saitoh T, Maehara N, Noguchi M (2014) Bark-carving and sap-obtaining behavior of *Trypoxylus dichotomus septentrionalis* on *Fraxinus japonica*. *Tohoku Journal of Forest Science* 19: 63–65. [In Japanese]
- Yoshimoto J, Nishida T (2007) Boring effect of carpenterworms (Lepidoptera: Cossidae) on sap exudation of the oak, *Quercus acutissima*. *Applied Entomology and Zoology* 42: 403–410. <https://doi.org/10.1303/aez.2007.403>

Supplementary material 1

Horned beetles

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Explanation note: *Dynastes hercules* adult male and female and *Trypoxylus dichotomus* male.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl1>

Supplementary material 2

Bark-carving behaviors of *D. hercules*, adult males mainly from lateral side

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl2>

Supplementary material 3

Bark-carving behaviors of *D. hercules*, adult males mainly from anterior

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl3>

Supplementary material 4

Measurements of *T. dichotomus* mandible break resistance strengths under load

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Measurement

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl4>

Supplementary material 5

Diagram of the direction (from the top to the bottom) of the load on mandible. View from the anterior

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Figure

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl5>

Supplementary material 6

Determination of the presence or absence of gear-like structures on mandibles and types of tips in horned beetles and other insects

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Table

Explanation note: a) +, presence; -, absence of gear-like structures. b) There was an individual variation. c) The chip of mandible was flat. d) The chip of mandible turned inward.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl6>

Supplementary material 7

Bark-carving behaviors of *D. hercules* adult females and a *Trypoxylus dichotomus* adult male

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Explanation note: Horned beetles did not use the projection of clypeus but did use mandibles for bark carving.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl7>

Supplementary material 8

Synchronous movements of adult *D. hercules* mandibles

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Explanation note: Movements of an intact head, a head after stripping the labium and maxillae, and a head after exposure of the gear structure; the gear-like structure was filed gradually to demonstrate the engagement point. Scenes were filmed from the ventral side.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl8>

Supplementary material 9

Comparison mandible muscles between *Trypoxylus dichotomus* horned beetles and the typical biting type insect *Locusta migratoria*

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Figure

Explanation note: **A** anterior view of *L. migratoria* **B** anterior view of male *T. dichotomus* **C** *L. migratoria* **D** *T. dichotomus*. To expose the mandible and its muscles the wall of the left half of the face was removed. Isolated mandibles with adductor and abductor muscles of *L. migratoria* (**E**) and *T. dichotomus* (**F**) Abbreviations: ad=adductor muscle; ab=abductor muscle. Scale bar 1 mm.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl9>

Supplementary material 10

The mouthparts of *Trypoxylus dichotomus* larva

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Figure

Explanation note: **A** ventral view; **B** To expose the posterior regions of mandibles, the labium and maxillae were removed. When the mandibles were open, the hind regions of mandibles did not engage and no gear-like structure was observed. Abbreviations: Ant = antenna; Mx = maxilla; Lb = labium; Md = mandible; mol = molar lobe. Scale bar 2 mm.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.supp10>

Supplementary material 11

Movements of larval *Dynastes hercules* mandibles

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Explanation note: When the larva bites a toothpick, both mandibles move synchronously, whereas manual closing of the left larval mandible was not accompanied by synchronous closing of the right mandible.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.supp11>

Supplementary material 12

Movements of adult *D. hercules* mandibles following breakage of gear-like structural regions

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Explanation note: The mandibular gear region was broken using a file and mechanical synchronous movements were abolished.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl12>

Supplementary material 13

Synchronous movements of adult *D. hercules* mandibles

Authors: Wataru Ichiishi, Shinpei Shimada, Takashi Motobayashi, Hiroaki Abe

Data type: Movie

Explanation note: To expose the mandibles, the male head horn was removed and the cranium was filed as much as possible. Footage was filmed from the dorsal side.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.813.29236.suppl13>

Descriptions of immature stages of four species of the genera *Graptus*, *Peritelus*, *Philopedon*, and *Tanymecus* and larval instar determination in *Tanymecus* (Coleoptera, Curculionidae, Entiminae)

Rafał Gosik¹, Peter Sprick², Michael G. Morris³

1 Department of Zoology, Maria Curie-Skłodowska University, Akademicka 19, 20–033 Lublin, Poland
2 Curculio-Institute e. V. (CURCI), Weckenstraße 15, 30451 Hannover, Germany **3** Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom

Corresponding author: Rafał Gosik (r.gosik@poczta.umcs.lublin.pl)

Academic editor: M. Alonso-Zarazaga | Received 12 October 2018 | Accepted 13 December 2018 | Published 7 January 2019

<http://zoobank.org/AF1D1300-BD4B-4C17-95B4-8567EFAD850C>

Citation: Gosik R, Sprick P, Morris MG (2019) Descriptions of immature stages of four species of the genera *Graptus*, *Peritelus*, *Philopedon*, and *Tanymecus* and larval instar determination in *Tanymecus* (Coleoptera, Curculionidae, Entiminae). ZooKeys 813: 111–150. <https://doi.org/10.3897/zookeys.813.30336>

Abstract

The mature larva and pupa of *Graptus triguttatus triguttatus* and the mature larva of *Peritelus sphaeroides* are described for the first time. The larvae of *Philopedon plagiatum* and *Tanymecus palliatus* are re-described. Five larval instars were determined in *Tanymecus*, thereby correcting doubtful data in the literature. The relationship between larval growth, number of larval instars, head width of the mature larva, and the adult weevil is explained using the example of *Tanymecus*. The nearly constant ratio of subsequent larval instars in head width ratio, termed “growth factor” and derived from Dyar’s ratio, is used for the determination of larval instars. Larval collecting and breeding data are discussed in relation to their significance for the clarification of life-cycles.

Keywords

Bionomics, Central European region, chaetotaxy, Curculionoidea, Entiminae, immature stages, larval instar determination, morphology, taxonomy, weevils

Introduction

In this continued contribution on larvae of the subfamily Entiminae Schönherr, 1823 we describe or redescribe the mature larvae of four further species (e.g., Sprick and Gosik 2014; Gosik et al. 2016, 2017) and the pupa of one species. They represent four different tribes: Byrsopagini Lacordaire, 1863 (= Alophini LeConte, 1874), Cneorhinini Lacordaire, 1863, Peritelini Lacordaire, 1863, and Tanymecini Lacordaire, 1863 (Alonso-Zarazaga et al. 2017). They also allow some insight into the morphological diversity of Central European Entiminae larvae.

In the present paper we describe for the first time the mature larva and pupa of *Graptus triguttatus* (Fabricius, 1775). For this species, Van Emden (1952) provided a description of the first instar larva, eggs and oviposition habit. Dudich (1921) provided two host plant records, *Beta vulgaris* L. and *Symphytum officinale* L., and some data about oviposition and egg morphology, but no relevant information about larval or pupal stages was given. For *Philopodon plagiatus* (Schaller, 1783) there is a description of the mature larva by Van Emden (1952), but only the head capsule and the right pedal lobe were illustrated. A study of distribution and biology of this species in Great Britain was published by Morris (1987).

From the tribe Peritelini we describe the mature larva of *Peritelus sphaeroides* Germar, 1824 for the first time. The pupa was already described by Gosik and Sprick (2013). We do not know of any description of a Central European species in this tribe. Van Emden (1952) and Rosenstiel (1987) characterized the larvae of two North American Peritelini genera, *Nemocestes* Van Dyke, 1936 and *Peritelinus* Casey, 1888.

Despite the frequency and abundance of *Tanymecus palliatus* (Fabricius, 1787), and the good characterization of its development (Hoffmann 1963; Dieckmann 1983), there is no detailed description of the larval instars of this species. Only Znamenskij (1927), in his keys to soil insects, depicted the habitus and last abdominal segment. But this source is not readily available, and we received only a few pages of this work through the kindness of Vitaliy Nazarenko. These studies had been carried out after damage by this species to sunflower and beet fields in Ukraine and southern Russia in the 1920s and 1930s. To our knowledge, the most complete description of a *Tanymecus* Germar, 1817 larva was published by Catrinici (1944) for *T. dilaticollis*. It is supplemented by Van Emden (1952) with a description of the mature larva of *T. palliatus* (without figures) and a description of the North American *T. confusus* (“or very near”). The pupa of *T. palliatus* was already described by Gosik and Sprick (2013).

The aim of this paper is to describe the mature larvae of the four Entiminae species mentioned before and to give some examples about how to use data from larval descriptions for the determination of larval instars and for the study of life-cycles. An important prerequisite for studying life-cycles is to have correctly identified larvae, which is often difficult and a main reason why life-cycles of Entiminae weevils, apart from some noxious *Otiorhynchus* and *Sitona* species, are usually little known.

Materials

Specimens of three of the four species studied were collected in the field under certain plants and usually at the same sites where adults were previously collected. Larval instars of the fourth species, *Peritelus sphaeroides* Germar, 1824, were obtained in captivity by breeding in an air-conditioned room (see Gosik et al. 2016). Two searches for preimaginal stages at the field site where adults of this species were known to occur, were unsuccessful. Number of specimens examined, date and places of collecting are given ahead of the description of each species. As “mature” we regard the larvae with the largest head capsule widths (most closely corresponding to head size of pupa and adult of the species). We also take into consideration results of measurements (if available) provided by other authors.

Methods

All specimens studied were fixed in 75% ethanol and examined under an optical stereomicroscope (Olympus SZ 60 and SZ11) with calibrated oculars. Measurements of larval instars were made for: body length (BL), body width (BW) (usually at abdominal segment I or II), width (HW) and height (HH) of the head capsule (see Fig. 18). In pupae, body length (BL), body width (BW) (at the level of middle legs) and width of pronotum (= thorax) (THW) were measured.

The observations and measurements were conducted using a light compound microscope with calibrated oculars. Drawings and outlines were made using a drawing tube (MNR-1) installed on a stereomicroscope (Ampliwal) and processed by computer software (Corel Photo-Paint X7, Corel Draw X7). Photos were taken with an Olympus E-M10 or using an Olympus BX63 microscope and processed by Olympus cellSens Dimension software. The larvae selected for pictures using SEM (scanning electron microscope) were at first dried in absolute ethyl alcohol (99.8%), rinsed in acetone, treated by CPD procedure (critical point drying) and then gold-plated. For the examination of selected structures a TESCAN Vega 3 SEM was used. General terminology and chaetotaxy follow Anderson (1947), May (1994), Marvaldi (1997, 1998a, 1998b, 1999) and Skuhrovec et al. (2015), with terminology for antennae following Zaharuk (1985), May (1994) and Marvaldi (1998).

Morphological abbreviations:

Abd. 1–10 – abdominal segments 1–10, **Th. 1–3** – thoracic segments 1–3, **at** – antenna, **clss** – clypeal sensorium, **st** – stemmata, **Se** – sensorium, **sa** – sensillum ampullaceum, **sb** – sensillum basiconicum, **lr** – labral rods, **ur** – urogomphus; setae: **als** – anterolateral, **ams** – anteromedial, **as** – alar (larva), **as** – apical (pupa), **cls** – clypeal, **d** – dorsal, **des** – dorsal epicranial, **dms** – dorsal malar, **ds** – discal (pupa), **ds** – dorsal

(larva), *eps* – epipleural, *es* – epistomal, *eus* – eusternal, *fes* – femoral, *fs* – frontal, *les* – lateral epicranial, *ligs* – ligular, *lrs* – labral, *ls* – lateral, *lsts* – laterosternal, *mbs* – malar basiventral, *mds* – mandibular, *mes* – median, *mps* – maxillary palp, *os* – orbital, *pas* – postantennal, *pda* – pedal, *pds* – postdorsal, *pls* – posterolateral, *pes* – postepicranial, *pfs* – palpiferal, *pms* – postlabial, *prms* – prelabial, *prns* – pronotal, *prs* – prodorsal, *ps* – pleural, *rs* – rostral, *sos* – superorbital, *ss* – spiracular, *stps* – stipal, *sts* – sternal, *ts* – terminal, *v* – ventral (pupa), *ves* – ventral (larva), *vms* – ventral malar, *vs* – vertical.

We follow Trnka et al. (2015) and Skuhrovec et al. (2015) who counted in weevils 3 pairs of *ams* and 2 pairs of *mes*. Position of the distal pair of *mes* is still questionable and some other authors (e.g. May 1994; Marvaldi 1998) reported for weevil larvae 2 pairs of *ams* and 3 pairs of *mes*, and they regarded *ams*₁ as *mes*₃.

All these specimens are deposited in the collection of the Department of Zoology, Maria Curie-Skłodowska University (Lublin, Poland). In Table 4 the chaetotaxy of the larvae is given. If necessary, head width of adults was measured directly behind eyes.

Larval instar determination is based on Dyar's law (Dyar 1890), which had been developed and refined by Leibe et al. (1980), Rowe and Kok (1985) and Sprick and Gosik (2014). For the instar determination only data of L₁ larvae and of mature larvae are needed. We explain in several steps how this method works, define the 'growth factor' (based on Dyar's ratio), use it in all detail in *Tanymecus dilaticollis* and show how to find the best approximation of the factor that determines larval growth.

Results

Graptus triguttatus triguttatus

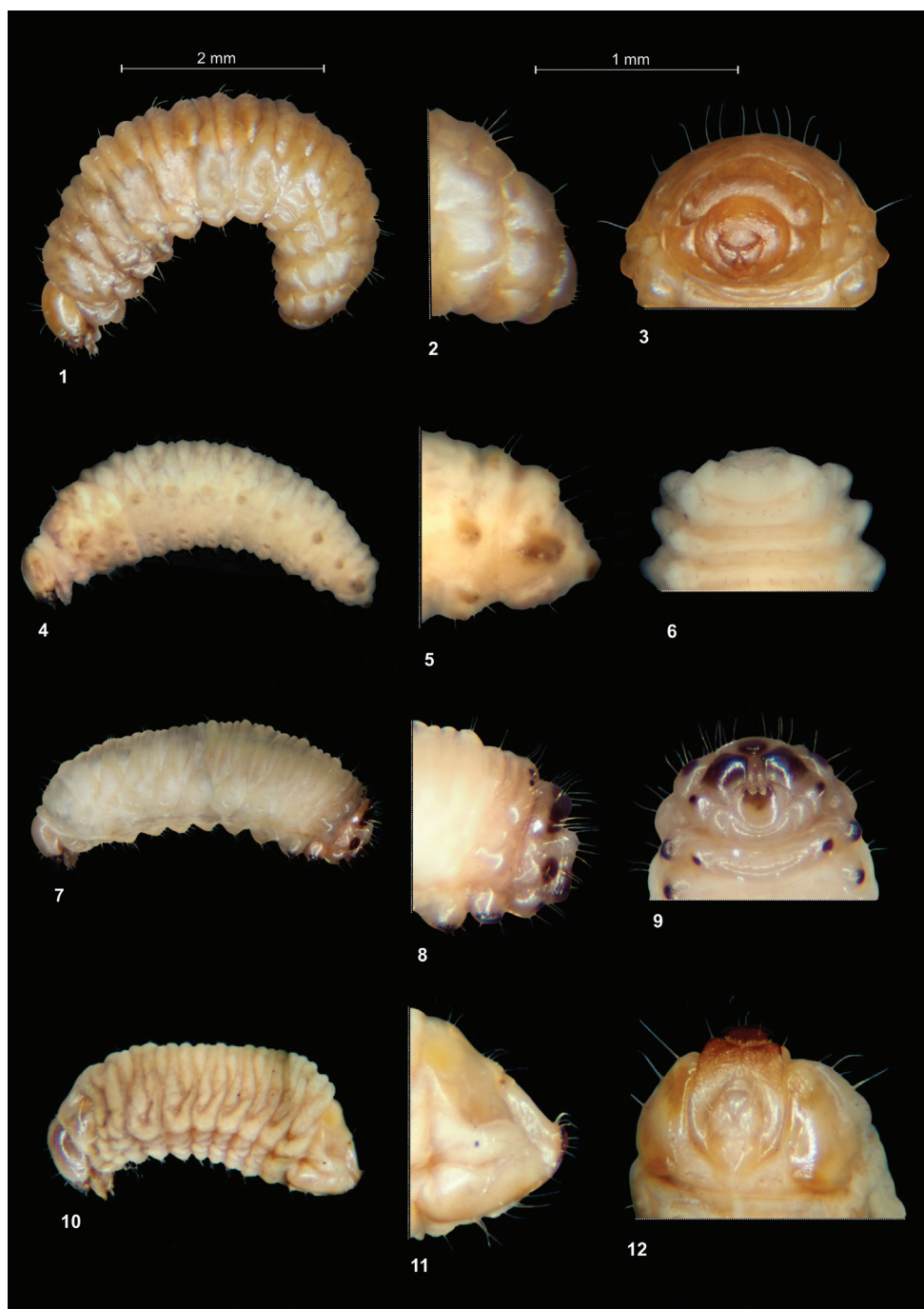
Specimens examined. 5 premature larvae: Germany, Niedersachsen, Braunschweig, apple orchard and meadow in the area of Julius-Kühn-Institut (JKI), all under *Plantago lanceolata* L.; 10.01.2013: 3 ex., 18.04.2013: 1 ex., 24.05.2013: 1 ex.

22 mature and penultimate instar larvae: same site as previously noted, 10.1.2013: 5 ex., 18.04.2013: 7 ex., 03.05.2013: 7 ex., 24.05.2013: 1 ex., 10.06.2013: 2 ex.

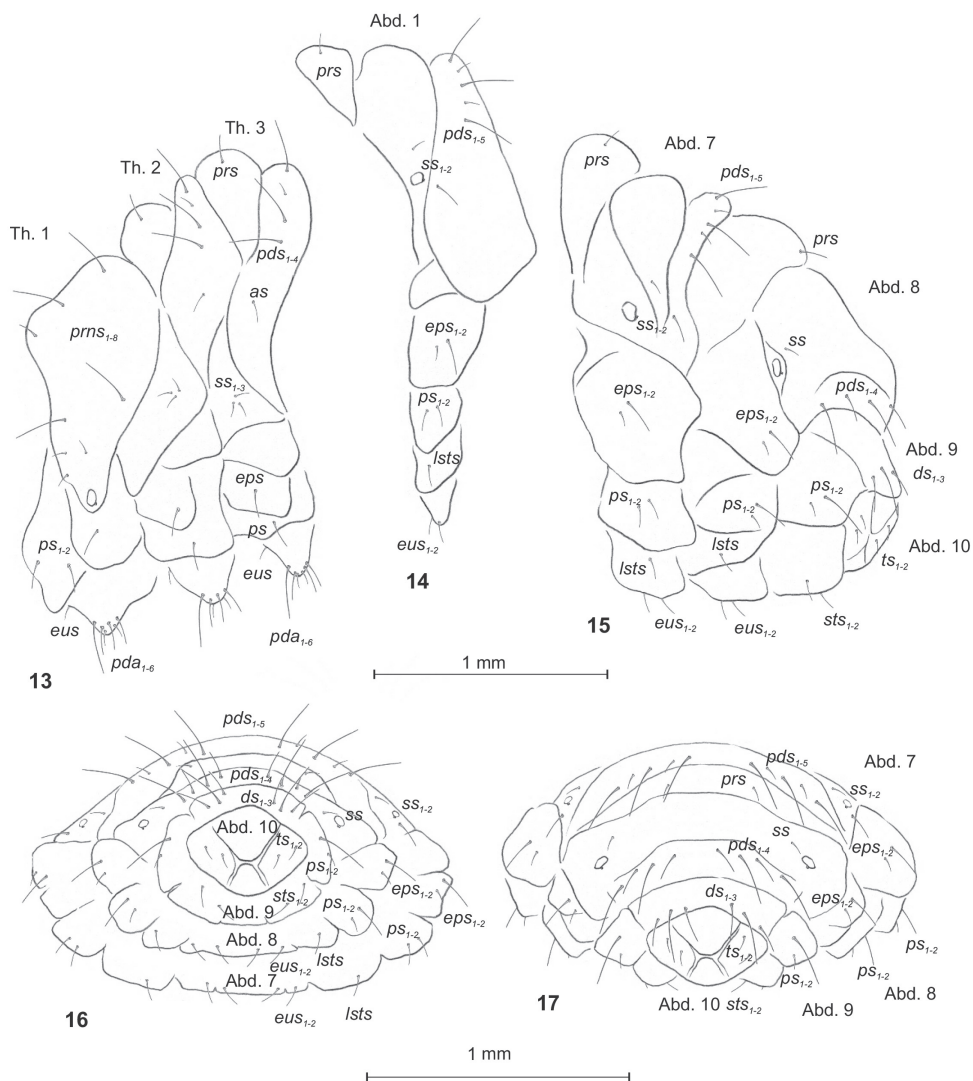
3 pupae: Germany, Niedersachsen, Braunschweig, JKI, apple orchard, meadow; 2 mature larvae, collected on 24.05. and 10.06.2013, both had developed into the pupal stage in breeding boxes in the laboratory in Hannover on 20.06.2013. Another mature larva from the same site, collected on 03.05.2013, developed into the pupal stage before 27.05.2013.

Description of the mature larva. Body length: 6.8–8.5 mm, body width: 3.0–3.4 mm, head width: 1.53–1.55 mm, head height: 1.35–1.37 mm.

Body (Figs 1–3). Moderately slender, curved, rounded in cross section. Prothorax slightly narrower than mesothorax; metathorax as wide as mesothorax. Abdominal segments 1–6 of almost equal length; 7–9 decreasing gradually to the terminal body part; 10 reduced to 4 anal lobes with the largest in dorsal and the smallest in ventral position, lateral lobes of equal size (Fig. 3). Spiracles (of thoracic and abdominal segments



Figures 1–12. *Graptus triguttatus* mature larva. **1** Habitus **2** Last abdominal segments lateral view **3** Last abdominal segments ventral view *Peritelus sphaeroides* mature larva. **4** Habitus **5** Last abdominal segments lateral view **6** Last abdominal segments ventral view *Philopodon plagiatum* mature larva **7** Habitus **8** Last abdominal segments lateral view **9** Last abdominal segments ventral view *Tanymecus palliatus* mature larva **10** Habitus **11** Last abdominal segments lateral view **12** Last abdominal segments ventral view.



Figures 13–17. *Graptus triguttatus triguttatus* mature larva, habitus and chaetotaxy. **13** Thoracic segments, lateral view **14** First abdominal segment lateral view **15** Abdominal segments 7–10 lateral view **16** Abdominal segments 7–10 ventral view **17** Abdominal segments 7–10 dorsal view. Abbreviations: Th. 1–3 – thoracic segments 1–3, Abd. 1–10 – abdominal segments 1–10, setae: *as* – alar, *ps* – pleural, *eps* – epipleural, *ds* – dorsal, *lts* – laterosternal, *eus* – eusternal, *pda* – pedal, *pds* – postdorsal, *prns* – pronotal, *prs* – prodorsal, *ss* – spiracular, *sts* – sternal, *ts* – terminal.

1–8) annular with 2 vestigial airtubes. Chaetotaxy well developed, setae capilliform, variable in length, dark yellow to brown. Each side of prothorax (Fig. 13) with 8 *prns* of unequal length: 5 of them placed on the weakly visible premental sclerite, next 3 short setae close to spiracle; 2 *ps* and 1 *eus*. Meso- and metathorax (Fig. 13) on each side with 1 short *prs*, 4 *pds*, variable in length: first, third and fourth long, second very short, 1

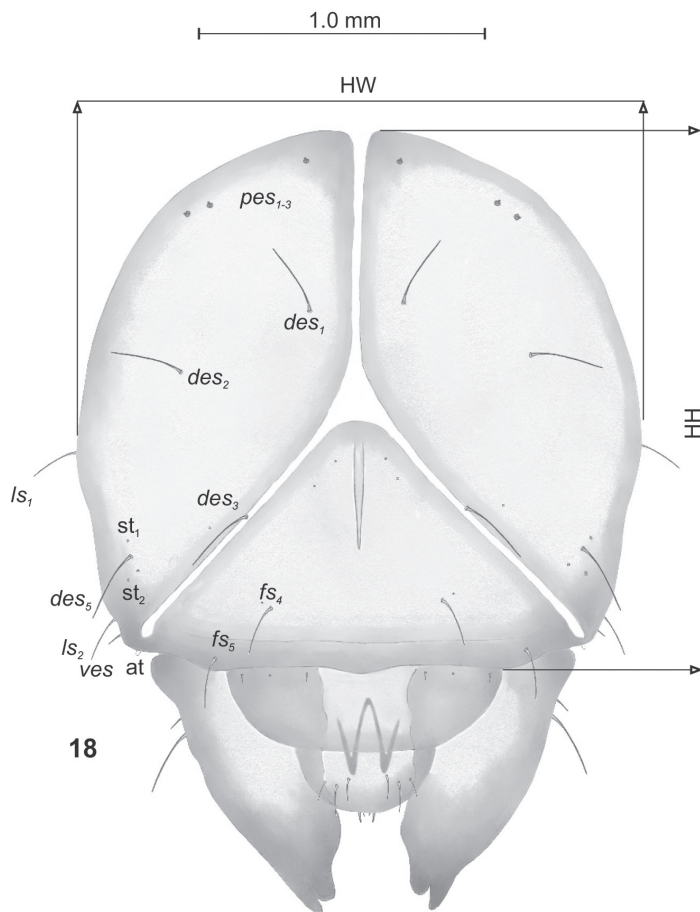


Figure 18. *Graptus triguttatus triguttatus* mature larva, head, frontal view. Abbreviations: at – antenna, HW – head width, HH – head height, st – stemmata, setae: *des* – dorsal epicranial, *fs* – frontal, *les* – lateral epicranial, *pes* – postepicranial, *ves* – ventral.

short *as*, 3 short *ss*, 1 moderately long *eps*, 1 moderately long *ps* and 1 *eus*. Each pedal area of thoracic segments with 6 *pda*, variable in length. Abd. 1–7 (Figs 14–17) on each side with 1 short *prs*, 5 *pds* variable in length (first, third and fifth long, second and fourth short) and arranged along the posterior margin of each segment, 1 minute and 1 short *ss*, 2 *eps* and 2 *ps* of various length, 1 *lts* and 2 short *eus*. Abd. 8 (Figs 15–17) on each side with 1 short *prs*, 4 *pds* variable in length (first and third moderately long, second and fourth long) and arranged along the posterior margin of the segment, 1 minute *ss*, 2 *eps* and 2 *ps* of various length, 1 *lts* and 2 short *eus*. Abd. 9 (Figs 15–17) on each side with 3 *ds* (dorsal setae), first moderately long, second and third long, all located close to the posterior margin of the segment, 1 long and 1 minute *ps* and 2 short *sts*. Each lateral anal lobe (Abd. 10) with a pair of minute setae.

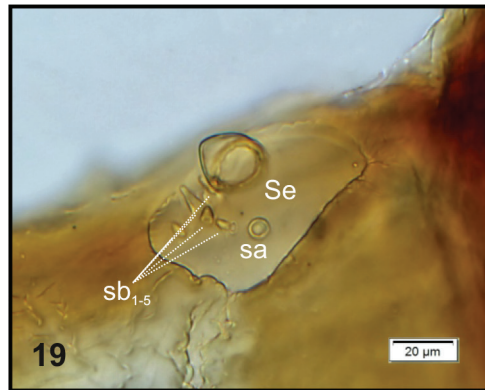


Figure 19. *Graptus triguttatus triguttatus* mature larva, right antenna. Abbreviations: Se – sensorium, sa – sensillum ampullaceum, sb – sensillum basiconicum.

Head (Fig. 18). Light to dark yellow, oval, frontal suture distinct, Y-shaped, endocarina present, reaching to middle of frons. Setae on head capilliform; $des_{1, 2, 3, 5}$ equal in length; des_1 and des_2 located in the central part of epicranium, des_3 placed on frontal suture, des_5 located anterolaterally; $fs_{4, 5}$ equal in length, fs_4 located anteromedially, fs_5 anterolaterally, close to epistome; les_1 and les_2 equal in length, less than half the length of des_3 ; ves short, poorly developed. Postepicranial area with 3 very short pes (Fig. 18). Two weakly visible stemmata close to des_5 . Antennae (Fig. 19) located at the end of frontal suture; antennal segment membranous, bearing sensorium (Se) conical, almost as wide as long, located medially, and 6 sensilla of different types: 1 sa and 5 sb. Labrum (Fig. 20) almost semicircular, anterior margin rounded; 3 pairs of lrs , different in length, lrs_1 and lrs_2 very long, lrs_3 moderately long; lrs_1 placed medially, lrs_2 anteromedially, lrs_3 anterolaterally. Clypeus (Fig. 20) trapezoid, its anterior margin slightly concave, covered with asperities; 2 pairs of cls short, located postero-medially; $clss$ clearly visible, placed medially between cls . Epipharynx (Fig. 20) with 3 pairs of finger-shaped als of almost equal length; 3 pairs of ams : ams_1 and ams_3 rod-shaped, very short, ams_2 finger-like, very long; 2 pairs of rod-shaped mes of various lengths: first pair placed medially, second pair anteriorly, very close to ams . Surface of epipharynx smooth. Labral rods elongate, converging posteriorly. Mandibles (Fig. 21) curved, narrow, with slightly divided apex (teeth of various lengths). There is an elongate protuberance on the cutting edge between the apex and the middle of the mandible; both mds capilliform, different in length, placed transversely. Maxilla (Figs 22–24) with 1 $stps$ and 2 pfs of equal length; mala with 7 finger- or rod-like dms of almost equal size, 4 vms , varied in length and all shorter than dms ; mb s short. Maxillary palpi with 2 palpomeres, basal with short mps ; distal palpomere apically with a group of sensilla, each palpomere with a pore. Basal palpomere distinctly wider than distal, both of almost equal length. Prelabium (Fig. 24) cup-like with 1 moderately long $prms$, located medially. Ligula with 3 pairs of minute $ligs$. Premental sclerite clearly visible, trident-shaped, posterior extension with acute apex. Labial palpi 2-segmented; apex of distal palpomere with some sensilla; each palpomere with

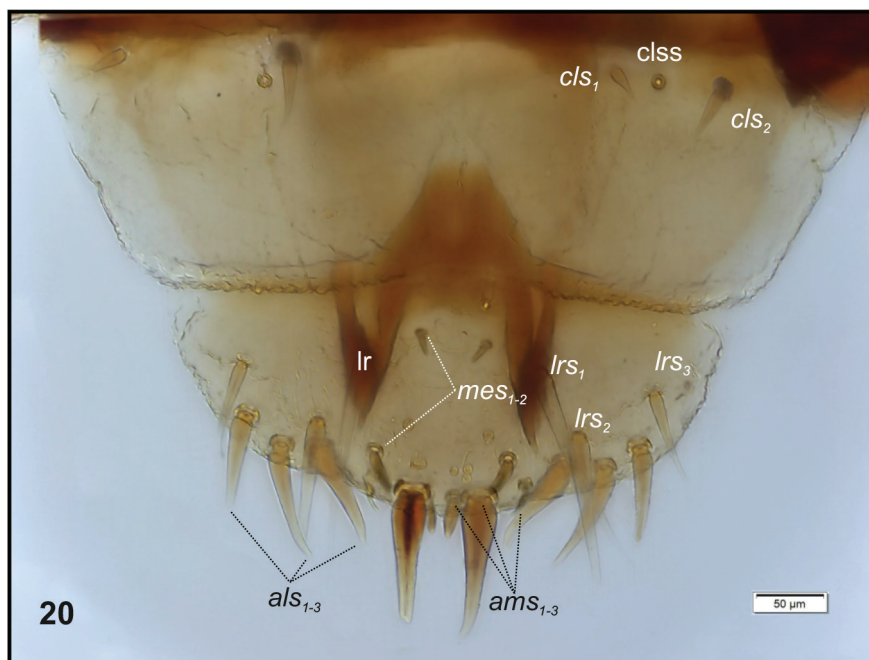


Figure 20. *Graptus triguttatus triguttatus* mature larva, clypeus, labrum and epipharynx. Abbreviations: cls – clypeal sensorium, lr – labral rods, setae: als – anterolateral, ams – anteromedial, cls – clypeal, lrs – labral, mes – median.

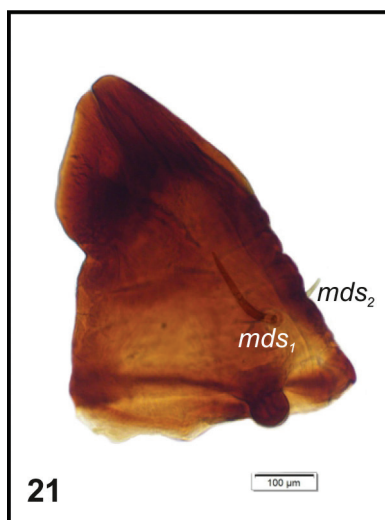
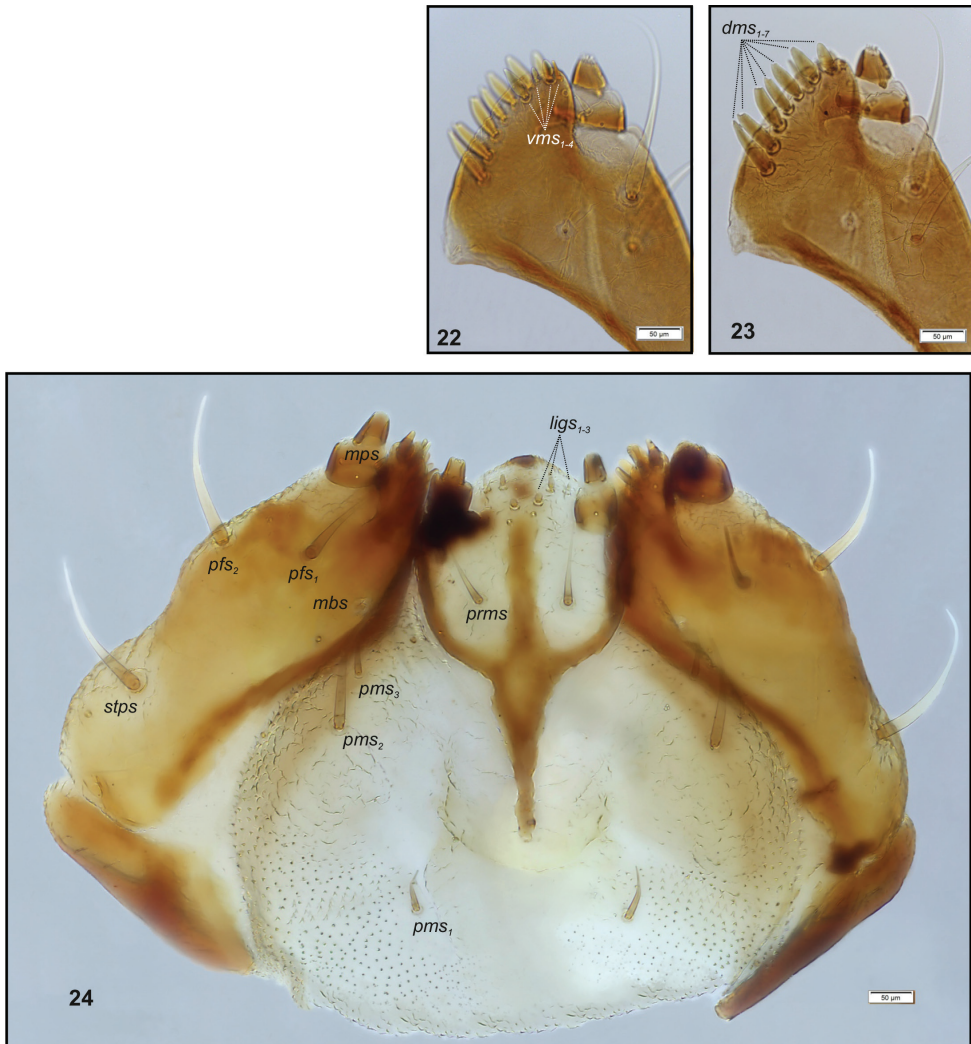


Figure 21. *Graptus triguttatus triguttatus* mature larva, right mandible. Abbreviations: mds – mandibular seta.

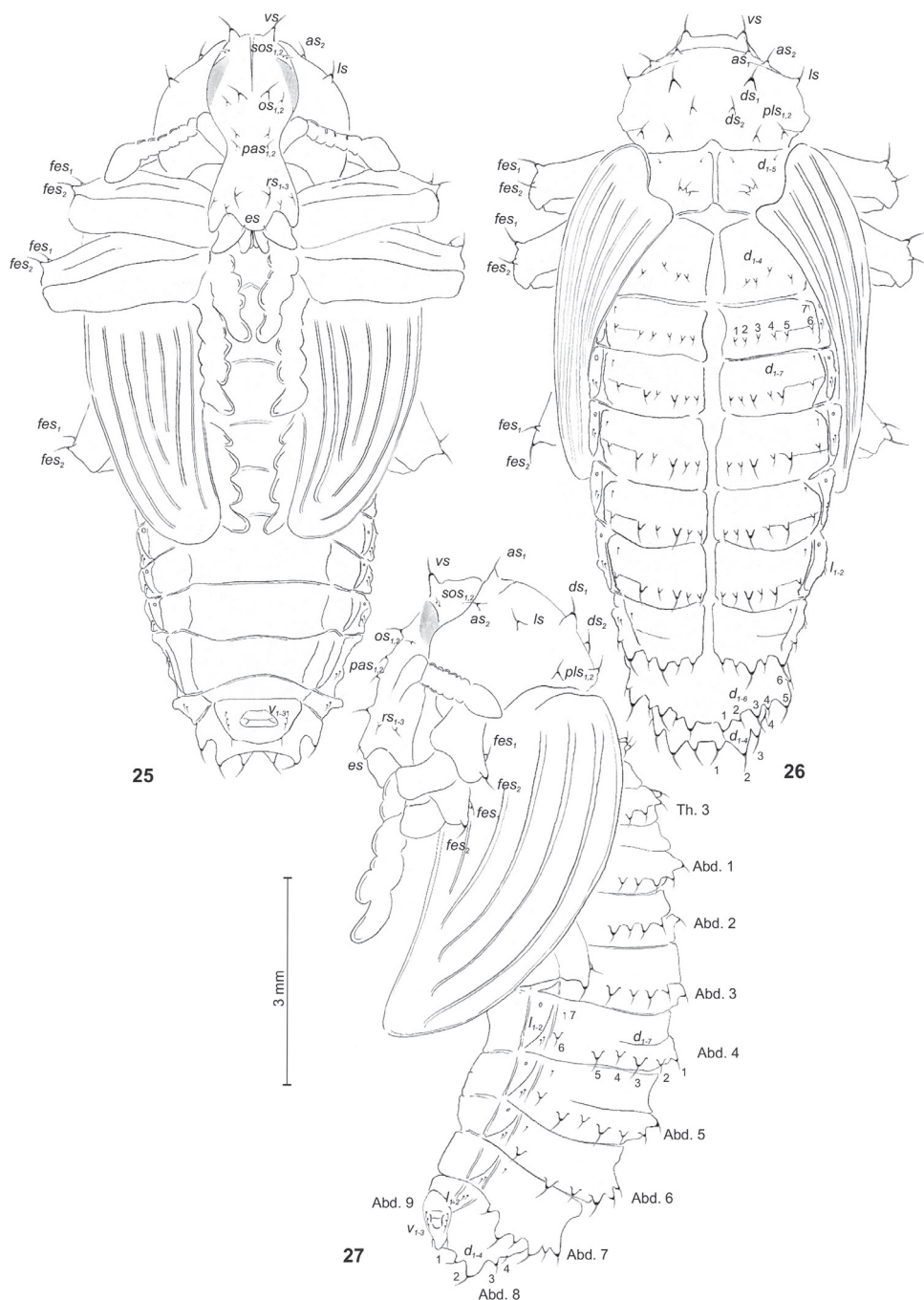
a pore. Basal palpomere distinctly wider than distal, both of almost equal length. Postlabium (Fig. 24) with 3 capilliform *pms* (postlabial setae), the first pair located anteromedially, the remaining 2 pairs posterolaterally; *pms*₁ and *pms*₃ very short, *pms*₂ twice as long as others.



Figures 22–24. *Graptus triguttatus triguttatus* mature larva, body parts. **22** Right maxilla apical part ventral aspect **23** Right maxilla apical part dorsal aspect **24** Maxillolabial complex ventral aspect. Abbreviations: *dms* – dorsal malar, *ligs* – ligular, *mbs* – malar basiventral, *mps* – maxillary palp, *pfs* – palpiferal, *prms* – prelabial, *pms* – postlabial, *stps* – stipal, *vms* – ventral malar.

Description of the pupa. Body length (σ , φ): 7.5–9.0 mm; body width (at level of mesocoxae): 3.8–4.5 mm; width of thorax: 2.0–2.3 mm.

Body moderately slender, straight, yellowish. Cuticle smooth (Figs 25–27). Rost-rum short, 1.2 times as long as wide, extended beyond procoxae. Antennae moderately long and slender. Pronotum almost 1.8 times as wide as long. Abdominal segments 1–4 of almost equal length, segments 5–7 decreasing gradually, 8 semicircular, 9 distinctly smaller than previous segments. Urogomphi absent (Figs 25–27).



Figures 25–27. *Graptus triguttatus triguttatus* pupa. **25** Ventral **26** Dorsal **27** Lateral view. Abbreviations: Th. 1–3 – pro-, meso- and metathorax, Abd. 1–9 – abdominal segments 1–9, ur – urogomphus, setae: *as* – apical, *d* – dorsal, *ds* – discal, *es* – epistomal, *fes* – femoral, *l*, *ls* – lateral, *os* – orbital, *pas* – postantennal, *pls* – posterolateral, *rs* – rostral, *sos* – superorbital, *v* – ventral, *vs* – vertical.

Chaetotaxy well developed, setae variable in length and shape: spine-like or capilliform, dark yellow to brown, usually located on visible protuberances. Head capsule and rostrum include 1 *vs*, 2 minute *sos*, 1 spine-like and 1 minute *os*, 2 *pas*, 3 *rs* of varied sizes and 1 minute *es*. Except *sos* and *es*, all setae of the head and rostrum are placed on protuberances. Pronotum with 2 *as*, 1 *ls*, 2 *ds* and 2 *pls*. All setae of pronotum spine-like, of equal size (only *ds*₁ slightly larger than others); all setae placed on protuberances. Mesothorax with 2 minute setae placed anteromedially and 3 spine-like setae placed medially. Metathorax with 4 spine-like setae placed medially. Abdominal segments 1–7 with 7 pairs of *d*_{1–7}: *d*_{1–6} short, spine-like, placed on protuberances, in lines along the posterior margin of segments, *d*₇ short, capilliform, placed anterolaterally, and 2 minute *l*_{1–2}. Setae no. 3 and no. 5 increasing gradually from segment 2 to 7. Segment 8 with 4 pairs of spine-like setae of varied lengths (*d*_{1–4}), placed on protuberances, in lines along the posterior margin of the segment. Seta no. 2 distinctly larger than others. Segment 9 with 3 pairs of short, capilliform *v*_{1–3}. Each apex of femora with 2 *fes*, spine-like and of various length.

Peritelus sphaeroides

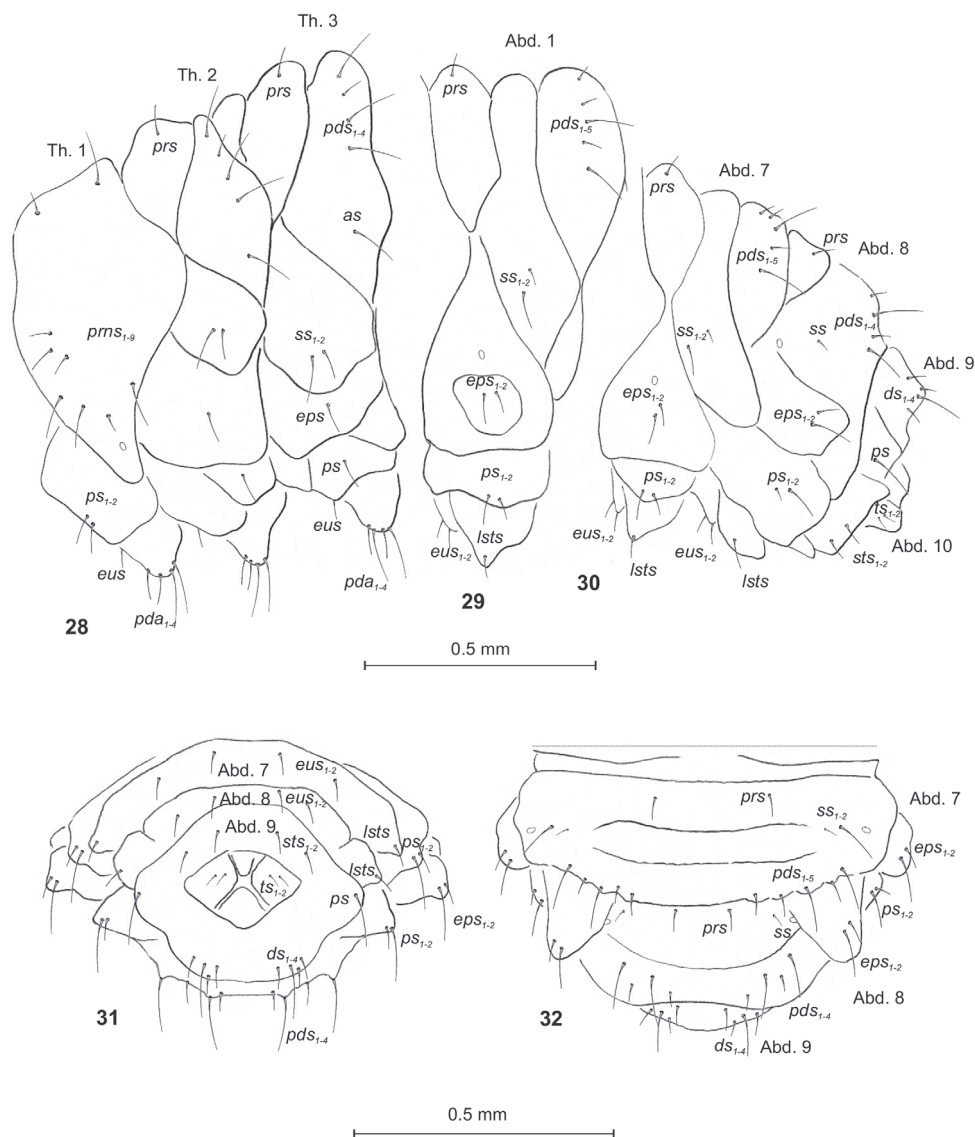
Specimens examined. Rearing was started on 02.05.2012 in the climate chamber of JKI in flowerpots with mainly *Euonymus fortunei* (Turcz.) Hand.–Mazz. and one with *Prunus laurocerasus* L. Adults had been collected 5 days previously in a hedgerow with ornamental shrubs in the JKI area.

3 premature larvae: flowerpot with *Euonymus fortunei*, climate chamber, JKI, 13.12.2012: 2 ex. These specimens were bred to produce pupae and transferred to Hannover for regular pupal control. As there was no further development, they were taken out on 25.01.2013; flowerpot with *Prunus laurocerasus*, climate chamber of JKI, 14.03.2013: 1 ex.

12 mature larvae: flowerpot with *Euonymus fortunei*, JKI, climate chamber, 24.08.2012: 1 ex. (the first mature larva after 3 months and 3 weeks of development), 01.11.2012: 2 ex., do., 13.12.2012: 5 ex. (4 of them were used for regular pupae control; as there was no pupation, they were taken out on 25.01.2013), 14.03.2013: 2 ex., flowerpot with *Prunus laurocerasus*, JKI, climate chamber, 14.03.2013: 2 ex.

Description of mature larva. Body length: 6.5–7.7 mm, body width at the widest part (level of first abdominal segment): 2.0–2.5 mm, head width: 1.10–1.17 mm, head height: 0.90–1.00 mm.

Body (Figs 4–6). Slender, curved, rounded in cross section. Prothorax slightly smaller than mesothorax; metathorax as wide as mesothorax. Abdominal segments 1–6 of almost equal length; 7–9 decreasing gradually to the terminal parts of the body; 10 reduced to 4 anal lobes of various sizes (ventral lobe the smallest, dorsal the largest) (Fig. 6). Spiracles (of thoracic and abdominal segments 1–8) annular. Chaetotaxy well developed; setae capilliform, variable in length, dark yellow to brown. Each side of prothorax (Fig. 28) with 9 *prns* of different length; 2 *ps* and 1 *eus*. Meso- and metathorax (Fig. 28)



Figures 28–32. *Peritelus sphaeroides* mature larva, habitus and chaetotaxy. **28** Thoracic segments lateral view **29** First abdominal segment lateral view **30** Abdominal segments 7–10 lateral view **31** Abdominal segments 7–10 ventral view **32** Abdominal segments 7–10 dorsal view Abbreviations: Th. 1–3 – thoracic segments 1–3, Abd. 1–10 – abdominal segments 1–10, setae: *as* – alar, *ps* – pleural, *eps* – epipleural, *ds* – dorsal, *lsts* – laterosternal, *eus* – eusternal, *pda* – pedal, *pds* – postdorsal, *prms* – pronotal, *prs* – prodorsal, *ps* – spiracular, *sts* – sternal, *ts* – terminal.

on each side with 1 moderately long *prs*, 4 *pds*, variable in length (first, third and fourth long, second short), 1 long *as*, 2 moderately long *ss*, 1 moderately long *eps*, 1 moderately long *ps* and 1 *eus*. Each pedal area of thoracic segments with 4 *pda*, variable in length. Abd. 1–7 (Fig. 27) on each side with 1 short *prs*, 5 *pds*, varied in length (first, second

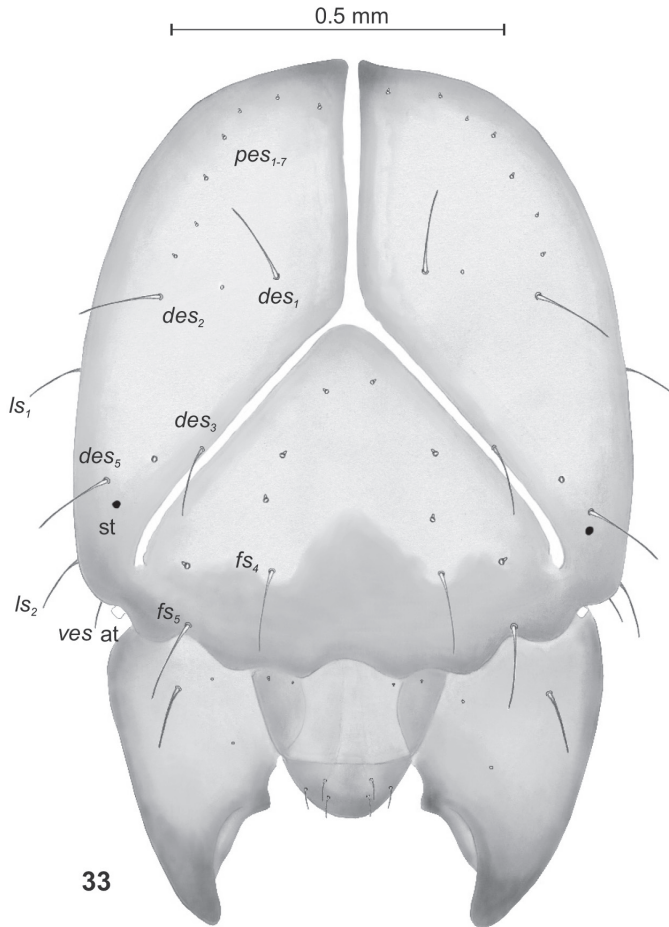


Figure 33. *Peritelus sphaeroides* mature larva, head frontal view. Abbreviations: at – antenna, st – stemmata, setae: *des* – dorsal epicranial, *fs* – frontal, *les* – lateral epicranial, *pes* – postepicranial, *ves* – ventral.

and fourth very short, third and fifth long) and arranged along the posterior margin of each segment, 1 minute and 1 long *ss*, 2 *eps* and 2 *ps* of varied lengths, 1 *lsts* and 2 short *eus*. Abd. 8 (Figs 30–32) on each side with 1 short *prs*, 4 *pds* of varied length (first and third moderately long, second and fourth long) and all arranged along posterior margin of the segment, 1 minute *ss*, 2 *eps* and 2 *ps* of various length, 1 *lsts* and 2 short *eus*. Abd. 9 (Figs 30–32) on each side with 4 *ds* (dorsal setae): first, second and fourth short, third moderately long, all located close to posterior margin of the segment, 1 long *ps* and 2 short *sts*. Each lateral anal lobe (Abd. 10) with a pair of minute terminal setae (*ts*).

Head (Fig. 33). Light to dark yellow, slightly narrowed bilaterally, frontal suture distinct, Y-shaped, endocarina absent. Setae on head capilliform; *des*_{1, 2, 3, 5} equal in length; *des*₁ and *des*₂ located in the central part of epicranium, *des*₃ placed on frontal suture, *des*₅ located anterolaterally; *fs*_{4, 5} almost equal in length, *fs*₄ located anteromedially, *fs*₅ anterolaterally, close to epistome; *les*₁ and *les*₂ equal in length, slightly shorter than *des*₁.

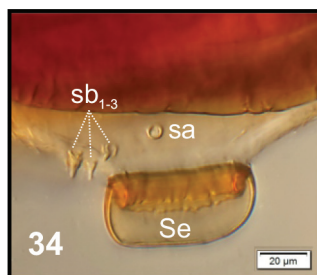
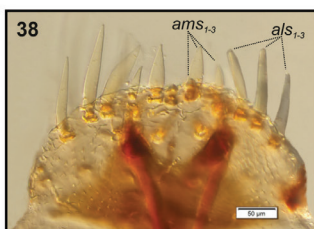
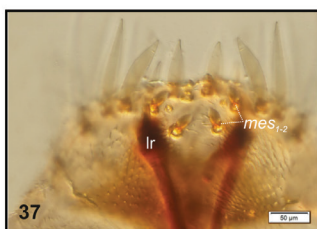
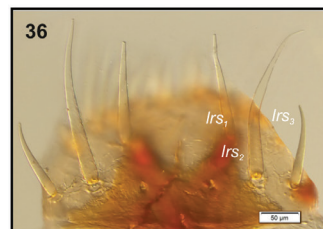
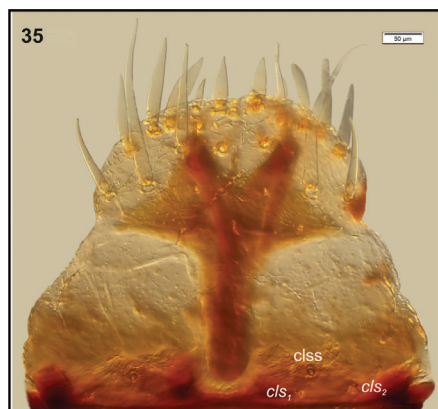
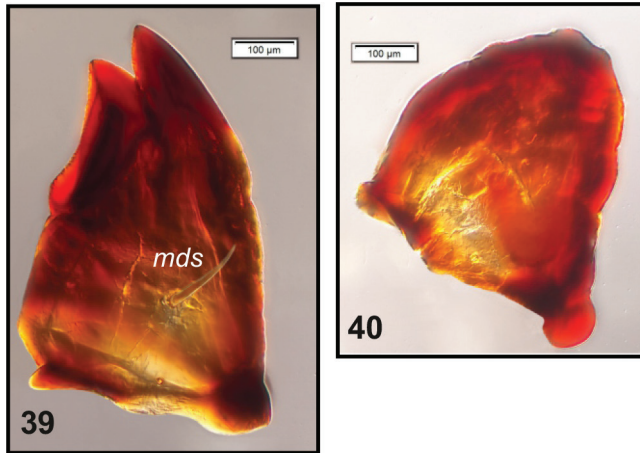


Figure 34. *Peritelus sphaeroides* mature larva, right antenna. Abbreviations: Se – sensorium, sa – sensillum ampullaceum, sb – sensillum basiconicum.



Figures 35–38. *Peritelus sphaeroides* mature larva, body parts. **35** Clypeus and labrum **36** Clypeus **37, 38** Epipharynx. Abbreviations: clss – clypeal sensorium, lr – labral rods, setae: als – anterolateral, ams – anteromedial, cls – clypeal, lrs – labral, mes – median.

Postepicranial area with 7 very short *pes*. Single *ves* very short (Fig. 33). Stemmata poorly visible, located close to *des_5*. Antenna (Fig. 34) located at the end of frontal suture; antennal segment membranous, bearing cushion-like sensorium (Se), located medially and 4 sensilla of different types: 1 ampullaceum (sa) and 3 basiconica (sb). Clypeus (Fig. 35) trapezoid, anterior margin of clypeus slightly emarginate at the inside; 2 pairs of *cls* very short, located posteromedially; *clss* clearly visible, placed medially between *cls*. Labrum (Figs 35, 36) almost semicircular, anterior margin rounded; 3 pairs of *lrs* of different length, *lrs_1* and *lrs_3* moderately long, *lrs_2* very long, all *lrs* reaching behind anterior margin of

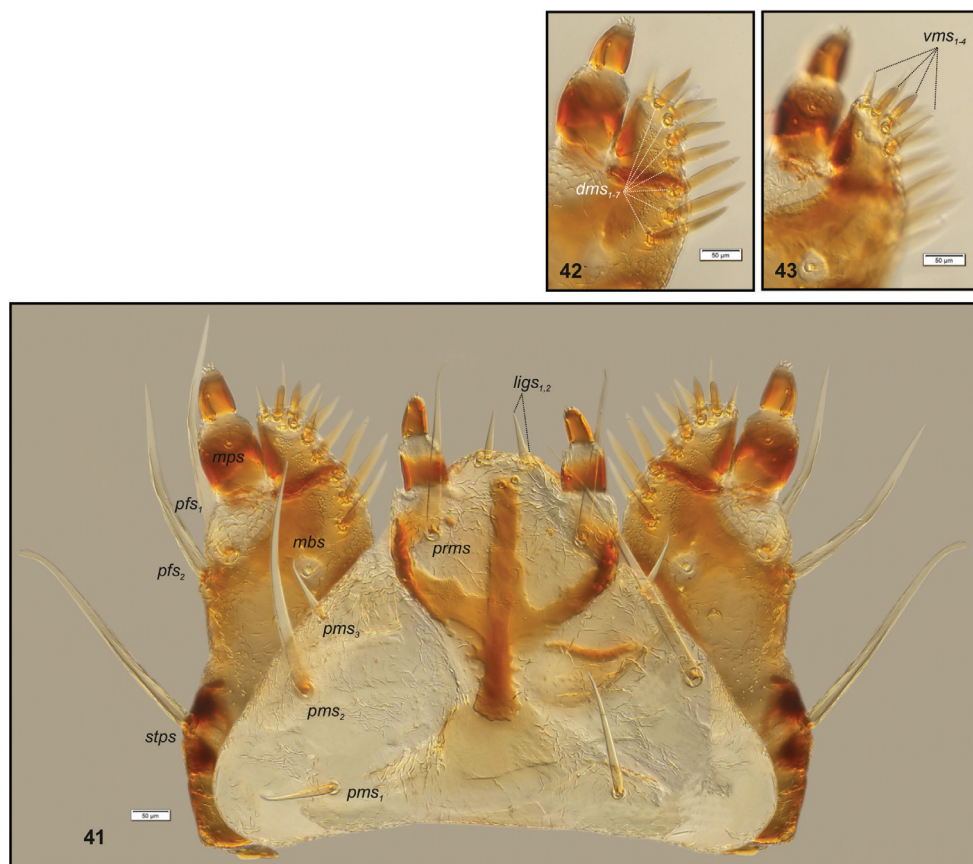


Figures 39, 40. *Peritelus sphaeroides* mature larva, right mandible. **39** Typical **40** Worn out. Abbreviations: *mds* – mandibular seta.

labrum; *lrs*₁ placed medially, *lrs*₂ anteromedially, *lrs*₃ anterolaterally. Epipharynx (Figs 37, 38) with 3 pairs of finger-shaped *als* of almost equal length; 3 pairs of *ams*: *ams*₁ and *ams*₃ rod-shaped, very short, *ams*₂ finger-like, very long; 2 pairs of rod-shaped *mes*, equal in length. Surface of epipharynx covered with asperities. Labral rods elongate, converging posteriorly. Mandibles (Figs 39, 40) elongate, narrow, with divided apex (teeth variable in length). There is a protruding additional tooth on the cutting edge between the apex and the middle of the mandible; single *mds* capilliform, moderately long. These characters can disappear due to intensive feeding and gradual wear and tear of mandibles (Fig. 40). Maxilla (Figs 41–43) with 1 *stps* and 2 *pfs* of equal length; mala with 7 finger-like *dms* (Fig. 42) and 4 *vms*, all of varied length, the latter only slightly shorter than *dms* (Fig. 43); *mb*s short. Maxillary palpi with 2 palpomeres, basal with short *mps*; distal palpomere apically with a group of sensilla, each palpomere with a pore. Basal palpomere distinctly wider and longer than distal. Prelabium (Fig. 41) cup-like with 1 very long *prms*, located medially. Ligula with 2 pairs of *lgs*: first relatively long, second minute. Premental sclerite clearly visible, trident-shaped, posterior extension with thickened apex. Labial palpi 2-segmented; apex of distal palpomere with some sensilla; each palpomere with a pore. Basal palpomere distinctly wider and slightly longer than distal. Postlabium (Fig. 41) with 3 capilliform *pms*, the first pair located anteromedially, the remaining 2 pairs posterolaterally: *pms*₁ moderately long, *pms*₂ twice as long as *pms*₁ and *pms*₃ short.

Philopedon plagiatum

Specimens examined. 6 premature larvae: Germany, Niedersachsen, Hannover-Vahrenheide, Kugelfangtrift, nutrient-poor sandy grassland, collected at sparsely grown sites under *Plantago lanceolata*, 02.10.2011: 3 ex., 11.11.2011: 3 ex.

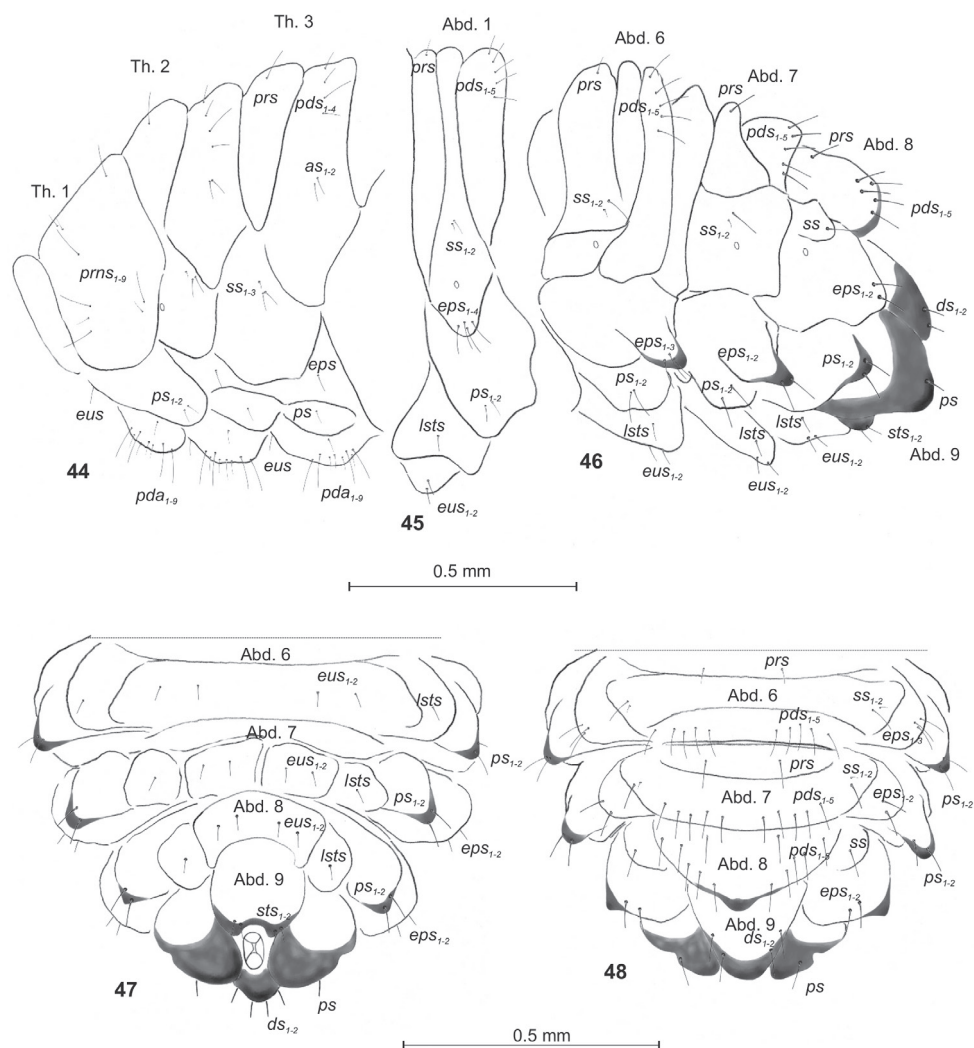


Figures 41–43. *Peritelus sphaeroides* mature larva, body parts. **41** Maxillolabial complex ventral aspect **42** Right maxilla apical part dorsal aspect **43** Right maxilla apical part ventral aspect. Abbreviations: setae: *dms* – dorsal malar, *ligs* – ligular, *mbs* – malar basiventral, *mps* – maxillary palp, *pfs* – palpi-feral, *prms* – prelabial, *pms* – postlabial, *stps* – stipal, *vms* – ventral malar.

3 mature larvae: same site as before, 02.10.2011: 1 ex., 11.11.2011: 1 ex.; Denmark, Syddanmark, Emmerlev Klev near Højer, moraine at the sandy sea shore of the North Sea, collected on 13.08.2015: 1 ex., between the roots of *Plantago maritima* L. subsp. *maritima*, very probably; *P. lanceolata* was also present nearby.

Description of the mature larva. Body length: 6.5–8.2 mm, body width at the widest part (level of first abdominal segment): 2.4–3.4 mm, head width: 0.97–1.03 mm, head height: 0.75–0.83 mm.

Body (Figs 7–9). Slender, elongate, slightly narrowed bilaterally dorso-ventrally. Prothorax slightly smaller than mesothorax; metathorax as wide as mesothorax. Abdominal segments 1–7 of almost equal length. Abdominal segment 8 wide, flattened posteriorly, with conical lateral lobes. Abdominal segment 9 strongly reduced, consisting of 4 well-isolated lobes, ventral almost rounded, lateral conical, dorsal semicircular. Abdominal segment 10 consists of 4 anal lobes of almost equal size. Anus located ter-



Figures 44–48. *Philopodon plagiatus* mature larva, habitus and chaetotaxy. **44** Thoracic segments lateral view **45** First abdominal segment lateral view **46** Abdominal segments 7–10 lateral view **47** Abdominal segments 7–10 ventral view **48** Abdominal segments 7–10 dorsal view. Abbreviations: Th. 1–3 – thoracic segments 1–3, Abd. 1–10 – abdominal segments 1–10, setae: *as* – alar, *ps* – pleural, *eps* – epipleural, *ds* – dorsal, *lst* – laterosternal, *eus* – eusternal, *pda* – pedal, *pds* – postdorsal, *prms* – pronotal, *prs* – prodorsal, *sp* – spiracular, *sts* – sternal.

minally, covered by lobes of abdominal segment 9. Apical parts of lateral lobes of the segments 6–8 and all lobes of segment 9 darkly sclerotized (Figs 46–48). Spiracles (of thoracic and abdominal segments 1–8) annular. Chaetotaxy well developed, setae capilliform, variable in length, yellowish to brown. Each side of prothorax (Fig. 44) with 9 *prms* of unequal length, placed on the weakly sclerotized pronotal sclerite; 2 *ps* and 1 *eus* very short. Meso- and metathorax (Fig. 44) on each side with 1 moderately long

prs and 4 *pds*, variable in length (first, second and fourth short, third moderately long), 2 short *as*, 3 minute (various in length) *ss*, 1 moderately long *eps*, 1 short *ps* and 1 *eus*. Each pedal area of thoracic segments with 9 *pda*, variable in length. Abd. segment 1–8 (Figs 45–48) on each side with 1 short *prs* and 5 *pds*, almost equal in length, arranged along the posterior margin of each segment, 1 minute and 1 long *ss* (segment 8 with 1 minute *ss* only), 4 *eps* (segment 6 with 3 *eps*, segments 7 and 8 with 2 *eps*) and 2 *ps*, equal in length, 1 *lts* and 2 short *eus*. Abdominal setae increase slightly and gradually from segment 1 to 8. Abd. segment 9 (Figs 46–48) on each side with 2 moderately long *ds*, located near the posterior margin of the segment, 1 moderately long *ps* and 2 short *sts*. Anal lobes without setae.

Head (Fig. 49). Greyish to light yellow, suboval, slightly oblate bilaterally; frontal suture almost invisible, endocarina absent. Setae on head capilliform; *des*_{1, 2, 3, 5} long, equal in length, *des*₄ very short; *des*₁ and *des*₂ located in the central part of epicranium, *des*₃ and *des*₄ placed on epicranium close to *des*₃, *des*₅ located anterolaterally; *fs*_{4, 5} equal in length, *fs*₃ very short; *fs*₃ placed medially, *fs*₄ located anteromedially, *fs*₅ anterolaterally, close to epistome; *les*₁ and *les*₂ equal in length, slightly shorter than *des*₁. Single *ves* moderately long. Postepicranial area with 4 very short *pes* (Fig. 49). Antenna (Figs 50, 51) located at the end of frontal suture; antennal segment membranous, bearing sensorium usually cushion-like, truncate at apex (Se) (Fig. 50) or occasionally conical-like (Fig. 51), and 5 sensilla: 2 ampullacea (*sa*) and 3 basiconica (*sb*). Labrum (regular) (Figs 52, 53) (deformed) (Fig. 54) almost semicircular, anterior margin doubly sinuate; 3 pairs of *lrs* almost equal in length; *lrs*₁ placed medially, *lrs*₂ anteromedially, *lrs*₃ anterolaterally. Clypeus (Figs 52, 53) trapezoid, anterior margin of clypeus straight; 2 pairs of *cls* almost as long as *lrs-cls*₁ located posterolaterally, *cls*₂ posteromedially; *clss* clearly visible placed posteriorly, between *cls*. Epipharynx (Fig. 53) with 3 pairs of finger-shaped *als* of various lengths; 3 pairs of rod-shaped *ams*: *ams*₁ and *ams*₃ short, *ams*₂ moderately long; 2 pairs of rod-shaped *mes*, equal in length, both placed anteromedially very close to *ams*. Surface of epipharynx (especially close to margin) covered with asperities. Labral rods short, rounded, slightly converging posteriorly. Mandibles (Fig. 55) curved, narrow, with divided apex (teeth different in length). There is an elongated protuberance on the cutting edge between the apex and the middle of the mandible; both *mds* capilliform, almost equal in length. Maxilla (Figs 56–58) with 1 *stps* and 2 *pfs* of equal length; mala with 8 finger- or rod-like *dms* (Fig. 57) and 4 *vms*, both varied in length (Fig. 58); *mbs* very short. Maxillary palpi with 2 palpomeres, basal with short *mpps*; distal palpomere apically with a group of sensilla, each palpomere with a pore. Basal palpomere wider than distal, both of almost equal length. Prelabium (Fig. 56) cup-like with 1 long *prms*, located medially. Ligula with 2 pairs of *ligs*, various in length. Premental sclerite clearly visible, trident-shaped (median branch weakly sclerotized), posterior extension with elongated apex. Labial palpi 2-segmented; apex of distal palpomere with some sensilla; each palpomere with a pore. Basal palpomere slightly wider and shorter than distal. Postlabium (Fig. 56) with 3 capilliform *pms*, the first pair located anteromedially, the remaining 2 pairs posterolaterally; *pms*₁ minute, *pms*₂ very long, *pms*₃ moderately long. Surface of postlabium and stipes partially covered with asperities.

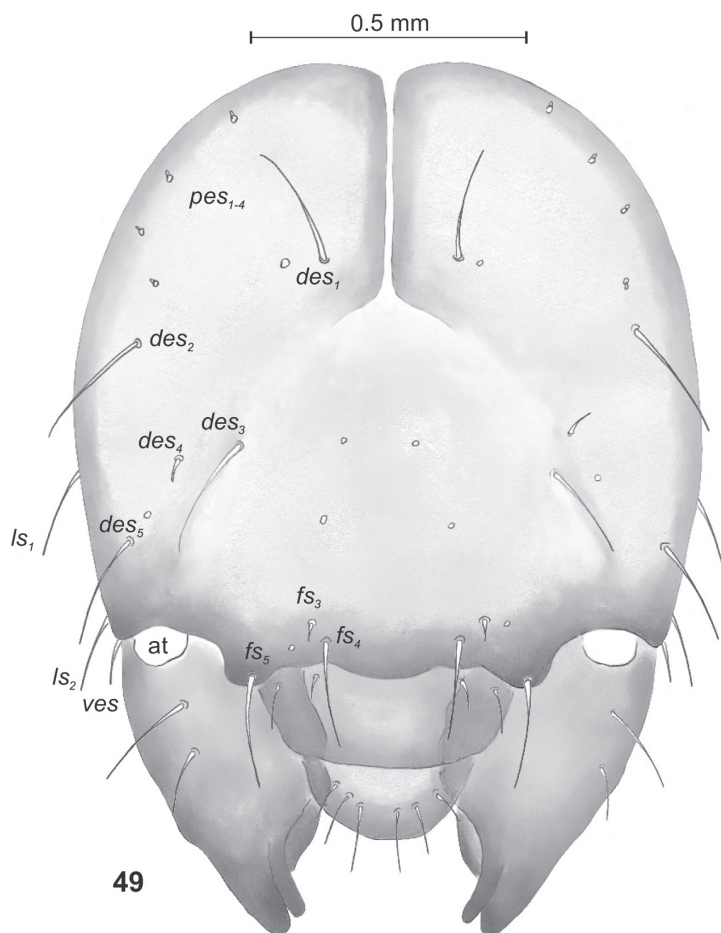
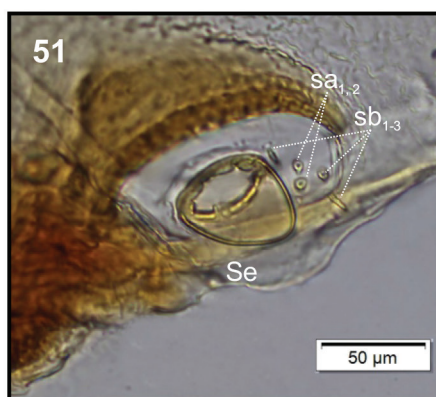
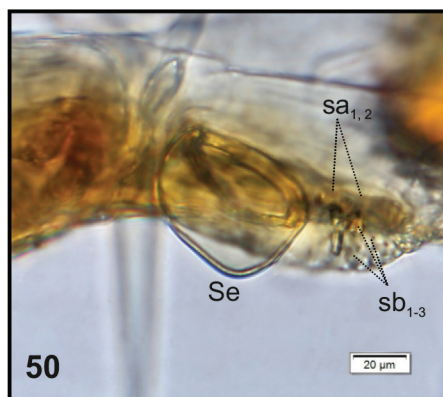
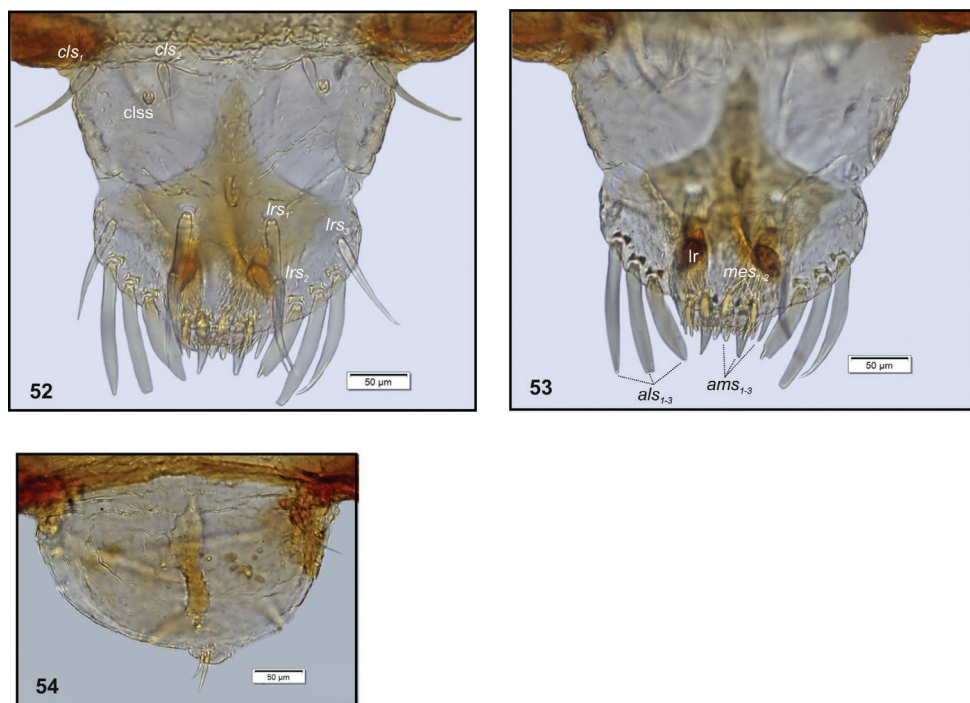


Figure 49. *Philopedon plagiatus* mature larva, head, frontal view. Abbreviations: at – antenna, setae: des – dorsal epicranial, fs – frontal, les – lateral epicranial, pes – postepicranial, ves – ventral.



Figures 50–51. *Philopedon plagiatus* mature larva, right antenna. **50** Cushion-like **51** Conical-shaped. Abbreviations: Se – sensorium, sa – sensillum ampullaceum, sb – sensillum basiconicum.



Figures 52–54. *Philopodon plagiatum* mature larva, body parts. **52** Clypeus and labrum **53** Epipharynx **54** Clypeus and labrum, deformed. Abbreviations: *clss* – clypeal sensorium, *lr* – labral rods, setae: *als* – anterolateral, *ams* – anteromedial, *cls* – clypeal, *lrs* – labral, *mes* – median.

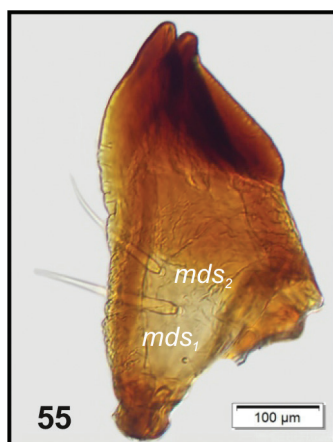
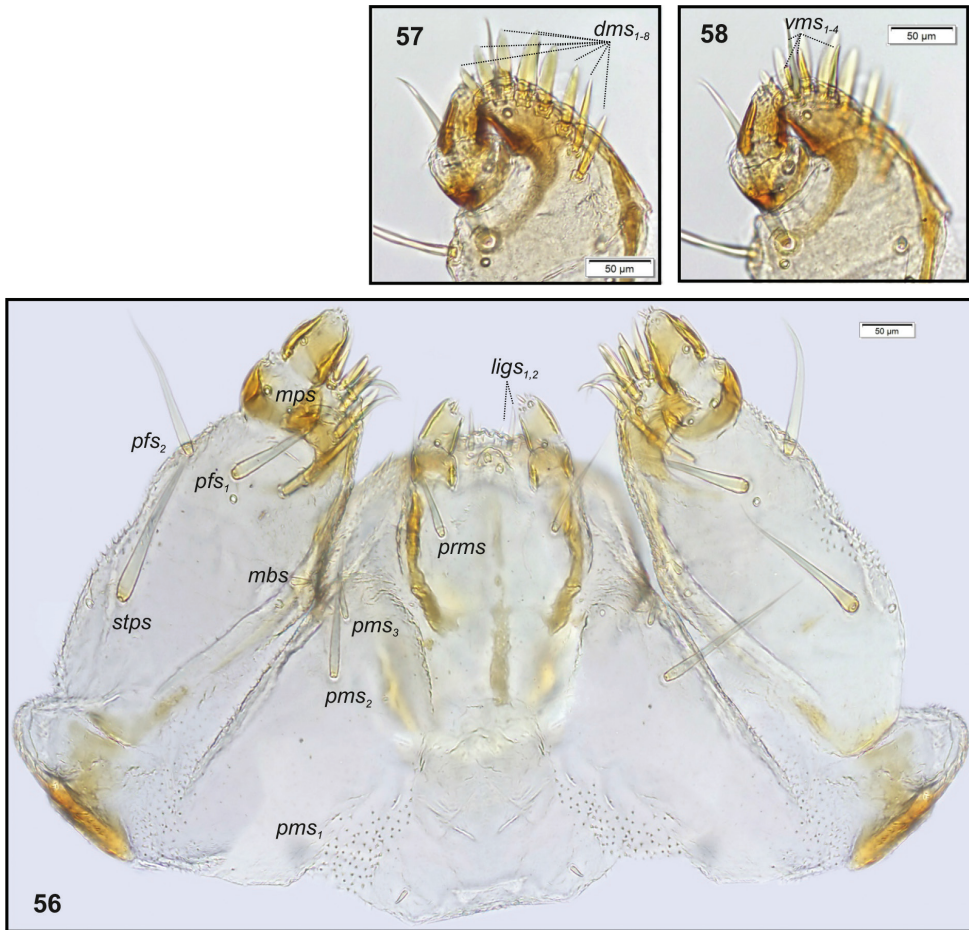


Figure 55. *Philopodon plagiatum* mature larva, left mandible. Abbreviations: *mds* – mandibular seta.



Figures 56–58. *Philopodon plagiatus* mature larva, body parts. **56** Maxillolabial complex ventral aspect **57** Right maxilla apical part dorsal aspect **58** Right maxilla apical part ventral aspect. Abbreviations: setae: *dms* – dorsal malar, *lig*s – ligular, *mbs* – malar basiventral, *mps* – maxillary palp, *pfs* – palpiferal, *prms* – prelabial, *pms* – postlabial, *stps* – stipal, *vms* – ventral malar.

Tanymecus palliatus

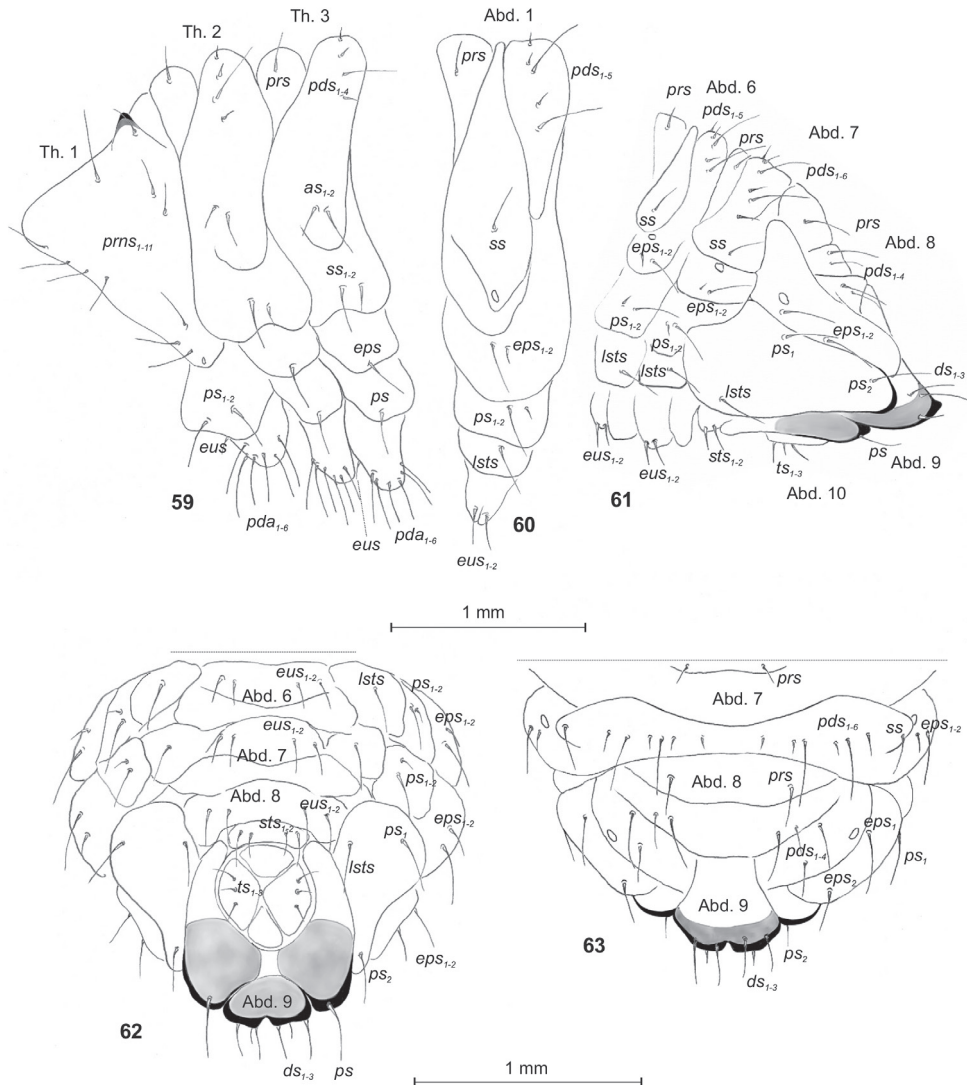
Specimens examined. 3 mature larvae: Germany, Brandenburg, Cottbus: Kittlitz, collected on 09.08.2011 in a permanent field of *Medicago sativa* L., together with a *Tanymecus* pupa and many larvae of *Otiorhynchus ligustici* (Linnaeus, 1758).

12 first instar larvae: A female collected in the field (Kittlitz) laid eggs on 17.05.2012 in the laboratory in Hannover. Larvae, hatched from eggs on 31.05.2012, were used for this study.

Description of the mature larva. Body length: 8.3–10.0 mm, body width at the widest part (level of first abdominal segment): 2.5–3.2 mm, head width: 1.6–1.8 mm, head height: 1.4–1.6 mm.

Body (Figs 10–12). Moderately stout, slightly curved, rounded in cross section. Prothorax slightly smaller than mesothorax; metathorax as wide as mesothorax. Prothorax with a pair of dark sclerotized, conical protuberances, placed dorsally, near margin with mesothorax (Fig. 59). Spiracles (of thoracic and abdominal segments 1–8) annular. Abdominal segments 1–7 of almost equal length, segment 8 wide, flattened posteriorly, with conical lateral lobes. Abdominal segment 9 strongly reduced, consisting of 4 well-isolated lobes, of which the lateral lobes are the biggest; segment 10 consists of 4 anal lobes of various size. Anus located ventrally (Figs 11, 12, 62). Chaetotaxy well developed, setae capilliform, variable in length, greyish or yellowish. Each side of prothorax (Fig. 59) with 11 *prns* of almost equal size (8 of them placed on the weakly visible premental sclerite, next 3 close to the spiracle; 2 *ps* moderately long and 1 short *eus*. Meso- and metathorax on each side with 1 moderately long *prs* and 4 *pds* of various lengths (first, second and fourth very short, third long), 1 moderately long and 1 short *as*, 1 moderately long and 1 short *ss*, 1 *eps* and 1 *ps*, both moderately long, 1 short *eus*. Each pedal area of thoracic segments with 6 *pda*, almost equal in length. Abd. 1–7 (Figs 60–63) on each side with 1 moderately long *prs* and 5 *pds*, of various lengths (first, second and fourth very short, third and fifth very long; Abd. 7 with 6 *pds*), arranged along the posterior margin of each segment, 1 long *ss*, 2 *eps* and 2 *ps*, both of different lengths, 1 *lts* and 2 moderately long *eus*. Abd. 8 (Figs 61–63) on each side with 1 moderately long *prs*, 4 relatively elongate *pds*, 2 *eps* and 2 *ps*, both of different lengths, 1 *lts* and 2 *eus*. Abd. 9 (Figs 61–63) on each side with 3 *ds*, first and third moderately long, second short, all located close to the posterior margin of the segment, 1 medium *ps* and 2 short *sts*. Each lateral anal lobe (Abd. 10) with 3 short setae (*ts*_{1–3}).

Head (Fig. 64). Greyish or light yellowish, suboval, frontal suture distinct, Y-shaped, endocarina absent. Setae on head capilliform; *des*_{1, 2, 3, 5} equal in length; *des*₁ and *des*₂ located in the central part of epicranium, *des*₃ placed on frontal suture, *des*₅ located anterolaterally; *fs*_{4, 5} both as long as *des*₁, *fs*₄ located anteromedially, *fs*₅ anterolaterally, near epistome; *les*₁ and *les*₂ equal in length, only slightly shorter than *des*₅; *ves*₁ and *ves* almost as long as *les*. Postepicranial area with 5 very short *pes* (Fig. 64). Antenna (Fig. 65) located at the end of frontal suture; antennal segment membranous, bearing cushion-like sensorium (Se), located medially, and 6 sensilla of various types: 5 ampullacea (*sa*) and 1 basiconicum (*sb*). Labrum (Fig. 66) narrow, anterior margin slightly sinuate; 3 pairs of *lrs*, *lrs*₁ and *lrs*₃ moderately long, *lrs*₂ long; *lrs*₁ anteromedially, *lrs*₂ medially, *lrs*₃ laterally. Clypeus (Fig. 66) twice as long as than labrum, anterior margin of clypeus straight; 2 pairs of *cls*: *cls*₁ as long as *lrs*₂, *cls*₂ less than half the length of *cls*₁, both located posteromedially; *cls* clearly visible, placed medially between *cls*. Epipharynx (Fig. 66) with 4 pairs of finger-shaped *als* of equal length; 3 pairs of *ams*: *ams*₁ rod-shaped, very short, *ams*₂ and *ams*₃ moderately long, finger-like; 2 pairs of short, rod-shaped *mes*, both placed medially, between labral rods. Surface of epipharynx smooth. Labral rods short, converging posteriorly. Mandibles (Fig. 67) curved, narrow, with slightly divided apex (teeth of various length). There is an additional tooth on the cutting edge in the middle of the mandible; both *mds* capilliform, different in length. Maxilla (Figs 68–70) with



Figures 59–63. *Tanymecus palliatus* mature larva, habitus and chaetotaxy. **59** Thoracic segments lateral view **60** First abdominal segment lateral view **61** Abdominal segments 7–10 lateral view **62** Abdominal segments 7–10 ventral view **63** Abdominal segments 7–10 dorsal view. Abbreviations: Th. 1–3 – thoracic segments 1–3, Abd. 1–10 – abdominal segments 1–10, setae: *as* – alar, *ps* – pleural, *eps* – epipleural, *ds* – dorsal, *lsts* – laterosternal, *eus* – eusternal, *pda* – pedal, *pds* – postdorsal, *prms* – pronotal, *pr*_s – prodorsal, *sp*_s – spiracular, *sts* – sternal, *ts* – terminal.

1 *stps* and 2 *pfs* of equal length; mala with 7 rod-like *dms* of various size (Fig. 69) and 4 capilliform *vms* variable in length (Fig. 70); *mb*s short. Maxillary palpi with 2 palpomeres, basal with short *mps*; distal palpomere apically with a group of sensilla, each palpomere with a pore. Basal palpomere distinctly wider and slightly longer than distal. Prelabium (Fig. 68) almost rounded with 1 long *prms*, located medially. Ligula with 2 pairs of *lgs* of various length. Premental sclerite clearly visible tri-

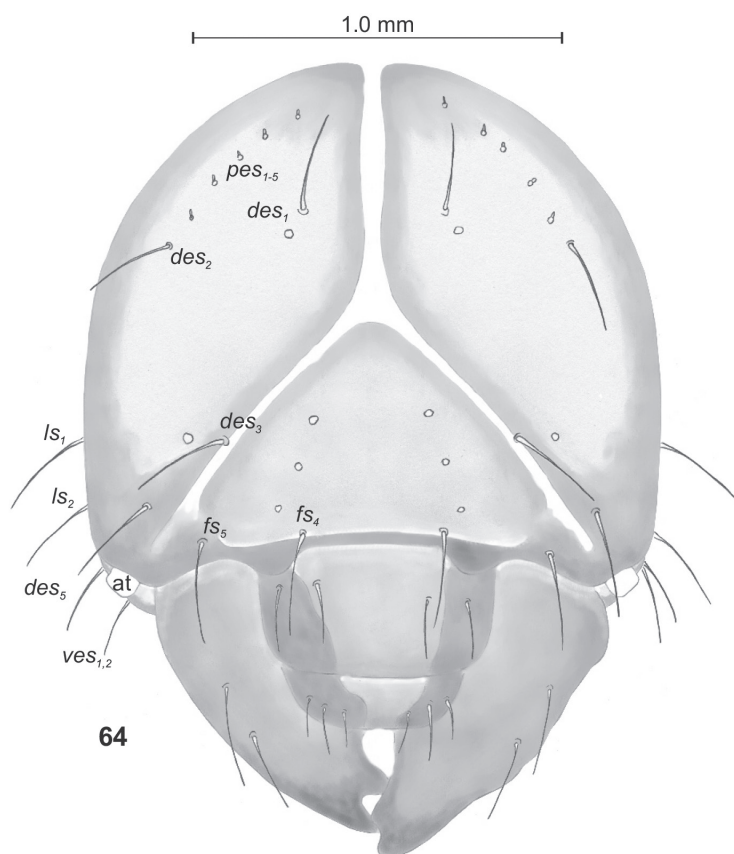


Figure 64. *Tanymecus palliatus* mature larva, head, frontal view. Abbreviations: at – antenna, setae: *des* – dorsal epicranial, *fs* – frontal, *les* – lateral epicranial, *pes* – postepicranial, *ves* – ventral.

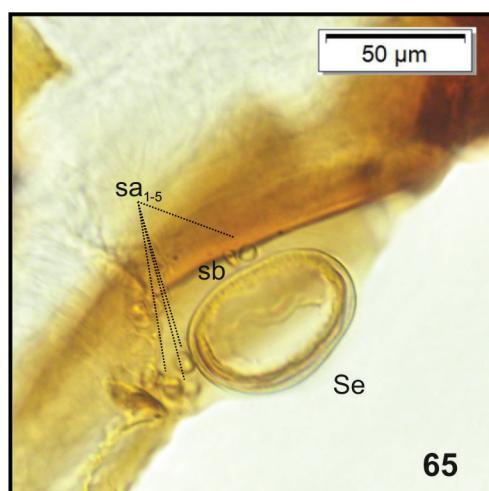


Figure 65. *Tanymecus palliatus* mature larva, body parts, right antenna. Abbreviations: Se – sensorium, sa – sensillum ampullaceum, sb – sensillum basiconicum.

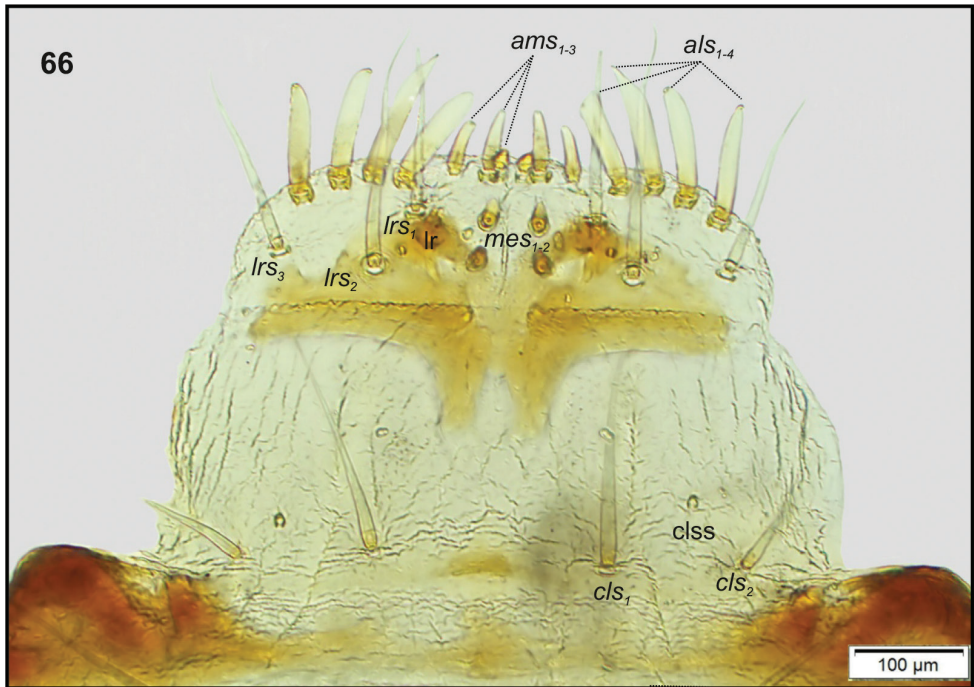
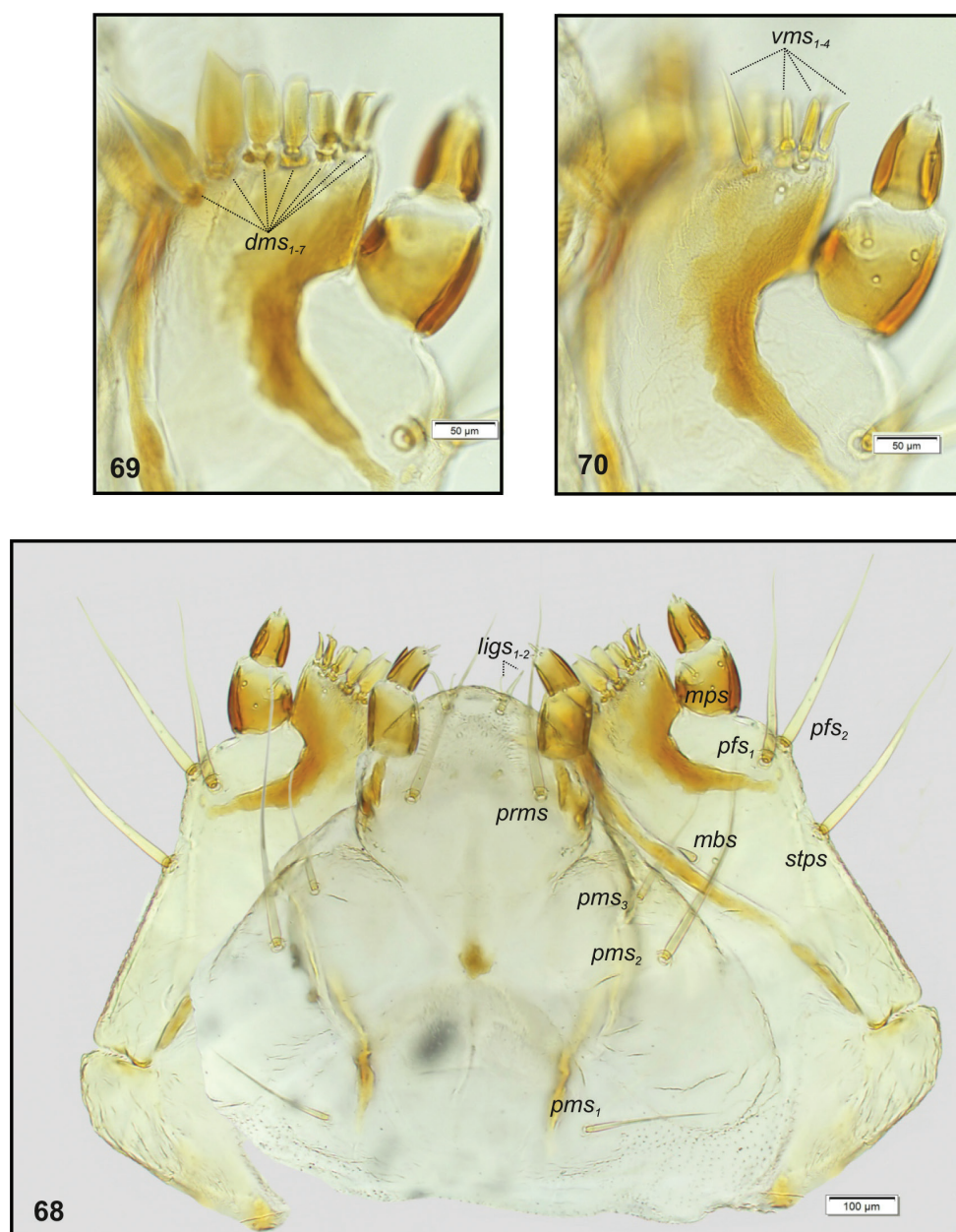


Figure 66. *Tanymecus palliatus* mature larva, body parts, clypeus, labrum and epipharynx. Abbreviations: clss – clypeal sensorium, lr – labral rods, setae: als – anterolateral, als – anteromedial, cls – clypeal, lrs – labral, mes – median.



Figure 67. *Tanymecus palliatus* mature larva, left mandible. Abbreviations: mds – mandibular seta.

den-shaped, median branch and posterior extension weakly sclerotized. Labial palpi 2-segmented; apex of distal palpomere with some sensilla; each palpomere with a pore. Basal palpomere wider and longer than distal. Postlabium (Fig. 68) with 3



Figures 68–70. *Tanymecus palliatus* mature larva, body parts. **68** Maxillolabial complex ventral aspect **69** Right maxilla apical part dorsal aspect **70** Right maxilla apical part ventral aspect. Abbreviations: setae: *dms* – dorsal malar, *ligs* – ligular, *mbs* – malar basiventral, *mps* – maxillary palp, *pfs* – palpiferal, *prms* – prelabial, *pms* – postlabial, *stps* – stipal, *vms* – ventral malar.

capilliform *pms*, the first pair located anteromedially, the remaining 2 pairs posterolaterally; *pms*₁ and *pms*₃ long, *pms*₂ very long. Only posterior margin of postlabium covered with fine asperities.

Differentiation of described species

- | | | |
|---|---|---|
| 1 | Abdominal segment 9 about regular structure (type “A” Van Emden 1950) | 2 |
| – | Abdominal segment 9 strongly reduced, consisting of four well-isolated lobes (type “B” Van Emden 1950)..... | 3 |
| 2 | Abdominal segment 9 with 3 <i>ds</i> , each pedal area with 6 <i>pda</i> , meso- and metathorax with 3 <i>ss</i> each, Se conical-like | <i>Graptus triguttatus triguttatus</i> |
| – | Abdominal segment 9 with 4 <i>ds</i> , each pedal area with 4 <i>pda</i> , meso- and metathorax with 2 <i>ss</i> each, Se cushion-like | <i>Peritelus sphaeroides</i> |
| 3 | Abdominal segment 9 with 2 <i>ds</i> , each pedal area with 9 <i>pda</i> , meso- and metathorax with 3 <i>ss</i> each, abdominal segments 1–6 with 3–4 <i>eps</i> , clypeus almost as wide as labrum..... | <i>Philopodon plagiatum</i> |
| – | Abdominal segment 9 with 3 <i>ds</i> , each pedal area with 6 <i>pda</i> , meso- and metathorax with 2 <i>ss</i> each, abdominal segments 1–6 with 2 <i>eps</i> , clypeus twice as long as labrum | <i>Tanymecus palliatus</i> |

The number of larval instars in *Tanymecus*

There are some strange statements about the number of larval instars in the larval stage of species of genus *Tanymecus*. Hoffmann (1963), who relied on authors from the former Soviet Union, reported about 10 larval instars in *T. palliatus*, which was already commented by Dieckmann (1983) as a ‘for weevils surprising fact’.

In *T. dilaticollis* Gyllenhal, 1834, Catrinici (1944) determined six larval instars. She reported that larval head width increased up to the fourth larval instar, decreased in the fifth and increased again in the sixth instar to nearly the same value as in the fourth. This sounds really strange and has to be taken with caution and tested with new observations. This was also the reason for Van Emden (1952) to propose four larval instars for *T. dilaticollis*.

For the exact determination of the number of larval instars we summarized and assessed our own measuring data and added data from literature, if necessary (Tables 1, 2).

Due to the dubiousness of the number of larval instars in *T. dilaticollis* given by Catrinici (1944) and Van Emden (1952) we used measuring data for the head width (HW) of adults of both species and of mature larvae of *T. palliatus* to assess the HW of the mature larva of *T. dilaticollis*. This ratio should be rather similar in two species of the same genus. Hence, the value calculated in this way for the HW of the mature larva of *T. dilaticollis* is 1.51 mm.

We also needed to determine the number of larval instars for both species: there are data for L₁ and for mature larvae, and in *T. dilaticollis* there are also measurements for several instars, even if (especially in the higher instars) the data are doubtful.

The determination of larval instars is mainly based on the method of Dyar (1890) and has been used by several authors, even if apparently not known to all scientists who have dealt with larvae. There are several publications about weevil larvae where this

Table 1. Head width measuring data of the species studied. Results in mm; ⁿ – number of specimens measured, in adults behind eyes; L₁ – first instar larva; ML – mature larva; *: an assignment to this instar is doubtful. A transfer to ‘mature larvae’ would change the average value only slightly; **: data from Gosik and Sprick (2013). Data from literature in italics.

Species	Larval instars			Pupa	Adult
	L ₁ larvae	Premature larvae	Mature larvae		
<i>Graptus triguttatus</i> (L ₁ data from Van Emden 1952)	0.33 ¹ ; 0.34 ² ; 0.35 ¹	0.57 ² ; 0.60 ³ ; 1.00 ¹ ; 1.07 ² ; 1.17 ¹	1.37 ³ ; 1.40 ² ; 1.43 ¹ ; 1.47 ² ; 1.50 ⁶ ; 1.53 ⁴ ; 1.55 ¹	1.13 ¹ ; 1.17 ¹ ; 1.20 ¹	1.05 ¹ ; 1.15 ¹ ; 1.2 ¹ ; 1.25 ³ ; 1.3 ³ ; 1.4 ¹ ; 1.45 ¹ ; 1.5 ¹
Mean value ($\bar{x}$)	0.34	0.806	1.471	1.167	1.283
<i>Peritelus sphaeroides</i>	–	–	0.87 ¹ ; 0.90 ² ; 1.10 ³ ; 1.13 ³ ; 1.17 ⁴	1.00 ¹ ; 1.05 ¹	0.75 ¹ ; 0.85 ¹ ; 0.95 ² ; 1.0 ² ; 1.025 ¹ ; 1.2 ¹
Mean value ($\bar{x}$)	–	–	1.083	1.025	1.025
<i>Philopodon plagiatum</i>	–	0.60 ¹ ; 0.67 ⁴ ; 0.73 ¹	0.97 ¹ ; 1.00 ¹ ; 1.03 ¹	–	1.05 ¹ ; 1.1 ² ; 1.225 ¹ ; 1.25 ¹ ; 1.375 ¹ ; 1.4 ²
Mean value ($\bar{x}$)	–	0.668	1.00	–	0.966
<i>Philopodon plagiatum</i> (from Van Emden 1952)	–	0.57 ² ; 0.61 ¹ ; 0.63 ¹ ; 0.64 ¹ ; 0.68 ¹ ; 0.72 ¹ ; 0.75 ¹ *; 0.79 ¹ *	0.86 ¹ ; 0.89 ² ; 0.96 ² ; 1.00 ¹ ; 1.07 ² ; 1.08 ¹ ; 1.1 ² ; 1.14 ¹ ; 1.17 ¹ ; 1.18 ¹ ; 1.21 ²	–	–
Mean value ($\bar{x}$)	–	0.662	1.056	–	–
<i>Tanymecus dilaticollis</i>	–	–	–	–	1.30 ²
Mean value ($\bar{x}$)	–	–	–	–	1.30
<i>Tanymecus palliatus</i>	0.34 ¹ ; 0.37 ² ; 0.38 ⁵ ; 0.39 ² ; 0.40 ²	–	1.62 ¹ ; 1.70 ¹ ; 1.80 ¹	–	1.275 ¹ ; 1.425 ¹ ; 1.5 ¹ ; 1.55 ¹ ; 1.6 ¹
Mean value ($\bar{x}$)	0.380	–	1.717	–	1.470

Table 2. Head width measuring data of *Tanymecus* larvae and adults for larval instar determination. *: collected together with *T. palliatus* pupae; **: calculated from the ratio of adult's HW to HW of the mature larva.

Instar	Mean value (mm)	Specimens measured	Source
<i>Tanymecus dilaticollis</i>			
L ₁	0.35	12 larvae	Catrinici (1944)
L ₂	0.56	7 larvae	Catrinici (1944)
L ₃	0.77	5 larvae	Catrinici (1944)
L ₄	1.27	4 larvae	Catrinici (1944)
Mature larva	1.51**	–	calculated value
Adult weevil	1.30	2 adults	own data
<i>Tanymecus palliatus</i>			
L ₁	0.38	12 larvae	own data (see Tab. 1)
Mature larva*	1.71	3 larvae	own data (see Tab. 1)
Mature larva	1.71	5 larvae	Van Emden (1952)
Adult weevil	1.47	5 adults	own data

ratio was applied. We preferred to use Dyar's ratio⁻¹ and called it Growth Factor (GF) as it corresponds more to the natural development.

In *Mitoplinthus caliginosus* (Fabricius, 1775) (subfamily Molytinae), after comparison of the growth factors 1.35, 1.4 and 1.5, the best approximation was found

with a value of 1.4 for head capsule width (Sprick and Gosik 2014). This value agrees with Dyar's ratio of 0.714. Rowe and Kok (1985) gave a Dyar's ratio value for *Rhinocyllus conicus* (Frölich, 1792) (subfamily Lixinae) larvae of 0.65 (this agrees with a GF of 1.538). In agreement Leibe et al. (1980) determined the ratios of each instar of two populations of *Sitona hispidulus* (Fabricius, 1777) (subfamily Entiminae, tribe Sitonini) and reported Dyar's values between 0.642 and 0.739. The median value of these data is by our calculation 0.6995 (GF = 1.43). These data show that there are rather different values for larval growth and that there are also differences between the growth of different larval instars.

For larval instar determination in *Tanymecus* we tested four values between 1.4 and 1.5 to achieve the best approximation of larval growth. We started with the L₁ larva that we received from egg-laying of adult weevils (head width 0.38 mm) and calculated the subsequent instars with the selected GF values until 1.71 mm, the head width of the mature larvae, was achieved. For this procedure, five steps were needed. Higher GF values, as for example 1.538 in *Rhinocyllus conicus*, were excluded because of the reduced number of larval instars in this rather distantly related subfamily (Table 3).

From Table 2 it can easily be seen that both species have 5 larval instars. The best approximation is achieved with a GF of 1.44 in *Tanymecus dilaticollis* and 1.46 in *T. palliatus* (i.e. Dyar's ratio of 0.694 and 0.685, respectively). The small difference may be due to the absent HW variation of the two available adult *T. dilaticollis* specimens that showed both the same value and hence do not represent the HW variation of the population. Furthermore it can be stated that the values of Catrinici (1944) are beginning to seem doubtful from the fourth larval instar onward.

For this approximation it is only necessary to know the head width of the L₁ larva and that of the last instar. And the HW of the last instar can be assessed from the HW of the adult weevil, as it is shown in Table 2. In adults HW was always measured directly behind the eyes to avoid an excessive importance of prominent eyes, which could be a problem in genera such as *Strophosoma* Billberg, 1817 (see Gosik et al. 2017) or in species such as *Tanymecus dilaticollis*. Larval growth, number of larval instars and size of the adults' head width (and therefore size of adults, too) are in a very close relationship to each other. The same may be true for the HW of the pupa.

An instar determination is also possible for *Graptus triguttatus*. According to Van Emden (1952), the head capsule width of the L₁ larva is 0.34 mm (average of three larvae; Table 1). The application of a GF of 1.45 shows a good approximation with the measuring values given in Table 1: 0.34 mm × 1.45 (repeatedly) = 0.493 mm (L₂), 0.715 mm (L₃), 1.037 mm (L₄) and finally 1.503 mm (L₅). Thus, *Graptus triguttatus* has also 5 larval instars, and the premature larvae from Table 1 may represent L₂ and L₄ larvae. There is a great variation in adults' head width in this species ranging from 1.05 mm to 1.5 mm (Table 1). This agrees with the span between the last larval instars and nearly achieves the supposed growth factor of 1.45, so that the assignment of a certain larva to the right instar is doubtful in extremely sized specimens, the more as head width variation becomes larger with instar and size. A similar size variation was observed in *Peritelus sphaeroides* adults and *Philopodon plagiatum* larvae (Table 1).

Table 3. Larval instar determination for *Tanymecus dilaticollis* and *T. palliatus*. All measuring data in mm; initial data bold, calculated data in italics; target data of the approximation bold and in italics.

<i>Tanymecus palliatus</i>			
Growth factor (to be tested)	1.40	1.45/1.46	1.50
L ₁ (measured)	0.38	0.38	0.38
L ₂ (calculated)	<i>0.532</i>	<i>0.551/0.555</i>	<i>0.57</i>
L ₃ (calculated)	<i>0.745</i>	<i>0.799/0.810</i>	<i>0.855</i>
L ₄ (calculated)	<i>1.042</i>	<i>1.158/1.183</i>	<i>1.283</i>
L ₅ (calculated)	<i>1.460</i>	<i>1.680/1.727</i>	<i>1.924</i>
Mature larva (measured)	1.71	1.71	1.71
<i>Tanymecus dilaticollis</i> *			
Growth factor (to be tested)	1.40	1.44/1.45/1.46	1.50
L ₁ (measured)	0.35	0.35	0.35
L ₂ (measured)	0.56	0.56	0.56
L ₂ (calculated)	<i>0.49</i>	<i>0.504/0.508/0.511</i>	<i>0.525</i>
L ₃ (measured)	0.77	0.77	0.77
L ₃ (calculated)	<i>0.686</i>	<i>0.726/0.736/0.746</i>	<i>0.7875</i>
L ₄ (measured)	1.27	1.27	1.27
L ₄ (calculated)	<i>0.960</i>	<i>1.045/1.067/1.089</i>	<i>1.181</i>
L ₅ (calculated)	<i>1.345</i>	<i>1.505/1.55/1.590</i>	<i>1.772</i>
Mature larva (calculated; see Tab. 1)	1.51	1.51	1.51

*: measuring data from Catrinici (1944).

In *Peritelus sphaeroides* and *Philopedon plagiatum* an instar determination is impossible due to the absence of L₁ head width data. It can only be concluded from the data for premature larvae in *Philopedon plagiatum* (Table 1) that these data represent the penultimate instar. Opposite to *Graptus* and *Tanymecus*, the HW of *Philopedon* adults is greater than in mature larvae. In *Peritelus sphaeroides* the HW of adults is slightly smaller than in mature larvae, but not significantly.

Discussion

I. Significance of morphological features of larvae for the relationship between genera and higher taxa

Philopedon belongs to genera with abdominal type 'B' larvae together with *Strophosoma* and *Tanymecus* (Van Emden 1950, 1952). The main feature is the presence of a flattened dorso-ventral abdominal segment 9. Furthermore this feature (unique among weevils) may suggest an unknown kind of relationship between these genera (Gosik et al. 2017). Nevertheless, there are some morphological differences between larvae of type B, for example, the abdominal segment 10 is almost covered by segment 9 in *Philopedon*, whereas segment 10 is integrated with segment 9 and forms a sclerotized

Table 4. Number of setae in mature larvae of *Graptus triguttatus triguttatus* (a), *Peritelus sphaeroides* (b), *Philopodon plagiatum* (c), *Tanymecus palliatus* (d).

Part of body	Setae	a	b	c	d
Prothorax	<i>prns</i>	8	9	9	11
	<i>ps</i>	2	2	2	2
	<i>eus</i>	1	1	1	1
	<i>pda</i>	6	4	9	6
Meso-, metathorax	<i>prns</i>	1	1	1	1
	<i>pds</i>	4	4	4	4
	<i>as</i>	1	1	2	2
	<i>ss</i>	3	2	3	2
	<i>eps</i>	1	1	1	1
	<i>ps</i>	1	1	1	1
	<i>eus</i>	1	1	1	1
	<i>pda</i>	6	4	9	6
	<i>prns</i> 1-7	1	1	1	1
	<i>prns</i> 8	1	1	1	1
Abdominal segments 1–8	<i>pds</i> 1-7	5	5	5	5*
	<i>pds</i> 8	4	4	5	4
	<i>ss</i> 1-7	2	2	2	1
	<i>ss</i> 8	1	1	1	0
	<i>eps</i> 1-7	2	2	3-4	2
	<i>eps</i> 8	2	2	2	2
	<i>ps</i> 1-7	2	2	2	2
	<i>ps</i> 8	2	2	2	2
	<i>lsts</i> 1-7	1	1	1	1
	<i>lsts</i> 8	1	1	1	1
	<i>eus</i> 1-7	2	2	2	2
	<i>eus</i> 8	2	2	2	2
Abdominal segment 9	<i>ds</i>	3	4	2	3
	<i>ps</i>	2	1	1	1
	<i>sts</i>	2	2	2	2
Abdominal segment 10	<i>ts</i>	2	2	0	3
Head	<i>des</i>	4	4	5	4
	<i>fs</i>	2	2	3	2
	<i>ls</i>	2	2	2	2
	<i>pes</i>	3	7	4	5
	<i>ves</i>	1	0	1	2
Mouthpart	<i>cls</i>	2	2	2	2
	<i>lrs</i>	3	3	3	3
	<i>mes</i>	2	2	2	2
	<i>als</i>	3	3	3	4
	<i>ams</i>	3	3	3	3
	<i>mds</i>	2	1	2	2
	<i>des</i>	7	7	8	7
	<i>ves</i>	4	4	4	4
	<i>pfs</i>	2	2	2	2
	<i>stps</i>	1	1	1	1
	<i>pbs</i>	1	1	1	1
	<i>mps</i>	1	1	1	1
	<i>pms</i>	3	3	3	3
	<i>prms</i>	1	1	1	1
	<i>ligs</i>	3	2	2	2

* only abdominal segment 7 with 6 *pds*.

ventral surface in *Strophosoma* and *Tanymecus*. The dorsal lobe of segment 9 is largest in *Strophosoma*, whereas the ventral lobe is largest in *Tanymecus*.

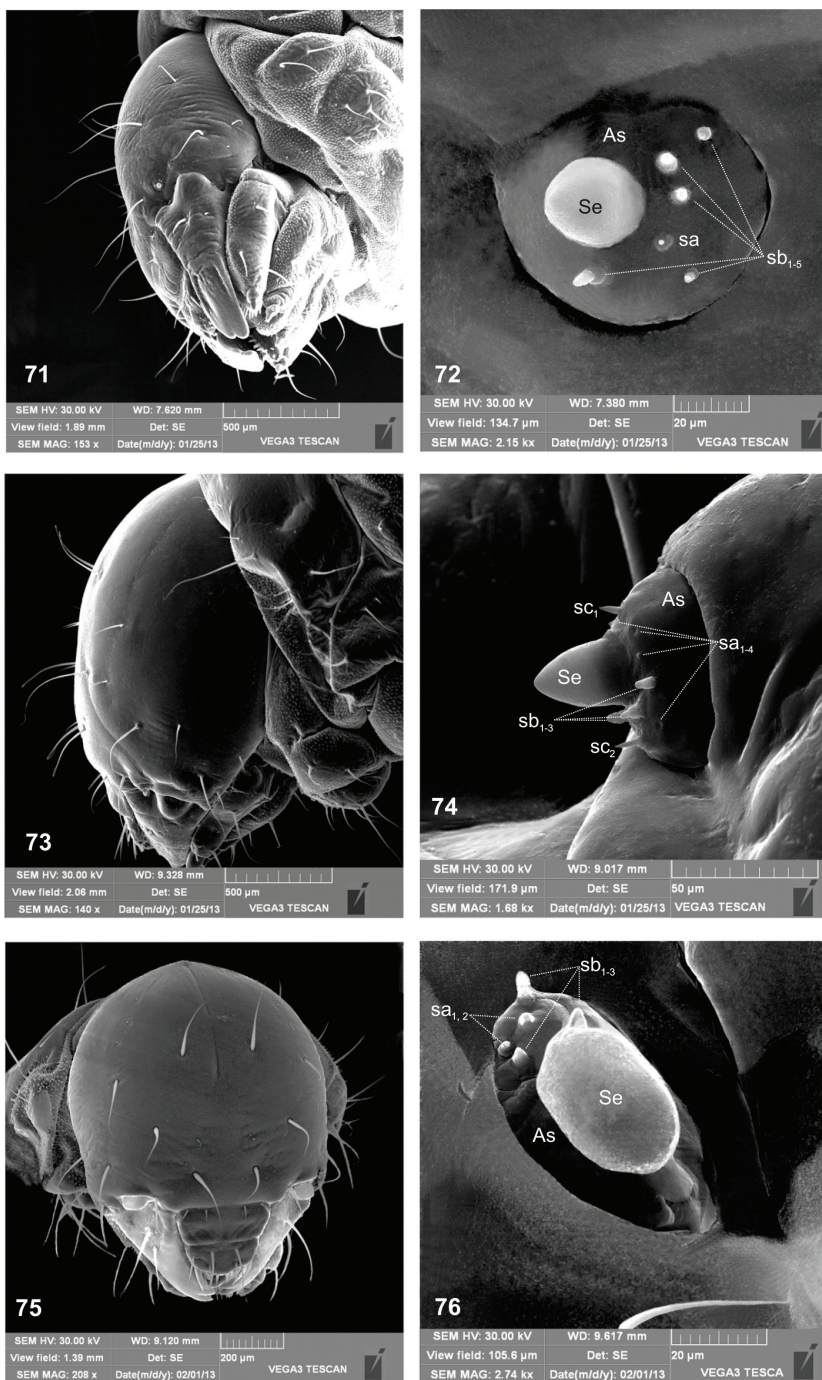
According to Van Emden (1952), the chaetotaxy of the mature larva of *Tanymecus palliatus* is only slightly different from L_1 , namely: only six setae instead seven on the pedal lobe and lack of microseta des_4 . It is worth mentioning that the proportion between (and arrangement of) setae shows the same order on L_1 as on mature larvae, a finding that is not common in Entiminae (Fidler 1936; Gosik and Sprick 2012b; Gosik et al. 2016, 2017). To the characters listed by Van Emden (1952) as typical for the genus *Tanymecus* we can add that the clypeus is twice as long as labrum (Fig. 66).

The mature larva of *Philopodon plagiatus* is described by Van Emden (1952). The only exception is cls : rather short on L_1 versus extremely long on the mature larva; cls as long as lrs_1 is not observed in other Entiminae.

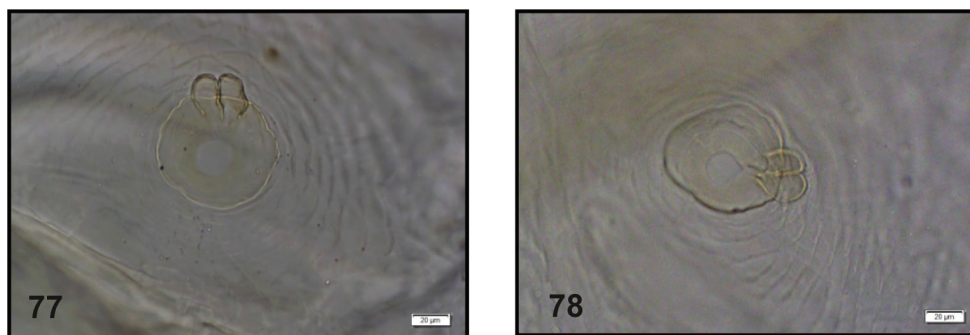
In *Graptus triguttatus* the shape of the body, number and proportion of setae on L_1 (according to Van Emden 1952) and of the mature larva (present paper) are almost identical. The tribe Byrsopagini (with the genus *Graptus* Schönherr, 1823) is currently removed from the subfamily Molytinae Schönherr, 1823 to the subfamily Entiminae Schönherr, 1823 (Thompson 1992; Marvaldi 1997, 1998a; Alonso-Zarazaga and Lyal 1999; Löbl and Smetana 2013). From two apomorphies, most typical for Entiminae (4 vms and cushion-like sensorium) in the sense of Marvaldi (1997, 1998a, 1998b, 2003), the larva of *Graptus* possesses only this first character (4 vms). But 4 vms , observed constantly on larvae of all Entiminae, were also recorded on several other weevil taxa (e.g., Tychinii Gistel, 1848; Skuhrovec et al. 2015). On the other hand, the number of setae and shape of the larval body of *G. triguttatus* seem to be more similar to the Entiminae than to the Molytinae. But the fact that different larval stages and the pupae were found between the roots of dicotyledonous plants, outside of any plant tissues, supports the placement of *Graptus* (and Byrsopagini) in the subfamily Entiminae (Thompson 1992; Marvaldi 1997, 1998a; Alonso-Zarazaga and Lyal 1999); even taking into account some small differences between the description of the mature larva of *G. triguttatus* presented by us and descriptions of L_1 presented by Van Emden (1952) and Marvaldi (1998a) (e.g., the lack of ts on L_1 versus lateral lobes of mature larva with a pair of ts). On the other hand, the rest of characters listed by Marvaldi (1998a) (e.g., 1 as ; 4–6 pds on Abd. 8; sensillum cluster between mes_1 and mes_2 ; mandibles with bidentate apex; four anal lobes) have been mentioned in both descriptions.

From morphological data, the taxonomic position of Byrsopagini is apparently not so clear, but its placement within Entiminae is more plausible than in Molytinae; this is in line with the results of the cladistic and phylogenetic analyses performed by Marvaldi (1997, 1998a) and Stüben et al. (2015).

Moreover, a detailed analysis of the structure of antennae disclosed only ostensible similarities between *Graptus* and Molytinae, e.g. *Mitoplinthus caliginosus* described by Sprick and Gosik (2014). First of all, the larva of *M. caliginosus* shows a larger number and variety of sensilla than the *Graptus* larva. The shape of the antennal sensorium is quite variable in both genera as well. Finally, the sensorium on *Mitoplinthus* is elongate and pointed, whereas it is stout and rounded in *Graptus* (Figs 71–76). The presence of



Figures 71–76. *Graptus triguttatus triguttatus*, mature larva. **71** Head **72** Antenna *Mitoplinthus caliginosus* mature larva **73** Head **74** Antenna, *Philopodon plagiatus* mature larva **75** Head **76** Antenna. Abbreviations: as – antennal segment, Se – sensorium, sa – sensillum ampullaceum, sb – sensillum basiconicum, sc – sensillum chaeticum.



Figures 77, 78. *Graptus triguttatus triguttatus*, mature larva. **77** Thoracic spiracle **78** Abdominal spiracle.

a conical instead of a cushion-like sensorium in *Graptus* larvae is difficult to explain. Van Emden (1952) described the larva of *Philopodon plagiatum* with “broader and more rounded” sensoria. The same type of structure was found in larvae obtained during this study (Fig. 51).

Van Emden (1950) did not recognize any kind of spiracles of the *Graptus triguttatus* larva, and finally marked them as “?”. At first view they could be recognized as bicameral, but under high magnification (40×) they turned to appear annularly (Figs 77, 78).

The description of the pupa of *Graptus triguttatus* did not provide any relevant features that could confirm a replacement of this genus in the Entiminae. It seems to be especially difficult to distinguish them from other subfamilies due to the large diversity of both, shape and chaetotaxy, and the absence of features strictly characteristic of Entiminae pupae (Gosik and Sprick 2012a, 2013). The absence of urogomphi in pupae of *G. triguttatus* corresponds with pupae of some species of *Otiorhynchus* Germar, 1822 with strongly reduced urogomphi (Gosik and Sprick 2012a) and with the pupa of *Liophloeus tessulatus* (Müller, 1776), which does not bear urogomphi at all. Unfortunately, pupae of Molytinae are also characterized by variable development of urogomphi: they are well developed in the genera *Pissodes* Germar, 1817 and *Trachodes* Germar, 1824 (Scherf 1964) but almost absent in *Homalinotus* Schönherr, 1823 (De Oliveira Lira et al. 2017).

II. Some aspects of biology

Graptus triguttatus. In January and April 2013 many larvae of different instars were found, and most of them were already mature (Figs 85–87), which indicates hibernation in a higher instar and no development in this early time of the year. In May and June the proportion of mature larvae continued to grow, and pupae were also recorded. We conclude that teneral adults will be present from July onward. The biology is nevertheless poorly known. Dudich (1921) stated that adult weevils are mainly present from mid-April to mid-May. These findings are difficult to explain. If the species directly overwinters in soil after emerging from the pupa, the presence of winter larvae



Figures 79–87. Habitats, host plants, digging sites and larvae of soil-dwelling weevils. **79** Habitat of *Philopodon plagiatus* in Southwestern Denmark, sandy sea shore of the North Sea **80** Habitat of *P. plagiatus* in Hannover-Vahrenheide, Kugelfangtrift, sand path with *Plantago lanceolata* **81** Digging site of *P. plagiatus*, sandy soil with *P. lanceolata* at the Kugelfangtrift **82** Larva of the penultimate instar of *P. plagiatus*, found in the field in October, just molted, with light head capsule **83, 84** Larva of *Peritelus sphaeroides*, bred in flowerpots with *Euonymus fortunei* in a climate chamber **85, 86** Host plant (*Plantago lanceolata*) and digging site of *Graptus triguttatus triguttatus* under *P. lanceolata* in an occasionally mown meadow of the JKI area in Braunschweig **87** Mature or penultimate instar larva of *Graptus triguttatus triguttatus*, found in the field in Braunschweig in April.

of mainly higher instars is unexpected, and if the species appears on the soil surface in summer after reproduction, then the maximum of adults in April and May is hard to explain. It seems probable that there is a high degree of overlap between different generations; the life-cycle has still to be clarified.

Peritelus sphaeroides. According to Hoffmann (1963) and Dieckmann (1980), eggs are deposited in April and May and the new generation of adults emerges from June to August. Our data, with mature larvae from the end of August (Figs 83, 84), November and December (most larvae) until March of the following year, and two pupae found in December (Gosik and Sprick 2013), may suggest that there is a much longer period of larval development than given by the sources cited above. Probably there is a degree of overlap between the generations, as it was suggested for *Graptus triguttatus* and as it was often found in *Otiorhynchus* species (e.g., Gosik et al. 2016). Apparently, there is a need for regular search of larvae and pupae in the field to clarify the life-cycle.

Philopodon plagiatum. A description of the pupa is not available, even though the species attracted attention occasionally in beet fields, horticultural crops and pine plantations in sandy areas (Figs 79–82) (e.g., Dieckmann 1980; Brendler et al. 2008). Although teneral adults were found mainly in April (Dieckmann 1980), the period of pupation cannot be ascertained from this fact. In several species, such as *Otiorhynchus raucus* or *O. singularis*, pupation occurs in mid-summer and adults overwinter with smooth cuticula in their pupal chambers until next spring (Gosik et al. 2017). Morris (1987) found a young adult weevil with mandibular appendages in September. The fact of a long development and overlap of generations, as also suggested for the two species treated before, *Graptus triguttatus* and *Peritelus sphaeroides*, can be directly concluded from the data on *P. plagiatum* given by Dieckmann (1980). Morris' observations support these findings; he also stated that the species does not have a simple life-cycle, and he already supposed that it develops over two years. This seems to be probable and the best explanation for the data presented previously.

Acknowledgements

We thank Dr Martin Hommes for the chance to use a climate chamber in the Julius-Kühn-Institute (JKI) in Braunschweig, and Manfred Lehmann and Dr Jörn Lehmhus (JKI) for the presentation of the sites of *Tanymecus palliatus* and *Graptus triguttatus*, where these species occurred in high numbers. Vitaliy Nazarenko provided some pages from a hard to obtain source with an old description of larva of *T. palliatus*.

References

- Alonso-Zarazaga MA, Lyal CHC (1999) A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera) (Excepting Scolytidae and Platypodidae). Entomopraxis, Barcelona, Spain, 315 pp.

- Alonso-Zarazaga MA, Barrios H, Borovec R, Bouchard P, Caldara R, Colonnelli E, Gültekin L, Hlaváč P, Korotyaev B, Lyal CHC, Machado A, Meregalli M, Pierotti H, Ren L, Sánchez-Ruiz M, Sforzi A, Silfverberg H, Skuhrovec J, Trýzna M, Velázquez de Castro AJ, Yunakov NN (2017) Cooperative Catalogue of Palaearctic Coleoptera Curculionoidea. Sociedad Entomológica Aragonesa, Monografías electrónicas SEA, 8. <http://www.sea-entomologia.org> [accessed November 10, 2017]
- Anderson WH (1947) A terminology for the anatomical characters useful in the taxonomy of weevil larvae. *Proceedings of the Entomological Society of Washington* 49(5): 123–132.
- Brendler F, Holtschulte B, Rieckmann W (2008) Grauer Kugelrüssler *Philopodon plagiatu* Schaller (Syn. *Cneorhinus*). Zuckerrübe, Krankheiten, Schädlinge, Unkräuter. Agroconcept, Bonn, Gelsenkirchen, 130.
- Catrinici C (1944) Die Bestimmung der Larvenstadien des Rüsselkafers *Tanymecus dilaticollis* Gyll. *Bulletin Section Scientia Academia Romania* 26: 626–635.
- Dieckmann L (1980) Beiträge zur Insektenfauna der DDR: Coleoptera – Curculionidae (Brachycerinae, Otiorhynchinae, Brachyderinae). *Beiträge zur Entomologie* 30(1): 145–310.
- Dieckmann L (1983) Beiträge zur Insektenfauna der DDR: Coleoptera – Curculionidae (Tanymecinae, Leptopiinae, Cleoninae, Tanyrhynchinae, Cossoninae, Raymondionyminae, Bagoinae, Tanysphyrinae). *Beiträge zur Entomologie* 33(2): 257–381.
- Dudich E (1921) Zur Biologie des *Alophus triguttatus* F. *Entomologische Blätter* 17(4–6): 62–64.
- Dyar HG (1890) The number of molts of lepidopterous larvae. *Psyche* 5: 420–422. <https://doi.org/10.1155/1890/23871>
- Emden FI Van (1950) Eggs, egg-laying habits and larvae of short-nosed weevils. Eighth International Congress of Entomology, Stockholm 1948: 365–372.
- Emden FI Van (1952) On the taxonomy of *Rhynchophora* larvae: Adelognatha and Alophinae (Insecta: Coleoptera) *Proceedings of the Zoological Society of London* 122(3): 651–795. <https://doi.org/10.1111/j.1096-3642.1952.tb00248.x>
- Fidler H (1936) On the first instar larvae of some species of *Otiorrhynchus* found on strawberries, with notes on their biology. *Bulletin of Entomological Research* 27: 369–376. <https://doi.org/10.1017/S0007485300058211>
- Gosik R, Sprick P (2012a) Morphology and identification of the pupae of seven species of the genus *Otiorrhynchus* Germar, 1822 (Coleoptera, Curculionidae, Otiorhynchini). *Zootaxa* 3483: 39–57. <https://doi.org/10.11646/zootaxa.3731.4.2>
- Gosik R, Sprick P (2012b) Larval morphology of *Otiorrhynchus ligustici*, *O. porcatus* and *O. salicicola* (Coleoptera, Curculionidae, Otiorhynchini). *Deutsche Entomologische Zeitschrift* 59: 301–316. <https://doi.org/10.1002/mmnd.201200025>
- Gosik R, Sprick P (2013) Morphology and identification of the pupae of several species of soil-dwelling broad-nosed weevils from Central Europe (Coleoptera, Curculionidae, Entiminae). *Zootaxa* 3731(4): 445–472. Incl. Erratum: *Zootaxa* 3745(2): 299–300. <https://doi.org/10.11646/zootaxa.3731.4.2>
- Gosik R, Sprick P, Skuhrovec J, Derús M, Hommes M (2016) Morphology and identification of the mature larvae of several species of the genus *Otiorrhynchus* (Coleoptera, Curculionidae, Entiminae) from Central Europe with an update of the life history traits. *Zootaxa* 4108(1): 1–67. <https://doi.org/10.11646/zootaxa.4108.1.1>

- Gosik R, Sprick P, Czerewko K (2017) Morphology of the larvae of three Central European *Strophosoma* Billberg, 1820 (Coleoptera, Curculionidae, Entiminae) species. *Deutsche Entomologische Zeitschrift* 64: 27–42. <https://doi.org/10.3897/dez.64.11446>
- Hoffmann A (1963) *Entomologie appliquée à l'agriculture*. Tome I. Coléoptères. Second volume. *Phytophagoidea* (suite et fin) (Chrysomelidae – Curculionidae – Attelabidae – Scolytidae et Platypodidae). Sous-famille des Otiorrhynchinae. Paris, Masson, 878–1360.
- Leibee GL, Pass BC, Yeargan KV (1980) Instar determination of Clover Root Curculio, *Sitona hispidulus* (Coleoptera: Curculionidae). *Journal of the Kansas Entomological Society* 53(3): 473–475.
- Löbl I, Smetana A (2013) *Catalogue of Palaearctic Coleoptera*. Vol. 8. Curculionoidea II. Brill, Leiden/Boston, 700 pp.
- Marvaldi AE (1997) Higher level phylogeny of Curculionidae (Coleoptera: Curculionoidea) based mainly on larval characters, with special reference to broad-nosed weevils. *Cladistics* 13: 285–312. <https://doi.org/10.1111/j.1096-0031.1997.tb00321.x>
- Marvaldi AE (1998a) Larvae of Entiminae (Coleoptera: Curculionidae): tribal diagnoses and phylogenetic key, with a proposal about natural groups within Entimini. *Entomologica Scandinavica* 29: 89–98. <https://doi.org/10.1163/187631298X00212>
- Marvaldi AE (1998b) Larvae of South American Rhytirrhinae (Coleoptera: Curculionidae). *The Coleopterists Bulletin* 52(1): 71–89.
- Marvaldi AE (1999) Morfología larval en Curculionidae. *Acta Zoológica Lilloana* 45: 7–24.
- Marvaldi AE (2003) Key to larvae of the South American subfamilies of weevils (Coleoptera, Curculionoidea). *Revista Chilena de Historia Natural* 76: 603–612. <https://doi.org/10.4067/S0716-078X2003000400005>
- May BM (1994) An introduction to the immature stages of Australian Curculionoidea. In: Zimmerman EC (Ed.) *Australian Weevils*. Volume II. Brentidae, Eurhynchidae, Apionidae and a chapter on immature stages by Brenda May. CSIRO, Melbourne, 1–755.
- Morris MG (1987) The distribution and ecology of *Philopodon plagiatus* (Schaller) (Col.: Curculionidae), with particular reference to inland records. *Entomologist's Record and Journal of Variation* 99: 11–20.
- Oliveira Lira A De, Rosado-Neto GH, Marques MI, Oliveira De Sous W (2017) A first description of the larva and pupa of the coconut palm borer *Homalinotus depressus* (Linnaeus, 1758) (Coleoptera: Curculionidae: Molytinae) and a discussion about the terminology for immature forms. *Zootaxa* 4311(4): 589–599. <https://doi.org/10.11646/zootaxa.4311.4.10>
- Rosenstiel RG (1987) Larval taxonomy of some Polydrosinae and Entiminae (Coleoptera: Curculionidae). *Miscellaneous Publications of the Entomological Society of America* 67: 1–64.
- Rowe DJ, Kok LT (1985) Determination of larval instars, and comparison of field and artificial diet-reared larval stages of *Rhinocyllus conicus* (Col.: Curculionidae). *Virginia Journal of Science* 36(4): 277–280.
- Scherf H (1964) Die Entwicklungsstadien der mitteleuropäischen Curculioniden (Morphologie, Bionomie, Ökologie). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* 506: 1–335.
- Skuhrovec J, Gosik R, Caldara R, Košťál M (2015) Immatures of Palaearctic species of the weevil genus *Sibinia* (Coleoptera, Curculionidae): new descriptions and new bionomic data

- with suggestions on their potential value in a phylogenetic reconstruction of the genus. *Zootaxa* 3955(2): 151–187. <https://doi.org/10.11646/zootaxa.3955.2.1>
- Sprick P, Gosik R (2014) Biology and morphology of the mature larva of *Mitoplinthus caliginosus caliginosus* (Curculionidae, Molytinae). Snudebiller. Studies on Taxonomy, Biology and Ecology of Curculionoidea 15 (229): 1–10. <https://www.curci.de/?beitrag=229>
- Stüben PE, Schütte A, Bayer C, Astrin JJ (2015) The Molecular Weevil Identification Project (Coleoptera: Curculionoidea), Part II. Towards an Integrative Taxonomy. Snudebiller. Studies on taxonomy, biology and ecology of Curculionoidea 16(237): 1–294. [including No. 238: table with phylogenetic relations. Curculio-Institute, Mönchengladbach. <https://www.curci.de/?beitrag=237> and https://curci.de/data/snudebiller/sn16/btr_238/16g_text238.pdf]
- Thompson RT (1992) Observations on the morphology and classification of weevils (Coleoptera, Curculionoidea) with a key to major groups. *Journal of Natural History* 26: 835–891. <https://doi.org/10.1080/00222939200770511>
- Trnka F, Stejskal R, Skuhrovec J (2015) Biology and morphology of immature stages of *Adosomus roridus* (Coleoptera: Curculionidae: Lixinae). *Zootaxa* 4021: 433–446. <https://doi.org/10.11646/zootaxa.4021.3.3>
- Zacharuk RY (1985) Antennae and sensilla. In: Kerkut GA, Gilbert LI (Eds) *Comparative Insects Physiology, Chemistry and Pharmacology* 6. Pergamon Press, Oxford, 69 pp.
- Znamenskij AV (1927) Posobie dlja Proisvodstva Obsledovanija Entomofauni Potschvi 595 (7). S.S.U. Pravlenija Sacharotresta, Kiew, 3–72. [extract pp. 22–24] [In Russian]