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Taxonomic status and behavioural documentation of the troglobiont *Lithobius matulici* (Myriapoda, Chilopoda) from the Dinaric Alps: Are there semiaquatic centipedes in caves?

László Dányi[1], Gergely Balázs[2], Ivan Hadrián Tuf[3]

1 *Department of Zoology, Hungarian Natural History Museum, Baross u. 13, H-1088 Budapest, Hungary* **2** *Department of Systematic Zoology and Ecology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary* **3** *Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, CZ-77900 Olomouc, Czech Republic*

Corresponding author: *László Dányi* (laszlodanyi@gmail.com)

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Abstract

Lithobius matulici Verhoeff, 1899 is redescribed based on type material and newly collected specimens. *Strandiolus jugoslavicus* Hoffer, 1937, described from another cave in the same region in Bosnia and Hercegovina, is presented as a junior subjective synonym of *L. matulici* (syn. nov.). *L. matulici* is shown to be most closely related to *Lithobius remyi* Jawłowski, 1933, type species of the subgenus *Thracolithobius* Matic, 1962. The completeness of the chitin-lines on the forcipular coxosternite is discussed as a promising character for interspecific differentiation within Lithobiomorpha. Documentation of hitherto unknown semiaquatic behaviour in *L. matulici* and other cave-dwelling centipede species from Herzegovinian-, Montenegrin- and Pyrenean caves is presented.

Keywords

Balkan Peninsula, biospeleology, cave, Lithobiomorpha, redescription, semiaquatic lifestyle, synonymy

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Introduction

Many species of lithobiomorph centipedes have been described from European caves during the 19th and 20th centuries (e.g. Verhoeff 1899; Matic and Dărăbanţu 1968), as well as more recently (e.g. Negrea and Minelli 1994; Iorio 2009, 2015; Stoev et al. 2013; Akkari et al. 2017). The degree of cave adaptation in the morphology of these species is rather variable: while some of them still have ocelli and rather short appendages similar to those in epigeic species, other taxa present highly troglomorphic characters, such as being completely blind and having strongly elongated legs and antennae (Folkmanová 1940; Lewis 1981). Regarding the Dinaric Mountains on the Balkan Peninsula and considering only the species with functionally articulated tarsi, five species variously placed in six genera/subgenera have been described as belonging to the latter, troglomorphic group: *Lithobius* (*Oligobothrus*) *matulicii* [sic] Verhoeff, 1899; *Strandiolus jugoslavicus* Hoffer, 1937; *Mesobothrus troglomontanus* Folkmanová, 1940; *Lithobius* (*Troglolithobius*) *sketi* Matic & Dărăbanţu, 1968; and *Lithobius* (*Thracolithobius*) *remyi* Jawłowski, 1933. In addition to their troglomorphic features, all of these taxa might be considered as troglobionts according to the definition of Sket (2008), as they have only been found in caves and never in surface (epigean) habitats. Most of these species are known only from their original description and only from their one or two type locality cave(s) in South Herzegovina, Montenegro, and North Albania (Fig. 1). When revising the taxonomy of the above mentioned (sub)genera, Stoev (1997) concluded that probably none of these are natural taxa and synonymised *Strandiolus* Hoffer, 1937, *Hemibothrus* Folkmanová, 1946 (replacement name for *Mesobothrus* Folkmanová, 1940 due to homonymy) and *Troglolithobius* Matic, 1967 under *Lithobius* Leach, 1814 (s.s.). Regarding *L. matulici*, *S. jugoslavicus*, *M. troglomontanus*, and *L. sketi* he stated that: "It will be no great surprise if the four Balkan „species" are in fact highly variable cave populations of one or two species. Only additional collecting and/or type revision can settle this problem." (Stoev 1997: 90).

Just as suggested more than 20 years ago, freshly collected specimens from that area combined with the study of type material allowed us to revise one of these species, *L. matulici*, and to show that one of the others, *S. jugoslavicus*, is its junior subjective synonym.

Some morphological and behavioural characters not highlighted in earlier descriptions are discussed here in detail:

1. The posteriorly rounded form of the 14th tergite might indicate a close relation of *L. matulici* to members of the subgenus *Thracolithobius* Matic, 1962 (Zapparoli and Edgecombe 2011);

2. The completeness of the chitin-line on the forcipular coxosternite is an important specific character within several genera in Geophilomorpha (Bonato et al. 2011), but until now, it has not been used in Lithobiomorpha. Our unpublished preliminary studies show that this character is also probably useful for interspecific differentiation in this group, as it seems to have different character states (i.e. incomplete, or complete – as in *L. matulici*) which are stable within species;

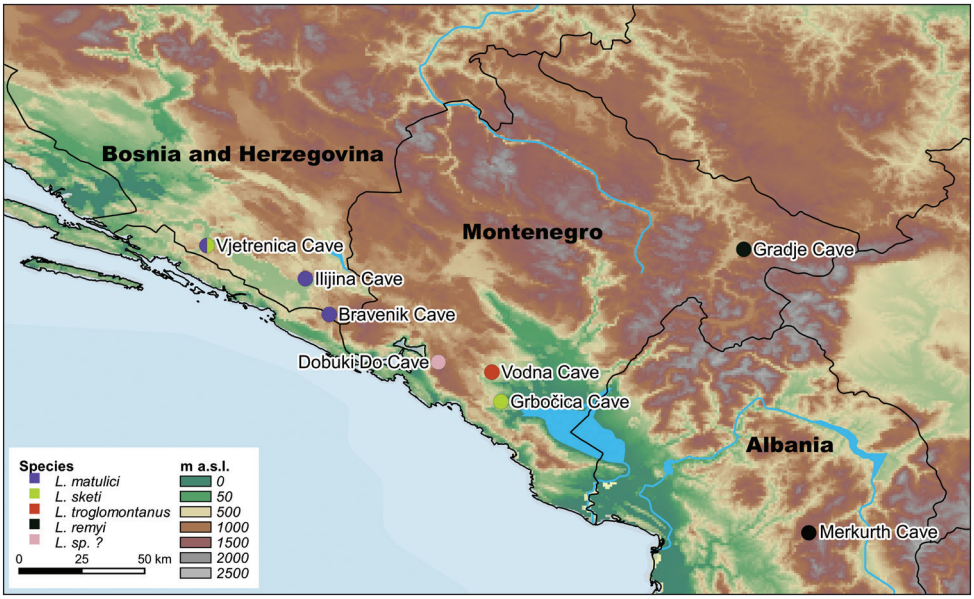


Figure 1. Occurrences of blind *Lithobius* species in the South Dinaric Alps.

3. An amphibious lifestyle in freshwater has not been reported for lithobiomorph centipedes yet, and there is only one species with such behaviour within Chilopoda as a whole. Documentation of underwater activity in cave-dwelling species is presented here, from which at least one is ascertained to be *L. matulici*; another observation made in a Pyrenean cave indicates that this behaviour might be actually rather widespread among cave-dwelling centipedes, similarly as in troglobiont millipedes, where a few amphibious species are already known (Enghoff 1985).

Material and methods

For light microscopy, specimens from Bravenik Cave (Bosnia and Herzegovina, Grab (near Trebinje), 42°35.97'N, 18°25.29'E) were cleared in a mixture of lactic acid and glycerol (3:1) on temporary slides. Two specimens were later cleared also in potassium-hydroxide and mounted in Euparal on permanent slides (all deposited in the Myriapoda Collection of the Hungarian Natural History Museum, Budapest, Hungary: inventory numbers HNHM chilopr-377–378; HNHM chilo-6330). Slides were examined under a Leica DM 1000 microscope equipped with a drawing tube for preparing line drawings. The map for Figure 1 was generated with QGIS version 3.2.2. (QGIS Development Team 2018).

Terminology for external anatomy follows Bonato et al. (2010).

The following abbreviations are used in the text and tables: a—anterior, C—coxa, D—dorsal, F—femur, m—median, p—posterior, P—prefemur, T—tibia, t—trochanter, V—ventral.

Taxonomic part

Class Chilopoda Latreille, 1817
Order Lithobiomorpha Pocock, 1895
Family Lithobiidae Newport, 1844
Subfamily Lithobiinae Newport, 1844
Genus *Lithobius* Leach, 1814

Lithobius (*Lithobius*) *matulici* Verhoeff, 1899

Lithobius (*Oligobothrus*) *Matulicii* [sic] Verhoeff 1899: 452, figs II, III, V (original description)
Lithobius (*Oligobothrus*) *Matulicii* [sic] Verhoeff: Verhoeff 1900: 158, 167 (in key; new data)
Lithobius (*Lithobius*) *matulicii* [sic] Verhoeff: Verhoeff 1937: 196 (in key); Matic 1960: 447 (in key)
Lithobius (*Troglolithobius*) *matulicii* [sic] Verhoeff: Matic 1967: 90 (erection of the new subgenus *Troglolithobius*); Matic and Dărăbanţu 1968: 211, figs 4a–4g, tab. 4 (redescription); Lewis 1981: 106 (mentions enlarged Tömösváry organ); Kos 1992: 357 (in list)
Lithobius (*s.s.*) *matulici* Verhoeff: Folkmanová 1946: 64 (in key, emendation); Stoev 1997: 90 (synonymisation of *Troglolithobius*); Zapparoli and Edgecombe 2011: 377 (only mentions)
Strandiolus jugoslavicus Hoffer 1937: 429, figs 1–10 (syn. nov.) (original description, erection of new genus); Jeekel 2005: 31 (in list)
Lithobius jugoslavicus (Hoffer): Stoev 1997: 90 (synonymisation of *Strandiolus*)

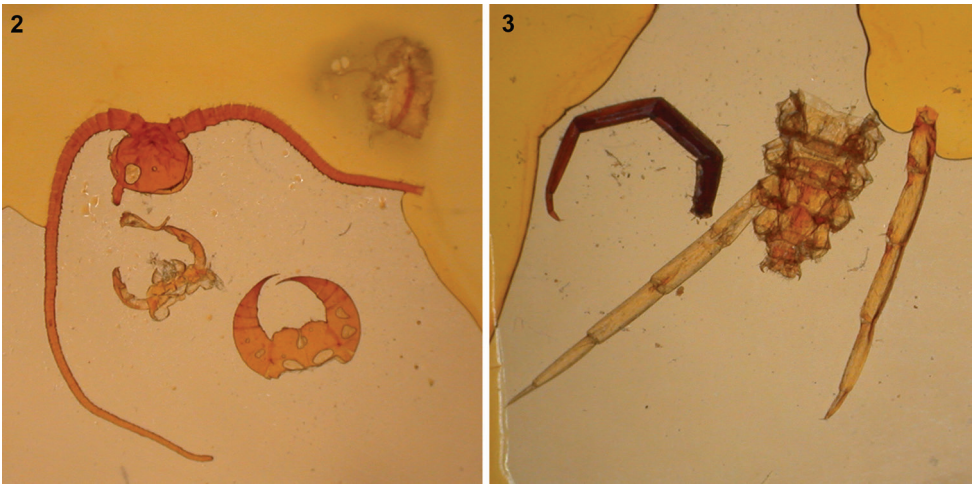
Remark on the origin of name. The species was dedicated to Lucijan von Matulić (teacher at a high school in Trebinje and founder of the first Speleological Society in Bosnia and Herzegovina in Trebinje in 1911), thus it was emended to "*matulici*" by Folkmanová (1946).

Type locality. Ilijina Pećina (as "Elias Höhle bei Trebinje" in the original description (Verhoeff 1899)) 42°43.63'N, 18°20.17'E. (Type locality of *S. jugoslavicus*: Vjetrenica Cave – as "grotte sur le mont 'Brencovac' près de Zavala en Popovo polje" in the original description (Hoffer 1937), 42°50.752'N, 17°59.028'E).

Material examined. Type material: female holotype on two slides (Slide No. 266 and 267) housed by the Museum für Naturkunde, Berlin. The slides were mounted in Canada balsam, but in an inappropriate way since they are partially dried out (Figs 2, 3). Such drying may probably happen because of the mixing of the Canada balsam with a diluting-agent, like glow-oil or xylene, at a too high of a level.

Slide No. 266: cephalic capsule, mandibles, maxillae, forcipules and forcipular tergite, half of the 1st leg-bearing segment's tergite (Fig. 2).

Slide No. 267: posterior part of body from 12th segment, legs missing except right 14th leg and the 15th pair detached. Right ultimate leg was probably not macerated in



Figures 2, 3. Holotype of *Lithobius matulici* Verhoeff, 1899 on slides from the Museum für Naturkunde, Berlin **2** slide No. 266 **3** slide No. 267.

any clearing agents before slide mounting, since the muscles are well visible inside (Fig. 3). All the other parts cleared, probably via potassium hydroxide, because their muscles were dissolved.

Other material examined. 2 ♀ (HNHM chilo-6330, HNHM chilopr-377), 1 subadult ♀ (HNHM chilopr-378): Bosnia and Herzegovina, Bravenik Cave, Grab (near Trebinje), 42°35.97'N, 18°25.29'E, 20.07–20.09.2008, leg. Roman Lohaj.

Further data. A subadult female of 12 mm from the type locality cave (Verhoeff 1900; not studied). Two males and three females from the Vjetrenica Cave (type locality of male *Strandiolus jugoslavicus*) (Matic and Dărăbanţu 1968; not studied).

Diagnosis. A *Lithobius* Leach, 1814 species (subgenus *Lithobius* Leach, 1814) of a length about 14–26 mm; with long antennae of 76–110 articles, reaching the posterior end of tergites 8–9 when folded backwards; ocelli absent; Tömösváry's organ large, with a diameter 0.08–0.1 times of the length of the cephalic plate; 2+2–3+4 obtuse and short teeth on dental margin of forcipular coxosternum, porodonts large, about 2.8–3 times longer and 1.3–2 times broader than teeth; chitin-lines on the forcipular coxosternite reaching the posterior margin of coxosternite; posterior part of 14th tergite without setae-bearing area in both sexes; legs 1–13 with long anterior and posterior accessory spines; 14th and 15th pairs of legs without accessory spines, without secondary sexual characters, and with the following plectrotaxy 15: -,-,(m)p,-,-/-m,mp,m,- and 14: -,-,(m)p,-,-/-m,mp,m,-; 3,4,4,3–5,5,5,5 coxal pores arranged in a single row; female gonopods with 2+2 spurs on first article, gonopodal claw bipartite.

Redescription based on material examined and on literature. Where differences between specimens from different caves occur, they are highlighted at the given characters.

Body length 14–26 mm (holotype 21.5 mm according to the original description; specimens from Vjetrenica Cave 20–26 mm (26 mm in holotype of *S. jugoslavicus*),

specimens from Bravenik Cave 14–17 mm). Coloration yellowish-white in alcohol. The whole cuticle is thin and rather soft, almost transparent, wrinkled on the cephalic plate and tergites (wrinkling not mentioned for specimens from Vjetrenica Cave). Cephalic plate, forcipules and body without punctae. Cephalic plate as broad as tergite 8, about as broad as long (1.96 mm long and 2.28 mm wide in holotype, but width obviously affected there by flattening at slide-mounting; Fig. 2). Ocelli missing. Tömösváry's organ very large, with diameter 0.08–0.1 times of the length of the cephalic plate, placed on the ventral to anterolateral margin of cephalic pleurite. Antennae composed of 76–110 articles (in holotype right antenna with 106 articles, left antenna broken and distal part missing; 85–88 articles in holotype of *S. jugoslavicus* and 106–110 in other specimens from Vjetrenica Cave), long (7.8–18.5 mm, 13.5 mm in holotype, 18.5 mm in holotype of *S. jugoslavicus*), reaching the posterior end of tergites 8–9. Most articles short, probably from secondary segmentation, with only one whorl of setae (number of whorl of setae not documented in specimens from Vjetrenica Cave but proportion of antennal articles illustrated as the same in Hoffer 1937: fig. 1). Forcipular coxosternite broad, with 2+2–3+4 obtuse and short teeth (usually 3+3 as in the holotype (Fig. 5), in the holotype of *S. jugoslavicus* and four other specimens from Vjetrenica Cave, and in the specimen HNHM chilo-6330 from Bravenik Cave; 3+4 in only one specimen from Vjetrenica Cave according to Matic and Dărăbanţu (1968: fig. 4c), 2+2 in specimens HNHM chilopr-377–378 (Fig. 4) from Bravenik Cave; porodonts stout and strong, about 2.8–3 times longer and 1.3–2 times broader than teeth; dentate part of the coxosternite concave, shoulder of coxosternite broad (Figs 4, 5); chitin-lines reaching the posterior margin of coxosternite (Fig. 4). Lateral edges of trochanteroprefemur and part of coxosternite extended beyond cephalic plate. Calyx of poison gland 6.5–7 times as long as wide, about ¼ situated in distal half of forcipular tibia (Figs 4, 6) (not known for specimens from the Vjetrenica Cave). Forcipular tergite narrower than cephalic plate with a ratio of about 0.8 (in holotype of *S. jugoslavicus* similar ratio according to Hoffer 1937: fig. 1, but about 1.1 in his fig. 8; 0.85 for another specimen from the same Vjetrenica Cave according to Matic and Dărăbanţu (1968: fig. 4b)). Lateral sides of body rather parallel, only slightly broadened at tergites 8–10. Tergites 3, 5, 8, 10, 12 and 14 posteriorly rounded, without protuberances; posterior end of tergite 14 semicircular (less pronounced in younger specimens from Bravenik Cave (Fig. 8), almost perfect in the female holotype (Fig. 7) and in the male holotype of *S. jugoslavicus* illustrated by Hoffer (1937: fig. 1)). Sternites 1–10 longer than broad, sternites 11–15 shorter than broad (sternites 1–11 missing and not documented in holotype). Sternite 15 in female trapeziform, posterolaterally narrower than anterolaterally, with straight posterior border, in male longer than broad according to Hoffer (1937: fig. 10, from Vjetrenica Cave, not documented from other caves). Legs elongated, 14–15th without modifications. Length of leg articles of holotype (in mm): leg 14: trochanter+prefemur = 1.7, femur = 2.0, tibia = 2.2, tarsus 1 = 2.0, tarsus 2 = 0.8; legs 15: trochanter+prefemur = 1.6–1.7, femur = 2.1–2.2, tibia = 2.2–2.4, tarsus 1 = 2.0–2.1, tarsus 2 = 0.8–0.9. Right ultimate leg of holotype with tarsus

Table 1. *Lithobius matulici* Verhoeff, 1899. Plectrotaxy of holotype, legs 1–13 missing.

Leg pairs	Ventral					Dorsal			
	C	t	P	F	T	C	P	F	T
14–15	–	m	mp	m	–	–	mp	–	–

Table 2. *Lithobius matulici* Verhoeff, 1899. Plectrotaxy of a young female (HNHM chilopr-377) from Bravenik Cave, Grab (near Trebinje), Bosnia and Herzegovina (brackets indicate spines present asymmetrically).

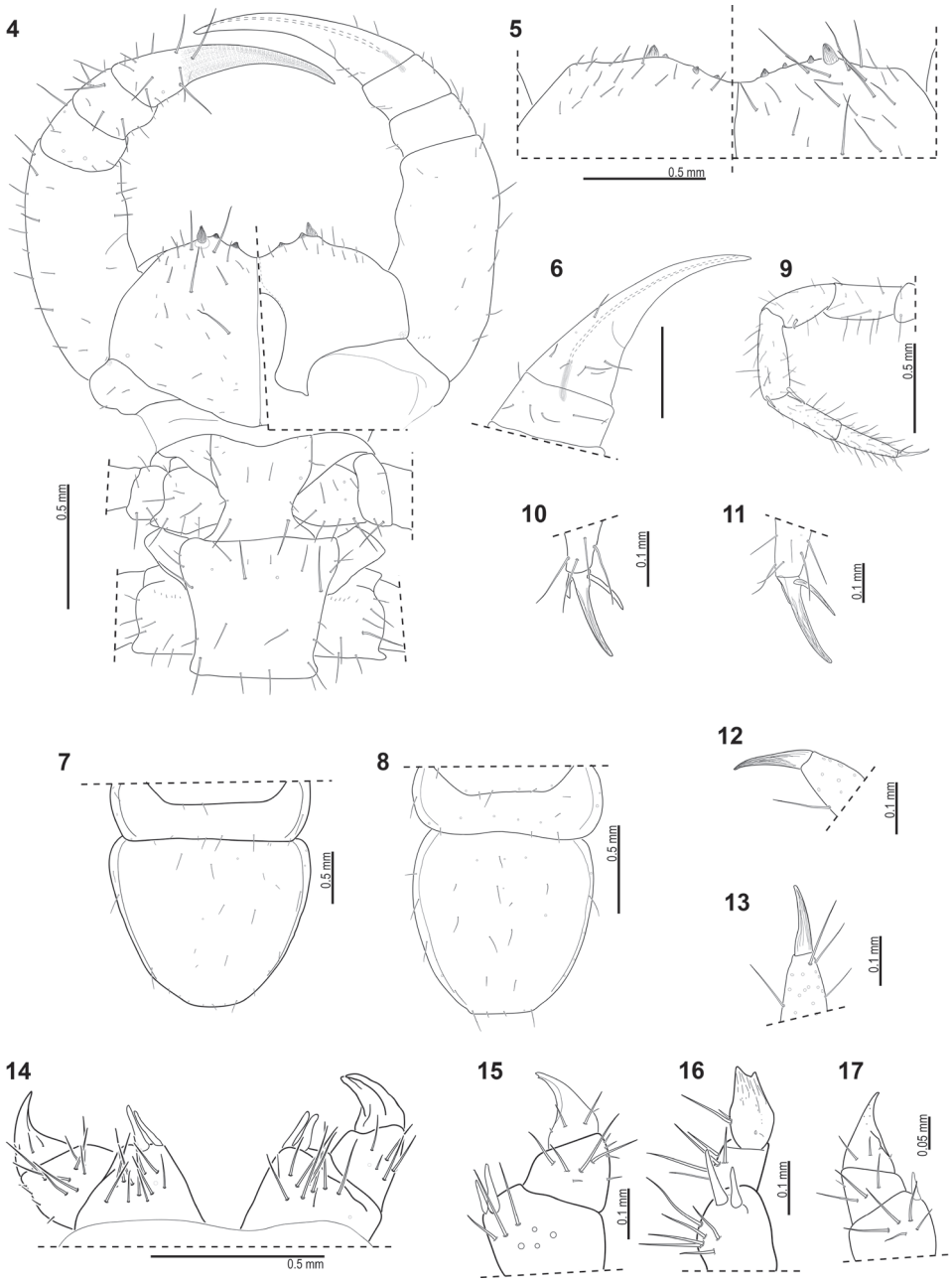
Leg pairs	Ventral					Dorsal			
	C	t	P	F	T	C	P	F	T
1–12	–	–	–	m	m	–	–	–	a
13	–	m	mp	m(p)	m	–	p	–	a
14–15	–	m	mp	m	–	–	mp	–	–

Table 3. *Lithobius matulici* Verhoeff, 1899. Plectrotaxy of adults combined from all available data (brackets indicate spines missing in some cases).

Leg pairs	Ventral					Dorsal			
	C	t	P	F	T	C	P	F	T
1	–	–	–	(m)[†]	m	–	–	–	a
2–11	–	–	–	m	m	–	–	–	a
12	–	–	(mp)[‡]	m(p)[‡]	m	–	–	–	a
13	–	(m)[§]	(mp)[‡]	m(p)[‡]	(m)[†]	–	(p)[†]	–[┘]	a
14	–	m	mp[¶]	m[¶]	–[¶]	–	(m)[‡]p	–[┘]	–
15	–	m	mp	m	–	–	(m)(p)[#]	–[┘]	–

[†]Absent in *S. jugoslavicus* according to Hoffman (1937), but present in specimens from the same cave according to Matic and Dărăbanţu (1968: table 3).
[‡]Present in *S. jugoslavicus* according to Hoffman (1937), but absent in specimens from the same cave according to Matic and Dărăbanţu (1968: table 3).
[§]Present in only one specimen from Bravenik Cave (see Table 2).
[|]The presence of spines on femora instead of prefemora in Matic and Dărăbanţu (1968: table 3) is most probably a typing or printing error, i.e. marking the spines in the wrong column of the table.
[¶]The ventral plectrotaxy given for leg 14 by Matic and Dărăbanţu (1968: tab. 3), -,m,m,mp,m, i.e. more spines on femur than on prefemur is very unusual in *Lithobius*, thus a printing error in the table might be suspected.
[#]Only spine "p" present in specimens from the Vjetrenica Cave according to Matic and Dărăbanţu (1968: table 3). Only one spine in *S. jugoslavicus* from the same cave according to Hoffman's (1937) plectrotaxy table, which is spine "m" according to the illustration in the same work (Hoffman 1937: fig. 1). Both spines "p" and "m" present in the holotype of *L. matulici* and in the specimens from Bravenik Cave.

2 having an 'articulated' appearance (Fig. 3), although only collapsed as an artefact (probably caused during the mounting). Leg plectrotaxy as in Tables 1–3 (differences between cave populations given in footnotes), spines 1–6VmF and 1VmT missing in the subadult female of ~11 mm (HNHM chilopr-378). Legs 14–15 with claws of usual proportions, without accessory spines (Figs 12, 13); legs 1–13 with elongated claws and with elongated anterior and posterior accessory spines (Figs 10, 11), relative length



Figures 4–17. *Lithobius matulici* Verhoeff, 1899 (holotype 5–7, 14; HNHM chilopr-377 4, 8–13, 15–16; HNHM chilopr-378 17) **4** forcipules and trunk segments 1–2, left side of forcipules with ventral view, right side with dorsal view **5** coxosternal dentation, left side with dorsal view, right side with ventral view **6** tarsungulum and forcipular tibia of the holotype (ventral view) **7–8** tergites 13–14 **9** right leg 1 (anterior view) **10** claw of right leg 1 (anterior view) **11** claw of right leg 13 (anterior view) **12** claw of right leg 14 (posteriomedial view) **13** claw of left leg 15 (posteromedial view) **14** gonopods of holotype **15** female gonopod (lateral view) **16** female gonopod (anterior view) **17** subadult female gonopod (right, lateral view).

of accessory spines highest on legs 11–12: about 0.5 of claw's length for the anterior and 0.3 for the posterior spine (from Vjetrenica Cave Hoffer (1937: fig. 6) illustrated for leg 13 ratios of about 0.4 in both spines, while Matic and Dărăbanţu (1968: fig. 4g) illustrated for leg 10 ratios of 0.8 and 0.2). 3,4,4,3–5,5,5,5 coxal pores arranged in one line. In the original description Verhoeff (1899) mentioned 2(+1),3,4,3 as number for coxal pores in the holotype, but in fact it is 4,4,4,3 on legs 12–15 respectively; in *S. jugoslavicus* only legs 14–15 were documented with 5 and 4 coxal pores respectively (Hoffer 1937: fig. 10; in the text erroneously mentioned 4 and 5 respectively, which would be an unusual pattern in Lithobiomorpha). For the specimens from the same Vjetrenica Cave Matic and Dărăbanţu (1968) mentioned 5,5,5,5 coxal pores, while in the specimens from Bravenik Cave we found 3,4,4(5),3(4).

Female first genital sternite longer than wide, with 22–40 evenly scattered setae (40 in holotype; not known in specimens from Vjetrenica Cave); posterior border almost straight (Fig. 14) (not known in specimens from Vjetrenica Cave). Female gonopods with thin setae and 2+2 elongated spurs on first article (holotype in Fig. 14; unequal spurs in younger adults as in Figs 15, 16; 1+1 in a subadult specimen in Fig. 17). Lateral side of female gonopods with 7–12 moderate to long setae on first article, 5–8 setae on second and 1 or 2 setae on third article, arranged as in Figures 14–16 (only 4 setae on first article in a specimen from Vjetrenica Cave according to Matic and Dărăbanţu (1968: fig. 4d) but their drawing is probably inaccurate in this detail); dorsal side of gonopod with about 4 weak spines on second article and 1–3 minute spines on third article (Figs 14–16), medial side of female gonopods without setae (not known in specimens from Vjetrenica Cave). Gonopodal claw bipartite (on left gonopod of holotype (Fig. 14) misinterpreted by Verhoeff (1899: fig. V) as tripartite); medial tip smaller than lateral (Fig. 16).

Remarks on synonymy. *Strandiolus jugoslavicus* was described by Hoffer (1937) on a single male specimen from the Vjetrenica Cave ("grotte sur le mont 'Brencovac' près de Zavala en Popovo polje", 42°50.752'N 17°59.028'E) without comparison with *Lithobius matulici* Verhoeff, 1899, known from another cave only about 32 km away. The depository of the type is unknown, and it was not found at the National Museum in Prague (Dolejš 2015) where that part of Hoffer's material is housed that we know to exist. However, the original description is very detailed, supplemented with illustrations, and fits in every important character with Verhoeff's original description, but also with the holotype of *matulici*, as well as the fresh material studied by us. It also fits the five topotypic specimens described by Matic and Dărăbanţu (1968). The fact that Matic and Dărăbanţu (1968) identified these topotypic specimens as *L. matulici* (without any notes on *S. jugoslavicus*) also supports our conclusion that *S. jugoslavicus* is a subjective junior synonym of *L. matulici* (syn. nov.). Because also neighbouring caves might be completely isolated from each other, high-level genetic separation of cave populations might occur even in cases where no morphological differences of the specimens are obvious. Future molecular studies might easily support our decision based on morphology.

Taxonomic remarks. The posteriorly semicircular form of the 14th tergite has not been highlighted for this species by the earlier authors, although it was illustrated by

Hoffer's (1937: fig. 1) drawing on the habitus of the holotype of *S. jugoslavicus* and Verhoeff (1899) mentioned that the posterior corners of the tergites 3, 5, 8, 10, 12, and 14 are exceptionally strongly rounded. It is present in the holotype of *matulici* (Fig. 7) and in our fresh specimens as well. Matic and Dărăbanţu (1968) seem to have overlooked this character, as they only mentioned that the tergites are without posterior triangular projections. Hoffer (1937) characterised the tergites as of oval in shape, but for more details he referred to his drawing with the holotype which depicts tergite 14 with rounded posterior margin.

The shape of the 14th tergite seems to indicate a close relation of *L. matulici* to the members of the subgenus *Thracolithobius* Matic, 1962 (Zapparoli and Edgecombe 2011), especially to its type species, *Lithobius remyi*, described from the Gradje Cave (Montenegro), which is only 95–150 km from the known occurrences of *L. matulici*, and also reported from the North Albanian Merkurth Cave (Stoev 1996). As the posteriorly semicircular form of the 14th tergite is the key character defining *Thracolithobius*, we could consider *L. matulici* as member of this subgenus, but we refrain to do for reasons of nomenclatural stability. Including *L. matulici* in *Thracolithobius* would result in a situation in which the generic name *Strandiolus* Hoffer, 1937 would became a subjective senior synonym of *Thracolithobius* Matic, 1962 according to the principle of priority (ICZN 1999: Art. 23), because its type species, *Strandiolus jugoslavicus* Hoffer, 1937, is synonymised in the present paper under *L. matulici* (see above). *Strandiolus* was synonymised under *Lithobius* (s.s.) by Stoev (1997) (also proposed earlier informally and without explanation by Folkmanová (1946) in a key) because its differential characters are either actually common in *Lithobius* (s.s.) – three 'claws' on legs 1–13, reduced leg plectrotaxy, notched lateral edges of head, absence of tergal projections, form of maxillae II – or adaptations to the cave environment – absence of ocelli, elongation of legs and narrow anterior sternites, depigmentation, high number of antennal articles – and as such of no taxonomical importance. Meanwhile, *Thracolithobius* Matic, 1962 is considered as a valid subgenus (Stoev 1997; Shelley 2006; Ćurčić et al. 2008; Zapparoli and Edgecombe 2011) with three species – *L. dacicus* Matic, 1959, *L. inexpectatus* Matic, 1962, *L. remyi* Jawlowski, 1933 – but the monopyhly of this group might be questioned. The only common character defining this subgenus is the shape of the 14th tergite, a character that however has already been proven to vary at the inter(sub)specific level in *Lithobius* (Andersson 1979) and in another lithobiomorph genus, *Eupolybothrus* (Stoev et al. 2013; Akkari et al. 2017). Apart from this character, the members of the subgenus seem to be rather different in several other features (e.g. presence/absence of ocelli and a wart-like structure on forcipular tarsungulum) and *L. matulici* differs actually from the members of *Thracolithobius* even in an aspect of the 14th tergite: the rounded shape is present in *matulici* also in females, while it is known only from males in the other species. Although at least *L. remyi* and *L. matulici* seem to be similar also in some other features (lack of ocelli, strong porodonts, coxosternal dentation) this may be also due to convergent adaptation to a similar lifestyle in cave environments.

According to this, we can expect that molecular studies will prove *Thracolithobius* to be polyphyletic with its members spread among different clades of *Lithobius* (s.l.), which would result in its synonymisation under *Lithobius* (s.s.); and this would be the

case again even if its name would be changed here to the older name *Strandiolus*. In case future molecular studies give an opposite result (i.e. monophyly of *Thracolithobius* including *L. matulici*), *Strandiolus* might be revalidated.

Differential diagnosis. Among the *Lithobius* species with a posteriorly rounded tergite 14, *L. matulici* seems to be most similar to *L. remyi*, but differs from that species in size (11–13 mm in *remyi*, 14–26 mm in *matulici*), number of antennal articles (56–64 in *remyi*, 76–110 in *matulici*), and the shape of the female gonopodal claw (tripartite in *remyi*, bipartite in *matulici*). From *L. dacicus*, *L. matulici* differs in size (about 12 mm in *dacicus*, 14–26 mm in *matulici*), number of antennal articles (37–61 in *dacicus*, 76–110 in *matulici*), coxosternal dentation (2+2–3+4 small and obtuse teeth in *matulici*, 2+2 well developed teeth in *dacicus*), and completeness of coxosternal chitin-lines (not reaching the posterior margin of the coxosternite in *dacicus*, reaching it in *matulici*). *Lithobius inexpectatus* is distinguished from *L. matulici* by having 12–14 ocelli (missing in *matulici*), by the coxosternal dentation (2+2–3+4 small and obtuse teeth and very strong porodonts in *matulici*, 2+2 larger teeth and slender porodonts in *inexpectatus*), the number of antennal articles (42 in *inexpectatus*, 76–110 in *matulici*), the presence of accessory spines on legs 14–15 (absent in *matulici*), the shape of the female gonopod claw (tripartite in *inexpectatus*, bipartite in *matulici*), and plectrotaxy (1–15VaF, 1–13VaT, 1–14VpT, 8–15DaP, 1–15DpP, 1–13DaF, 3–15DpF and 3–15DpT present in *inexpectatus*, missing in *matulici*).

Although no rounded form of tergite 14 is known for it, *L. sketi* was stated to be very similar to *L. matulici*, and they also co-occur in Vjetrenica Cave (Matic and Dărăbanţu 1968). The two species are readily distinguished by the accessory spines on the 14–15th legs (present in *sketi*, missing in *matulici*), by the number and arrangement of coxal pores (5–9 per coxa arranged in 2 partly irregular rows in *sketi*, 3–5 per coxa in a single row in *matulici*), the female gonopods (1+1 spurs and simple claw in *sketi*, 2+2 spurs and bipartite claw in *matulici*), and their plectrotaxy (1–13VpP, 1–15DaP, 1–15DpP, 1–14DaF, 1–15DpF and 2–15DpT present in *sketi*, missing in *matulici*).

Semiaquatic behaviour. One lithobiomorph specimen was found in July 2014 while one of the authors, G. Balázs, was diving in Vjetrenica Cave. The specimen was in a water-filled part of the cave (Donje Vjetrenica), freely and consciously walking on the underwater bottom at a depth of 3 metres, at a distance of about 30 metres from any terrestrial microhabitats (i.e. chambers with air). This specimen was without any signs of distress (no spasms, no enfeeblement). There was no flood in the cave at that time, the water was still (not flowing), and thus a simple flushing away of the specimen from the water's edge might be ruled out. This individual spent another 2 hours in the water, while kept captured by the diver and escaped later during photographic documentation. In the photograph (Fig. 18), the 14th tergite of the specimen seems clearly rounded posteriorly, and thus it can be considered as *L. matulici* with confidence. Similar cases of lithobiomorph specimens on the bottom of water (puddles) in caves were photo-documented in Montenegro (Dobuki Do: 42°25.739'N 18°48.716'E: August 2006, Zsolt Polacsek in litt., Figs 19, 20; July 2018, Márton Mede in litt.), and from a cave in North Spain (Tibia-Fresca Cave System, 19 July 2016, see supplementary file 1: Video 1; Zsolt Polacsek in litt.).

Figures 18–20. *Lithobius* specimens from Dinaric caves **18** living *Lithobius* cf. *matulici* specimen of ca. 25 mm length from the Vjetrenica Cave (Bosnia and Herzegovina) (photo by Gergely Balázs) **19–20** *Lithobius* sp. under water in the Dobuki Do Cave (Montenegro) (photos by Zsolt Polacsek).

Key for the Dinaric *Lithobius* species without ocelli:

1 Tarsus 1–13 biarticulated **2**
– Tarsus 1–13 single **4**
2 Claw of ultimate and penultimate legs simple, without accessory claw **3**
– Claw of ultimate and penultimate legs with accessory claw .... ***L.* (*Lithobius*) *sketi* Matic & Dărăbanţu, 1968**
3 Number of antennal articles 62–64; female gonopodal claw tripartite; posterior half of tergite 14 in males with setaceous field and with or without a swelling ..... ***L.* (*Thracolithobius*) *remyi* Jawłowski, 1933**
– Number of antennal articles 76–110; female gonopodal claw bipartite; posterior half of tergite 14 in males without setaceous field or swelling .... ***L.* (*Lithobius*) *matulici* Verhoeff, 1899**
4 Antennae composed of 20 (21) or fewer articles .... ***L.* (*Monotarsobius*) *zveri* (Matic & Stenzer, 1977)**
– Antennae composed of more than 23 articles **5**
5 Antennae composed of 30–38 articles ..... ***L.* (*Sigibius*) *reiseri* Verhoeff, 1900**
– Antennae composed of 24–28 articles .... ***L.* (*Sigibius*) *apfelbecki* Verhoeff, 1900**

Discussion

Chitin-line. A suture extending posteromedially from the coxosternal condyle of the forcipule in lithobiomorphs corresponds in position to the chitin-line of geophilomorphs. These two structures are a little different in their construction in the two groups and are either a strongly sclerotised narrow stripe in Geophilomorpha or a weak suture in Lithobiomorpha according to Bonato et al. (2010). However, a weak suture is also present along the stripe in geophilomorphs, and weak sclerotisation is present along the suture also in lithobiomorphs (orig. obs.). Thus, the homology of the two structures seems probable, and we prefer to also use this established term (Bonato et al. 2010) in Lithobiomorpha, just as it has already been used by Latzel (1880).

While the chitin-line is an incomplete suture (i.e. not reaching the posterior margin of the coxosternite) in several lithobiomorph species, it is complete in *L. matulici*. Our preliminary unpublished studies reveal that a complete chitin-line is probably not rare at all (e.g. in *Lithobius forficatus* (Linnaeus, 1758), *Lithobius microps* Meinert, 1868, and *Lithobius burzenlandicus* Verhoeff, 1931). The states of this character seem to be stable within species, as well as in specimens of different age which promises that it might be useful for some cases of interspecific differentiation.

Semiaquatic behaviour. Semiaquatic behaviour in terms of actively and regularly moving into the water has never before been reported for lithobiomorphs, but even for Myriapoda as a whole there have been few examples. In the following paragraphs a

short overview is given (for Chilopoda as well as for millipedes), starting from observation of animals actively seeking water to species enduring inundation out of necessity in flood-prone areas.

Only two publications mention active semiaquatic behaviour in Chilopoda. One is the only report of centipedes entering freshwater on their own free will (Armitage 1982). This short paper reports on several specimens of the geophilomorph *Strigamia maritima* (Leach, 1817) found on two occasions in a small stream in England, where they possibly entered the water to hunt for caddisfly larvae (Armitage 1982). The other case is of a scolopendromorph specimen which was possibly hunting underwater (Moraes and Chagas-Júnior 2009). The centipede was found dead in a sea anemone which had probably caught it under water.

A semiaquatic lifestyle is more frequently noted for millipedes. Some species have been reported from under stones in streams in France (Causard 1903) and Australia (Burrows et al. 1994), and one species in South America is known to be able to live submerged for several months in subadult stadia (Adis 1986). Three additional, possibly highly water-adapted species have been reported from Guyana and from widely dispersed Atlantic and Pacific islands (Golovatch and Kime 2009). From caves there are several millipede species described as semiaquatic, for example some julids and polydesmids in the Italo-Balkan region of Europe (Adis et al. 1997; Enghoff et al. 1997; Antić et al. 2017). These cave millipedes enter water on purpose, spend a long time submersed, and have modified mouthparts, which are probably adapted to filtering and screening suspended organic particles from the water (Adis et al. 1997). Similar mouthparts are also known from some other cave-dwelling millipede species from the Caucasus and Papua New Guinea (Enghoff 1985), suggesting that semiaquatic behaviour might be more common in diplopods than generally acknowledged.

Some observations show centipedes to choose swimming as a way of escape when attacked or disturbed. Zulka (1991) published the first observations of this for *Lithobius curtipes* (C.L. Koch, 1847) and *Lamyctes emarginatus* (Newport, 1844), which entered water from objects standing out of surrounding water when he tried to catch them. Even when there are terrestrial pathways for escape, some species or at least specimens chose water: Siriwut et al. (2016) mentioned an individual of *Scolopendra cataracta* Siriwut, Edgecombe & Panha, 2016 that entered a stream to escape from the collector, and the same behaviour was observed by one of the authors (I.H. Tuf pers. obs.) in *Lithobius forficatus* (Linnaeus, 1758) and *Lithobius mutabilis* L. Koch, 1862.

Probably the most frequent reasons for myriapods to come into contact with water are tides and floods. From tide-affected seashores there are numerous reports of more than 40 centipede taxa (see review by Barber 2009, 2011). Almost all of these are geophilomorphs, many of which are considered as real halophiles with adaptations to submergence (Binyon and Lewis 1963; Barber 2011), while the recorded ubiquitous lithobiomorph species do not appear to be truly halophilic; the only exception might be *Lithobius ellipticus* Takakuwa, 1939 (Barber 2009). Several millipede species are able to survive river floods by living actively under floodwaters for weeks (Golovatch

and Kime 2009), while some centipedes have dormant submerged egg stage (e.g. *Lamyctes adisi* Zalesskaja, 1994 (Zalesskaja 1994) and *Lamyctes emarginatus* (Zulka 1991; Zerm 1997)). The centipede *Lithobius curtipes* is able to survive under water for more than one week under experimental conditions (Tufová and Tuf 2005), while in an experiment *Scolopendra subspinipes* Leach, 1816 was found to swim on the water surface, probably as a strategy for escape during floods (Lewis 1980). Another scolopendromorph, *Edentistoma octosulcatum* Tömösváry, 1882, does not swim, but in an experiment by Lewis (1980) simply walked along the bottom when inundated. *L. matulici* and related species inhabit caves where flash floods are common and which have active streams of highly fluctuating water levels, depending on the precipitation and/or snow melting at the surface region above them (Spahić 2015). In addition to the necessity of tolerating submergence during floods, the ability to submerge voluntarily and to move under water might be also useful in moving between parts of the cave that are separated by water. Semiaquatic behaviour might be potentially highly adaptive in caves also for another reason: in subterranean habitats food sources are limited and an expansion of the prey spectrum with the inclusion of the aquatic biota can help a terrestrial predator increase its fitness. This might be highly significant, especially when aquatic biota (e.g. *Niphargus* amphipods) represents the main part of the available biomass of possible prey, like in the caves discussed here (Gergely Balázs pers. obs.; Márton Mede in litt.). Due to similar conditions and forces, such adaptation might be hypothesized to emerge in parallel multiple times in different caves, just like in some hydrophilous millipede taxa (Enghoff 1985).

Two other cave-dwelling *Lithobius* (s.s.) species from the Dinaric Mountains. *Lithobius sketi* Matic & Dărăbanţu, 1968 was described as belonging to the subgenus *Troglolithobius* Matic, 1967 (junior synonym of *Lithobius* according to Stoev 1997), which included also *L. matulici* at that time, and it was stated to be very similar to that species. Although no rounded edge of tergite 14 is known for *L. sketi*, additional studies are needed to verify this character in this species. *Lithobius troglomontanus* (Folkmanová, 1940) was described from Vodna Cave (Vodna Pećina), Montenegro, but it is missing from the list of Mitić et al. (2007). Although Kos (1992) considered *L. troglomontanus* closely related to *L. matulici* and *L. sketi* and also as belonging to the subgenus *Troglolithobius* Matic, 1967, it seems to be different indeed from *L. matulici* in the shape of tergite 14 (with cornered posterior edges). It shares several characters with *L. sketi*, i.e. the structure of female gonopods (unipartite gonopodal claws, 1+1 spurs) and the arrangement of the coxal pores (smaller pores forming a second row), but they do differ in several important characters. Some of these differences (small Tömösváry's organ, short ultimate legs and antennae) actually show *troglomontanus* to be morphologically not very cave-adapted, and thus, surface collecting around the type locality cave might prove it to be not a real troglobiont species. Based on the differences, we consider *L. troglomontanus* and *L. sketi* to be two valid species, but molecular phylogenetic studies are needed on each of these cave taxa to clarify their actual relation to each other and to the members of other subgenera within *Lithobius*.

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Supplementary material 1

Lithobiomorph specimen under water in the Tibia-Fresca Cave System (North Spain) (video by Zsolt Polacsek)

Authors: László Dányi, Gergely Balázs, Ivan Hadrián Tuf

Data type: multimedia

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Taxonomy of the thelyphonid genus *Typopeltis* Pocock, 1894, including homology proposals for the male gonopod structures (Arachnida, Thelyphonida, Typopeltinae)

Gabriel Seraphim[1], Alessandro Ponce de Leão Giupponi[1], Gustavo Silva de Miranda[2]

1 *Laboratório de Referência Nacional em Vetores das Riquetsioses, LIRN-IOC-FIOCRUZ. Manguinhos, 21040-360, Rio de Janeiro, RJ, Brazil* **2** *Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington D.C., USA*

Corresponding author: *Alessandro Ponce de Leão Giupponi* (agiupponi@gmail.com)

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Abstract

The genus *Typopeltis* Pocock, 1894 is poorly known regarding its systematics, natural history, and distribution, despite important taxonomic advances during the 1990s. Currently, only 13 species are known from East Asia, including areas in south China, Japan, Vietnam, Laos, Thailand, and Taiwan. In this work, we describe and illustrate a new species of *Typopeltis* from Vietnam and provide a new description for the male of *T. guangxiensis* Haupt & Song, 1996. Additionally, we describe and illustrate the female gonopod of *T. guangxiensis* for the first time and propose a new homology hypothesis for the male gonopod parts. The male of *T. laurentianus* **sp. n.** is characterized by the unique patellar apophysis that presents a smooth texture and no spines. *Typopeltis laurentianus* **sp. n.** is the third species of this genus to be described from Vietnam.

Keywords

Asian fauna, taxonomy, Uropygi, Vinegaroons, whip scorpion

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Introduction

The order Thelyphonida Latreille, 1804 (also known as whip scorpions) is a conspicuous, yet small arachnid order with only 124 living species in 15 genera described so far (Zhang 2013, Barrales-Alcalá et al. 2018). Despite not being extremely diverse, the group is quite old and is estimated to have originated around 333 mya in tropical Pangea (Clouse et al. 2017). The oldest Thelyphonida fossil is from 318 mya and currently only seven fossils are known (Dunlop et al. 2008, Wolfe et al. 2016). Recent estimates of time divergence indicate an increase in diversification rates during the Cretaceous (Clouse et al. 2017).

Thelyphonida is currently composed of one family (Thelyphonidae) and four subfamilies: Hypoctoninae Pocock, 1899, Mastigoproctinae Speijer, 1933, Thelyphoninae Lucas, 1973 and Typopeltinae Rowland & Cooke, 1973. The classification history of the order goes back to Pocock (1899), who divided the family Thelyphonidae into two subfamilies, Thelyphoninae and Hypoctoninae (Pocock 1899). The two subfamilies were defined by the presence or absence of a keel between the medial and lateral eyes, respectively. Gravely (1916) set up an organization scheme that would become the current classification of the order, although some of the names were given only afterwards (such as Mastigoproctinae Speijer, 1933 and Typopeltinae Rowland & Cooke, 1973). Gravely (1916) divided Thelyphoninae into three groups, Mastigoproctinae and Typopeltinae, and a new one characterized by a strongly modified patellar process. This last group became the currently defined subfamily Typopeltinae, which includes the single genus *Typopeltis* Pocock, 1894 (Rowland and Cooke 1973).

The genus *Typopeltis* is endemic to Asia, with 13 valid species (including the *nomen dubium T. amurensis* (Tarnani, 1889) from Russia). Six of these species are known from Southeast Asia: two from Vietnam (*T. harmandi* Kraepelin, 1900; *T. soidaoensis* Haupt, 1996), three from southern China (*T. vanoorti* (Speijer, 1936); *T. sinensis* (Butler, 1872); *T. guangxiensis* Haupt & Song, 1996) and one from Laos (*T. magnificus* Haupt, 2004) (Haupt 1996, 2004a, Haupt and Song 1996, Harvey 2003). Several areas in East and Southeast Asia remain unsampled or undersampled, such as Cambodia and Thailand (with no records of *Typopeltis* at all), and Laos (with one record). Not only is there little information regarding the group's distribution, but the systematics of *Typopeltis* is also still in its infancy. The greatest contributor to the understanding of the genus was Joachim Haupt (Haupt and Song 1996, Haupt 2004a, 2009); however, several details of the morphology of the species were not addressed and continue to be unknown, such as the form of the male gonopod.

The genus *Typopeltis* can be easily recognized by the presence of a marked keel between the lateral and median eyes and by the absence of a suture dividing the abdominal tergites (Rowland and Cooke 1973). The males have a well-developed patellar apophysis and no projection on sternite III (Rowland and Cooke 1973). Females have modifications of the tarsomeres of leg I (antenniform), but according to Gravely (1916) this character can vary depending on the age and reproductive period of the specimen. In addition, the females have clear modifications of sternite II (genital plate) compared to males. The trochanter spines, despite being conspicuous, are not used in

the taxonomy of the group because they vary considerably, with differences between the right and left pedipalps of a single individual (Gravely 1916).

Not much is known regarding the phylogenetic relationships of *Typopeltis* species. In a molecular phylogeny of Thelyphonida, Clouse et al. (2017) included only one named species of *Typopeltis*, *T. crucifer*, which was recovered as sister to what was most likely an unnamed species in the same genus from Vietnam. Interestingly, Typopeltinae was recovered as being more closely related to Thelyphoninae and Mastigoproctinae than to Hypoctoninae.

In this work, we aim to contribute to the understanding of the morphological characters of *Typopeltis* by describing and illustrating a new species from Vietnam. In addition, we provide the first description of the male of *T. guangxiensis* Haupt & Song, 1996, provide detailed images of the female of that species, and present a hypothesis of homology of the male gonopod structures based on Giupponi and Kury (2013). Our homology hypothesis is made based on the consistent sister group relationship between Amblypygi and Thelyphonida (e.g. Ballesteros and Sharma 2019). With this, we intend to set the basis for the evaluation of new characters in future morphological phylogenetic studies.

Material and methods

Specimens were identified based on Rowland and Cooke (1973) and Haupt (1996). The description was adapted from Haupt (1996), Víquez and Armas (2007), Giupponi and Vasconcelos (2008), Villarreal and Giupponi (2009). The descriptions were made with NIKON SMZ745 and LEICA MZ15 stereomicroscopes. Photographs were made with a Leica M205C and Leica Application Suite V. 4.7 software. Scanning electron microscope (SEM) images were produced in a JEOL JSM-6390LV. The map was made with ArcGIS 10.3. All images have been edited with Adobe Photoshop CS6 and Adobe InDesing CS6.

Acronyms:

Fi = fistula; **GO** = genital operculum; **LaM** = lamina medialis; **LoD** = lobus dorsalis; **LoL1** = lobus lateralis primus; **LoL2** = lobus lateralis secundus; **PI** = processus internus; **Me** = Mensa (**new name**); **Fu** = Fulcrum (**new name**); **RS** = receptaculum seminis; **CCh** = circulus chitinosus (**new name**); **ACh** = arcus chitinosus.

MNRJ Museu Nacional, Rio de Janeiro, Brazil (the thelyphonid specimens were on loan from the collection when the Museum burned in 2018, so the material survided the incident; Dr. Adriano B. Kury);

CAVAISC Coleção de Artrópodes Vetores Ápteros de Importância em Saúde das Comunidades FIOCRUZ, Rio de Janeiro, Brazil (Dr. Marinete Amorim);

MNHN Muséum national d'Histoire naturelle, Paris, France (Dr. Mark Judson);

CAS California Academy of Sciences, San Francisco, USA (Dr. Darell Ubick).

Results

Taxonomy

Thelyphonidae Lucas, 1835
Typopeltinae Rowland & Cook, 1973
***Typopeltis* Pocock, 1894**

Type species:

Typopeltis: *Typopeltis crucifer* Pocock, 1894, by original designation.
Gipopeltis: *Typopeltis harmandi* Kraepelin, 1900, by original designation.
Teltus: *Teltus vanoorti* Speijer, 1936, by monotypy.

List of *Typopeltis* species:

1 – *T. amurensis* (Tarnani, 1889) (Russia), *nomen dubium;* 2 – *T. cantonensis* Speijer, 1936 (China); 3 – *T. crucifer* Pocock, 1894 (Japan, Taiwan); 4 – *T. dalyi* Pocock, 1900 (Thailand); 5 – *T. guangxiensis* Haupt & Song, 1996 (China); 6 – *T. harmandi* Kraepelin, 1900 (Vietnam); 7 – *T. kasnakowi* Tarnani, 1900 (Thailand); 8 – *T. magnificus* Haupt, 2004 (Laos); 9 – *T. sinensis* (Butler, 1872) (China); 10 – *T. soidaoensis* Haupt, 1996 (Thailand, Vietnam); 11 – *T. stimpsonii* (Wood, 1862) (Japan); 12 – *T. tarnanii* Pocock, 1902 (Thailand); 13 – *T. vanoorti* (Speijer, 1936) (China); 14 – *T. laurentianus* sp. n. (Vietnam).

***Typopeltis laurentianus* sp. n.**
http://zoobank.org/BE3AADFF-51A5-4F27-B039-346F0A280271

Type material. ***Holotype*** male: VIETNAM: Hà Tĩnh, 18.355240, 105.886949, 1998 (MNRJ 08243). ***Paratypes***: VIETNAM: Hà Tĩnh, 18.355240, 105.886949, 1998 (2 males, MNRJ 08243); Hà Tĩnh, 18.355240, 105.886949, 1997 (1 male, 1 female, MNRJ 08242); Quang Binh: Phong Nha-Kẻ Bàng National Park, 17.590802, 106.283344, 2001 (1 male, MNHN AR-UR-2 [ex MNRJ 08244]; 1 female, CAS, CASENT 9081667 [ex MNRJ 08245]); Vĩnh Phúc, 17.590802, 106.283344, x.1980, leg. R. Boistel (2 females, MNRJ 08246).

Etymology. Species name *laurentianus* (laurentiana, laurentianum) is a Latin adjective after our friend, the distinguished Franco-Brazilian arachnologist Wilson Lourenço. The Latin form of Portuguese Lourenço is Laurentius (genitive Laurentiī), a noun of the second declension, cognate of English Lawrence or French Laurent. The ICZN allows authors of new species to choose the Latin version of contemporary names derived from Latin, which may be more euphonic than the modern counterparts.

Diagnosis. Males (about 35 mm in total length without flagellum) larger than females (see measurements); males with patellar apophysis very long, with narrow base and apex, broader in the middle, with a small antero-posterior curve. Patellar apophysis without spines in the trunk or the terminal portion, with a smooth integument texture that differs from all the other species of *Typopeltis*. The male gonopod is simple, delimited by a sclerotized curved cuticle (posterior apex of **Fi**), with inverted trapezoid shape with rounded edges. The female gonopod has a bulbous **RS** with a wide base and a well marked **CCh**.

Description. (Holotype male) ***Colouration (in alcohol).*** Reddish-brown. Carapace darker on anterior region than posterior region. Abdomen slightly yellowish. Pedipalps dark red, lighter in females lighter. Median eyes dark, almost black, lateral eyes yellow.

Carapace (Figs 1A, 2A). With thick granules of irregular shapes homogeneously covering whole surface, granules interspaced. Lateral keel with one seta on each anterior end, next to median eyes; posterior end of keels above lateral triad of eyes, keel extends from posterior to anterior region of carapace; keels divided by median ocular ridge. Carapace has depression extending from posterior region of median ocular tubercle to region above subtriangular fovea. Median eye tubercle elevated, with well-marked ridge between eyes. Chelicerae with several setae in ventral region and on cheliceral claw. Cheliceral claw curved inwards, with thick base and narrow apex, and with short keel, smaller than half length of tooth (Fig. 3A).

Sternum (Figs 1B, 2B). Typical of order, tri-segmented; inconspicuous mesosternum.

Opisthosoma (Figs 1E, G; 2E, G). Pleura divided by crest of granules from tergites I–VIII; tergites without suture. Sides with fine granules (Figs 1E, 2E). Subcircular ommatoids present (Figs 1G, 2G). Flagellum with 38 articles (female paratype) and 36 in holotype (broken).

Pedipalps (Figs 1C, D; 2C, D). **Coxa** without accessory tooth, with few setae. **Trochanter** punctated with granules covering dorsal surface. Four spines in dorso-mesal region (I <II <III <IV), spines I-III as broad as or broader than long, conical, with broad base and acute apex; spine IV geminate with spine III, with long setae, conical; apex rhomboid and bigger than double size of spine III. Two small spines close to articular condyle (trochanter-femur), spines smaller than mesal spine I (Fig. 1C). Ventral region with thick ridge all along joint with femur, ending mesally with two small conical spines, broader than long (Fig. 1D). **Femur** unarmed and covered with shallow pores concentrated on outer margin (Fig. 1D). Ventro-mesal region with reduced rhombus spine (almost a granule), conical, broader than long, surrounded by long setae (Fig. 1C). In females, ventro-mesal spine well developed, twice longer than wide, with very sharp, curved tip and broad base. Two small conical spines dorsally (I < II); in males these spines reduced to two small granules, clearly homologous to spines present in females. **Patella** covered by pores, especially on ectal face, with few setae; several setae mesally. Patellar apophysis almost as long as patella, with large non-terminal (median) expansion on external margin of apophysis (like large hump); unprecedented smooth texture and slight curvature in ventral direction on terminal portion. Apophysis with spatulated shape with slight concavity ventrally. Ventral face without spines. Females with two coni-

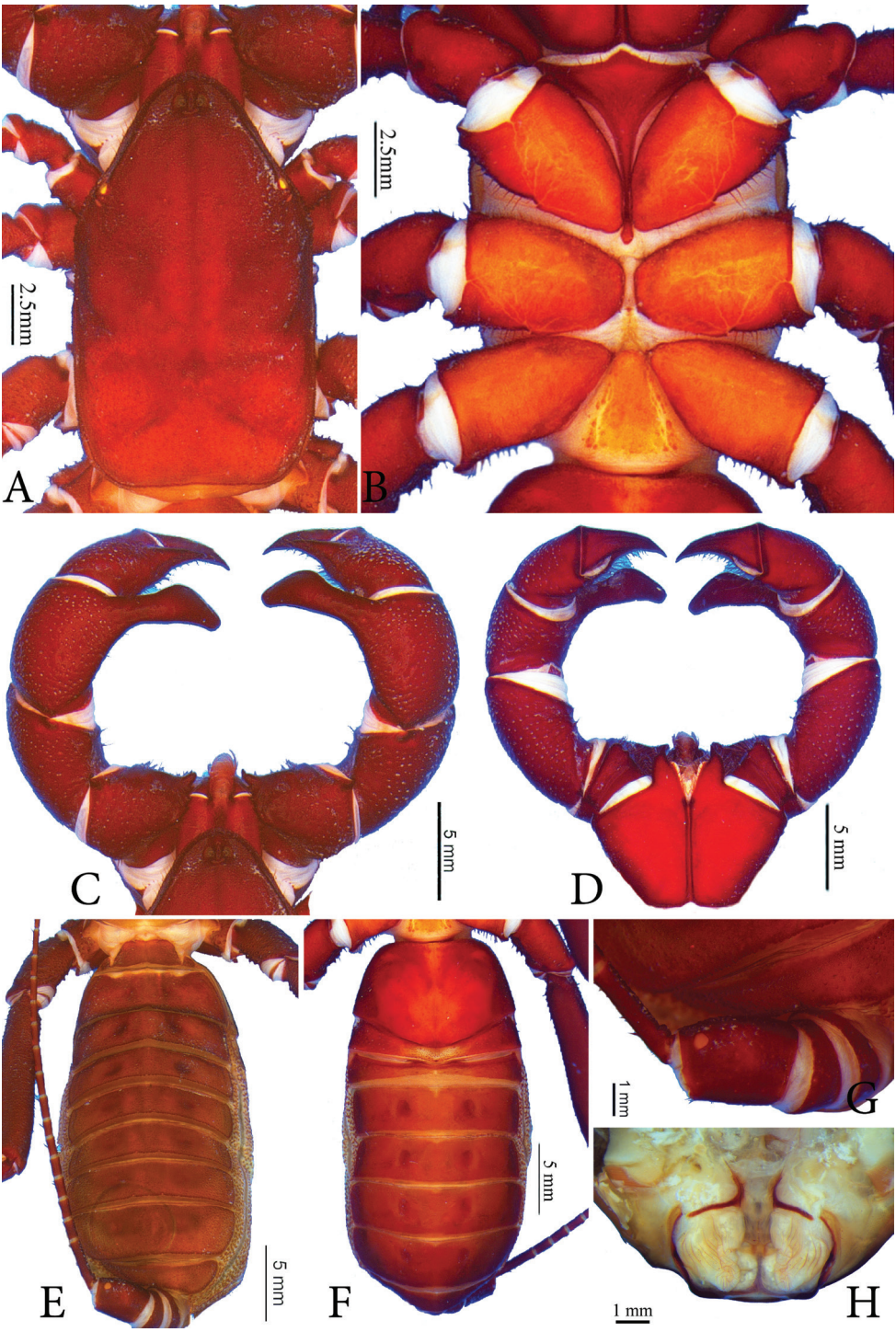

Figure 1. *Typopeltis laurentianus* sp. n., holotype (male). **A** Carapace **B** sternum **C** pedipalps (dorsal) **D** pedipalps (ventral) **E** opisthosoma (dorsal) **F** opisthosoma (ventral) **G** ommatoid **H** gonopod.

Figure 2. *Typopeltis laurentianus* sp. n., paratype (female). **A** Carapace **B** sternum **C** pedipalps (dorsal) **D** pedipalps (ventral) **E** opisthosoma (dorsal) **F** opisthosoma (ventral) **G** ommatoid **H** gonopod. **CCh** = circulus chitinosus; **ACh** = arcus chitinosus.

cal spines of subequal size in dorso-mesal view (Fig. 2C); spines as broad as long, with broad base and sharp tip, most anterior at base of apophysis. Ventral apophysis with reduced ventro-mesal spine in distal position (Fig. 2D). Patellar apophysis well developed, but slightly smaller than length of patella, conical, tapering towards apex; with single spine on mesal surface, positioned just before apex; row of spines with three or four small subequal basal spines on ectal face, followed by median series of four spines increasing in size; second series of spines larger than double first row of spines; distal series composed of three spines, with middle ones larger than two others. Two rows of setae at edges of ventral region, absent in males. **Tibia** covered by pores, large concentration of setae (in mesal view), more than in femur and patella. Tibial apophysis conically-shaped, broad base, acute apex, with series of dorsal spines. In ventro-mesal view with two small spines, most apical rhombic and double the size of previous one; penultimate spine with conical shape, with wide base. **Tarsus** covered by long setae, with greater predominance on mesal surface. With longitudinal series of ventral rhomboid spines and another dorsal series.

Leg I. Eight tarsomers (variation: seven to nine), first very short (like small ring), second, third and last larger than others (I <II> III–VII <VIII); size and number of tarsomers can vary if leg is regenerated. Apical portion of tibia with two dorsolateral tricobothria, absent in femur and patella. Femur covered with thick granules, patella and tibia with smooth appearance. All articles covered with setae dorsally and ventrally.

Legs II–IV. Trochanter and femur with granules. Coxa, tibia and tarsus smooth, last two with concentration of setae. With dorso-apical tricobothrium on tibia; ventro-apical region with thin, acuminate spur. Basitarsus with two spurs, one mesal and other ectal; ventral region with two longitudinal rows with four or five spiniform setae. Distitarsus divided into three tarsomers (I> II <III), length of tarsomere I equal or greater than II + III. Tarsomere I with two longitudinal rows with eight spiniform setae. Tarsomeres II and III similar to previous, but with three and four setae, respectively.

Sternite (Figs 1F, 2F). Genital plate about 1.5 times wider than long, with irregularly distributed setae and accumulated pores on sides. Other ventrites mostly smooth.

Male Gonopod (Figs 1H; 3B, C, 3D). **LoL1** broader than long, reniform, with thin longitudinal sclerotized wrinkles, slightly curved and sinuous in terminal portion (Fig. 1H); **Fi** with sclerotized borders and inverted trapezoid shape with rounded edges. **LoD** with strongly sclerotized acute projection positioned above all other gonopod structures. **LoL2** globose, soft, partially covered by **LoL1**; **LaM** as two parallel plates originating in **Me** and supported by **Fu** (Fig. 3C). **Me** subtriangular and covered by denticles (Fig. 3D). ***Female Gonopod*** (Fig. 2H) with seminal receptacle (**RS**) of bulbous shape, with base slightly narrower than more dilated distal portion; longer than wide; concave chitinous arc with two sclerotized chitinous rings at base of **RS**. Two well-sclerotized structures on sides of chitinous arch, very long and thin, slightly curved inwards, with base wider than apex.

Measurements. (holotype male before brackets, variation inside brackets).

Prosoma: 13.6 mm (length) [12.0–13.6 mm], 8.0 mm (width) [7.1–8.0 mm]; Opisthosoma: 19.4 mm (length) [17.5–19.4mm], 10.7 mm (width) [8.3–10.7mm]; **Pedipalp:** Trochanter: 4.2 mm [3.7–4.2 mm]; Femur: 4.0 mm [4.0–4.4mm]; Patella: 5.4 mm [4.8–5.4 mm]; Patellar apophysis: 4.4 mm [4.0–4.4 mm]; Tibia: 4.1 mm [3.0–4.1 mm]; Tibial apophysis: 1.7 mm [1.6–1.8 mm]; Tarsus: 3.1 mm [2.6–3.1 mm].

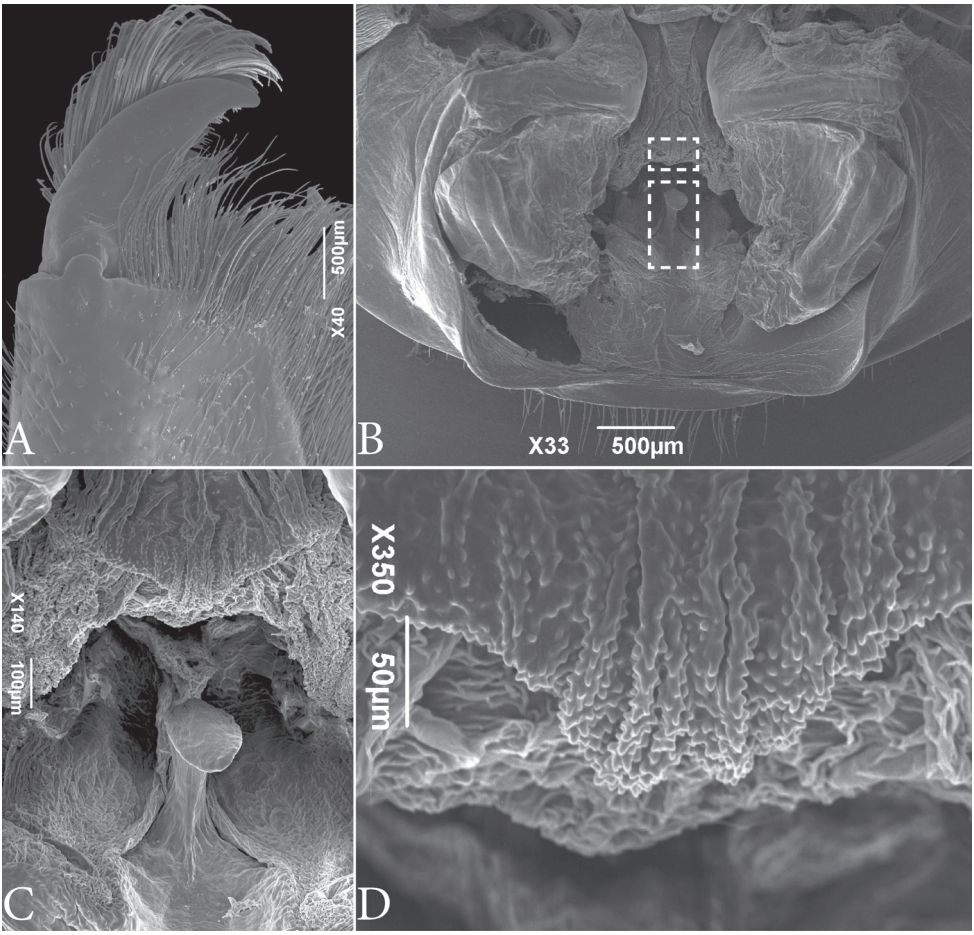

Figure 3. SEM images of *Typopeltis laurentianus* sp. n., paratype (male). **A** Chelicerae (right) **B** gonopod. Details in dashed rectangles are shown in images **C** (lower rectangle) and **D** (upper rectangle). **C** Fulcrum (**Fu**) detail **D** Mensa (**Me**) detail.

Typopeltis guangxiensis Haupt & Song, 1996

Studied material. CHINA: Guangxi: Nanning: Gao Feng Park, 22.955023, 108.365636, 136 m, 13.vii.2016, leg. A. Giupponi, A. Kury, I. Kury & C. Zhang (3 females, MNRJ 08249); same locality, 13.vii.2016, leg. A. Giupponi, A. Kury, I. Kury & C. Zhang (3 juvenile males, 2 juvenile females, MNRJ 08250). Fangshenggang: Shi Wan Danshan National Park, 21.90538, 107.90366, 276 m, 11–12.vii.2016, leg. A. Giupponi, A. Kury, I. Kury & C. Zhang (1 male, MNRJ 08251); same locality, 11–12.vii.2016, leg. A. Giupponi, A. Kury, I. Kury & C. Zhang (2 juvenile males, MNRJ 08252).

Emended diagnosis (after Haupt 1996). Males (about 30 mm in total length without flagellum and chelicerae) larger than females (see measurements); very long patellar apophysis with narrow base and almost straight, blunt tip with three small blunt terminal projections. Male gonopod trapezoidal, **LoL1** reniform with longitudinal sclerotized

streaks, **Me** square, covered by denticles, with four longitudinal crests partially formed by collapsed spines (observable only in SEM). Female gonopod with bulbar **RS** with wide base and well-marked **CCh** with large bevel in upper inner portion; **ACh** concave.

Description. ***Colouration (in alcohol).*** Male blackish red, carapace colour becoming lighter from anterior to posterior region. Pedipalps darker in relation to body; legs II–IV lighter compared to carapace. Middle eyes black; lateral eyes yellow. Females slightly lighter than males, more reddish in general.

Carapace (Fig. 4A). Granules without specific pattern. Keel present between median and lateral eyes. Deep line flanked by granules from posterior region of median eyes to slightly above fovea. Chelicerae similar to *T. laurentianus*, but setae apparently thinner and denser ventrally. Chelicerae claw curved inwards, with thick base and narrow apex, with keel longer than half-length of chelicerae claw (Fig. 5A).

Sternum (Fig. 4B). Typical tri-segmented sternum; inconspicuous mesosternum.

Tergites (Fig. 4E, G). Acute granules present at posterior border of each tergite, absent in small central region of posterior border of tergites I–V. Ommatoids subcircular.

Pedipalps (Fig. 4C, D). **Coxa** covered in setae, with higher concentration in ventro-apical and latero-apical portions. **Trochanter** dorsally armed with 5 spines; spines I, II, III increasing in size, facing inwards; spine IV more than twice larger than others, rhomboid and paired with spine III in apical position; spine V smallest, rhombus (I <II <III <IV> V) (Fig. 4C). Two short, broad-based subequal spines ventrally (Fig. 4D). **Femur** dorsal face covered in thick granules, with few ectal setae and single reduced mesal spine (Fig. 4C). One ectal-ventral spine much larger than dorsal one (about four times) (Fig. 4D). **Patella** dorsal face with many pores with no apparent pattern, patellar apophysis slender, with small spines on anterior face, enlarged apex with three globular expansions (Fig. 4C). Ventral face without spines (Fig. 4D). **Tibia** dorsally armed with large conical apophysis with sharp tip, slightly smaller than tibia, covered in setae, with two longitudinal series of small spines, dorsal series with eight small spines, ventral series with fourteen spines (Fig. 4C). Ventrally armed with two ectal conical spines, distal almost double the size of subdistal (Fig. 4D). **Tarsus** armed dorsally with longitudinal series of fourteen small spines (Fig. 4C), armed ventrally with ten spines (Fig. 4D).

Leg I (antenniform) with nine tarsomers, first very short (as small ring); second, third and last tarsomers longer than others. Number of tarsomeres may vary if there is regeneration of tarsomeres. Apical portion of tibia with two dorsolateral trichobothria, absent on femur and patella. Femur covered with thick granules, patella and tibia with smooth appearance. All articles covered with setae, dorsally and ventrally. ***Legs II–IV.*** Trochanter and femur with granules. Coxa, tibia and tarsus smooth, last two with concentrated setae. Dorsal-apical trichobothrium present on tibia, thin acuminate spur on ventro-apical region of tibia. Basitarsus with two spurs, one mesal and one ectal; ventral region with two longitudinal rows of five spiniform setae. Distitarsus divided into three tarsomers (I> II <III), I equal or greater than II + III. Tarsomere I with two longitudinal rows of nine to ten spinniform setae each. Tarsomeres II and III with similar structure, but with three and four setae, respectively.

Sternites (Fig. 4F). Genital plate wider than long (one and a half times wider than long). Sternite mostly smooth, with granules concentrated on sides. Sternite II with

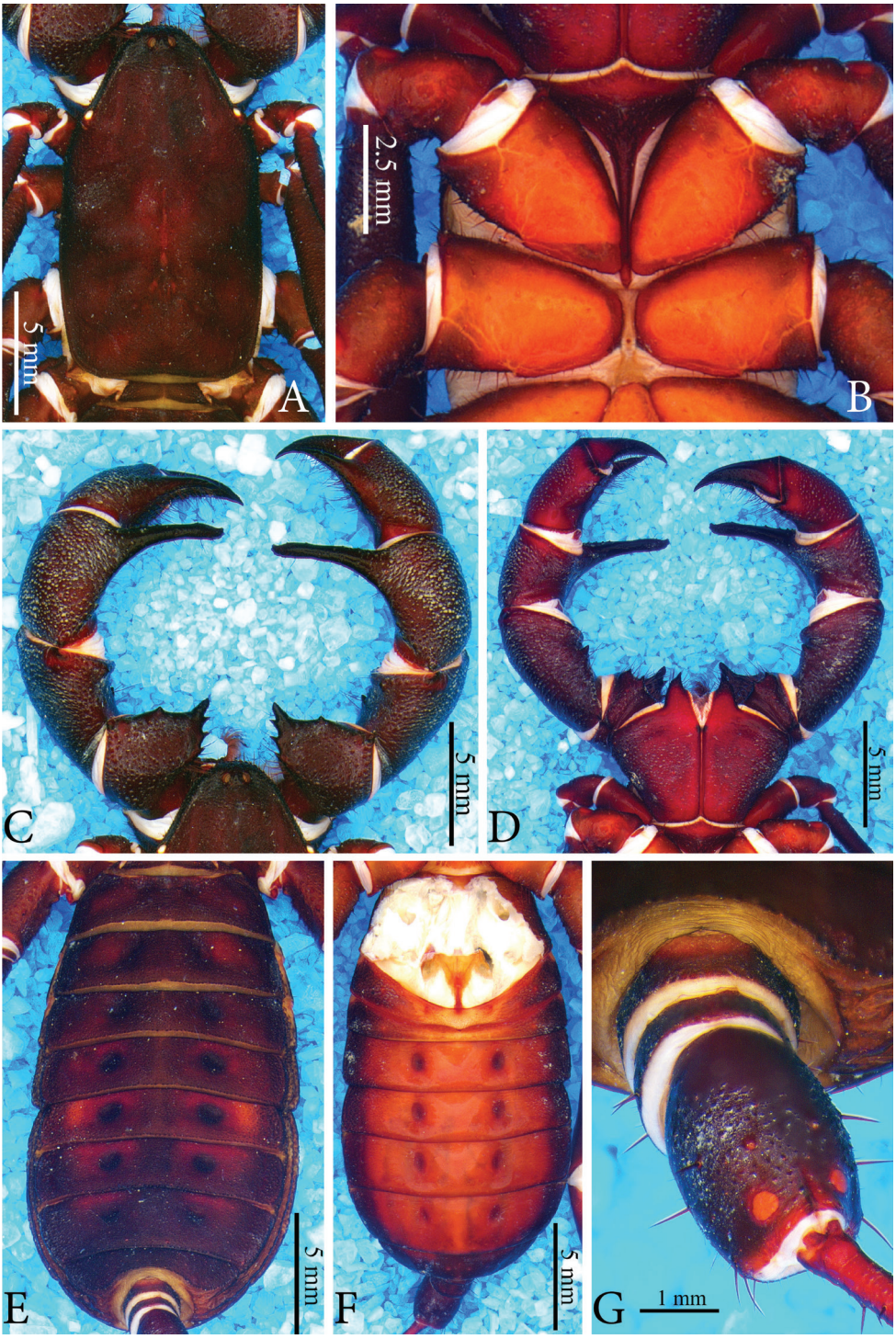

Figure 4. *Typopeltis guangxiensis* (male). **A** Carapace **B** sternum **C** pedipalps (dorsal) **D** pedipalps (ventral) **E** opisthosoma (dorsal) **F** opisthosoma (ventral) **G** ommatoids.

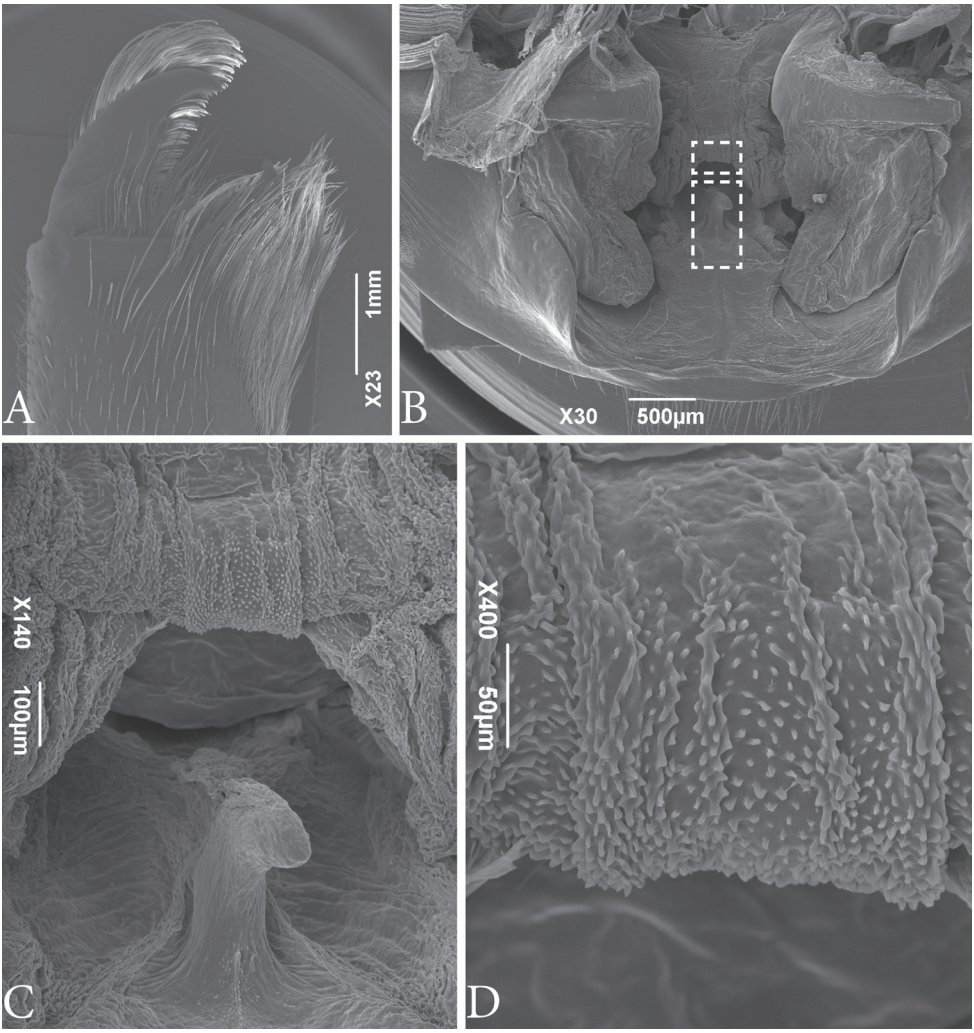

Figure 5. SEM images of *Typopeltis guangxiensis* (male). **A** Chelicerae (right) **B** gonopod. Details in dashed rectangles are shown in images **C** (lower rectangle) and **D** (upper rectangle). **C** Fulcrum (**Fu**) detail **D** Mensa (**Me**) detail.

large number of setae and central acute granule on posterior margin. Flagellum with thirty-eight articles.

Male gonopod (Fig. 5B, C, D). **LoL1** broader than long, reniform, with thin longitudinally sclerotized striations, slightly curved and sinuous in terminal portion; **Fi** with sclerotized borders, with inverted trapezoid shape with rounded edges. In basal portion, **Fi** and **LoD** are not fully fused. **LoL2** globose and partially covered by **LoL1**; **LaM** as two parallel plates, originating in **Me** and supported by **Fu** (Fig. 5C). **Me** square, covered by denticles, with four longitudinal crests formed partially by collapsed spines (Fig. 5D). ***Female gonopod*** (Fig. 6H), **RS** of bulbous shape, with base narrower than more dilated portion, not much longer than wide; **ACh** chitinous, concave, with two **CCh** sclerotized

Figure 6. *Typopeltis guangxiensis* (female). **A** Carapace **B** sternum **C** pedipalps (dorsal) **D** pedipalps (ventral) **E** opisthosoma (dorsal) **F** opisthosoma (ventral) **G** ommatoids **H** gonopod. **CCh** = circulus chitinosus; **ACh** = arcus chitinosus.

at base of **RS**, with large chamber in upper inner portion. On sides of **Ach**, the two long and thin sclerotized structures (observed in *T. laurentianus* sp. n.) are absent.

Natural history. Collected on the ground of forested areas in the outskirts of Nanning (Guangxi, China), living under logs and stones in shady and humid places. The specimens were abundant in habitats associated with human disturbance, such as roadsides, trails, and abandoned constructions in the forest.

Measurements. (male)

Prosoma (length): 14.0 mm; Prosoma (width): 8.0 mm; Opisthosoma (length): 16.8 mm; Opisthosoma (width): 10.3 mm. **Pedipalp-** Trochanter: 4.3 mm; Femur: 3.5 mm; Patella: 5.6 mm; Patellar apophysis: 4.2 mm; Tibia: 4.0 mm; Tibial apophysis: 2.1 mm; Tarsus: 3.8 mm. **Genital Plate-** Length: 6.0 mm; Width: 9.0 mm.

Discussion

The taxonomy of Southeast Asian whip scorpions was greatly advanced by Haupt (1996, 2004a, b, 2009) and Haupt and Song (1996). However, due to the large geographical extension of the region, a lot of work still needs to be done to fully comprehend the diversity of thelyphonids in the area. The genus *Typopeltis* alone has a large distribution covering China, Japan, Laos, Russia, Taiwan, Thailand and Vietnam. Currently, only 14 species are known in the genus. *Typopeltis laurentianus* sp. n. is the third species of the genus described from Vietnam (Fig. 7). The others are *T. harmandi,* known only from females from the southern tip of the country, and *T. soidaoensis*, which has also been recorded from Thailand.

Typopeltis laurentianus sp. n. differs from the others in the genus by the unique shape of the patellar apophysis of the male. In *T. soidaoensis* the apophysis is thinner, tapering uniformly, with a dorso-ventral curvature and small apical digitiform structures (see Haupt 1996: fig. 1d), whereas in the species from southern China, *T. guangxiensis* and *T. cantonensis*, the apophysis is straight, thin and has a blunt tip armed with apical denticles (Fig. 4C, D for *T. guangxiensis*; see Haupt and Song 1996: fig. 2d for *T. cantonensis*). In *T. magnificus*, which occurs on the Laos border, the apophysis is similar to that of *T. soidaoensis*, but it is thinner and longer. Females in general have thinner patellar apophyses than males (see Haupt 2004: figs 1, 3).

Typopeltis commonly have a notable expansion in the terminal region of the patellar apophysis of the male pedipalps (secondary sexual dimorphism), which is generally armed with spines, digitiform projections or large granules (see *T. crucifer*, *T. dalyi*, *T. niger*, *T. stimpsonii*, and *T. tarnanii*) (Haupt 1996, Haupt and Song 1996). The other species, despite having some type of apical structure in the patellar apophysis of males, do not have the pronounced expansion. *Typopeltis laurentianus* sp. n., on the other hand, has a great median expansion, located only on the external margin of the apophysis, like a large hump, in addition to having an unprecedented smooth texture. The apophysis presents a slight curvature in the final portion in the ventral direction (Fig. 1C, D), as was observed in *T. kasnakowi* and *T. vanoorti* (Haupt 1996, Haupt and Song 1996),

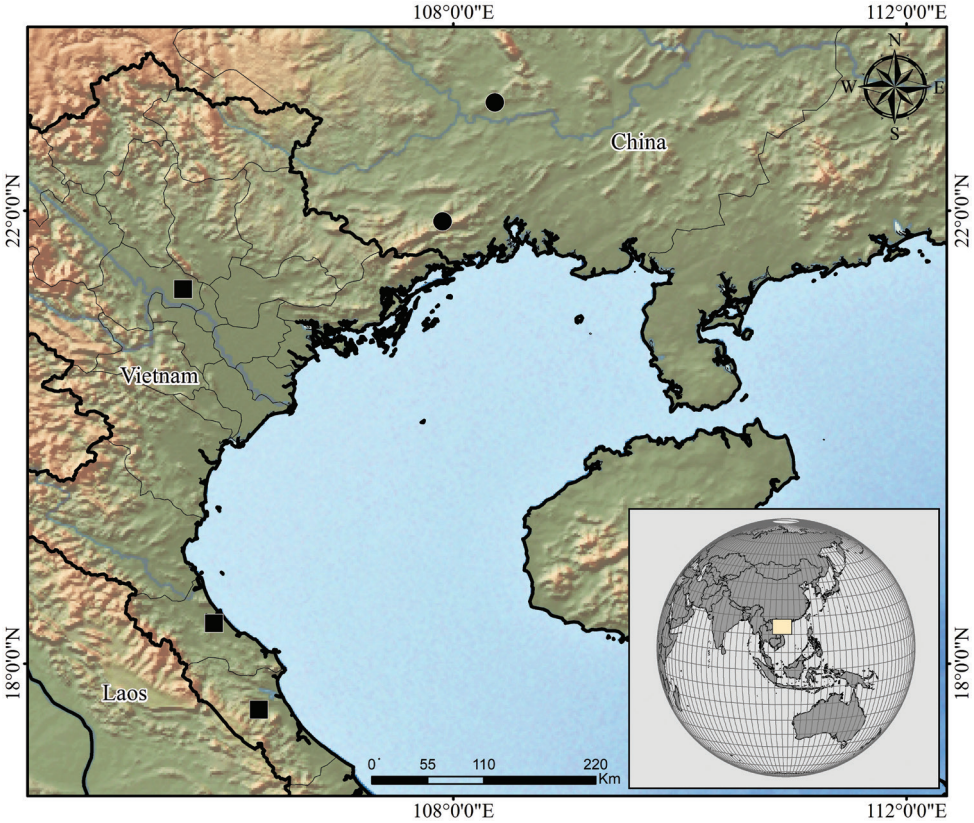

Figure 7. Distribution map of *Typopeltis laurentianus* sp. n. (black squares) and *T. guangxiensis* (black circles).

where only the terminal region is curved. *Tylopeltis stimpsonii*, *T. soidaoensis*, *T. magnificus* and *T. tarnanii* differ from the new species by having a more pronounced ventral curvature that is not restricted to the terminal portion. According to Haupt (1996) and Haupt and Song (1996), *T. crucifer* has the curve facing the tibia, while *T. cantonensis* has a straight apophysis, characteristic also for *T. guangxiensis* (Fig. 4C, D). The species *T. amurensis*, *T. harmandi* and *T. kasnakowi* are only known from female specimens.

The keel of the carapace in *T. laurentianus* sp. n. does not reach the front of the middle eyes (Fig. 1A), a character described in Haupt (1996) and Haupt and Song (1996) for most species of *Typopeltis,* with the exception of *T. dalyi*, *T. crucifer*, *T. niger* and *T. stimpsonii*. In *T. magnificus*, Haupt (2004) does not comment on whether or not the keel reaches the median eye tubercle, but the illustration shows that it does not reach.

Male gonopods in Pedipalpi (Schizomida, Thelyphonida and Amblypygi) are formed by soft structures used to shape the extruded spermatophore. Those structures were studied in detail in amblypygids by Giupponi and Kury (2013), but are barely known in the other groups. Here we presented details of the male gonopod of thelyphonids for the first time with SEM images. This allowed us tentative homologies of the male genitalic structures between the two orders based on their position and shape

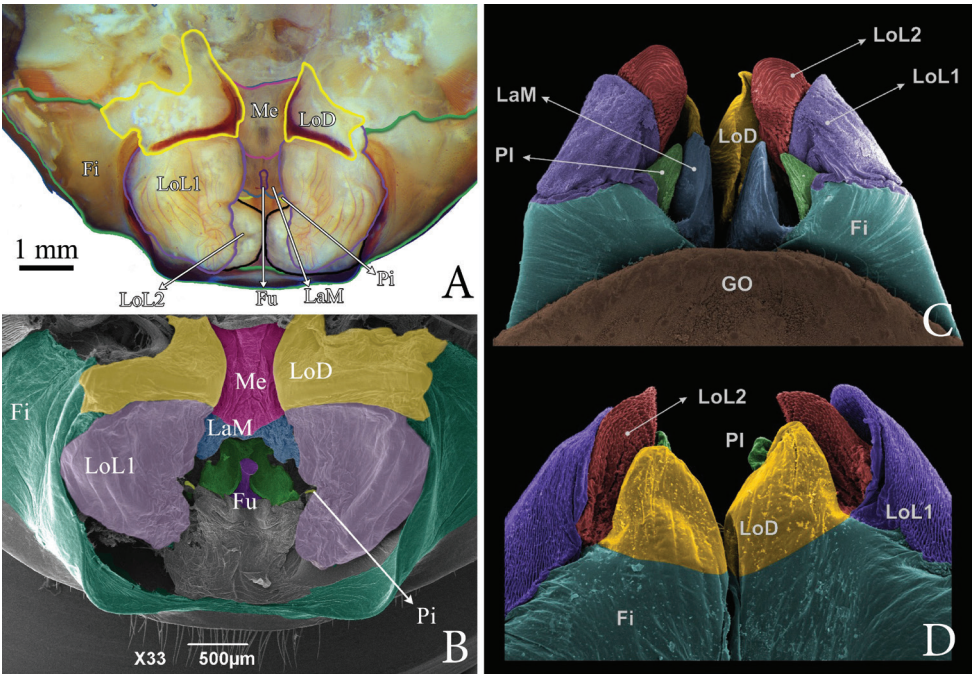

Figure 8. Comparison between gonopods of *T. laurentianus* sp. n. (**A, B**) and *Heterophrynus* sp. Pocock, 1894 (**C, D**). **A** Male gonopod of *T. laurentianus* in posterior-dorsal view **B** SEM of male gonopod of *T. laurentianus* in posterior-dorsal view **C** SEM of male gonopod of *Heterophrynus* sp. in ventral view **D** SEM of male gonopod of *Heterophrynus* sp. in dorsal view. **Fi** = fistula; **GO** = genital operculum; **LaM** = lamina medialis; **LoD** = lobus dorsalis; **LoL1** = lobus lateralis primus; **LoL2** = lobus lateralis secundus; **PI** = processus internus; **Me** = Mensa; **Fu** = Fulcrum.

(Fig. 8). The most external and fleshy tubes with a smooth surface are recognized as being a homologue of the *fistula* (**Fi**) of amblypygids. In thelyphonids, the distalmost apex of the *fistula* is chitinized and projects posteriorly. Dorsal to the **Fi** is a pair of chitinized projections, the *Lobus dorsalis* (**LoD**), that cover the other gonopod structures. Different from amblypygids, the **LoD** in Thelyphonida does not form a tube and does not cover the gonopod completely dorsally, thus allowing us to see the other parts of the gonopod. On the tip of the **Fi** two telescoping soft bodies are projected; they are the *Lobus lateralis primus* (**LoL1**), which has sclerotized wrinkles in its surface. Beneath **LoL1**, a pair of smooth soft bodies are present, the *Lobus lateralis secundus* (**LoL2**). Inner to **LoL2**, there is a soft blade from the *fistula*'s median-dorsal part, which is preceded by a leaf-like lamina, the *Lamina medialis* (**LaM**). Male gonopods of whip scorpions have structures unique to the group, such as the *Fulcrum* (**Fu**) and *Mensa* (**Me**). The **Fu** is sclerotized and seems to be a supporting structure. The **Me** covers the upper part of the **LaM**.

As far as we know, there are no published images of male gonopods of *Typopeltis*. Haupt (2009) published images of the gonopod of other genera of Thelyphonida and studied the structure superficially; in that work the sclerotized region here named LoD

was called "cuticular clasp". We prefer not to use the same name because the chitinized region appears to be stationary, and therefore does not work as a clasp. Additionally, no gonopod characters were used for taxonomy by Haupt (2009). In the SEM images of the gonopod illustrated here it is possible to identify ultrastructures not observable in traditional microscopy (Figs 3B, C, D; 5B, C, D; 8), such as the **Fu** and **Me**. While **Fu** seems to be a supporting structure of the **LaM** (which in the SEM images is collapsed, thus allowing the **Fu** to be observed), apparently it does not show differences between the two studied species. In the case of **Me**, there is a noticeable difference in integument texture and shape between the two species. In *T. guangxiensis* the general form of **Me** is similar to a square with four crests of rhombus spines separated by three areas of lamellar spines. In *T. laurentianus* sp. n. **Me** is subtriangular with more pronounced ridges, blunt, and often with paired teeth.

Females of *Typopeltis* do not have secondary sexual characters and are more homogeneous morphologically than the males. The identification of species based only on females is, therefore, more difficult. Additionally, informative diagnostic structures (such as the gonopod) are rarely depicted in scientific papers, making it even harder to use females to separate species. Only the female gonopods of *T. guangxiensis* and *T. crucifer* are known in the literature (Haupt and Song 1996, Haupt 2009). In some cases, it is possible to separate the females by evident characters of external morphology, such as the spines of the patellar apophysis on the pedipalps. In *T. laurentianus* sp. n. the mesal face has a row of spines with a distinct size relation (see description); on the ectal face, it has only one spine in the final third, besides the second dorsal-patellar spine that is practically at the base of the apophysis (Fig. 2C, D). *Typopeltis guangxiensis* (Fig. 6) has a particular size relation of spines on the mesal part of the patella; additionally, besides the two usual spines, females have two smaller (smaller than half of the other two) spines in the middle third (Fig. 6C, D). *Typopeltis magnificus* also has a specific size pattern in the mesal row; in the ectal face it has a third spine subequal to the others, in the medial third of the patellar apophysis.

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Description of a new species of *Rhinocoeta* Burmeister, 1842 (Scarabaeidae, Cetoniinae) from the South African Northern Cape

Renzo Perissinotto[1]

1 *School of Environmental Sciences, Nelson Mandela University, P.O. Box 77000, Port Elizabeth 6031, South Africa*

Corresponding author: *Renzo Perissinotto* (renzo.perissinotto@mandela.ac.za)

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Abstract

Rhinocoeta namaqua **sp. nov.** is recognised as a separate species from its closest relative, *R. cornuta* (Fabricius, 1781) after a review and close analysis of specimens recently collected in the semiarid region of the Northern Cape Province, South Africa. The new species can be readily separated from *R. cornuta* by the drastically reduced tubercle and associated depression on its anterior pronotal margin, particularly in the male. In addition, the general body shape of *R. namaqua* is more globose than that of *R. cornuta*, its average total length is larger, and its elytral costae are generally reduced and poorly visible, particularly at the level of the umbones. These characteristics make it practically impossible to separate the two sexes of *R. namaqua*, without inspection of the internal reproductive organs, as their external morphologies are virtually identical, unlike in *R. cornuta*. Finally, the aedeagal parameres of *R. namaqua* exhibit a narrower apex than those of *R. cornuta* and, in particular, lack the subapical hook-shaped lateral expansions that are so typical of all the other *Rhinocoeta* s. str. species. The new species appears to be restricted to specific bioregions of the Succulent and Nama Karoo biomes of the Northern Cape, and like all other species of the genus is generally found on or under mammal herbivore dung. Adult activity is limited to short periods immediately after rainfall events, during which individuals fly around and mate, but do not feed on either fruits or flowers.

Keywords

Afrotropical region, Cetoniinae, new species, Northern Cape, *Rhinocoeta,* Xiphoscelidina

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Introduction

The genus *Rhinocoeta* Burmeister, 1842 currently includes two subgenera, the nominal one with five recognised species and *R.* (*Haematonotus*) Kraatz, 1880 with three species (Holm and Marais 1992, Beinhundner 2017). On the basis of adult and larval morphology, it has been argued that the genus may phylogenetically be placed close to the subtribe Xiphoscelidina rather than the Cetoniina, and that it constitutes part of a relict lineage derived directly from the most primitive Cetoniinae (Krikken 1984, Holm 1992, Smith et al. 1998). However, preliminary genetic DNA analyses seem to indicate that it may actually be closer to the more modern Cetoniina than previously believed (Kouklík 2017, Šípek et al. 2016). Consequently, their perceived "relictual/primitive" characters may actually represent adaptations to their peculiar habitat (e.g., droughts, extreme seasonality, scarce vegetation) and to their unique lifestyle, characterized by short bursts of activity and inability to feed at the adult stage (Smith et al. 1998).

Close analysis of a series of specimens collected during the past two decades in the Namaqualand and Upper Karoo regions of the Northern Cape, South Africa, has revealed that a new species previously confused with *R. cornuta* (Fabricius, 1781) occurs in these semiarid regions. None of the four synonyms used in the past to refer to *R. cornuta* can in fact be associated with the populations of the western part of the Northern Cape that constitute the new species. High resolution photos, collection data and circumstantial evidence obtained from the ZMUK (Kiel, Germany), BMNH (London, UK), MNHN (Paris, France), have led to the conclusion that *Cetonia cornuta* Fabricius, 1781, *Scarabaeus arcas* Olivier, 1789, *Cetonia cornigera* Gmelin (in L.), 1790 and *Scarabaeus hispidolugubris* Voet, 1779 all fit the typical characteristics of the Cape south-western populations of *R. cornuta*. This is also consistent with the period of their description, the late 18th century, which coincided with the early exploration of the subcontinent. It would have been virtually impossible for the collectors of that period to have ventured beyond the colonial settlement of Cape Town and its immediate surroundings on the south and west coasts. Indeed, the remote Northern Cape populations remain very poorly sampled even in the modern era, with only approximately 20 specimens currently known for the new species here described.

Extensive observations made recently throughout the southern African region have also allowed for a better resolution of the distribution range and, particularly, the ecology of all the species of the nominal subgenus *Rhinocoeta*. This has prompted a reanalysis of the taxonomic position of *R. limbaticollis* (Péringuey, 1907), a rather enigmatic "species" described at the turn of the 19th century on the basis of one female specimen only.

Materials and methods

Specimens for this study were obtained through direct collections in the field during the period 1996–2018 (R Perissinotto and L Clennell legit), or from museum and private collections (as per list provided below). Fresh specimens were either caught in

flight using standard nets after rainfall events, or collected on or under dung accumulations of a variety of herbivorous mammal species. Holotype, lectotype, paratypes, and other specimens of *R. cornuta* were analysed from high-resolution photographic material submitted by the museum curators listed in the Acknowledgement section.

For the description of morphological characters, the terminology used by Krikken (1984) and Holm and Marais (1992) is followed in this study. Specimen total length and maximum width were measured using a Vernier calliper, from the anterior margin of the clypeus to the apex of the pygidium and at the widest point of the elytra, respectively. Photos of specimen dorsal and ventral habitus were taken with a Nikon CoolPix S9700 digital camera with macro setting, while photos of the male genitalia were obtained using a Nikon DigitalSight DS-Fi2 camera attached to a Nikon SMZ25 dissecting microscope. The background was removed from the photos using Microsoft Word 2010 (Picture Tools), in order to increase clarity of resolution. The Combine ZP Image Stacking Software by Alan Hadley (alan@micropics.org.uk) was used to obtain z-stacking composite images.

Repositories are abbreviated as follows:

BMPC Jonathan Ball and Andre Marais Private Collection, Cape Town, South Africa
BMNH Natural History Museum, London, United Kingdom
BMSA The National Museum, Bloemfontein, South Africa
GBPC Gerhard Beinhundner Private Collection, Euerbach, Germany
ISAM Iziko South African Museum, Cape Town, South Africa
ISNB Institut Royal des Sciences Naturelles, Brussels, Belgium
MNHN Muséum National d'Histoire Naturelle, Paris, France
SANC South African National Collection of Insects, Pretoria, South Africa
TGPC Thierry Garnier Private Collection, Montpellier, France
TMSA Ditsong National Museum of Natural History (formerly Transvaal Museum), Pretoria, South Africa
ZMUK Zoologisches Museum der Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Data on distribution, period of adult activity, and other biological information for *R. cornuta* were also obtained from Péringuey (1907), Holm (1992), Allard (1991), Sakai and Nagai (1998) and Beinhundner (2017). The key abbreviations for the provinces of the Republic of South Africa used within the text are as follows:

EC Eastern Cape
FS Free State
NC Northern Cape
WC Western Cape

Taxonomy

***Rhinocoeta namaqua* sp. nov.**
http://zoobank.org/AB13CDDD-DE3E-48F7-81C1-D1202F91E6A3
Figures 1, 3, 5

Diagnosis. The two species can be separated mainly on the basis of the male parameres, which in *R. namaqua* lack the spine-like expansions on the apico-lateral margins that are so typical of the parameres of *R. cornuta* (compare Figs 3 and 4).The new species can also be recognised by the virtual absence of the depression behind the pronotal tubercle, which on the other hand is very prominent in *R. cornuta*, especially in the male (compare Figs 1 and 2). The pronotal tubercle of *R. namaqua* is very short and blunt in both sexes, with the apical surface virtually flat (Fig. 1A). Conversely, in *C. cornuta* this is generally quite elevated in the male and in both sexes the apex is generally smoothly rounded (Fig. 2A). Essentially in *R. namaqua* it is very difficult, if not impossible, to separate males from females on the basis of external morphology alone, as the pronotal tubercle is similarly reduced and the associated depression is lacking in both sexes. These characters are, on the other hand, key towards the separation of the sexes in *R. cornuta*.

Further to this, the two closely related species also exhibit differences at the level of the elytral costae three and five which, with some notable exceptions, are largely obsolete in most specimens of *R. namaqua* but still noticeable in *R. cornuta*, at least in the proximal two thirds of the elytra, above the apical umbone (compare Figs 1 and 2). Additionally, the body shape of *R. namaqua* is remarkably more globose and its average length larger than that of *R. cornuta*, i.e., 20.3–24.6 mm versus 12.5–22.5 mm (Holm 1992, Beinhundner 2017), respectively.

Description of holotype male (Figs 1A–E, 3A–C). *Size*. Length 22.2; width 14.1 mm.

Body. Completely black and generally matte, with small shiny areas restricted to elytral suture, basal portion of costae, humeral callus and peri-scutellar area (Fig. 1A); globose with dense sculpture throughout dorsal surface and short, scattered yellowish setae on dorsal periphery, becoming longer and denser on lateral margins (Figs 1A, C, D).

Head. Black with dense but coarse sculpture throughout surface and poorly elevated vertical, median ridge on frons; with medium to long yellow-brown setae on frons, eye canthus and antennal pedicel and basal margin; clypeus bilobate and deeply concave, with lateral margins elevated but straight posteriorly and smoothly rounded anteriorly; antennal clubs black to dark brown, of normal cetoniine length, slightly longer than flagellum; pedicel black but flagellum dark brown.

Pronotum. Completely black, matte and virtually semicircular in shape, with apically flat tubercle at anterior margin and smooth angles at postero-lateral margins, forming straight line in front of scutellum; peritubercular depression poorly developed and barely noticeable; small, scattered round punctures on disc, becoming larger,

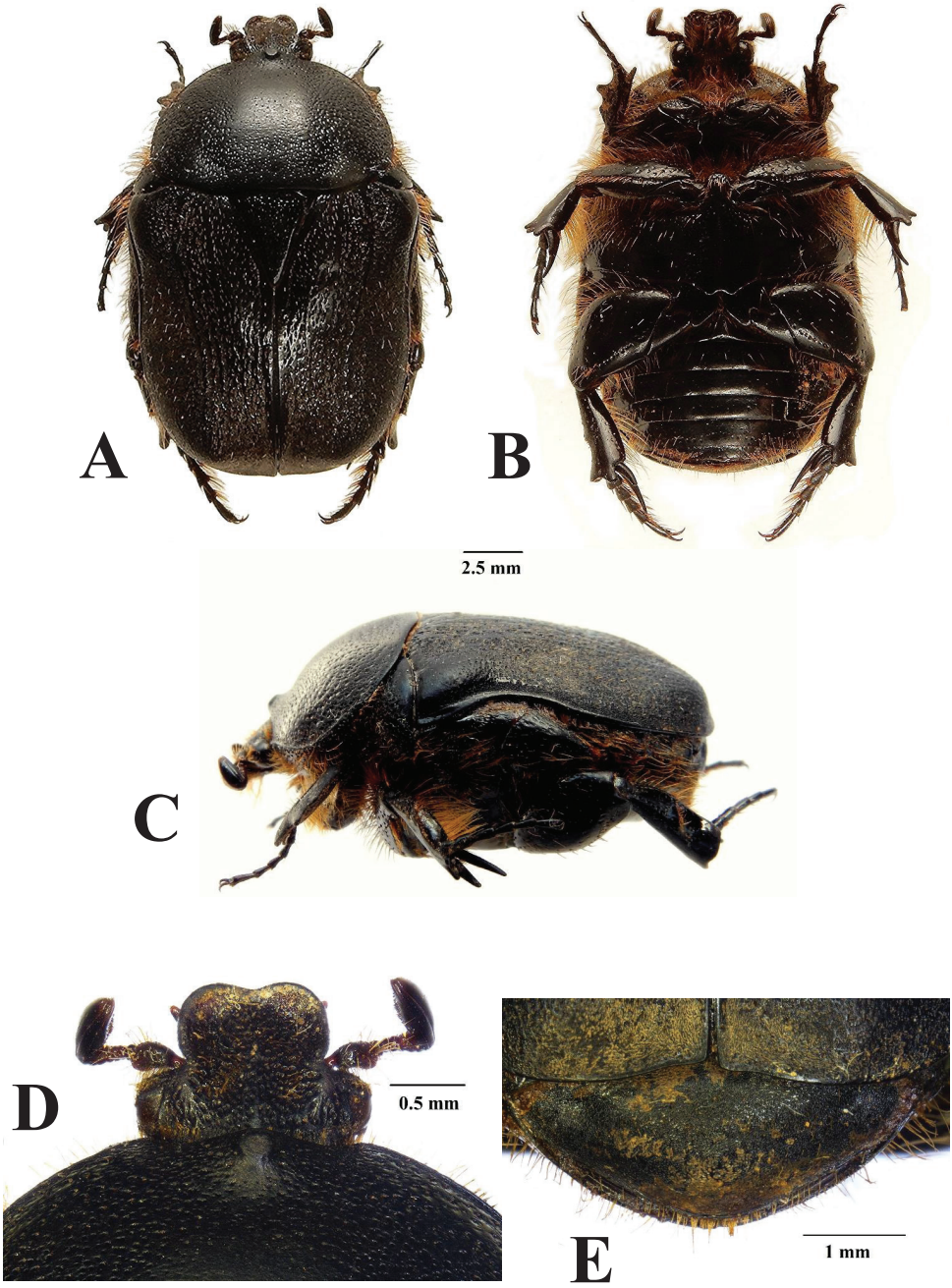

Figure 1. *Rhinocoeta namaqua* sp. nov: dorsal (**A**) ventral (**B**) and lateral (**C**) views of body habitus, with details of clypeus (**D**) and pygidium (**E**). Photographs by Lynette Clennell.

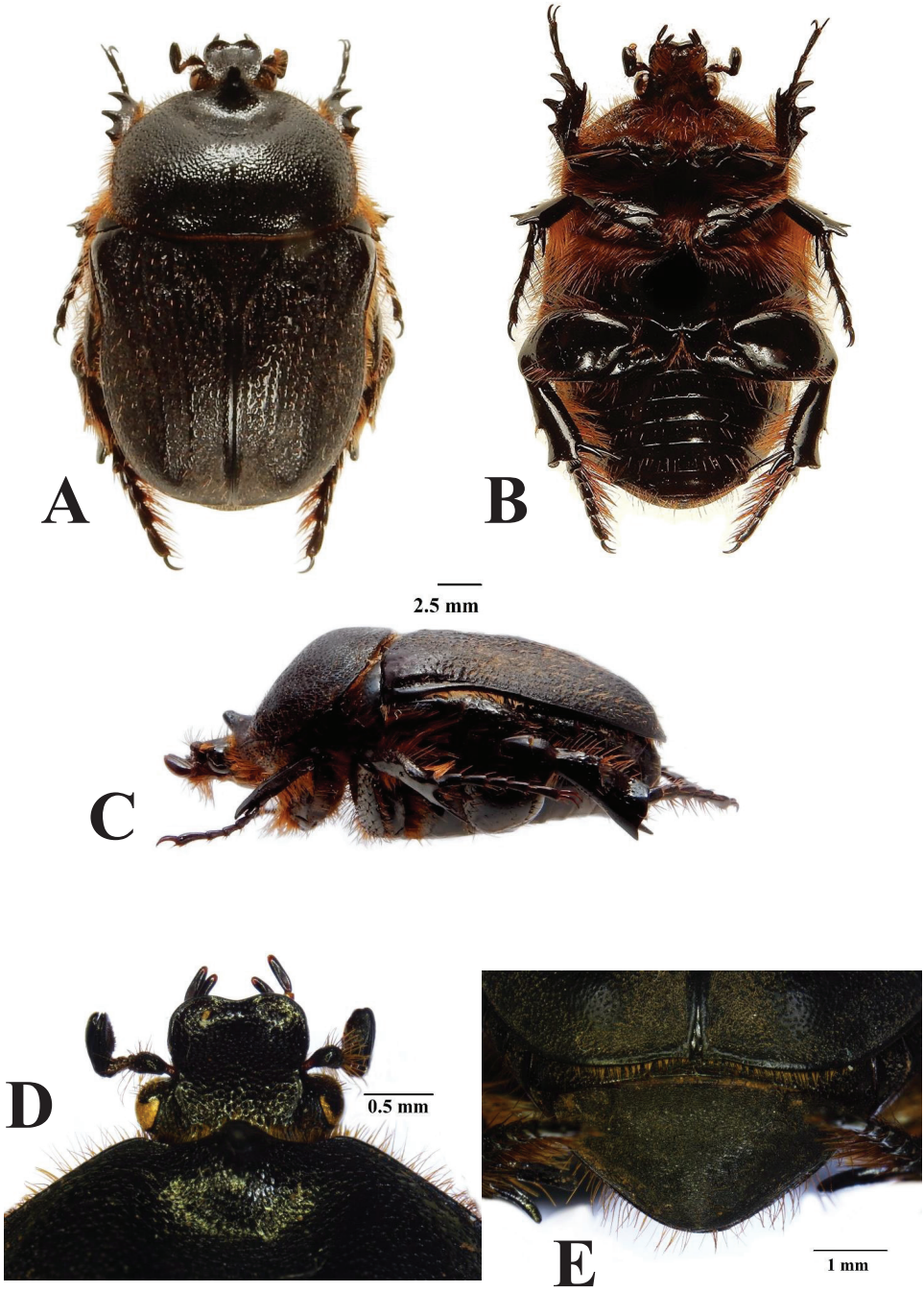

Figure 2. *Rhinocoeta cornuta* (Fabricius, 1781): dorsal (**A**) ventral (**B**) and lateral (**C**) views of body habitus, with details of clypeus (**D**) and pygidium (**E**). Photographs by Lynette Clennell.

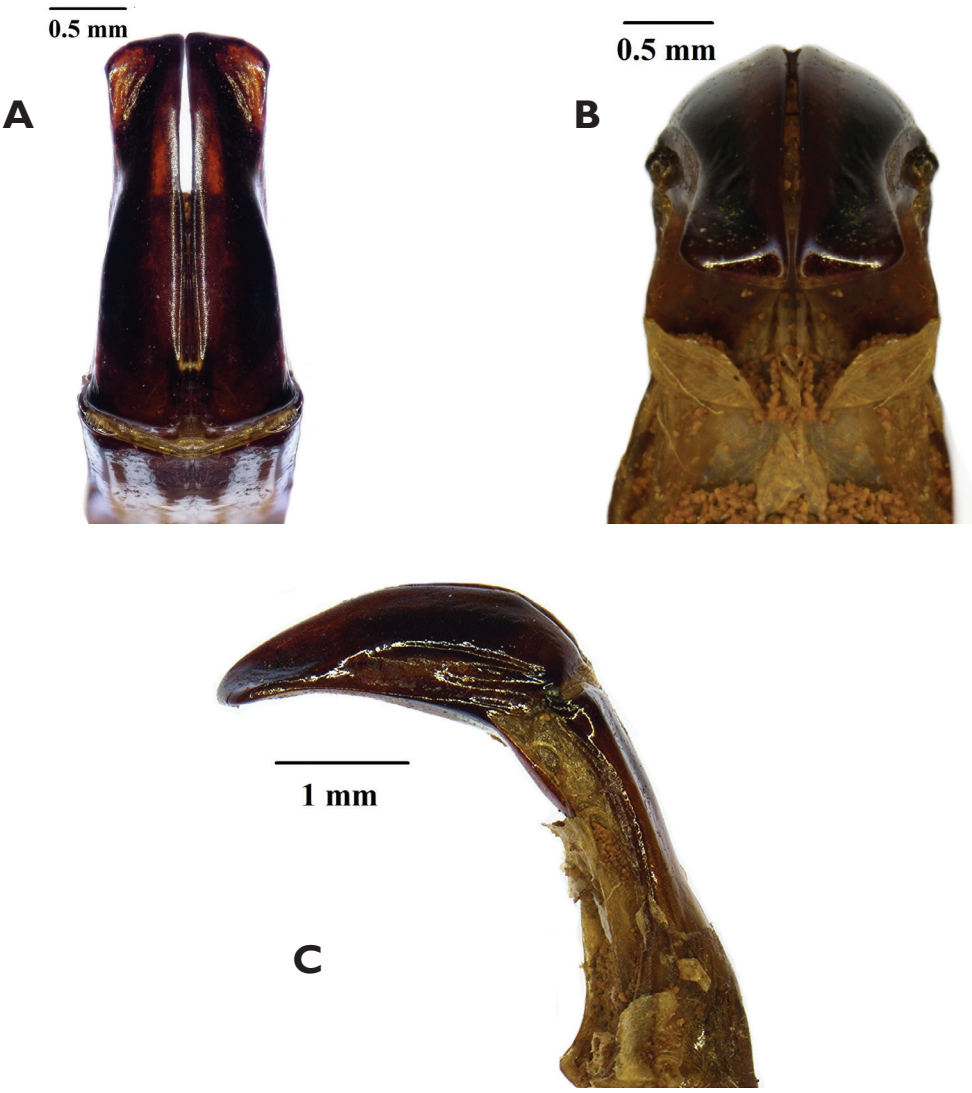

Figure 3. *Rhinocoeta namaqua* sp. nov: dorsal (**A**) frontal (**B**) and lateral (**C**) views of male aedeagus Photographs by Lynette Clennell.

denser and more elongate towards lateral and anterior margins; short, scattered yellow-brown setae on lateral and anterior declivities, becoming longer and denser at all margins except posterior (Figs 1A, C).

Scutellum. Black, isoscelic triangular with sharply pointed apex and deep but narrow lateral grooves; with scattered round to elongate punctures across the surface and short setae on basal margin only (Fig. 1A).

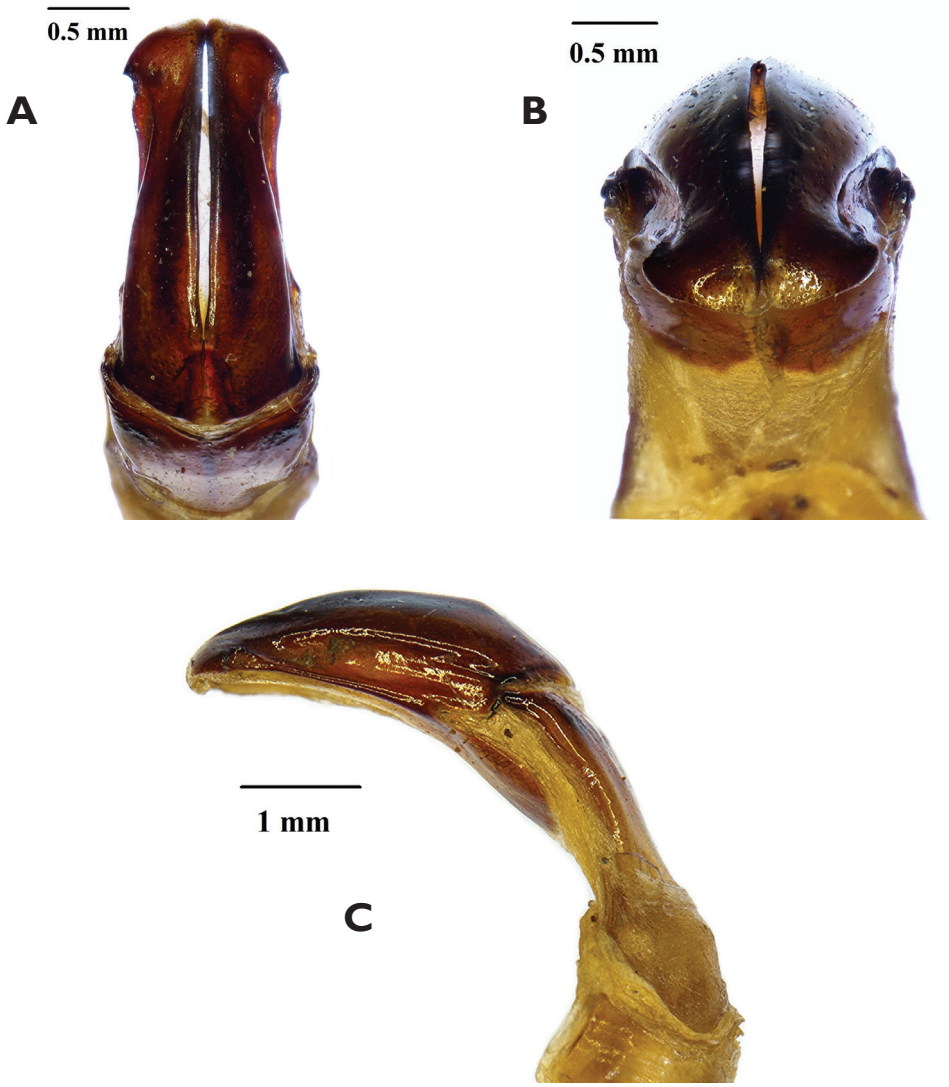

Figure 4. *Rhinocoeta cornuta* (Fabricius, 1781): Dorsal (**A**) frontal (**B**) and lateral (**C**) views of male aedeagus. Photographs by Lynette Clennell.

Elytron. With costae barely visible and shiny around sutural, periscutellar area and two basal thirds of third and fifth costae; rest of surface matte and densely sculptured with round to vertically elongate punctures, becoming rugose on lateral and apical declivities; with short, erect tawny-coloured setae scattered regularly across whole surface, except periscutellar area and umbones; with apices smoothly rounded and matching perfectly at sutural joint, without significant gap or spinal projections; both humeral and apical calluses pronounced (Figs 1A, B).

Pygidium. Uniformly black, broadly elliptical with dense and fine rugose sculpture; with moderate central convexity and shallow, symmetric baso-lateral depressions; bearing thin, long setae along entire apical margin, with denser cluster around apical point.

Legs. Short and robust, with typical fossorial adaptations; tarsal segments moderately developed but tibiae thickened and expanded laterally, with several spurs, spines and denticles; protibia tridentate, with third tooth substantially reduced; mesotibia short, reinforced with mid outer ridge, three apical spines and two spurs; metatibia short but extremely robust, with supporting diagonal outer ridge, one hypertrophic spade-like spine and two thick spurs, with proximal spur hypertrophic and reaching half distance of total metatarsal length (Figs 1A, B, C); femora equally robust and expanded, reaching hypertrophy in metalegs.

Ventral surface. Black and shiny, but overwhelmingly covered in long, dense tawny-coloured setae, except on ventral side of femora, metasternum and abdominal sternites; exhibiting small and sparse round sculpture throughout surface; mesometasternal lobe smoothly rounded and poorly protruding anteriorly, with regularly spaced round punctures and thin setae emerging at their centre; abdominal sternites flat to very slightly depressed around middle.

Aedeagus. Parameres with dorsal lobes laterally expanded, covering completely ventral lobes in dorsal view (Fig. 3A); exhibiting constriction towards apical third, then expanding again at apex; apex flattening abruptly, with lateral corners sharp, but not exhibiting spinal protrusion (Figs 3A, B); duck-bill shaped and smoothly curved in lateral view (Fig. 3C).

Description of female. Superficially, there is virtually no sexual dimorphism in this species, as its male lacks the deep depression around the pronotal tubercle, and the tubercle itself is normally short and blunt. This contrasts markedly with *R. cornuta*, where the male typically exhibits a well-developed tubercle (often hypertrophic) surrounded by a wide and deep depression on the anterior margin of the pronotum (Figs 2A, C, D). As a result, males and females of *R. namaqua* can only be separated by using a suite of secondary characters, especially the generally protruding pygidium and the slightly more convex abdominal sternites in the latter sex. The meso- and metatibial spurs are also substantially shorter in the female than in the male counterpart, particularly the proximal ones. The female is also more deeply and densely sculptured on the dorsal area, particularly on the pronotum, where small round punctures are uniformly distributed across its surface.

Distribution. All known records are from areas situated above the South African Great Escarpment, in the Succulent and Nama Karoo biomes of the Northern Cape Province (Fig. 5). The specific bioregions included in its range are the Namaqualand Hardeveld, the Trans-Escarpment Succulent Karoo and the Upper Karoo, respectively (Mucina and Rutherford 2006). Thus, the species appears to be a specialist of arid to semiarid environments.

Biology. Larval development seems to be linked to sandy soils, generally in or around dry riverbeds or in alluvional or erosion deposits. Adults have often been col-

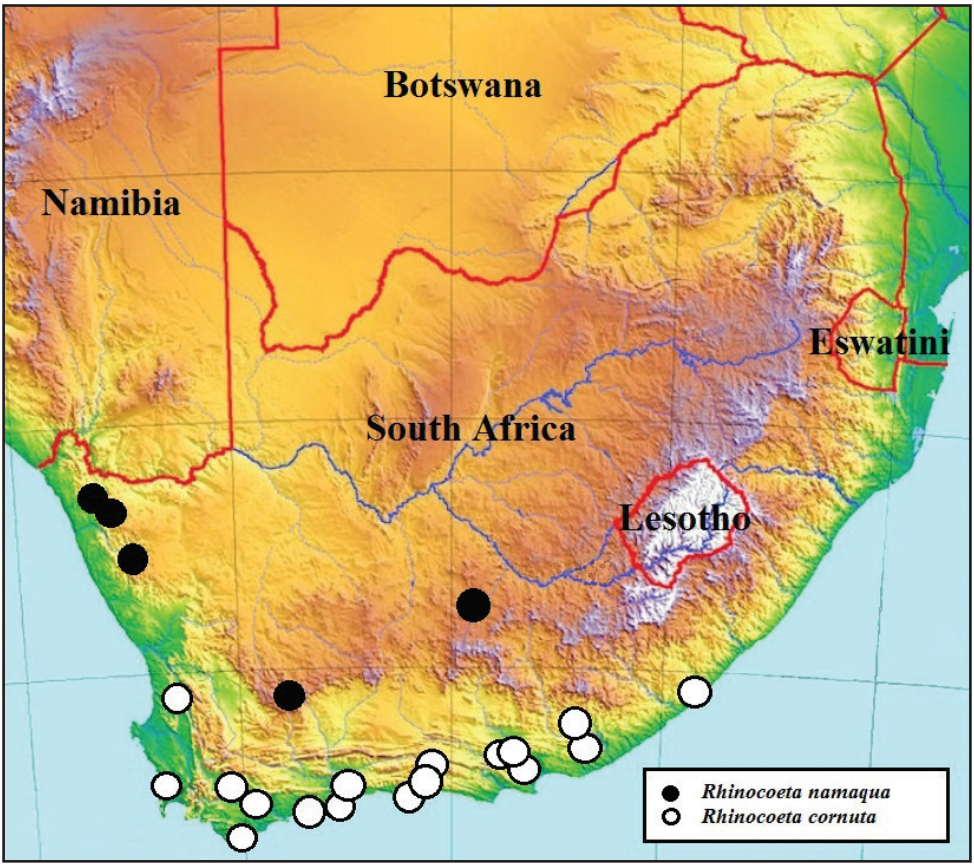

Figure 5. Known distribution range of *Rhinocoeta cornuta* (Fabricius, 1781) and *Rhinocoeta namaqua* sp. nov. within southern Africa (Map adapted from Mapsland: Copyright 2019 Mapsland).

lected in or under dung hills of herbivore mammals, including farmed goats and sheep. Its life cycle, therefore, appears to be similar to that of other, better-known members of the genus *Rhinocoeta*, e.g., *R. sanguinipes* (Smith et al. 1998), although its larval stages remain undescribed. Adult activity seems to be restricted to the austral summer, from December to March, and emergence from the soil has been observed to be linked to rainfall events (pers. obs.). No adult specimen has yet been recorded feeding, either on flowers, fruits or tree sapping flows and, thus, it is almost certain that their period of adult activity may be very short and sustained only by energy reserves accumulated during larval development (Smith et al. 1998, Perissinotto et al. 1999).

Derivatio nominis. The species is named after the semiarid Namaqualand region of South Africa (Northern Cape Province), where most known specimens were collected.

Remarks. There is no variability in the colouration within the series of specimens examined in this study. However, the elevation of elytral costae three and five varies substantially among specimens, with most exhibiting poorly elevated to obsolete costae, but a minority showing pronounced costae (e.g., paratype from De Aar). Within

the type series, the size ranges as follows: ♂ length 20.3 – 24.2 mm, width 13.2 – 14.4 mm (n = 10); ♀ length 23.3 – 24.6 mm, width 13.4 –14.6 mm (n = 6).

Type material. Holotype (♂): South Africa, NC, Goegap Nat Res, 30 Dec 1996, R Perissinotto & L Clennell (ISAM). Paratypes: 5♂♂ + 4♀, as above (BMPC); 1♂, South Africa, Northern Cape, Sutherland, Swaarweerberg 1683 m, 32°23'50.1"S, 20°34'39.3"E, 01 Jan 2008, AP Marais leg (BMPC); 3♂♂ + 2♀, South Africa, NC, Kamieskroon, 26 Jan 2018, R Perissinotto & L Clennell; 1 ind, South Africa, Northern Cape, Anenous, Namaqualand, 01/01/1911, CL Biden leg (ISAM: COL-A027336); 1♀, S.W. Africa, De Aar (50 milles au N d'Upington), 7-III-1950, H-J Brédo (ISNB: R.I.Sc.N.B., I.G. 18.284) [Reference to SW Africa and distance from Upington most likely erroneous; A Drumont, pers. comm.].

Updated and expanded identification key to the species of *Rhinocoeta* s. str. (revised after Holm 1992)

1 Body black, often with some brick-red areas; elytra sculptured with well-discernible round to crescent-shaped punctures.....**2**

– Body never bicolorous; elytra between costae finely and densely textured, without discernible crescent sculpture **3**

2 Scutellum with punctures elongate; pronotal punctures round; underside, pronotal sides and legs brick-red, but in westernmost populations entirely black; length: 17.5 – 24.9 mm; distribution: South Africa (WC, EC, NC, FS) and southwestern Namibia.....***R.* (*R.*) *sanguinipes* Gory & Percheron, 1833**

– Scutellum with round punctures; pronotal punctures crescent-shaped; body completely black or pronotal sides and elytral disc with variable degree of brick-red colouring; length: 19.5 – 24.0 mm; distribution: South Africa (EC, NC – central Karoo mountains, at altitudes > 1500 m).....***R.* (*R.*) *maraisi* Holm, 1992**

3 Pronotum with tubercle at middle of anterior margin, not forming depression around it; third and fifth elytral costae converging at apical umbone; length: 10.0 – 16.0 mm; distribution: South Africa (WC, NC, EC, FS), unconfirmed old records also in Namibia and Zimbabwe ..... ***R. armata* Boheman, 1860**

– Pronotum with prominent to moderate tubercle at middle of anterior margin, forming shallow depression to deep concavity around it; third and fifth elytral costae weakly elevated and becoming obsolete before reaching apical umbone.....**4**

4 Antero-median pronotal tubercle and associated depression showing marked sexual dimorphism, becoming hypertrophic and deep in male; aedeagal paramereres with latero-apical spinal expansion; length: 12.5 – 22.5 mm; distribution: South Africa (WC, EC – coastal lowlands and Cape Fold mountains) (Fig. 5) ....***R.* (*R.*) *cornuta* (Fabricius, 1781)**

– Antero-median pronotal tubercle and associated depression poorly developed and similar in both sexes; aedeagal paramereres without latero-apical spinal expansion; length: 20.3 – 24.6 mm; distribution: South Africa (NC – Namaqualand, Roggeveld and Upper Karoo) (Fig. 5) ..... ***R.* (*R.*) *namaqua* sp. nov.**

Discussion

The new description reported here brings to six the total number of species currently recognised within the nominal subgenus: *R. sanguinipes* (Gory & Percheron, 1833); *R maraisi* Holm, 1992; *R. armata* Boheman, 1860; *R. limbaticollis* (Péringuey, 1907), *R cornuta* (Fabricius, 1781) and *R. namaqua* sp. nov. (Holm 1992, Beinhundner 2017). There are then three further species in the subgenus *Haematonotus* Kraatz, 1880: *R.* (*H.*) *turbida* (Boheman, 1860), *R.* (*H.*) *hauseri* (Kraatz, 1896) and *R.* (*H.*) *leonardi* Beinhundner, 2013 (Beinhundner 2017). These have adequately been dealt with and thoroughly illustrated recently by Beinhundner (2013, 2017).

The issue of *R. limbaticollis*, however, remains a complex and unresolved one. It was described under a different genus, *Lipoclita*, by Péringuey (1907) mainly on the basis of its uncharacteristic mouthparts. These were regarded as fundamental by Péringuey (1907), but have subsequently been downgraded by Holm (1992), on the basis that mouthparts are variable and regressive, particularly in taxa such as all the members of the genus *Rhinocoeta* s. l. that do not feed at the adult stage. A close re-analysis of its key features reveals that *R. limbaticollis* largely resembles a typical dark/black female of *R.* (*H.*) *turbida* particularly in terms of protibiae, clypeus, antennal clubs and mesosternal process (Figs 6A–D), yet differs from this in terms of two key characters. These are: 1) the presence of cretaceous spots on the pygidium (Fig. 6D; although these are very regressive and not as developed as in males *R.* (*H.*) *turbida*); and 2) the lack of dense and coarse (even rugose on the pronotum) sculpture on the dorsal aspect (Fig. 6A).

Considering that this is unfortunately still the only specimen known for this "species", despite the extensive searches that were undertaken in the area of the type locality during the past 30 years, there seem to be only two options left regarding its identity. The first is that it indeed represents a separate species to *R.* (*H.*) *turbida*, very rare or even extinct, as suggested by Holm and Marais (1992). This is difficult to believe, because the broader area of its type locality has not been completely "transformed" by mining and agriculture. There are in fact still protected and virtually pristine reserves in that area (e.g., Vaalbos/Mokala National Park, Sandveld Nature Reserve, Vredefort Dome World Heritage Site). Also direct observations made on virtually all the species of this genus indicate that they are very adaptable and resilient to anthropogenic activities (pers. obs.). The second option is that the specimen represents a case of gynandromorphy of *R.* (*H.*) *turbida*, with a mixture of female (e.g., bidentate protibiae, convex abdomen) and male characters (small and scattered sculpture, cretaceous spots on pygidium). It is hoped that the formulation of these working hypotheses may trigger further research, both in the field and the lab, to finally resolve this issues with conclusive evidence. To facilitate this, high-resolution photos of the specimen are presented here for the first time, courtesy of ISAM Collections Manager, Aisha Mayekiso.

Since the revision of the genus *Rhinocoeta* by Holm (1992) and Holm and Marais (1992), much has been learned about the biology/ecology and geographic distribution of the five confirmed species now constituting the nominal subgenus. Smith et

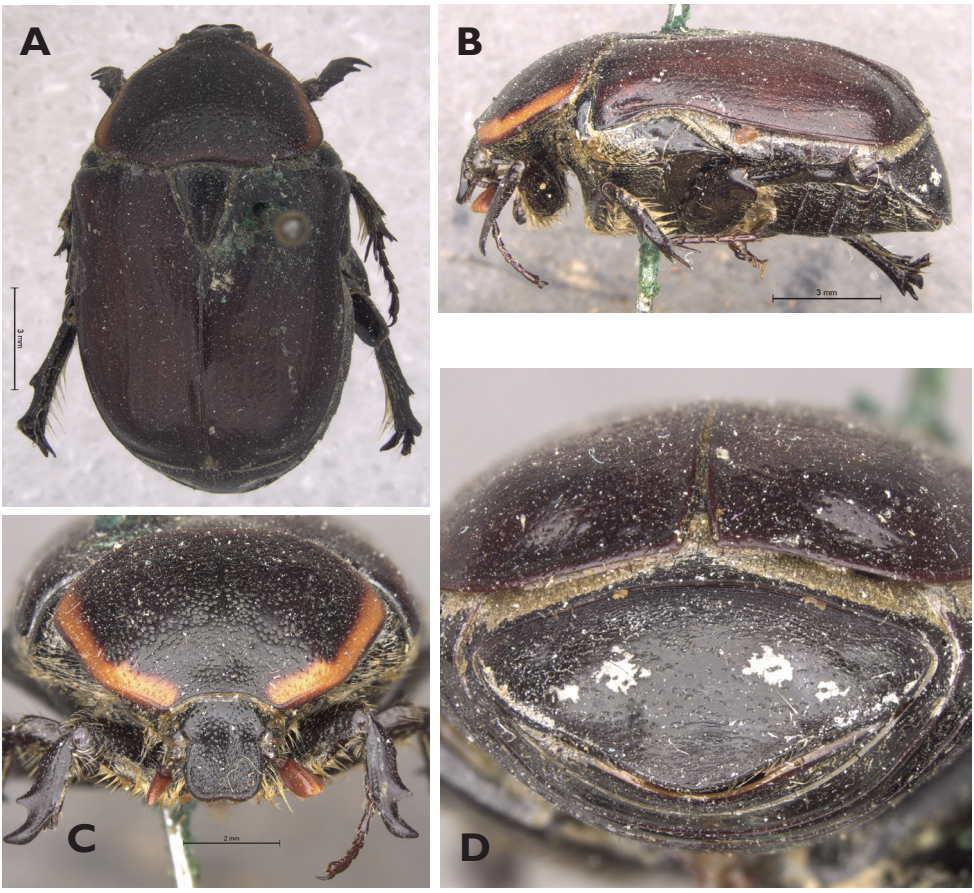

Figure 6. *Rhinocoeta limbaticollis* (Péringuey, 1907), Holotype ♀ **A** dorsal habitus **B** lateral view **C** head and pronotum **D** pygidium. Photographs by Aisha Mayekiso; copyright Iziko Museums of South Africa.

al. (1998) reported that adults of the genus are generally collected under dung pads of cattle or in dung middens of a large variety of indigenous antelopes. In the Winterberg range of the Eastern Cape, larvae, pupae, and eclosing adults of *R. sanguinipes* have been found under a bontebok (*Damaliscus dorcas dorcas*) dung midden (Smith et al. 1998). Still in the Eastern Cape, but in the Sneeuberg, adults of *R. maraisi* sdults were collected in cattle dung (Holm and Stobbia 1995).

More comprehensive investigations carried out in the last 20 years have revealed that the four larger species, i.e., *R. sanguinipes*, *R. maraisi*, *R. cornuta* and *R. namaqua*, all depend on herbivorous and insectivorous mammal dung for their development (pers. obs.). On the other hand, the larva of *R. armata* has been observed making subsurface tunnels in sandy soil, in order to drag detrital matter underground, including leaf litter and pieces of dung pellets, of kudu (*Tragelaphus strepsiceros*) for instance (pers. obs., P Malec and P Šípek, pers. comm.). *Rhinocoeta sanguinipes*

has been observed most frequently in large dung accumulations of klipspringer (*Oreotragus oreotragus*), but also in smaller dung deposits of aardvark (*Orycteropus afer*), red hartebeest (*Alcelaphus buselaphus caama*) and even domesticated goats, sheep and cows. *Rhinocoeta maraisi* seems to prefer dung droppings of mountain reedbuck (*Redunca fulvorufula*), Cape hare (*Lepus capensis*) and farmed horses and cows. *Rhinocoeta cornuta* on the Western and Eastern Cape south coast thrives on dung of farmed goats and sheep, but also of bontebok, hartebeest and other unidentified antelopes. Finally, the new species, *R. namaqua*, has so far been observed only on dung droppings of kudu and farmed sheep (pers. obs., J Ball and AP Marais, pers. comm.).

In terms of distribution, further to the ranges already reported in Holm (1992), Holm and Marais (1992) and Holm and Stobbia (1995), new data show that, apart from the Sneeuberge, *R. maraisi* occurs also in other major mountains of the Eastern and Northern Cape Karoo, such as the Bamboesberg, the Kikvorsberg and the Groot Tafelberg. The more widespread distribution of *R. sanguinipes* is confirmed, with populations occurring across the south-western and central parts of South Africa, as well as in southern Namibia. As previously reported by Holm (1992), the Namibian population exhibits entirely black ventral and dorsal habitus, and it has now been established that the westernmost South African populations also follow this colour pattern, generally lacking the typical brick-red pigmentation on ventral surface, legs, pygidium and pronotal lateral margins. Concerning *R. armata*, however, no evidence has been obtained in confirmation of the dubious distribution records of this species in Zimbabwe and Namibia, thus suggesting that the old references to these localities may be incorrect, as proposed earlier by Holm and Marais (1992). Finally, *R. cornuta* and *R. namaqua* seem to be clearly separated in their distribution by the Great South African Escarpment, with the first species restricted to the coastal lowlands and Cape Fold Mountains below it, while the second species has so far only been recorded in the north-western highlands above the escarpment (Fig. 5).

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Species review of the genus *Boreophilia* Benick from North America (Coleoptera, Staphylinidae, Aleocharinae, Athetini): Systematics, habitat, and distribution

Jan Klimaszewski[1], Derek S. Sikes[2], Adam Brunke[3], Caroline Bourdon[1]

1 *Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du PEPS, PO Box 10380, Stn. Sainte-Foy, Québec, QC, G1V 4C7, Canada* **2** *University of Alaska Museum, 1962 Yukon Drive, Fairbanks, Alaska 99775-6960, USA* **3** *Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, ON, K1A 0C6, Canada*

Corresponding author: *Jan Klimaszewski* (jan.klimaszewski@canada.ca)

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Abstract

Fourteen species of the genus *Boreophilia* Benick are now recognized in North America. *Boreophilia insecuta* (Eppelsheim), reported by Lohse (1990) from North America, is a misidentification of a new species, which is described here as *B. neoinsecuta* Klimaszewski, **sp. n.**, and the true *B. insecuta* (Epp.) does not occur in North America. An additional new species is found in Alaska, and described as *B. beringi* Klimaszewski & Brunke, **sp. n.** The following three species are synonymized (second name being valid): *Boreophilia herschelensis* Klimaszewski & Godin, 2012, with *Boreophilia vega* (Fenyes, 1920); *Boreophilia manitobensis* Lohse, 1990, with *B. caseyi* Lohse, 1990; and *B. angusticornis* (Bernahuer, 1907) with *B. subplana* (J Sahlberg, 1880), based on study of genital structures and external morphology. *Atheta gelida* J Sahlberg, 1887, and *Atheta munsteri* Bernhauer, 1902, considered as *Boreophilia* in recent publications, are transferred to the genus *Atheta* Thomson, subgenus *Dimetrota*. *Boreostiba piligera* (J Sahlberg) is transferred to *Boreophilia* based on morphology and the results of our phylogenetic analysis. *Boreophilia nearctica* is recorded from Alberta and *B. nomensis* is recorded from British Columbia for the first time. Each valid species is illustrated by color image of habitus, and black and white images of genitalia and tergite and sternite VIII. A new key to all Nearctic species of the genus is provided. DNA barcode data were available for nine of the 14 species, which we downloaded, analyzed, and used as additional evidence for the taxonomic conclusions reached herein.

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Keywords
Identification, nomenclature, North America, northern species, rove beetles, taxonomy

Introduction

Boreophilia G Benick, 1973, is a small athetine genus, comprising Nordic species distributed exclusively in the Palaearctic and Nearctic regions. There are 17 species recorded in the Palaearctic (Smetana 2004), and 14 in the Nearctic regions. Of these, six species have a circumpolar Holarctic distribution, but two species included by Smetana (2004) in this genus, *B. gelida* (J Sahlberg) and *B. munsteri* (Bernhauer), are here transferred back to the genus *Atheta* where they were originally described, on the grounds of morphology of genital characters, including sexual modification of male tergite VIII, which is unmodified in *Boreophilia*. Consequently, there are 15 Palaearctic and 14 Nearctic species, of which six are Holarctic. It is interesting that none of the species with elytra shorter than the pronotum (and presumably brachypterous), were shown to be Holarctic. Limited dispersal in these groups has likely led to allopatric speciation between Nearctic and Palaearctic populations (e.g., *B. piligera* and *B. beringi* sp. n.). The Holarctic species constitute ca. 24% of the total fauna of the genus, which is likely the highest percentage of Holarctic species among Nearctic aleocharine genera. This genus, like *Gnypeta* CG Thomson, another northern Holarctic genus, is a good target for monitoring climate warming and its effects on distribution and survival of Nordic species. We here update our knowledge on all recorded Nearctic species and synonymize four species from previous records (Lohse et al. 1990, Klimaszewski et al. 2012). Two Nearctic species are described as new. All Nearctic species are classified to species groups, which presumably reflect their close relationships.

In the past, there was confusion regarding some Nearctic species of *Boreophilia* because species of this genus have similar structures of the median lobe of the aedeagus and of the spermatheca, insufficient material was available for study, and a general poor knowledge of Palaearctic species in the Nearctic region. We have corrected these as much as the available material permitted and have provided better diagnoses for Nearctic species. We have also studied European material to compare with Nearctic specimens of selected Holarctic species. This resulted in additional synonymy and clarification as to the known distribution of many species in North America. Brundin's (1954) paper was very helpful to our study. This work was clearly ahead of its time, with perfectly accurate illustrations of the median lobe of the aedeagus and spermatheca of several European species, providing the best diagnostic characters at the species level. We hope to encourage other scientists to use species of this genus in monitoring the effects of climate change on species in Nordic environments.

Material and methods

Almost all specimens used in this study were dissected, and their genital structures examined. The genital structures were dehydrated in absolute ethanol and mounted in

Canada balsam on celluloid microslides, and pinned with the specimens from which they originated. The photographs of the entire body and the genital structures were taken using an image processing system (Nikon SMZ 1500 stereoscopic microscope; Nikon Digital Camera DXM 1200F; and Adobe Photoshop software).

Terminology mainly follows that used by Lohse et al. (1990) and Klimaszewski et al. 2018. The ventral part of the median lobe of the aedeagus is considered to be the part of the bulbus containing the foramen mediale, the entrance of the ductus ejaculatorius, and the adjacent venter (ventral part of the tubus of the median lobe) of the tubus; the opposite side is referred to as the dorsal part.

Depository abbreviations:

CBG	Centre for Biodiversity Genomics, Guelph, Ontario, Canada
CNC	Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada
DEI	Deutsches Entomologisches Institut, Eberswalde, Germany
LFC	Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Insectarium R Martineau, Quebec City, Quebec, Canada
NFRC	Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre Arthropod Collection, Edmonton, Alberta, Canada
MHNG	Muséum d'histoire naturelle, Genéve, Switzerland
NHMD	University of Copenhagen, Copenhagen, Denmark
RWC	Reginald Webster private collection, 24 Millstream Drive, Charters Settlement, New Brunswick, Canada
UAM	University of Alaska Museum, Fairbanks, Alaska, United States of America
USNM	United States National Museum (Smithsonian Institution), Washington D.C., United States of America
ZMH	Zoological Museum, Helsinki, Finland
ZMUO	Zoology Museum, University of Oulu, Oulu, Finland

DNA barcode data were downloaded from the BOLD website (http://www.boldsystems.org) after applying filters to exclude those flagged as misidentifications, those with sequence lengths under 100 bp, those with stop codons, and those flagged as contaminated. This resulted in sequence data for nine of the 14 species included herein. The amino acid based HMM BOLD aligner was used to align the data prior to download. Two sequences each of *Atheta cinnamoptera* and *Atheta munsteri* were used as outgroups. The latter species was also included to test its generic placement. This resulted in a dataset of 33 sequences. Of 654 base pairs in the alignment, 455 are constant, 19 are variable but parsimony uninformative, and 180 are parsimony-informative. Specimens of all included *Boreophilia* were identified to species via morphological study, or to genus for some females. These sequences came from a variety of projects (Table 1) and publications (Elven et al. 2010, Pentinsaari et al. 2014, Sikes et al. 2017). The NEXUS file with the alignment and resulting tree is available for download from https://doi.org/10.6084/m9.figshare.7822496.

Table 1. DNA voucher data with Process ID codes from the Barcode of Life Database (BOLD), BOLD BIN numbers, sequence length with number of 'n's indicated, GenBank accession codes, and locality data. See http://www.boldsystems.org for additional data associated with each.

Identification	Process ID	BIN	Seq. Length	GenBank	Country/Ocean, State/Province, Region, Sector, Exact Site, Lat, Lon
Atheta cinnamoptera	COLFC200-12	BOLD:ABW4507	658[0n]	KJ964314	Finland, Lapland, Lapponia kemensis pars orientalis, Sodankylae, Vuotso, 68.1117, 27.1862
	COLFC205-12	BOLD:ABW4507	614[0n]	KJ961954	Finland, Lapland, Lapponia kemensis pars orientalis, Sodankylae, Vuotso, 68.1117, 27.1862
Atheta munsteri	COLFA072-10	BOLD:AAJ9581	658[0n]	HM909090	Finland, Lapland, Lapponia enontekiensis, Enontekioe, 69.096, 21.138
	LEFIJ2464-14	BOLD:AAJ9581	658[0n]		Finland, Lapponia inarensis, Utsjoki, Skalluvaara, 69.802, 27.102
Boreophilia sp.	SAPIT188-08	BOLD:AAH0226	577[0n]		Canada, Manitoba, Churchill, 23 km E Churchill, Malcolm Ramsay Lake, road, Shrub community dominated by *Betula glandulosa*, 58.73, -93.8
	UAMIC2716-15	BOLD:ACU9385	407[0n]	KU874453	United States, Alaska, Nogahabara Dunes [Koyukuk NWR], 65.658, -157.476
	TWCOL345-09	BOLD:AAG4312	658[0n]	HM432945	Canada, Manitoba, Churchill, 4 km SE Churchill, Dene Village, 58.734, -94.112
Boreophilia eremita	COLFA420-12	BOLD:ABW4331	658[0n]	KJ963286	Finland, Northern Ostrobothnia, Ostrobottnia borealis pars australis, Kiiminki, 65.116, 25.829
	COLFB787-12	BOLD:ABW4331	658[0n]	KJ964811	Finland, Lapland, Lapponia inarensis, Utsjoki, Gaskabeaicohkka, 70.0088, 27.5069
	COLFE1022-13	BOLD:ABW4331	658[0n]	KJ965816	Finland, Ostrobottnia borealis pars borealis, Tornio, Alkunkarinlahti, 65.7811, 24.2119
	COLFB791-12	BOLD:ABW4331	583[0n]	KJ966458	Finland, Lapland, Lapponia inarensis, Utsjoki, Gaskabeaicohkka, 70.0088, 27.5069
	COLFB788-12	BOLD:ABW4331	582[0n]	KJ966313	Finland, Lapland, Lapponia inarensis, Utsjoki, Gaskabeaicohkka, 70.0088, 27.5069
	COLFB785-12	BOLD:ABW4331	567[2n]	KJ965976	Finland, Lapland, Lapponia inarensis, Utsjoki, Gaskabeaicohkka, 70.0088, 27.5069
Boreophilia fusca	COLFG320-14	BOLD:AAG4311	658[0n]		Finland, Lapponia inarensis, Inari, Kaamanen, 69.089, 27.184
	TWCOL344-09	BOLD:AAG4311	561[0n]	HM432944	Canada, Manitoba, Churchill, 4 km SE Churchill, Dene Village, 58.734, -94.112
Boreophilia vega	LFCAB223-15		407[0n]		Canada, Yukon Territory, Hershel Island, 69.571, -138.902
Boreophilia hyperborea	GBCL15075-13	BOLD:AAG4302	1000[1n]	GQ980933	Russia (specimen ZMUN:10002634)
	HMCOC722-09	BOLD:AAG4302	658[0n]	KJ203366	Canada, Manitoba, Churchill, 12 km S Churchill, Goose Creek Marina, Open substrate, 58.663, -94.166
Boreophilia islandica	LFCAB221-15	BOLD:AAH0226	407[0n]		Canada, Newfoundland and Labrador, Long Range Mountains, Portland Creek Hill,
Boreophilia nearctica	UAMIC2729-15	BOLD:ACU9385	658[0n]	KU874454	United States, Alaska, Naknek, 58.74, -157.064
	UAMIC2724-15	BOLD:ACU9385	613[0n]	KU874455	United States, Alaska, Selawik NWR, 66.561, -158.998
	LEPNG801-15	BOLD:ACU9385	658[0n]		Canada, Alberta, Plateau Mountain, 50.226, -114.555
	LEPNG802-15	BOLD:ACU9385	658[0n]		Canada, Alberta, Plateau Mountain, 50.226, -114.555
	LEPNG800-15	BOLD:ACU9385	407[0n]		Canada, Alberta, Plateau Mountain, 50.226, -114.555
Boreophilia nomensis	UAMIC2675-15	BOLD:ACU9384	658[0n]	KU874456	United States, Alaska, Thompson Pass, 61.137, -145.745
	SSKNA9232-15	BOLD:ACU9384	564[0n]	MG057964	Canada, British Columbia, Kinaskan Lake Provincial Park, Kinaskan Lake Trail, 57.532, -130.202

Identification	Process ID	BIN	Seq. Length	GenBank	Country/Ocean, State/Province, Region, Sector, Exact Site, Lat, Lon
Boreophilia sp.	UAMIC2676-15	BOLD:ACU9385	407[0n]	KU874457	United States, Alaska, Galena, Yukon Riv., W of town, 64.742, -156.98
	COLFC286-12	BOLD:ABX3767	658[0n]	KJ965200	Finland, Lapland, Lapponia inarensis, Inari, Saariselkae, 68.4214, 27.4396
Boreophilia subplana	COLFB810-12		407[0n]	KJ962674	Finland, Lapland, Lapponia inarensis, Utsjoki, Gaskabeaicohkka, 70.0069, 27.5357
	COLFB811-12		407[0n]	KJ963490	Finland, Lapland, Lapponia inarensis, Utsjoki, Gaskabeaicohkka, 70.0069, 27.5357
Boreophilia neoinsecuta	MOBIL8660-18		545[0n]		United States, Alaska, Anaktuvuk Pass, 68.1405, -151.741
	MOBIL8661-18		492[0n]		United States, Alaska, Anaktuvuk Pass, 68.1405, -151.741
Boreophilia piligera	COLFG746-14	BOLD:ACO9332	658[0n]		Finland, Lapponia enontekiensis, Enontekioe, Kilpisjaervi, Saana, 69.039, 20.854

To obtain a robust estimate of the mtDNA gene tree using these DNA barcode data, PartitionFinder2 (Lanfear et al. 2016) was used via the CIPRES Science Gateway (Miller et al. 2010) to obtain the best partitioning and modeling scheme. We used the following parameters for the cfg file: alignment = infile.phy, branchlengths = linked, models = all, model selection = aicc, search = greedy, with each codon position indicated as a separate partition. Mesquite v3.6 (Maddison and Maddison 2018), was used to export the original Nexus file to Phylip format for PartitionFinder. The best scheme chosen by PartitionFinder retained each codon position as a partition with first codon positions modeled using the TrN+I+G model, second positions modeled using the F81+I model, and third positions modeled using the GTR+G model. All DNA distances reported herein are uncorrected, p-distances. Minimum, average, and maximum distances were calculated in Excel from a distance matrix generated by PAUP 4.0a (build 164) (Swofford 2002). This data file is available at https://doi.org/10.6084/m9.figshare.7822508.

Bayesian and maximum likelihood phylogenetic analyses were conducted via the CIPRES portal using MrBayes v3.2.6 without the BEAGLE option (Ronquist et al. 2012) and Garli 2.0 (Zwickl 2006). Because MrBayes doesn't have the TrN model, for the first codon position we used the GTR model, which PartitionFinder selected for use with MrBayes. Two runs of four chains each were sampled for 8 million generations with samples taken every 1000 generations; the first 25% of the samples were discarded as burn-in, yielding 12,002 samples. The average standard deviation of the split frequencies was 0.003158 and the average Potential Scale Reduction Factor (Gelman and Rubin 1992a, b) was 1.000, thus indicating convergence had been reached. The sampling was considered adequate based on the average estimated sample sizes (ESS) of the parameters all being greater than 2000, as assessed by MrBayes. Also using the CIPRES portal, we ran 200 bootstrap replicates composed of four search replicates each using GARLI, with zero length branches collapsed. The resulting trees were imported into PAUP 4.0a (build 164) (Swofford 2002) to produce a 50% majority rule consensus tree, the node support values of which were transferred to the Bayesian consensus tree. An additional maximum likelihood analysis was conducted in IQTREE 1.6 (Nguyen et al. 2015) on an iMac (4 GHz i7, 16GB) to acquire alterna-

tive node support values, namely the ultrafast bootstrap of Hoang et al. (2017) and the SH-aLRT test of Guindon et al. (2010). The analysis was performed using the same partitioning scheme as used for GARLI and with the -spp option, which allows partition-specific rates, 500 search replicates, and other parameters set to defaults. Clade support was assessed using 1000 replicates of the ultrafast bootstrap and an SH-aLRT test with 1000 replicates. Nodes with support values of both UFB ≥ 95 and SH-aLRT ≥ 80 are considered well supported (Nguyen et al. 2015), nodes with one of UFB < 95 or SH-aLRT < 80 are considered weakly supported, and nodes with both UFB < 95 or SH-aLRT < 80 are considered unsupported.

Phylogenetic results

The resulting estimate of the mtDNA gene tree (Fig. 1) was relatively well resolved although a few relationships were obscured by polytomies or ambiguous due to low branch support values. The genus *Boreophilia,* as defined herein via morphology, was strongly supported as monophyletic (PP = 1.0, BS = 100, UFB = 100, SH-aLRT = 100). Notably, the species we transferred to *Boreophilia* (*B. piligera*) from *Boreostiba* was recovered within the clade of other *Boreophilia* while a species we transferred out of *Boreophilia* and into *Atheta* (*A. munsteri*) was recovered as the closest lineage to *Boreophilia*, with a long branch separating the two clades, thus supporting its exclusion from *Boreophilia*. Zero of the sampled Bayesian trees had *A. munsteri* nested within the *Boreophilia* clade, thus failing to reject the hypothesis that it is not a *Boreophilia* as morphologically defined herein. The *fusca* species group was supported as monophyletic with a strong posterior probability (0.98), ultrafast bootstrap (95%) and SH-aLRT support (82%) but relatively weak maximum likelihood bootstrap support (63%). The *subplana* species group, however, was not recovered as monophyletic due to its members and the *fusca* group emerging from a polytomy. Given the small size of the dataset, the *subplana* species group hypothesis remains ambiguous. All species with multiple specimens sampled were recovered as monophyletic with strong support (PP = 0.99 – 1.0, BS = 78–99% UFB = 84–100%, SH-aLRT = 89–100%) including two species, *B. hyperborea* and *B. fusca*, with samples from both the Nearctic and Palearctic. Nine of the ten species in our analysis are in BINs on BOLD (Table 1) with no species occurring in more than one BIN, and with no BIN holding more than one morphologically identified species.

Given the relatively small size of the dataset, in both taxon sampling and genetic data, we refrain from drawing any biogeographic conclusions based on these preliminary phylogenetic analyses. Additional genes including nuclear markers, greater specimen sampling within species, and addition of the missing *Boreophilia* species, would greatly improve our understanding of the evolution of these taxa.

Five specimens were female and could not be identified with certainty based on morphology alone (Fig. 1). Two of these (*B.* sp. TWCOL345_09 from Manitoba, Canada, and *B.* sp. COLFC286_12 from Finland) show branch lengths large enough to potentially be unique species not already represented in our dataset. The remaining

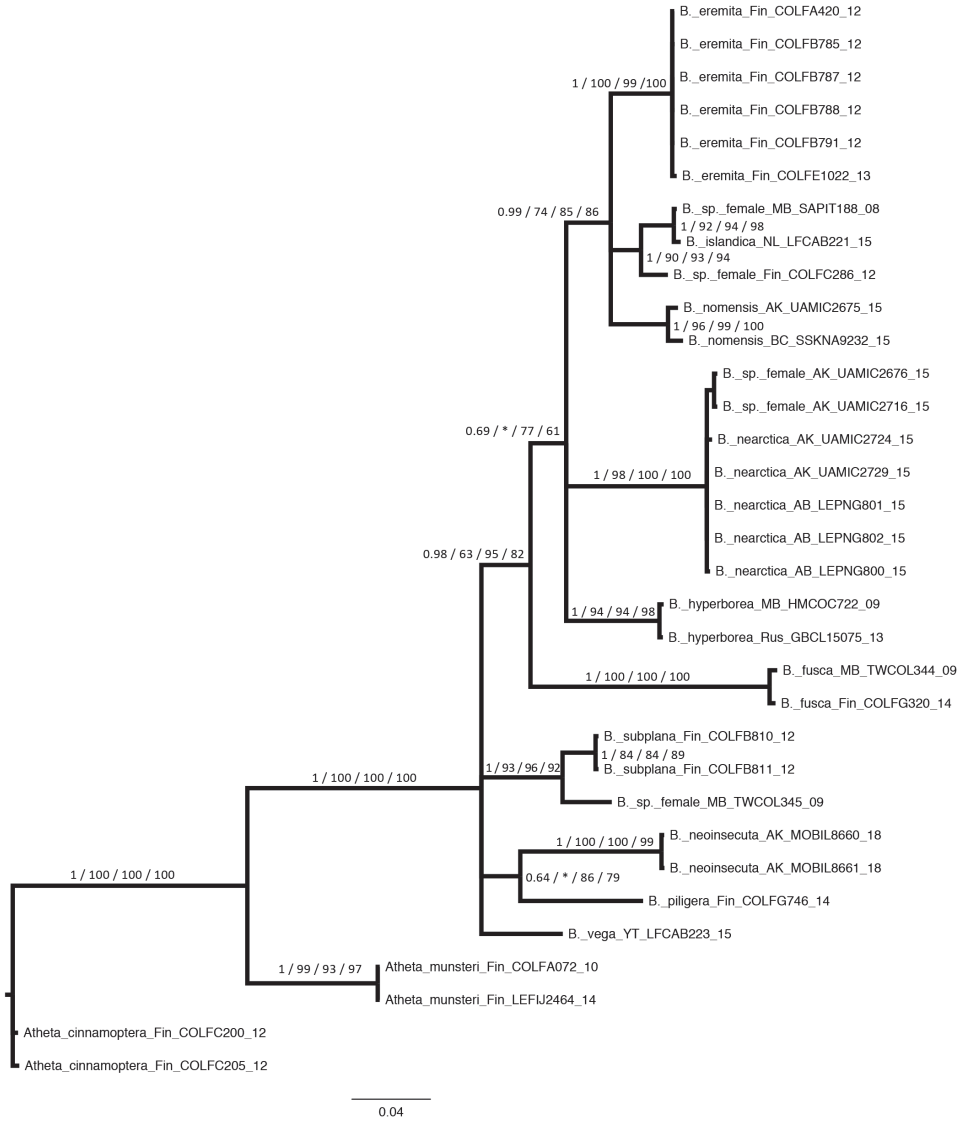


Figure 1. Fifty percent majority rule consensus phylogram from the Bayesian analysis with branch support values provided from left to right as: estimated posterior probabilities, maximum likelihood bootstrap proportions, ultrafast bootstrap values, and an SH-aLRT test values, with * = bootstrap values below 50%. Taxon identity is indicated for each sequence, followed by abbreviations of locality, and BOLD process IDs (see Table 1).

females, (*B.* sp. SAPIT188_08, from Manitoba, and *B.* sp. UAMIC2676_15 and *B.* sp. UAMIC2716_15, from Alaska) cluster with sequences obtained from specimens identified via morphology, indicating the Manitoba female is likely *B. islandica* and the two Alaskan females are likely *B. nearctica*. Further comments relevant to the phylogenetic results and each species' corresponding Barcode Index Numbers (BINs, Ratnasingham and Hebert 2013) on BOLD are listed under each species below.

Summary of DNA distances within and among species

A full spreadsheet of DNA distances and our calculations is archived at https://doi.org/10.6084/m9.figshare.7822508. We summarize the key findings here. Among *Boreophilia* species, the minimum uncorrected 'p' distance within a species (limited only to sequences identified to species via morphology) in our dataset was 0.00%, the mean within species distance was 0.280%, and the maximum within species distance was 1.072%. Surprisingly, this maximum distance was found between two Nearctic samples (*B. nomensis* from British Columbia versus *B. nomensis* from Alaska) rather than between Palearctic versus Nearctic conspecific samples (*B. fusca* from Finland versus *B. fusca* from Manitoba, Canada were 0.539% distant and *B. hyperborea* from Russia versus *B. hyperborea* from Manitoba, Canada were 0.155% distant). This maximum within species distance is not a result of one of these sequences being incomplete (the British Columbia sequence is only 564 bp long while the Alaska sequence is 658 bp long). When these two sequences were compared after excluding base pairs missing from the shorter sequence, so both were 564 bp long, their distance was 1.064%, which remains the maximum within species value.

The minimum among species distance was 4.589% (between *B. nomensis* from Alaska and *B. eremita* from Finland), a value more than four times larger than the maximum within species distance. The average distance among species was 8.436%, and the maximum distance among species of *Boreophilia* was 12.080% (between *B. fusca* from Finland and *B. nearctica* from Alaska).

Taxonomic review

Tribe Athetini Casey, 1910

(sensu Klimaszewski et al. 2018)

Genus *Boreophilia* Benick, 1973

Figs 1–119

Boreophilia Benick, 1973: 211; Lohse et al. 1990: 151; Gusarov 2003a, b: 81; Smetana 2004: 396; Schülke and Smetana 2015: 557; Klimaszewski et al. 2018. Type species: *Homalota islandica* Kraatz, 1857.

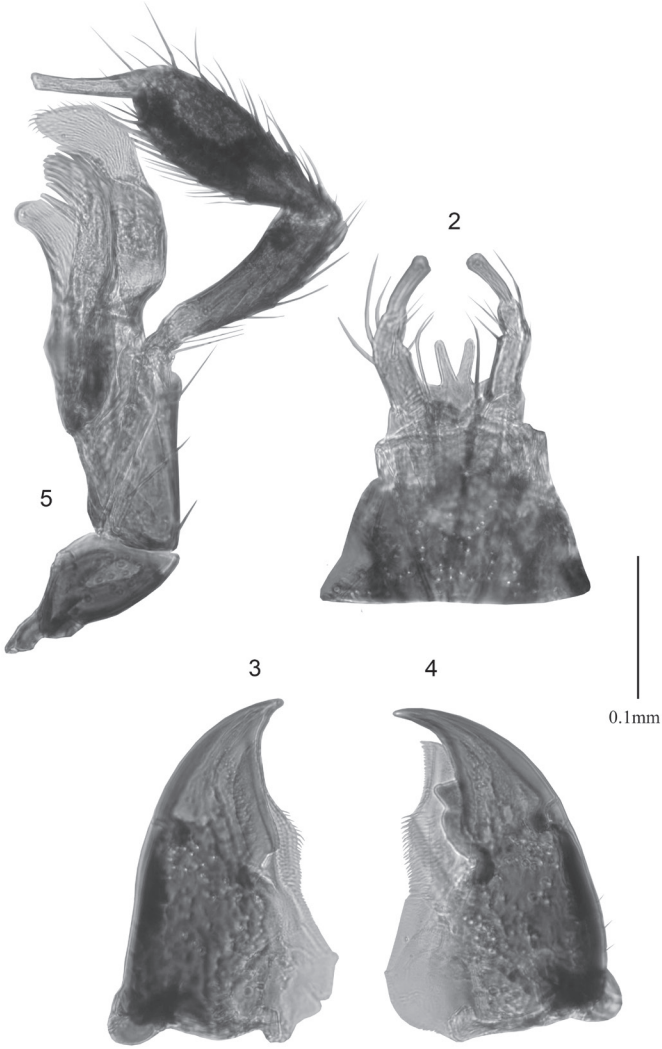
Diagnosis. *Boreophilia* may be distinguished from other athetines by the following combination of characters: body moderately narrow to broad, subparallel (Figs 6, 16, 24, 32, 43, 53, 57, 63, 71, 81, 84, 92, 100, 109, 117); head narrower or nearly as broad as pronotum (Fig. 6, 16, 24, 32, 43, 53, 57, 63, 71, 81, 84, 92, 100, 109, 117); eyes moderate in size, as long as or shorter than postocular region of head, tempora partially feebly carinate at base of head; antennae often reaching posterior margin of

elytra, antennomeres V-X subquadrate, slightly transverse, or elongate (Figs 6, 16, 24, 32, 43, 53, 57, 63, 71, 81, 84, 92, 100, 109, 117); ligula divided into two separate and diverging lobes (Fig. 2); mandibles simple (not bifid at apex as in *Schistoglossa*) (Figs 3, 4); maxillary palps with 4 palpomeres, terminal one needle-shaped (Fig. 5); pronotum transverse, broadest in the middle or in apical fourth, hind angles rounded, hypomeron clearly visible in lateral view; integument smooth and moderately glossy, weak microsculpture present, punctuation fine, pubescence on midline of disc directed anteriad at least in apical ¾, and elsewhere directed laterally; mesocoxae contiguous; median lobe of aedeagus broad, flattened latero-ventrally, bulbus enlarged and tubus narrow, short or moderately elongate, approximately triangularly shaped in dorsal view (Figs 9, 10, 18, 26, 35, 36, 46, 59, 60, 65, 74, 83, 86, 94, 102, 103, 111), crista apicalis narrowly elongate in most, internal sac with two large, elongate sclerites in bulbus, and additional smaller sclerites in apical part of internal sac; male tergite VIII entire, and not modified (Figs 11, 19, 27, 37, 47, 61, 66, 75, 87, 95, 104, 112); spermatheca diverse in shape, with elongate tubular capsule and sinuate stem, invagination of capsule small or absent (Figs 15, 23, 31, 41, 42, 51, 52, 56, 70, 79, 80, 91, 99, 108, 116, 120); cold loving species occurring in arctic habitats, in temperate regions usually confined to fens and bogs.

Checklist of species occurring in the Nearctic Region

(species list follows that in the text, synonyms indented, see Schülke and Smetana 2015 for strictly Palaearctic synonyms)

1. *Boreophilia eremita* (Rye, 1866). Fenyes 1920 (syn. of *Atheta islandica*); Lohse et al. 1990; Gusarov 2003a; Smetana 2004; Webster et al. 2012; Schülke and Smetana 2015; Klimaszewski et al. 2011, 2018.
   Holarctic species (Distribution: north and central Europe, Ireland, Ukraine, Russia – West and East Siberia and the Far East; Canada: LB, NB, MB; USA: AK).
2. *Boreophilia islandica* (Kraatz, 1857). Gusarov 2003; Smetana 2004; Klimaszewski et al. 2011, 2018.
   Holarctic species (Distribution: Fennoscandia, Estonia, Faeroe Islands, Great Britain, Iceland, Russia – North European Territory; Canada: LB, NF, NT, NU, YT; USA: AK).
3. *Boreophilia fusca* (CR Sahlberg, 1831). Bernhauer, 1909; Lohse et al. 1990; Gusarov 2003a; Smetana 2004; Schülke and Smetana 2015.
   Holarctic species (Distribution: Fennoscandia, Russia - North European Territory, West and East Siberia and the Far East; Canada: NT; USA: AK)
4. *Boreophilia hyperborea* (Brundin, 1940). Lohse et al. 1990; Gusarov 2003b; Smetana 2004; Ernst and Buddle 2013; Schülke and Smetana 2015.
   Holarctic species (Distribution: Fennoscandia, Greenland, Russia – North European Territory; Canada: NT, NU; USA: AK)



Figures 2–5. *Boreophilia islandica* (Kraatz), mouthparts: **2** mentum, labial palps and ligula **3, 4** mandibles **5** maxilla. Scale bar: 0.1 mm.

5. *Boreophilia nearctica* Lohse, in Lohse et al. 1990. Gusarov 2003b; Klimaszewski et al. 2011, 2018.
   Nearctic species (Distribution: Canada: LB, QC, MB, AB [new record], YT, NF; USA: AK).
6. *Boreophilia ovalis* Klimaszewski and Langor, in Klimaszewski et al. 2011. Klimaszewski et al. 2018.
   Nearctic species (Distribution: Canada: NF; USA: not recorded).
7. *Boreophilia nomensis* (Casey, 1910). Gusarov 2003a.
   Nearctic species (Distribution: Canada: YT, BC [new record]; USA: AK).

Boreophilia caseyiana Lohse, in Lohse et al. 1990. Gusarov 2003a (synonym of *B. nomensis*).

8. *Boreophilia venti* (Lohse), in Lohse et al. 1990.
   Nearctic species (Canada: YT; USA: AK [new record]).
9. *Boreophilia neoinsecuta* Klimaszewski, sp. n. Misidentified in Lohse et al. 1990 (as *B. insecuta*).
   Nearctic species (Distribution: Canada: MB, YT; USA: AK).
10. *Boreophilia beringi* Klimaszewski & Brunke, sp. n.
    Nearctic species (Distribution: USA: AK).
11. *Boreophilia subplana* (J Sahlberg, 1880). Brundin 1954; Lohse et al. 1990; Gusarov 2003a; Smetana 2004.
    Holarctic species (Distribution: Spitsbergen, Fennoscandia, Russia - West and East Siberia; Canada: NT, NU; USA: AK, NH).
    Boreophilia angusticornis (Bernhauer, 1907). Gusarov 2003a. **New synonymy**.
    Boreophilia plutonica (Casey, 1910). Gusarov 2003a: 83 (synonymy with *B. angusticornis*).
12. *Boreophilia caseyi* Lohse, in Lohse et al. 1990, Gusarov 2003b.
    Nearctic species (Distribution: Canada: MB, NU, YT; USA: AK).
    Boreophilia manitobensis Lohse, 1990, in Lohse et al. 1990. **New synonymy**.
    (Distribution: Canada: MB; USA: AK)
13. *Boreophilia vega* (Fenyes, 1920, as *Atheta*). Smetana 2004; Schülke and Smetana 2015.
    Holarctic species (Distribution: Russia - West and East Siberia, Far East, North Korea, Canada: YT; USA: not recorded).
    Boreophilia herschelensis Klimaszewski & Godin, 2012, in Klimaszewski et al. 2012. **New synonymy**.
14. *Boreophilia davidgei* Klimaszewski & Godin, in Klimaszewski et al. 2012.
    Nearctic species (Distribution: Canada: YT; USA: not recorded)

Species excluded from the genus *Boreophilia*

15. *Schistoglossa blatchleyi* (Bernhauer & Scheerpeltz, 1926) (replacement name for *Atheta caviceps* Blatchley 1910 nec Poppius 1908). Gusarov 2003a (as *Atheta*); Klimaszewski et al. 2009 (transferred to *Schistoglossa*).
    Nearctic species (Distribution: Canada: MB, NB, NWT, YT; USA: AK, IN).
    Boreophilia chillcotti Lohse, in Lohse et al. 1990. Gusarov 2003 (synonym of *S. blatchleyi*). Synonymy confirmed here.
    (Distribution: Canada: MB; USA: not recorded)
16. *Atheta* (*Dimetrota*) *gelida* J Sahlberg, 1887.Lohse et al. 1990 (as *Boreophilia*); Smetana 2004 (as *Boreophilia*); Schülke and Smetana 2015 (as *Boreophilia*); Klimaszewski et al. 2018 (as *Boreophilia*). Transferred back to *Atheta* here, subgenus *Dimetrota*, on basis of morphology of median lobe of aedeagus and spermatheca.
    Holarctic species (Distribution: Russia: Chukotka Peninsula; Canada: MB, NWT, QC, YT; USA: AK)

17. *Atheta* (*Dimetrota*) *munsteri* Bernhauer, 1902. Lohse et al. 1990 (as Dimetrota); Smetana 2004 (as *Boreophilia*); Bousquet et al. 2013 (as *Atheta* (*Dimetrota*)); Schülke and Smetana 2015 (as *Boreophilia*).
Holarctic species (Distribution: northern Europe, North Korea; Canada: MB, NT, YT; USA: AK)

Key to species of *Boreophilia* recorded from the Nearctic region

1 Body moderately narrow, elytra at base ca. as broad as maximum width of pronotum (Figs 6, 57, 71, 84) **2**

– Body broad, elytra at base distinctly broader than maximum width of pronotum (Figs 16, 24, 32, 43, 53, 63, 92, 100, 109, 117) **5**

2 Elytra at suture shorter than pronotum at midline (Figs 57, 84) **3**

– Elytra at suture as long as or longer than pronotum at midline (Figs 6, 71). **4**

3 Legs moderately long, hind legs much shorter that abdomen (Fig. 57); median lobe of aedeagus as illustrated (Figs 58–60), female unknown .... ***Boreophilia nomensis*** **(Casey)**

– Legs extremely long, hind legs almost as long as abdomen (Fig. 84); median lobe of aedeagus and spermatheca as illustrated (Figs 85, 86, 91) .... ***Boreophilia beringi*** **Klimaszewski & Brunke, sp. n.**

4 Tubus of median lobe of aedeagus without basal projection on each side in dorsal view (Fig. 74); capsule of spermatheca club-shaped and with small apical invagination, stem broadly looped posteriorly (Figs 79, 80) .... ***Boreophilia neoinsecuta*** **Klimaszewski, sp. n.**

– Tubus of median lobe of aedeagus with two basal projections on each side in dorsal view (Figs 9, 10); capsule of spermatheca elongate, tubular, and tapering apically, without apical invagination, stem narrowly looped posteriorly (Fig. 15) ..... ***Boreophilia eremita*** **(Rey)**

5 Maximum width of elytra one-fourth wider than pronotum (Figs 63, 100) **6**

– Maximum width of elytra one-fifth wider than pronotum (Figs 16, 24, 32, 43, 53, 92, 109, 117) **7**

6 Median lobe of aedeagus with ventral margin of tubus arcuate basally and apex broad and rounded in lateral view (Fig. 64); sclerites of internal sac broad, curved and with small median projection in lateral view (Fig. 64); capsule of spermatheca narrowly club-shaped, spherical apically, stem coiled posteriorly (Fig. 70) ..... ***Boreophilia venti*** **(Lohse)**

– Median lobe of aedeagus with ventral margin of tubus straight basally and apex broad and angular in lateral view (Fig. 101); sclerites of internal sac long, narrow and straight, without small median projection in lateral view (Fig. 101); capsule of spermatheca broadly club-shaped, hemispherical apically, stem hooked posteriorly (Fig. 108) ..... ***Boreophilia caseyi*** **Lohse**

7 Antennomeres VIII-X slightly to strongly elongate, less so in females (Figs 43, 53, 92) **8**

– Antennomeres VIII-X subquadrate to slightly transverse (Figs 16, 32, 109, 117) **10**

8 Body broadly oval, robust, flattened (Fig. 53); antennomeres VIII–X at least one-fourth longer than maximum width (Fig. 53); female sternite VIII rounded apically with strongly sinuate antecostal line (Fig. 55); capsule of spermatheca narrowed apically and slightly pointed laterad; stem short, strongly sinuate and looped posteriorly (Fig. 56) ..... ***Boreophilia ovalis*** **Klimaszewski & Langor**

– Body not as above (Figs 43, 92); antennomeres VIII–X less elongate; female sternite VIII and spermatheca not as above **9**

9 Median lobe of aedeagus narrow apically and slightly pointed in lateral view (Figs 44, 45); female sternite VIII truncate apically and slightly emarginate medially (Fig. 50); capsule of spermatheca evenly broad apically and evenly rounded at apex, stem broadly sinuate without posterior loop (Figs 51, 52) .. .. ***Boreophilia nearctica*** **Lohse**

– Median lobe of aedeagus broadly rounded apically in lateral view (Fig. 93), bulbus oval and broad apically in dorsal view (Fig. 94); sclerites of internal sac narrow (Figs 93, 94); capsule of spermatheca subspherical apically and apex rounded, stem narrow, sinuate, and narrowly looped posteriorly (Fig. 99) .... ***Boreophilia subplana*** **(J Sahlberg)**

10 Pronotum width to length ratio 1.3 (Figs 32, 109, 117) **11**

– Pronotum width to length ratio 1.4–1.5 (Figs 16, 24) **13**

11 Median lobe of aedeagus narrowly rounded apically in lateral view (Figs 33, 34), bulbus oval and narrowed apically in dorsal view (Figs 35, 36); sclerites of internal sac narrow (Figs 34, 36); capsule of spermatheca evenly broad apically, apex rounded, stem broad, sinuate, and looped posteriorly (Figs 41, 42) ..... ***Boreophilia hyperborea*** **(Brundin)**

– Genitalic structures not as above **12**

12 Capsule of spermatheca with apical part ovoid, apical invagination not apparent, stem narrow and hooked posteriorly (Fig. 120); male undescribed..... ..... ***Boreophilia davidgei*** **Klimaszewski & Godin**

– Capsule of spermatheca with apical part spherical, apical invagination present, stem moderately wide (Fig. 116); median lobe of aedeagus as illustrated (Figs 110, 111) ..... ***Boreophilia vega*** **(Fenyes)**

13 Tubus of median lobe of aedeagus with rounded baso-lateral projection in lateral view (Fig. 17); capsule of spermatheca with narrowly rounded apical part (Fig. 23) ..... ***Boreophilia islandica*** **(Kraatz)**

– Tubus of median lobe of aedeagus without basal projections in lateral view (Fig. 25); capsule of spermatheca with apical part narrowed and pointed laterad (Fig. 31) ..... ***Boreophilia fusca*** **(CR Sahlberg)**

Boreophilia fusca species group [modified from Lohse et al. 1990]

This group contains mostly broad and large species (except *B. nomensis*), and is defined based on similarity of median lobe of aedeagus and spermatheca. Bulbus of aedeagus is moderately broad to broad, oval in shaped in dorsal view, with two prominent and elongate structures bearing ventrally a short, angular projection (Figs 9, 10, 18, 26, 35, 36, 46, 60); tubus of median lobe is triangular in dorsal view (Figs 9, 10, 18, 26, 35, 36, 46, 60), and in lateral view arcuate or straight and in most species narrow apically (Figs 7, 8, 17, 25, 33, 34, 44, 45, 58). Spermatheca: capsule pitcher shaped basally and tubular apically, apical invagination lacking; stem long, strongly sinuate and looped or arcuate posteriorly (Figs 15, 23, 31, 41, 42, 51, 52, 56).

Species included: *Boreophilia eremita* (Rye), *B. islandica* (Kraatz), *B. fusca* (CR Sahlberg), *B. hyperborea* (Brundin), *B. nearctica* Lohse, *B. nomensis* (Casey), and *B. ovalis* Klimaszewski & Langor.

1. *Boreophilia eremita* (Rye, 1866)

BIN BOLD:ABW4331
Figs 6–15

Homalota eremita Rye, 1866: 123. Brundin 1953: 407 [as *B. hercynica*], Palm 1970: 260 [as *B. hercynica*], Lohse et al. 1990: 155, Smetana 2004: 396, Klimaszewski et al. 2011: 184, 2018: 566.

Atheta aluvialis Renkonen, 1936: 117. Smetana 2004: 396.

Cotypus. Finland: Muonio, Renkonen, *A. islandica* Kr., var. *alluvialis* Renk (MHNG) 1 male, 1 female.

Atheta smolkai Rybiński, 1902: 11. Smetana 2004: 396.

Diagnosis. Body moderately broad, forebody slightly and abdomen strongly glossy (Fig. 6); length 3.0–3.6 mm; dark brown to almost black, appendages light brown or reddish brown; antennomeres VIII-X subquadrate to slightly elongate; pronotum as long as elytra at suture, maximum width of pronotum slightly less than maximum width of elytra, pronotum in males is longer and less transverse that in females. **Male**. Tubus of median lobe of aedeagus with two, sharp basolateral projections on both sides in dorsal view (Figs 9, 10), and in lateral view ventral part of tubus produced angularly at base (Figs 7, 8), bulbus broadly oval in dorsal view, with two elongate narrow sclerites of internal sac (Fig. 9); tergite VIII arcuate apically (Fig. 11); sternite VIII elongate, parabolic apically (Fig. 12). **Female**. Spermatheca: capsule pitcher-shaped basally with elongate narrowly conical apical part without apical invagination, stem sinuate, narrowly looped posteriorly (Fig. 15); tergite VIII arcuate apically (Fig. 13); sternite VIII rounded apically, antecostal suture moderately sinuate (Fig. 14).

The median lobe of aedeagus of *B. eremita* is similar to that of *B. islandica*, but tubus is more elongate, narrower, less arcuate, and with basal projection angular in

lateral view (Figs 7, 8), in dorsal view tubus with two pointed latero-basal projections (Figs 9, 10). In *B. islandica*, the tubus is broader, shorter, more arcuate ventrally, and with basal arcuate projection rounded in lateral view (Fig. 17), in dorsal view tubus with two smaller and less angular basal projections (Fig. 18). Spermathecae of the two species is variable in shape and very similar, female tergite and sternite VIII are similar in shape but sternite VIII in *B. eremita* has feebly arcuate medial part of antecostal suture (Fig. 14), which is strongly sinuate in *B. islandica* in the majority of examined specimens (Fig. 22). On average, the body of *B. eremita* is narrower and elytra shorter than that of *B. islandica*.

Distribution. Holarctic species; recorded from north and central Europe, Ireland, Ukraine, Russia (west and east Siberia) and the Russian Far East; Canada: LB, NB, MB; USA: AK.

Collection data. Habitat: in NB – old silver maple forest with green ash and seasonally flooded marsh; silver maple swamp, margin of vernal pond, found in moist leaves. In AK - creekside/ocean beach confluence, under boards and drift wood; black and white spruce, willow; subalpine habitat with *Veratrum*, and *Calamagrositis*. Collecting methods: sifting litter, Lindgren funnel trap, Malaise trap, hand collecting. Collecting period: May to August.

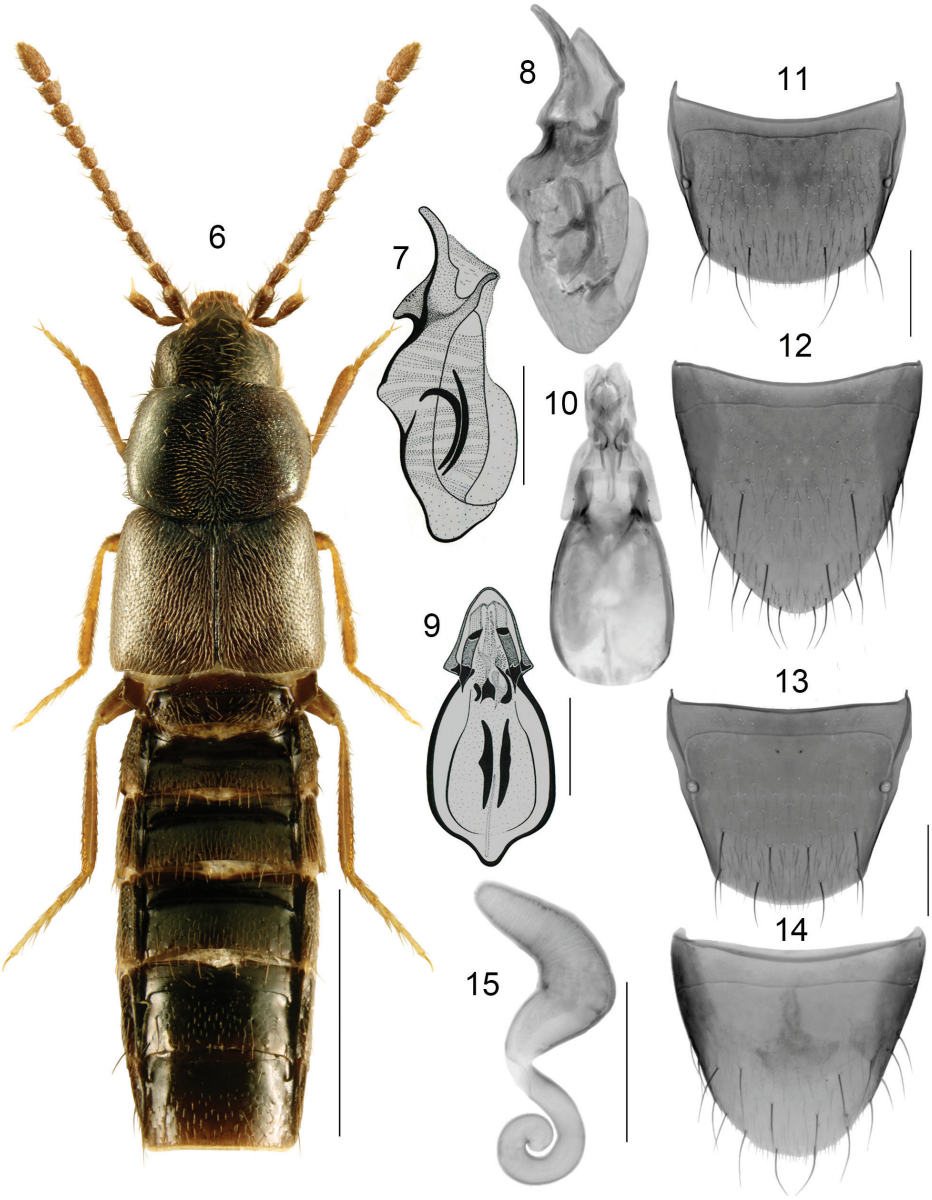
Additional material examined. NEARCTIC: Canada, New Brunswick, Queens Co., Grand Lake Meadows P.N.A., 45.8227N, 66.1209W, 19–31.V.2010, old Silver Maple forest with Green Ash and seasonally flooded marsh, Lindgren funnel trap (LFC) 1 male; New Brunswick, Queens Co., Canning Grand Lake near Scotchtown, 45.8762N, 66.1817W, 25.V.2006, Silver maple swamp, near lake margin, margin of vernal pond in moist leaves, RP Webster coll. (LFC) 1 female.

USA, Alaska, mi 110 Denali Hwy., Seattle Creek, 15.VII.1978, JM Campbell and S Smetana, coll. GA Lohse MHNG-1994 (MHNG) 1 male.

Females without male association, tentatively included in *B. eremita* [they may represent *B. eremita* or extreme narrow forms of *B. islandica*]: **USA**, Alaska, Naknek, 58.73973N, 157.0636W, creekside/ocean beach confluence, under boards and drift wood, hand collected, 10.VI.2007, D. Sikes, UAM100012293, UAM100012313, UAM100012315 (UAM) 3 females; Kenai Pen., Kalifonsky Bch. Near Kenai, 8.VI.78, A Smetana and E Becker (MHNG) 1 female.

PALEARCTIC: Finland: Muonio, Renkonen (MHNG) 6 females. **Germany**: b. Grönau, Lübeck, 10.V.34, G Benick (MHNG) 1 female [ident. uncertain]. **Russia**: Siberia, Tschimilcan, FI. Lenam. [Lena River], B. Poppius (MHNG) 1 male.

Comments. We have examined several European specimens identified as *B. hercynica*, which have the shape of median lobe of aedeagus and spermatheca similar to *B. eremita*, but the body color and the shape and proportions of forebody were different: body brown with dark brown head and pronotum and particularly elytra paler, and pronotum strongly transverse with sides broadly and evenly arcuate, elytra at suture slightly shorter than pronotum along midline. These specimens may represent extreme variation of *B. eremita* or a different and distinct species. Additional material is needed, and possibly DNA studies, to establish clear status of these specimens.



Figures 6–15. *Boreophilia eremita* (Rye): **6** habitus **7, 8** median lobe of aedeagus in lateral view **9, 10** median lobe of aedeagus in dorsal view **11** male tergite VIII **12** male sternite VIII **13** female tergite VIII **14** female sternite VIII **15** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

These specimens were labelled as follows: **Belgium**, Elsenborn, VIII.1931, Hohes Venn, coll. Benick (MHNG) 1 male; **Germany**, Brocken, Heinemann, *hercynica* s. mihi, det L Brundin (MHNG) 1 male; same label as before except: *islandica* Kr. (MHNG) 1 male; Brocken, Harz, 10.VI.1912, *islandica*, col. G Benick (MHNG) 1

male, Brocken, Heinemann (MHNG) 1 female, Brocken, 10–12.V.1913, Linke leg., *A. islandica* v. *hercynica* Renk., (MHNG) 1 female, Brockenkuppe, 26.VII.1916, coll. G Bennick (MHNG) 1 male; **Norway**, Kongberg [= Konsberg?], Münster, *islandica* Kr., coll. G Benick (MHNG) 1 female, Hammerfest, A Strand, *islandica* s. mihi, det. L Brundin (MHNG) 1 male, Norge, Skibotu, 22.VIII.1960, leg. Puthz, coll. Benick (MHNG) 1 male; **Scotland**, D Sharp, *hercynica* s. mihi det. L Brundin, *Metaxya eremita* Rye (MHNG) 2 males; Shetland Is., Mainland, Stany Fields nr. Wal Is., 13.VII.1963, ME Bacchus BM 1963-471, coll. G Benick (MHNG) 1 female; **Sweden**, Dorotea, Sa. Lappland, 30–31.V.1935, Bruce, *A. islandica*, coll. Benick (MHNG) 1 female.

DNA Barcode data. Our data included six sequences of *B. eremita* from Finland (four from Lapland and two from Northern Ostrobothnia), which grouped into BIN BOLD:ABW4331. BOLD reports these sequences have an average distance of 0.06%, a maximum distance of 0.18% and are 4.1% distant from their nearest neighbor.

2. *Boreophilia islandica* (Kraatz, 1857)

BIN BOLD:AAH0226
Figs 16–23

Homalota islandica Kraatz, 1857: 284. Smetana 2004: 396, Klimaszewski et al. 2011: 184, 2018: 567. **Syntypes**: Island, Krüper, coll. Kraatz, syntypus, *islandica* mihi (DEI) 1 male; 1 female. Examined.

Diagnosis. Body broad, forebody moderately and abdomen strongly glossy (Fig. 16); length 2.8–3.5 mm; uniformly black with paler, reddish brown appendages, or head, pronotum and VI-VII basal segments of abdomen dark brown, remainder of the body reddish brown, appendages light brown, sometimes elytra with some reddish tinge; antennomeres VIII-X subquadrate; pronotum as long as elytra at suture, maximum width of pronotum distinctly less than maximum width of elytra; elytral length variable, as long as pronotum or slightly longer. **Male**. Tubus of median lobe of aedeagus with two basolateral and slightly angular apically projections in dorsal view (Fig. 18), and one rounded projection in lateral view (Fig. 17); bulbus moderately broadly oval with two elongate sclerites of internal sac in dorsal view (Fig. 18); tergite VIII arcuate apically (Fig. 19); sternite VIII elongate, parabolic apically (Fig. 20). **Female**. Spermatheca: capsule pitcher-shaped basally with tubular apical projection moderately long and narrowed apically, stem coiled posteriorly, there is great variability in the shape of capsule (Fig. 23); tergite VIII arcuate apically (Fig. 21); sternite VIII rounded apically, antecostal suture strongly sinuate in most examined specimens (Fig. 22). For the differences between this and previous species see diagnosis of *B. eremita*.

Distribution. Holarctic species; recorded from Fennoscandia, Estonia, Faeroe Islands, Great Britain, Iceland, Russia (North European Territory); Canada: LB, NF, NT, NU, YT; USA: AK.

Collection data. Habitat [new data]: *Betula*, *Salix* litter; *Salix* tundra hillside; *Salix*/*Betula*/*Alnus*/grasses; black/white spruce, willow; vegetation at lakeshore pond; subalpine habitat with *Veratrum*, *Calamagrostis*, and *Leymus*, *Heracleum*, *Angelica*. Collecting methods: hanging Malaise trap, pitfall traps, sweeping with net. Collecting period: May to August.

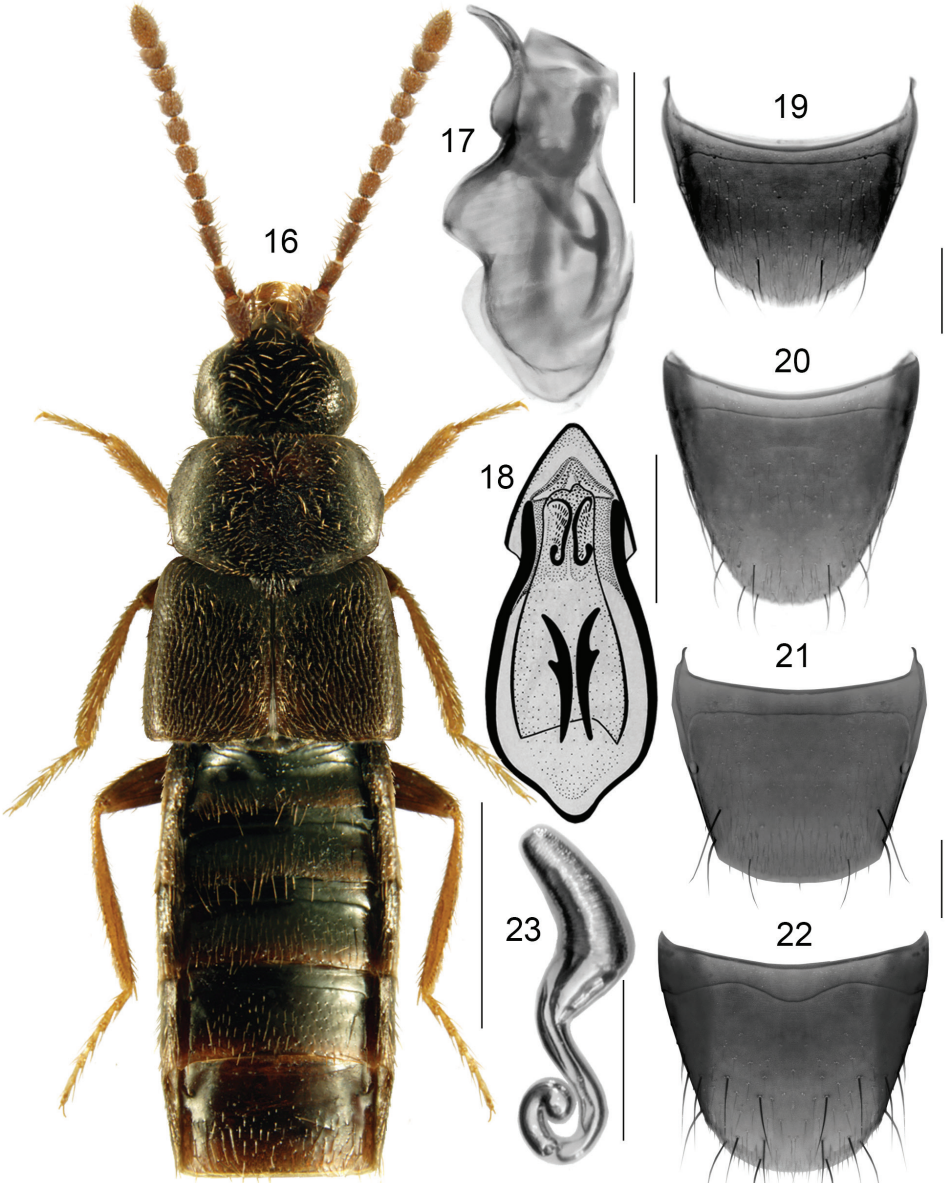
Comments. Females of this species may be confused with other species of *Boreophilia* and particularly those of closely related *B. eremita*. Associating females with males is considered here to be the most reliable way of identifying females of this and the previous species. At present, *B. islandica* is considered a somewhat variable species. Specimens vary from moderately robust and narrower to more robust and broader, with elytra as long as pronotum or slightly longer, all with the same morphology of genitalia. The BIN BOLD:ABX3767 formed a sister group to *B. islandica* in our analysis and was represented by a single female from Finland. The capsule of its spermatheca is curved at an angle of nearly 90 degrees and was among those shapes included in the illustrations of *B. islandica* by Palm (1970). BIN BOLD:ABX3767 may represent an undescribed species, or one of the described species not included in our DNA dataset, but corresponding males should be examined. A separate DNA based study is recommended to examine specimens of *B. islandica* sensu lato from a broad Holarctic distribution, including males and females.

Additional material examined. NEARCTIC: Canada, Newfoundland, Long Range Mts., Portland Cr. Hill, 12–13.VIII.1982, Belland, Larson, McDonald (LFC) 1 female; Northwest Territories, 10.VI.1956, R.E. Leech (LFC) 1 male; Northwest Territories, Aklavik, 14.VI.1956, RE Leech (MHNG) 1 male, 1 female.

USA, Alaska: Anaktuvuk Pass, 647 m el., 68.14049N, 151.74091W, +/- 250 m, *Salix*, sweeping, 19.V.2016, D Sikes, K Daly, UAM100427773 (UAM) 1 male; Alaska, Aguttu Is., 15 m el., 52.473083N, 173.59065E, +/- 30 m, beach, *Heracl. lanatum*, 5 pitfalls, 5–19. VI.2008, L Kenney, R Kaler, UAM100029353 (UAM) 1 male; Aiktak Is., 10–20 m el., 54.18549N, 164.8432W, +/- 50 m, *Leymus*, *Heracleum*, maritime tundra, 5 pitfalls, 17–31. VIII.2009, A.L. Larned, UAM100321897, UAM100321780 (UAM) 2 males; same data except: *Leymus*, *Heracleum*, *Angelica*, 11–29.VII.2009, UAM100321769 (UAM) 1 female; UAM100321861 (UAM) 1 female; 54.18356N, 164.82793W, +/- 1 km, maritime tundra, *Leymus*, *Heracleum*, 5 pitfalls, 9–24.VI.2009, AL Larned, UAM100322546 (UAM) 1 female; 24.VI-11.VII.2009, S. Sapora, UAM100323153, UAM100323220 (UAM) 2 females; 54.11036N, 164.50500W, +/- 5 m, streamside, *Leymus*, 2 pitfalls, 26.VI-14. VII.2008, BA Drummond, UAM100030104, UAM100030117 (UAM) 2 females.

Females without male association, tentatively included as *B. islandica*:

USA, Alaska: White Mtns. Rec. Area, 180 m el., 65.33469N, 146.83969W, +/- 10 m, b. & w. spruce, willow, hanging Malaise, 10–17.VI.2016, J Hagelin, UAM100407456 (UAM) 2 females; S. Chilkat Pen., pl. 19, 652 m el., 58.42509N, 135.14229W, +/- 30 m, subalpine, *Veratrum*, *Calamagrostis*, hand coll., 9.VII.2010, DS Sikes, UAM100277138 (UAM) 2 females; Kasatochi, 42 m el., 52.16869N, 175.52475W, +/- 34 m, under rocks, Talus, cliff base, 11.VI.2008, DS Sikes, UAM100031453, UAM100031445 (UAM) 2 females.



Figures 16–23. *Boreophilia islandica* (Kraatz): **16** habitus **17** median lobe of aedeagus in lateral view **18** median lobe of aedeagus in dorsal view **19** male tergite VIII **20** male sternite VIII **21** female tergite VIII **22** female sternite VIII **23** spermatheca. Scale bars: 1 mm (habitus); 0.2 mm (remaining).

PALEARCTIC: Czech Republic: Bohemia occ., Frant. Láznê-Soos, 1961, Smetana (MHNG) 2 females. **Denmark**: Helsinge, 6.V.1915, Häkan Lindberg (MHNG) 1 female. **Finland:** Kittilä, Renkonen (MHNG) 1 female; Lapp., Petsamo, 3.VII.1929, Häkan Lindberg (MHNG) 1 female; Oa Lappfiärd, 3.V.1944,

Harald Lindberg (MHNG) 1 female. **Germany:** I. b. Grönau, Lübeck, 27.III.12, G. Benick (MHNG) 1 female; Brocken, 15.V.32, Foln.…[not clear] (MHNG) 1 female; Brocken, Heineman (MHNG) 1 female; Brocken Harz, 15.VII.1935, Fehse, Thale, G Benick (MHNG) 1 female. **Island**: Island, VII.1969, G Benick (MHNG) 1 male. **Norvay**: Lakselv Po, A Strand (MHNG) 3 females. **Sweden**: Lule Lappmark, Vietas 20, 27.VI.1967, TE Leiler (MHNG) 1 female; Moen M. elev., A Strand (MHNG) 1 female; Ő Torne, T Palm (MHNG) 1 female; Imtl. Frost-viken, Palm (MHNG) 1 female.

DNA Barcode data. Our data included one sequence identified as *B. islandica* from Newfoundland and Labrador, Canada, and one sequence identified as *Boreophilia* sp. collected from Churchill, Manitoba, which are the only members of BIN BOLD:AAH0226. BOLD reports these sequences are 2.79% distant from their nearest neighbor.

3. *Boreophilia fusca* (CR Sahlberg, 1831)

BIN BOLD:AAG4311
Figs 24–31

Aleochara fusca CR Sahlberg, 1831: 371. Lohse et al. 1990: 152, Smetana 2004: 396.

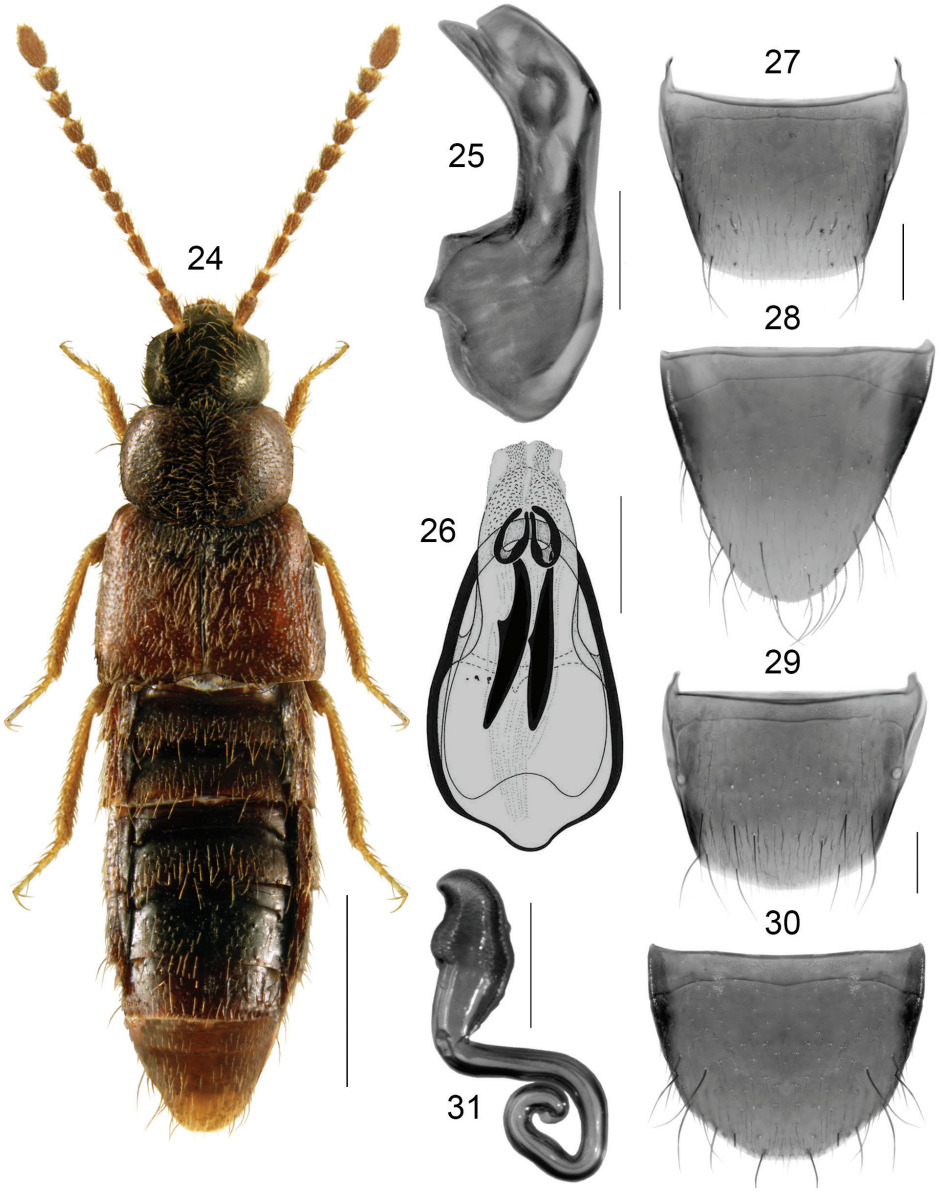
Diagnosis. Body broad, forebody moderately and abdomen slightly more glossy (Fig. 24); length 3.4–3.8 mm; head, pronotum and abdomen except for its apex dark brown, elytra dark-reddish brown, appendages light brown, or body entirely dark brown to almost black with tarsi reddish brown; antennomeres VIII-X subquadrate; pronotum shorter than elytra at suture, maximum width of pronotum distinctly less than maximum width of elytra. **Male**. Tubus of median lobe of aedeagus evenly arcuate laterally, apex narrow and slightly pointed in lateral view (Fig. 25), bulbus oval, broad basally and narrowed apically in dorsal view, and with two elongate sclerites of internal sac (Fig. 26); tergite VIII arcuate apically (Fig. 27); sternite VIII elongate, parabolic apically (Fig. 28). **Female**. Spermatheca: capsule pitcher-shaped basally with broadly tubular apical projection, moderately long and pointed apico-laterally, stem coiled posteriorly (Fig. 31); tergite VIII arcuate apically (Fig. 29); sternite VIII rounded apically, antecostal suture slightly sinuate (Fig. 30).

Distribution. Holarctic species; recorded from Fennoscandia, Russia (west and east Siberia) and the Far East; Canada: NT; USA: AK.

Collection data. Habitat: tundra. Collecting methods: not recorded in Nearctic region. Collecting period: June and July.

Additional material examined. NEARCTIC: Canada, NT, Aklavik, 16.VI.1956, EF Cashmann, fusca Sahlb. Det. Lohse (MHNG) 1 male; NT, Aklavik, 29.VI.1956, EF Cashmann, fusca Sahlb. Det. Lohse (MHNG) 1 female.

PALEARCTIC: Finland: Muonio, Renkonen, 2531, *A. fusca* Sahlb., Renkonen det., coll. G Benick (MHNG) 2 females. Country unspecified: Bjerkeng Schn. 21.VI.1912, coll. G Benick (MHNG) 1 male.



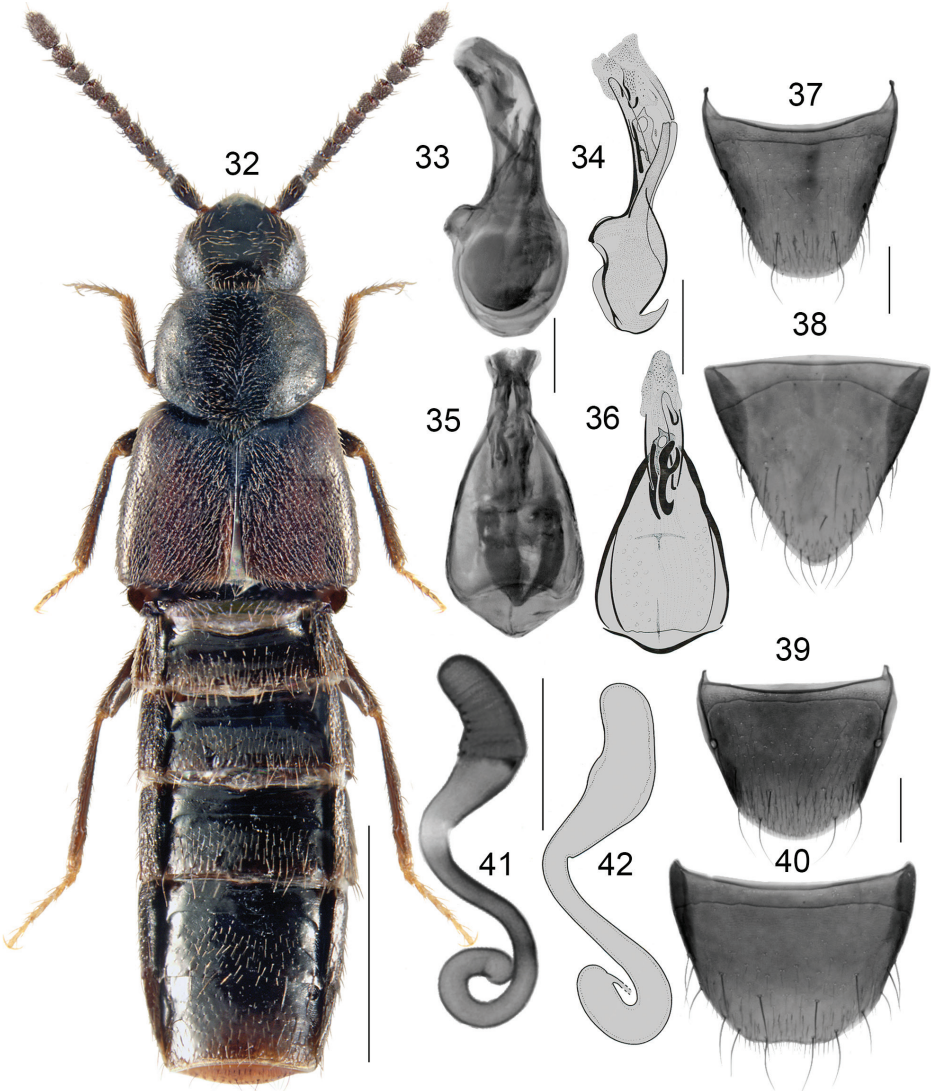
Figures 24–31. *Boreophilia fusca* (C.R. Sahlberg): **24** habitus **25** median lobe of aedeagus in lateral view **26** median lobe of aedeagus in dorsal view **27** male tergite VIII **28** male sternite VIII **29** female tergite VIII **30** female sternite VIII **31** spermatheca. Scale bars: 1 mm (for habitus); 2 mm (remaining).

DNA Barcode data. Our data included two sequences of *B. fusca*, one from Finland and one from Manitoba, Canada, which grouped into BIN BOLD:AAG4311. BOLD reports these sequences have an average and maximum distance of 0.54% and are 9.68% distant from their nearest neighbor.

4. *Boreophilia hyperborea* (Brundin, 1940)
Figs 32–42
BIN BOLD:AAG4302

Atheta hyperborea Brundin, 1940: 131. Lohse et al. 1990: 153, Smetana 2004: 396.

Diagnosis. Body broad, forebody glossy; length 2.8–3.5 mm; black with tarsi reddish brown (Fig. 32); antennomeres VIII-X subquadrate; pronotum as long as or slightly



Figures 32–42. *Boreophilia hyperborea* (Brundin): **32** habitus **33, 34** median lobe of aedeagus in lateral view **35, 36** median lobe of aedeagus in dorsal view **37** male tergite VIII **38** male sternite VIII **39** female tergite VIII **40** female sternite VIII **41, 42** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

shorter than elytra at suture, maximum width of pronotum distinctly less than maximum width of elytra. **Male**. Tubus of median lobe of aedeagus narrow, broadly arcuate in lateral view, apex narrow and rounded (Figs 33, 34), bulbus large, oval, broad basally and narrowed apically in dorsal view, and with two elongate sclerites (Figs 35, 36); tergite VIII arcuate apically (Fig. 37); sternite VIII elongate, parabolic apically (Fig. 38). **Female**. Spermatheca: capsule pitcher-shaped basally with evenly, broadly tubular apical projection, moderately long and rounded apically, stem sinuate, half-looped posteriorly (Figs 41, 42); tergite VIII broadly rounded apically (Fig. 39); sternite VIII rounded apically and truncate medially, antecostal suture straight medially and slightly sinuate laterally (Fig. 40).

Distribution. Holarctic species; recorded from Fennoscandia, Greenland, Russia (North European Territory); Canada: NT, NU; USA: AK.

Collection data. Habitat: tundra, under rocks. Collecting methods: hand collected from under rocks. Collecting period: June and July.

Additional material examined. NEARCTIC: Canada, NT, Barthurst Inl., Hiukitak River, 3.VIII.1966, GE Shewell, *B. hyperborea* Brn., det. GS Lohse (NHNG) 1 male.

USA, Alaska, Toolik Field Station, 724 m el., 68.6286N, 149.59772W, +/- 36 m, under rocks, 1- 3.VI.2008, D.S. Sikes, UAM100031281 (UAM) 1 male; Anaktuvuk Pass, 665 m el., 68.14059N, 151.74266W, +/- 200 m, *Salix*, tundra hillside, cobble, pollinator pitfall, 20.V.2016, D. Sikes, K. Daly, UAM100427687 (UAM) 1 female [tentative association].

PALEARCTIC: Norway, Vaalaasjö Andr. Strand, coll. G. Benick (NHNG) 1 female; Barviksmyren, W of Smelror, Varangerhalvøya, 22.VII.1998, V. Mahler (UCC) 1 female.

Greenland. Sdr. Strømfjord, 1.VII.1979, Brundin det. 1940 (NHMD) 1 female.

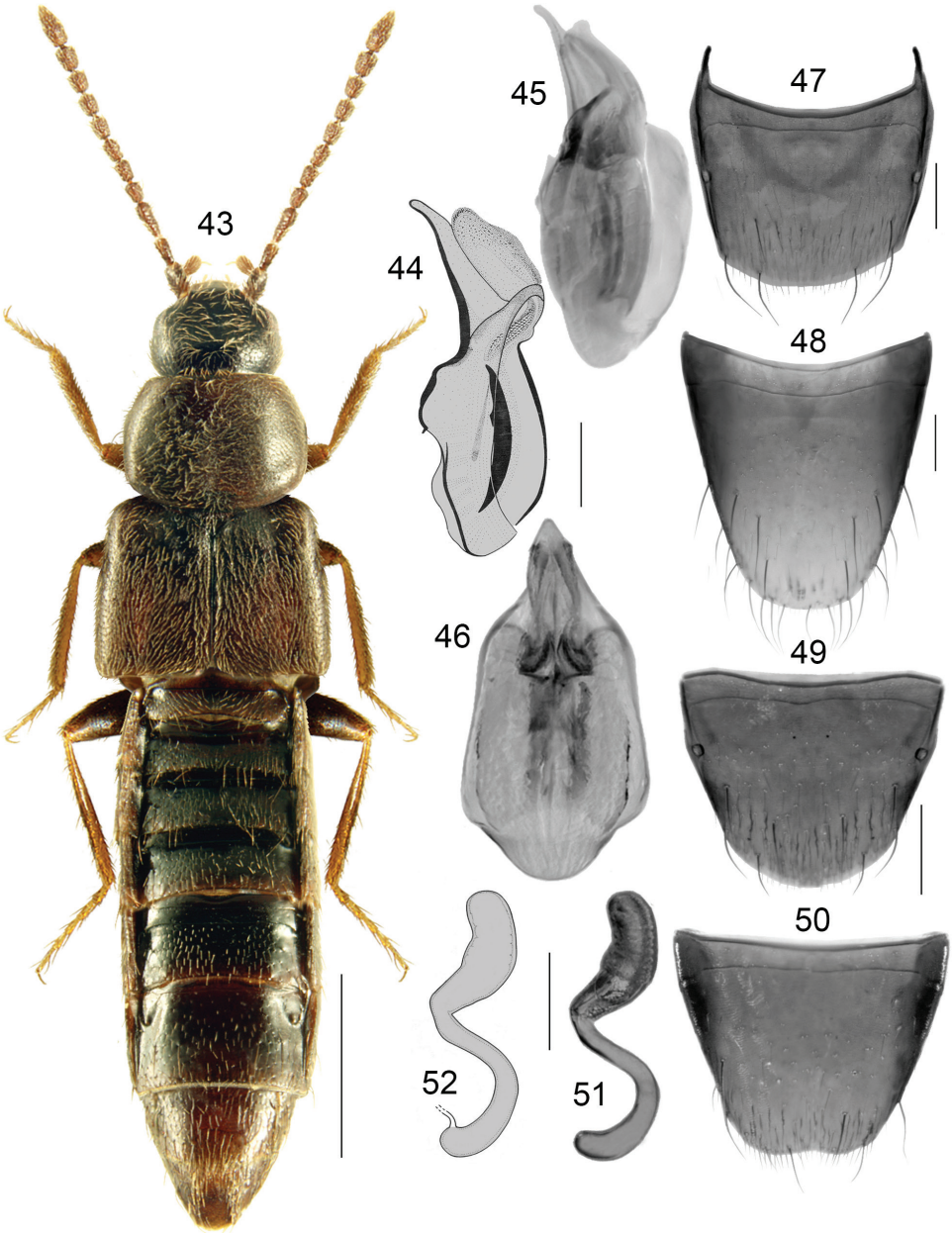
DNA Barcode data. Our data included two sequences of *B. hyperborea*, one from Russia and one from Manitoba, Canada, which grouped into BIN BOLD:AAG4302. BOLD reports these sequences have an average and maximum distance of 0.16% and are 6.82% distant from their nearest neighbor.

5. *Boreophilia nearctica* Lohse, 1990

BIN BOLD:ACU9385
Figs 43–52

Boreophilia nearctica Lohse, in Lohse et al. 1990: 153. Klimaszewski et al. 2011: 185, 2018: 567. **Paratype.** AK, mi 1252 Alaska Hwy., 7.VII.1968, *nearctica* nov. sp. Lohse, Paratype, Campbell and Smetana (NHNG) 1 male.

Diagnosis. Body moderately broad, forebody moderately glossy, abdomen slightly more so (Fig. 43); length 3.0–3.5 mm; head, pronotum and abdomen except for its apex dark brown, elytra reddish brown, appendages light brown, or body dark brown to almost black with elytra partially dark reddish brown and tarsi reddish brown; antennomeres VIII-X subquadrate to slightly elongate; pronotum ca. as long as elytra at suture, maximum width of pronotum distinctly less than maximum width of elytra.



Figures 43–52. *Boreophilia nearctica* Lohse: **43** habitus **44, 45** median lobe of aedeagus in lateral view **46** median lobe of aedeagus in dorsal view **47** male tergite VIII **48** male sternite VIII **49** female tergite VIII **50** female sternite VIII **51, 52** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

Male. Tubus of median lobe of aedeagus almost straight ventrally, apex narrow and pointed, slightly produced ventrally in lateral view (Figs 44, 45), bulbus broadly oval in dorsal view and with two elongate sclerites of internal sac (Fig. 46); tergite VIII arcuate

apically, slightly pointed medially (Fig. 47); sternite VIII elongate, parabolic apically (Fig. 48). **Female**. Spermatheca: capsule pitcher-shaped basally with broadly tubular apical projection, moderately long and rounded apically, stem sinuate and without posterior loop (Figs 51, 52); tergite VIII arcuate apically (Fig. 49); sternite VIII truncate apically and slightly emarginate medially, antecostal nearly straight medially and slightly sinuate laterally (Fig. 50).

Distribution. Nearctic species; recorded from Canada: AB [**new record**], LB, MB, QC, YT, NF; USA: AK.

Collection data. Habitat [new data]: black spruce forest; alpine meadow. Collecting methods: hanging Malaise trap, pitfall traps, hand collecting under rocks and litter. Collecting period: July to September.

Additional material examined. Canada, Alberta, Plateau Mountain, 50.226 -114.555, alpine meadow, under rocks and litter, 5.VII.2002, G Pohl and D Langor, 2 males, 1 female (CCDB-28541-D04, CCDB-28541-D05, CCDB-28541-D06) (NFRC); Newfoundland, Burnt Cape, site 10, 21U 0586332E, 5711616N, 51.54902N, 5.75489W, 24.VII.2003–7.VIII.2003 (LFC) 1 male; Burnt Cape, 55.755W, 51.549W, Coastal meadow, pitfall trap, 10–24.VII.2003, AM Hynes, Site 1–10 (LFC) 1 male; Yukon Territory, North Fork Pass, Ogilvie Mts.,3500', 17.VI.1962, RE Leech, paratype CNC No. 20308 (CNC) 1 male; North Fork Pass, Ogilvie Mts., 4100', 20.VI.1962, PJ Skitsko (CNC) 1 female; Quebec, Bonne Esperance, 14.VII.1929, WJ Brown, *nearctica* nov. sp. Lohse, Paratype (NHNG) 1 female.

USA, Alaska: Naknek, 58.73973N, 157.0636W, creek side/ocean beach confluence, under boards and drift wood, hand collected, 10.VI.2007, DS Sikes, UAM100012316 (UAM) 1 male; AK, Selawik NWR, 8 m el., 66.56137N, 158.99834W, +/- 304 m, *Spirea*, dwarf birch, 23–24.VI.2010, DS Sikes, UAM100283949 (UAM) 1 male; AK, Fairbanks, Bonanza Crk., 237 m el., 64.71922N, 148.38416W, +/- 10 m, birch, spruce, hanging Malaise trap, 6–13.V.2016, J Hagelin, UAM100407183 (UAM) 1 male; AK, Kenai Mts., Tern Lake Campground, 700', 18.Vi.1978, Smetana and Becker (NHNG) 1 male.

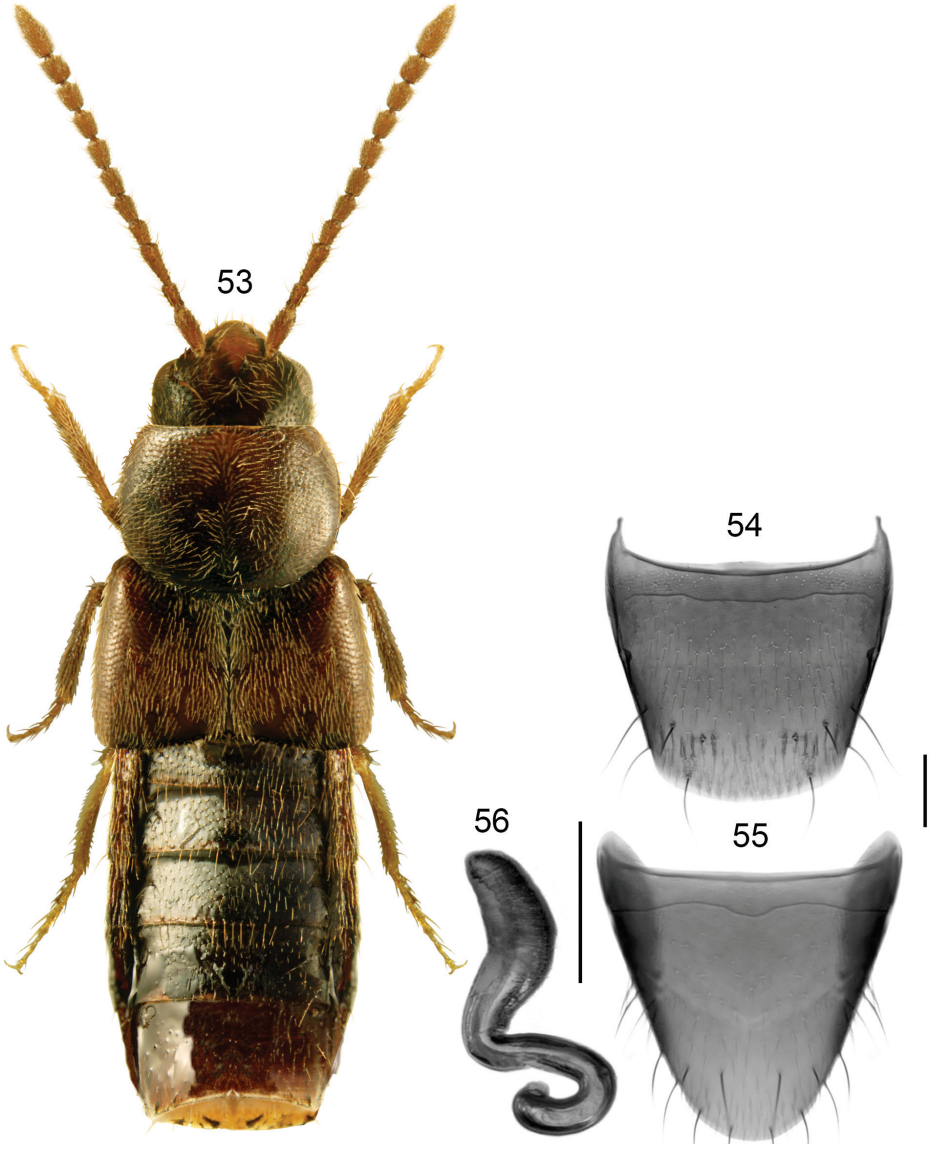
Comments. The southernmost record of this species in the Rockies of southern Alberta suggests that *B. nearctica* probably occurs continuously along the western cordilleras, at successively higher elevation sites with decreasing latitude.

DNA Barcode data. Our data included five sequences of specimens identified as *B. nearctica*, two from Alaska and three from Alberta, Canada, which grouped with two sequences identified as *Boreophilia* sp. into BIN BOLD:ACU9385. Our calculations indicate that the five sequences identified to species have an average distance of 0.14%, a maximum distance of 0.33% and are 6.37% distant from their nearest neighbor.

6. *Boreophilia ovalis* Klimaszewski & Langor, 2011

Figs 53–56

Boreophilia ovalis Klimaszewski & Langor, in Klimaszewski et al. 2011: 186. Klimaszewski et al. 2018: 568.



Figures 53–56. *Boreophilia ovalis* Klimaszewski and Langor: **53** habitus **54** female tergite VIII **55** female sternite VIII **56** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

Diagnosis. Body very broad, forebody moderately and abdomen strongly glossy (Fig. 53); length 3.0–3.5 mm; head, pronotum and abdomen except for its apex dark brown, elytra dark-reddish brown medially, appendages brown, or entire body dark brown to almost black and tarsi reddish brown; antennomeres VIII-X elongate; pronotum ca. as long as elytra at suture, maximum width of pronotum slightly less that the maximum width of elytra. **Male**. Unknown. **Female**. Spermatheca: capsule pitcher-shaped basally

with broadly tubular and slightly pointed apical part, stem short, strongly sinuate and looped posteriorly (Fig. 56); tergite VIII arcuate apically (Fig. 54); sternite VIII rounded apically, antecostal suture strongly sinuate medially (Fig. 55). Females of this species may be confused with those of *B. fusca*, from which they differ by distinctly elongate antennomeres VIII-X, more deeply medially sinuate antecostal suture of sternite VIII, and spermathecal capsule more evenly elongate and apex less pointed laterad.

Distribution. Nearctic species, recorded only from Canada: NF.

Collection data. Habitat: unspecified forest. Collecting methods: one female was captured in Malaise trap. Collecting period: June to September.

DNA Barcode data. Two specimens of this species, one being a paratype, were submitted for DNA barcoding but failed to generate DNA sequences (process IDs on BOLD: LFCAB222-15, NGSFT931-15).

7. *Boreophilia nomensis* (Casey, 1910)

BIN BOLD:ACU9384
Figs 57–62

Dinaraea nomensis Casey, 1910: 96. As *Boreophilia*: Lohse et al. 1990: 160; Gusarov 2003: 81. **Lectotype** (male): USA, Alaska, Nome (USNM). Designated by Gusarov 2003: 81.

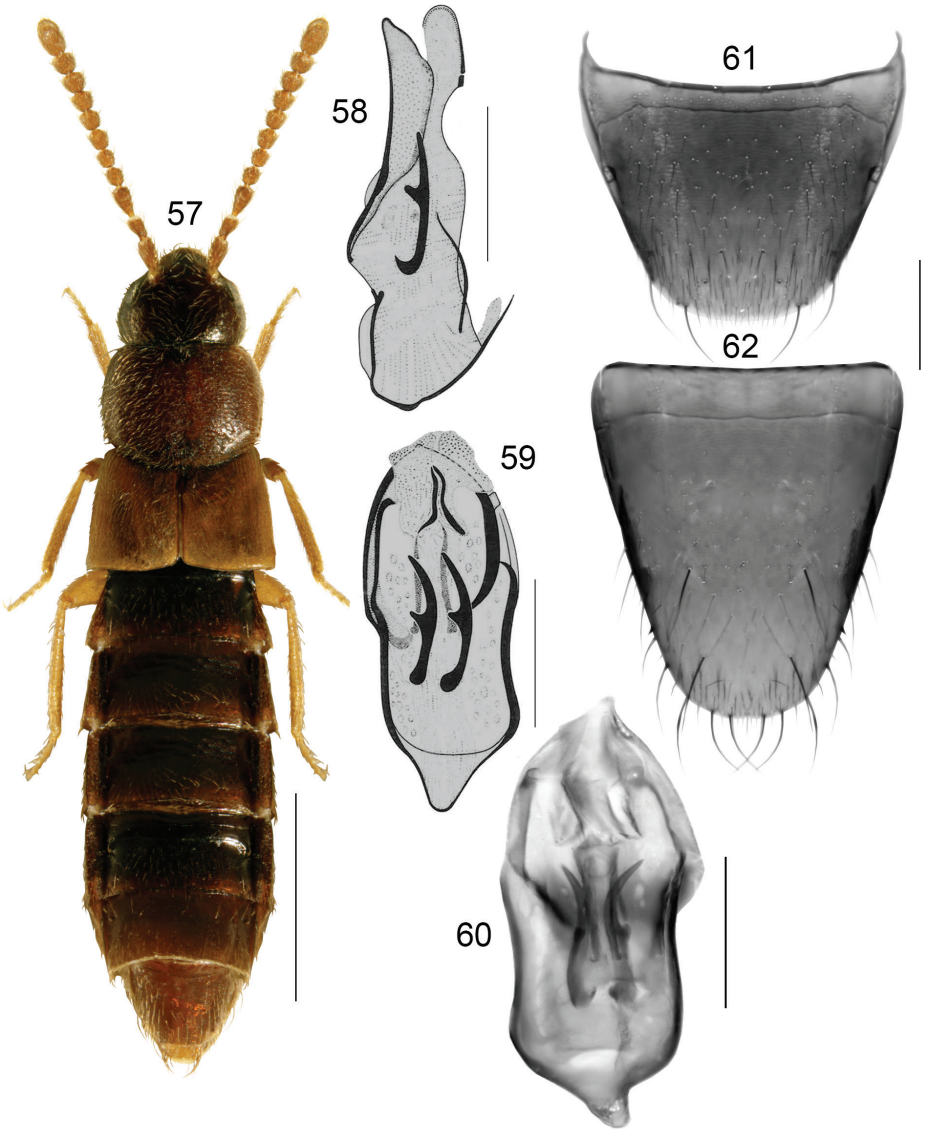
Boreophilia caseyiana Lohse 1990, in Lohse et al. 1990. Synonymized by Gusarov 2003. **Holotype** (male): Canada, Yukon Territory, North Fork Pass, Ogilvie Mts., 3500', 17.VI.1962, RE Leech, CNC No. 20312 (CNC).

Diagnosis. Body narrow, subparallel, moderately glossy, abdomen slightly more so (Fig. 57); length 3.0–3.8 mm; head, pronotum and abdomen dark brown, elytra reddish brown, legs paler, yellowish brown; antennomeres VIII–X subquadrate; pronotum longer than elytra at suture, maximum width of pronotum ca. the same as maximum width of elytra. **Male**. Tubus of median lobe of aedeagus almost straight (slightly arcuate) in lateral view, apex triangular in shape, slightly pointed (Fig. 58), bulbus broad in dorsal view and with two elongate narrow sclerites as illustrated (Figs 59, 60); tergite VIII arcuate apically (Fig. 61); sternite VIII elongate, parabolic apically (Fig. 62). **Female**. Undescribed.

Distribution. Nearctic species, recorded from Canada: YT, BC [new record]; USA: AK.

Collection data. Habitat: spruce and aspen forest with horsetail/shrub/grass undergrowth; edge of snowfield. Collecting methods: pitfall trap, hand collecting under rocks. Collecting period: June, July and August.

Additional material examined. Canada, British Columbia, Kinaskan Lake Provincial Park, Kinaskan Lake Trail, 57.532–130.202, 833 m, spruce and aspen forest, pitfall trap, 1.VIII.2014, BIObus 2014, (BIOUG24477-H04) (CBG). **USA,** Alaska, Thompson Pass, 61.137 -145.745, under rocks nr. snowfield, 28.VII.2010, DS Sikes and AB Sikes (UAM100288002) (UAM).



Figures 57–62. *Boreophilia nomensis* (Casey): **57** habitus **58** median lobe of aedeagus in lateral view **59, 60** median lobe of edeagus in dorsal view **61** male tergite VIII **62** male sternite VIII. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

DNA Barcode data. Our data included two sequences of specimens identified as *B. nomensis*, one from Alaska and one from British Columbia, Canada which grouped into BIN BOLD:ACU9384. BOLD reports these sequences have an average and maximum distance of 1.06% and are 4.64% distant from their nearest neighbor.

Boreophilia subplana species group

This newly defined group contains species defined by the similarity of the median lobe of aedeagus (Figs 64, 65, 72–74, 82, 83, 85, 86, 93, 94, 101–103, 110, 111), and capsule of spermatheca with a small apical invagination (Figs 70, 79, 80, 91, 99, 108, 116, 120). Bulbus of median lobe of aedeagus broad apically, oval in shape in dorsal view, with two prominent elongate structures of slightly different shape in different species (Figs 65, 74, 83, 86, 94, 102, 103, 111); tubus of median lobe of aedeagus arcuate or straight and broad or narrow in lateral view (Figs 64, 72, 73, 82, 85, 93, 101, 110); internal sac broadly open apically and supported by two narrow arcuate structures (Figs 65, 74, 83, 86, 94, 102, 103, 111). Spermatheca: capsule pitcher shaped basally and globular or ovoid apically with small apical invagination in most species; stem long strongly sinuate and looped or coiled posteriorly (Figs 70, 79, 80, 91, 99, 108, 116, 120).

Species included: *Boreophilia insecuta* (Eppelsheim), *B. neoinsecuta* Klimaszewski, sp. n., *B. beringi* Klimaszewski & Brunke, sp. n., *B. subplana* (J. Sahlberg), *B. caseyi* Lohse, *B. vega* Fenyes, *B. venti* (Lohse), and *B. davidgei* Klimaszewski & Godin.

8. *Boreophilia venti* (Lohse, 1990)

Figs 63–70

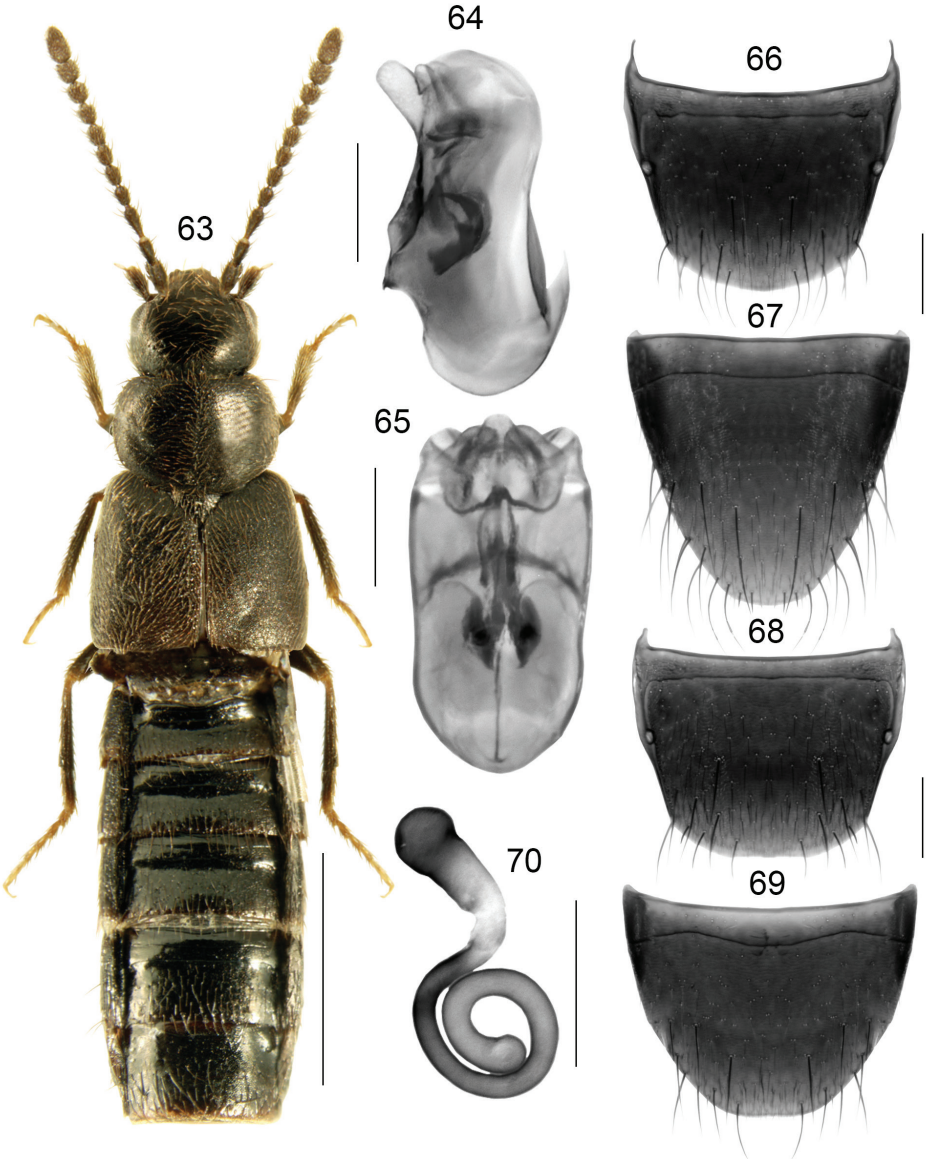
Dimetrota venti (Lohse), in Lohse et al. 1990: 183.

Holotype (male). Canada, Yukon Territory, British Mts., Windy Ridge, 450 m, 69.27N, 140.26W, 2.VII.1984, 84–47, sifting *Salix* litter, JM Campbell (CNC).

Paratypes. labeled as the holotype (CNC) 3 sex undetermined.

Diagnosis. Body narrowly subparallel, forebody moderately glossy, abdomen slightly more so (Fig. 63); length 3.0–3.4 mm; uniformly dark brown to black, appendages yellowish brown or reddish brown; antennomeres VIII-X subquadrate; pronotum ca. as long as elytra at suture, maximum width of pronotum slightly less than maximum width of elytra. **Male**. Tubus of median lobe of aedeagus arcuate basally, straight apically and produced ventrad in lateral view, apex broad and rounded in lateral view (Fig. 64), bulbus broad and with two large sclerites, each apically curved, sharply pointed, and with small median projection (Figs 64, 65); tergite VIII arcuate apically (Fig. 66); sternite VIII elongate, parabolic apically (Fig. 67). **Female**. Spermatheca: capsule club-shaped, tubular basally and rounded apically, with small apical invagination; stem sinuate and coiled posteriorly (Fig. 70); tergite VIII arcuate apically and truncate medially (Fig. 68); sternite VIII rounded apically and truncate medially, antecostal suture distinctly sinuate (Fig. 69). Spermatheca of this species is very similar to that of *B. neoinsecuta* (Figs 79, 80), but shape of apex of female sternite VIII are different in the two species (Figs 69, 78).

Distribution. Holarctic species, recorded from Europe, Finland; Asia, East and West Siberia, Mongolia; and North America: Canada: YT; USA: AK [**new record**].



Figures 63–70. *Boreophilia venti* (Lohse): **63** habitus **64** median lobe of aedeagus in lateral view **65** median lobe of aedeagus in dorsal view **66** male tergite VIII **67** male sternite VIII **68** female tergite VIII **69** female sternite VIII **70** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

Collection data. Habitat [new data]: *Salix* litter; *Salix* tundra hillside, lakeshore debris. Collecting methods: sifting *Salix* litter, pitfall traps, hand/aspirator collecting from under rocks. Collecting period: May and July.

Additional material examined. USA, Alaska, Anaktuvuk Pass, 640–680 m el., 68.14049N, 151.74091W,+/- 2 km in and around village, hand, forceps, 2–22.V.2016,

D Sikes, K Daly UAM100427681 (UAM) 1 female; Anaktuvuk Pass, 665 el., 68.14059N, 151.74266W, +/- 200 m, *Salix*, tundra hillside, cobble, pollinator pitfall, 20.V.2016, D Sikes, K Daly, UAM100427683 (UAM) 1 male; UAM100427684 (UAM) 1 female; UAM100427689 (UAM) 1 female; UAM100427693 (UAM) 1 male.

Comments. Lohse, in Lohse et al. 1990, described *Dimetrota venti* from Yukon. This species is extremely similar externally and genitally to *Boreophilia insecuta* described from Europe. The genitalia of *B. insecuta* were illustrated by Brundin (1954), and here, based on a specimen from Siberia (Figs 82, 83).

In *B. venti*, the tubus of the median lobe is distinctly arcuate in lateral view (Fig. 64), but in *B. insecuta* it is always straight basally (Fig. 82) and the two main sclerites are slightly different in shape (Figs 64, 65, 82, 83). However, the remaining genital characters are similar and females of the two species are not distinguishable morphologically.

9. *Boreophilia neoinsecuta* Klimaszewski, sp. n.

http://zoobank.org/364757AB-CD31-40E6-82E5-BEE1903B9621
BIN BOLD:ADR7545
Figs 71–80

Boreophilia insecuta sensu Lohse, in Lohse et al. 1990: 157. **Misidentification**.

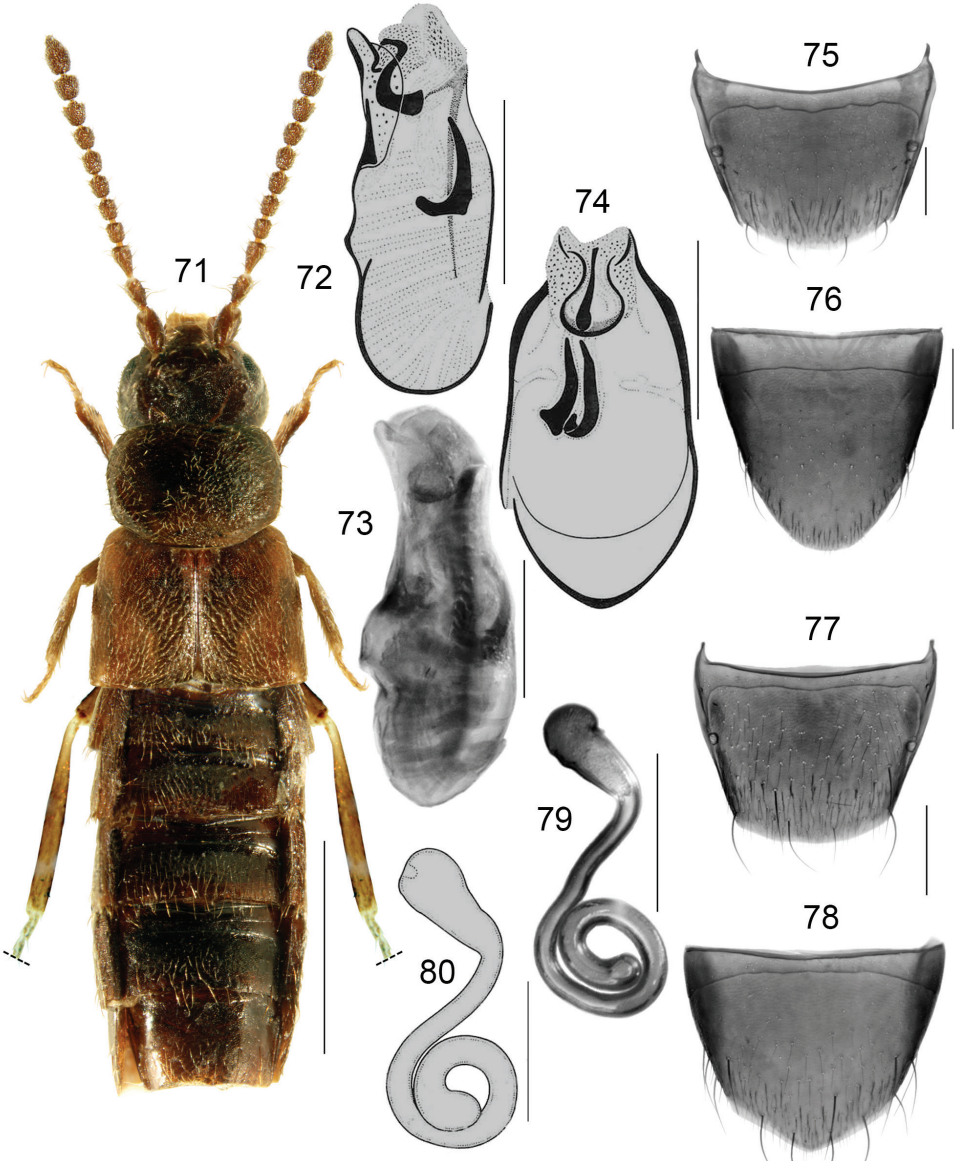
Holotype. (male): USA, Alaska, North Slope, Atkasuk, 17.VII.1978, B Vogel coll., B. insecuta det. Lohse (CNC).

Paratypes. USA, Alaska, Anaktuvuk Pass, 647 m el., 68.14049N, 151.74091W, +/- 250 m, under rocks, forceps/aspirators, 19.V.2016, D Sikes, K Daly, UAM100413204, UAM100413205, UAM100413207 (UAM) 3 females; Anaktuvuk Pass, 640–680 m el., 68.14049N, 151.74091W, +/- 2 km in and around village, hand/forceps, 20–22.V.2016, D Sikes, K Daly, UAM100388381 (UAM) 1 female.

Etymology. Derived from prefix *neo-* added to existing specific name *insecuta*, a closely related species.

Diagnosis. Body moderately broad, subparallel, forebody moderately glossy, abdomen slightly more so (Fig. 71); length 3.2–3.5 mm; head, pronotum and abdomen except for its apex dark brown, elytra reddish brown, mottled with some darker spots, appendages reddish brown; antennomeres VIII–X subquadrate; pronotum ca. as long as elytra at suture, maximum width of pronotum ca. equals to maximum width of elytra. **Male**. Tubus of median lobe of aedeagus almost straight laterally, apex narrow and rounded, bulbus broad and with two elongate and posteriorly bent sclerites (Figs 72, 73), in dorsal view median lobe elongate oval (Fig. 74); tergite VIII arcuate apically (Fig. 75); sternite VIII elongate, parabolic apically (Fig. 76). **Female**. Spermatheca: capsule club-shaped, moderately long and rounded apically, with small apical invagination; stem sinuate and coiled posteriorly (Figs 79, 80); tergite VIII arcuate apically (Fig. 77); sternite VIII produced apically and pointed medially, antecostal suture slightly sinuate laterally (Fig. 78).

Distribution. Nearctic, Canada: MB, YT: USA: AK.



Figures 71–80. *Boreophilia neoinsecuta* Klimaszewski: **71** habitus **72, 73** median lobe of aedeagus in lateral view **74** median lobe of aedeagus in dorsal view **75** male tergite VIII **76** male sternite VIII **77** female tergite VIII **78** female sternite VIII **79, 80** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

Collection data. Habitat: tundra, under rocks. Collecting methods: forceps/aspirator. Collecting period: May to July.

Comments. Lohse, in Lohse et al. (1990) reported *Boreophilia insecuta* (Eppelsheim) in North America from AK, MB, YT, as a Holarctic species. However, these records represented a different and undescribed Nearctic species, which is here described as *B. ne-*

oinsecuta sp. n. The two species, *B. insecuta* and *B. neoinsecuta*, have similarly shaped genitalia, which explains why they were confused. The true *B. insecuta* (Eppelsheim) was illustrated by Brundin (1954), and here (Figs 82, 83), and has a distinctly dilated apex of median lobe of aedeagus in lateral view (Fig. 82), while it is narrower in *B. neoinsecuta* (Figs 72, 73), and the two sclerites of internal sac of median lobe of aedeagus are broader and differently shaped than those of *B. neoinsecuta* (Figs 82, 83). Spermathecae of both species are very similar in shape, but female sternite VIII in *B. insecuta* is apically rounded and truncate medially, while in *B. neoinsecuta* is triangularly produced apically and pointed medially (Fig. 78).

DNA Barcode data. Our data included two sequences of *B. neoinsecuta* paratypes, both from Alaska which grouped into BIN BOLD:ADR7545. These sequences are 0.00% distant from each other and BOLD reports they are 7.23% distant from their nearest neighbor.

10. *Boreophilia beringi* Klimaszewski & Brunke, sp. n.

http://zoobank.org/2CC0CBCF-99F6-49FC-89D4-DBF5A2761DE2
Figs 84–91

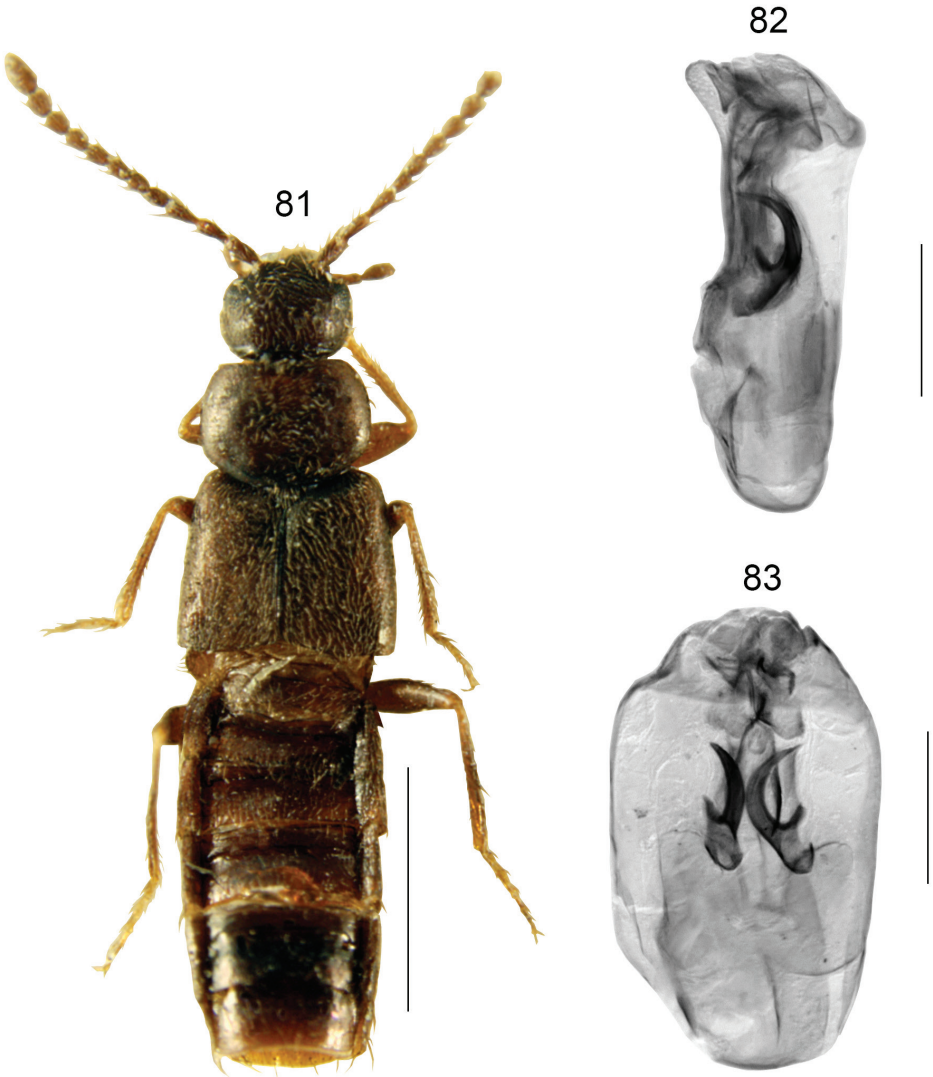
Holotype (male). USA, Alaska, Bering Land Bridge N. Pk., 413 m el, 65.83713N, 164.58995W, +/- 30 m snowfield, tundra, under rocks, on moss, 9.VII.2016, DS Sikes et al., UAM100418913 (UAM).

Paratypes. all labelled the same except: UAM100418886 (UAM) 1 male, UAM100418887 (UAM) 1 female, UAM100418892 (UAM) 1 female, UAM100418905 (UAM) 1 male, UAM100418912 (UAM) 1 female, UAM100418905 (UAM) 1 male.

Etymology. Named after Danish explorer Vitus Bering, whose name is shared with the species' type locality, Bering Land Bridge National Park, and 'Beringia', the area of adjacent Russia and Alaska that were previously connected multiple times during the past 1 million years.

Diagnosis. Body narrow, subparallel, glossy, abdomen slightly more so; microsculpture of forebody strong (Fig. 84); length 2.2–2.4 mm; head, head, pronotum and abdomen dark brown, elytra with reddish brown tinge, legs paler, yellowish brown; antennomeres VIII–X subquadrate; pronotum slightly longer than elytra at suture, maximum width of pronotum ca. the same as maximum width of elytra. **Male**. Tubus of median lobe of aedeagus straight basally and produced ventrad apically in lateral view, apex triangular in shape, distinctly pointed ventrad (Fig. 85), bulbus broad in dorsal view and with two elongate sclerites, strongly narrowed at one end as illustrated (Fig. 86); tergite VIII broadly arcuate apically (Fig. 87); sternite VIII elongate, narrowly rounded apically (Fig. 88). **Female**. Spermatheca: capsule club-shaped, moderately long and rounded apically, with small apical invagination; stem sinuate and coiled posteriorly (Fig. 91); tergite VIII arcuate apically (Fig. 89); sternite VIII broadly rounded apically, antecostal suture strongly sinuate medially (Fig. 90).

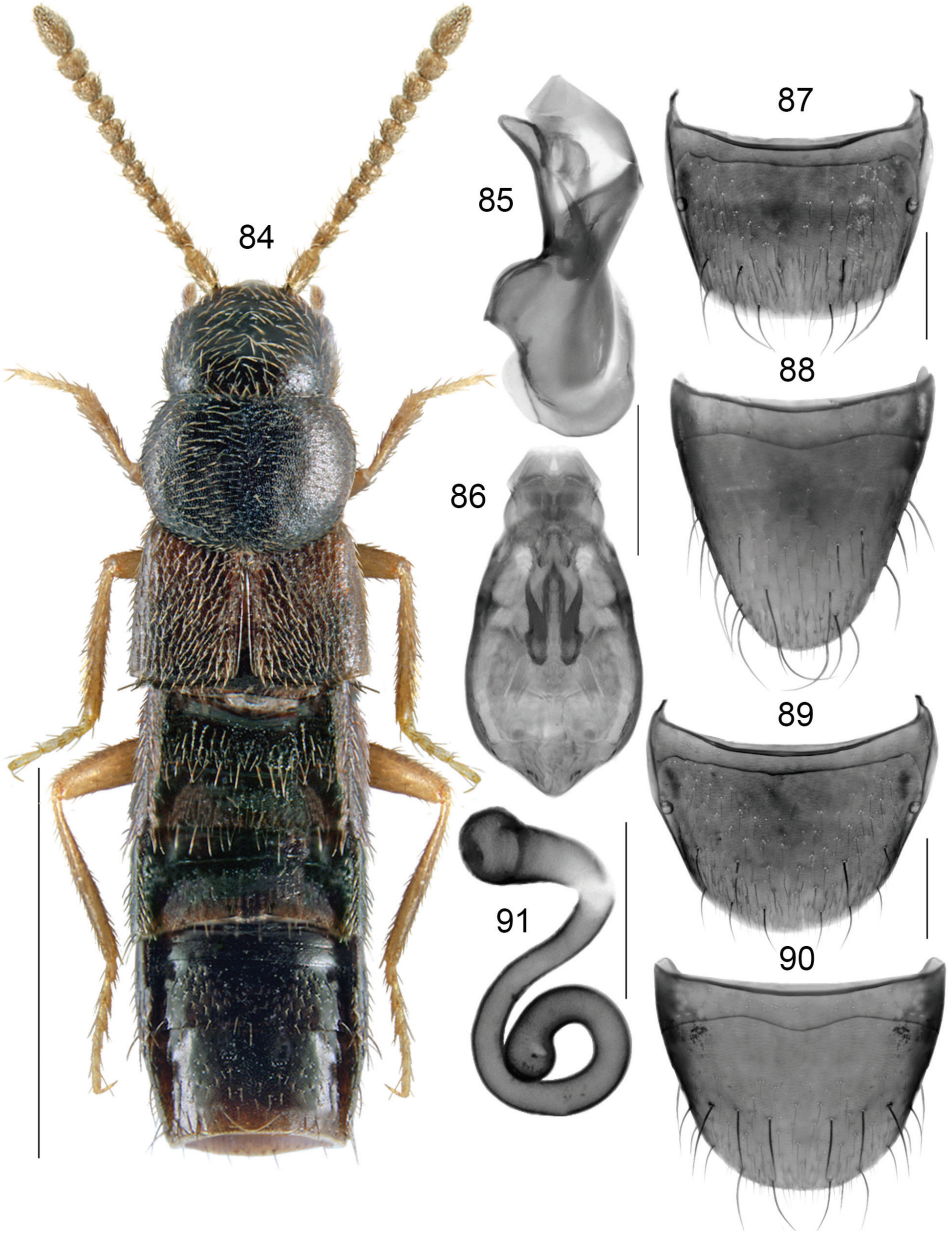
Distribution. Nearctic, USA: AK.



Figures 81–83. *Boreophilia insecuta* (Eppelsheim) [specimen from Siberia, Russia]: **81** habitus **82** median lobe of aedeagus in lateral view **83** median lobe of aedeagus in dorsal view. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

Collection data. Habitat: snowfield, tundra, under rocks, on moss. Collecting methods: aspirating from moss. Collecting period: July.

Comments. We here compared Palaearctic *Boreostiba piligera* (J. Sahlberg), two males from Finland (ZMUO, NHMD), with our new species from Alaska. The two species are very similar externally but may be distinguished by the different morphology of the median lobe of aedeagus. In *B. piligera*, the apical part of the tubus is more weakly deflexed ventrad and distinctly more elongate. The sclerites of the internal sac



Figures 84–91. *Boreophilia beringi* Klimaszewski and Brunke, sp. n.: **84** habitus **85** median lobe of aedeagus in lateral view **86** median lobe of aedeagus in dorsal view **87** male tergite VIII **88** male sternite VIII **89** female tergite VIII **90** female sternite VIII **91** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

are also more elongate and quite differently shaped (less like a talon and more even in thickness along their length). Therefore we conclude that these two populations represent sibling species.

11. *Boreophilia subplana* (J Sahlberg, 1880)

Figs 92–99

Atheta subplana J Sahlberg, 1880: 90. Lohse et al. 1990: 159; Gusarov 2003: 83 [as *B. angusticornis*]; Smetana 2004: 396.

Diagnosis. Body moderately broad, strongly glossy, abdomen slightly more so (Fig. 92); length 2.8–3.5 mm; black, elytra with some reddish tinge, tarsi yellowish brown; antennomeres VIII-X slightly elongate; pronotum shorter than elytra at suture, maximum width of pronotum distinctly less than maximum width of elytra. **Male**. Tubus of median lobe of aedeagus almost straight and slightly produced ventrad in lateral view, apex broad and rounded (Fig. 93), bulbus broad and with two elongate sclerites of distinctive shape (Figs 93, 94); tergite VIII truncate or slightly concave apically (Fig. 95); sternite VIII elongate, parabolic apically (Fig. 96). **Female**. Spermatheca: capsule broadly club-shaped, moderately long and rounded apically, with small apical invagination; stem sinuate, straight medially, and looped posteriorly (Fig. 99); tergite VIII arcuate apically (Fig. 97); sternite VIII triangularly produced apically and pointed medially, antecostal suture arcuate, straight medially (Fig. 98).

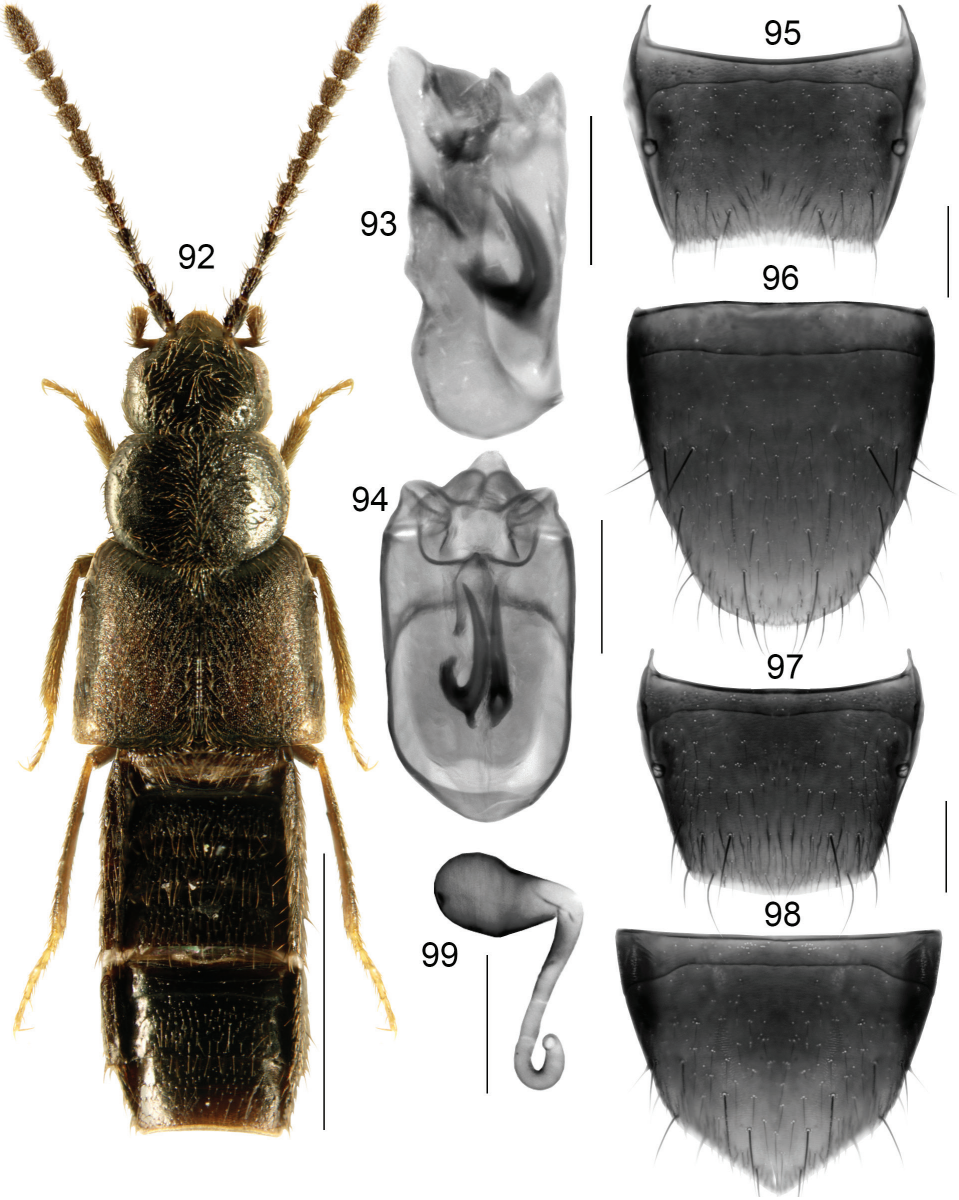
Distribution. Holarctic species, recorded from Spitsbergen, Fennoscandia, Russia (west and east Siberia); Canada: NT, NU; USA: AK, NH.

Collection data. Habitat: *Salix* leaf litter, tundra hillside, Black spruce forest, brackish shoreline, under rocks, wrack. Collecting methods: Malaise traps, aspirating from under rocks/cobbles, sweeping low vegetation, pitfall traps. Collecting period: June to August.

Additional material examined. NEARCTIC: Canada, NT, Muskox L., NWT, 64.45N, 108.10W, 2.VIII.1953, JG Chillcott, *Boreophilia subplana* Sahlb. Det. GA Lohse (MHNG) 1 male; Spence Bay, NWT, 2.VII.1951, *Boreophilia subplana* Sahlb. Det. GA Lohse (MHNG) 1 sex?

USA, Alaska: Anaktuvuk Pass, 647 m el., 68.14049N, 151.74091W, 19.V.2016, +/- 250 m, *Salix* leaf litter, Berlese funnel, D Sikes, K Daly, UAM100432806, UAM100432826, UAM100432833, UAM100431905, UAM100431909, UAM100413051, UAM100413054, *Salix*, sweeping, UAM100427774 (UAM) 4 males, 4 females; Anaktuvuk Pass, 665 m el., 68.14059N, 151.74266W, 20.V.2016, +/- 200 m, *Salix*, tundra hillside, pollinator pitfall, UAM100427686, UAM100427688, UAM100427690, UAM100427691, UAM100427692 (UAM) 2 males, 3 females; Tetlin NWR, Alaska Hwy, 63.36124N, 141.96761W, +/- 10 m, 13–24.VII.2015, Black spruce, Malaise, J Hagelin, UAM100391356 (UAM) 1 male; Tetlin NWR, Alaska Hwy site 2, 523 m el, 63.08456N, 141.96761W, +/- 10 m, 13–24.VII.2015, Black spruce, hanging Malaise, J Hagelin, UAM100390752 (UAM) 1 female; Kotzebue, 1 m el, 66.89151N, 162.5933W, +/- 100 m el, brackish shoreline, under rocks, wrack, 6.VII.2016, D Sikes, K Daly, UAM100420026 (UAM) 1 female; Anaktuvuk Pass, Eleanor Lk., 649 m el., 68.14742N, 151.71854W, +/- 100 m lake shore, pond, sweep, dipnet, 20.V.2016, D Sikes, K Daly, UAM100427750, UAM100427751 (UAM) 2 males.

PALEARCTIC: Russia, Polarnyi Ural, c. Tobols. Gyub. [ernia], F Zajzew, 5.VI.1909, A. subplana, det. Benick (MHNG) 1 male.



Figures 92–99. *Boreophilia subplana* (J Sahlberg): **92** habitus **93** median lobe of aedeagus in lateral view **94** median lobe of aedeagus in dorsal view **95** male tergite VIII **96** male sternite VIII **97** female tergite VIII **98** female sternite VIII **99** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

Comments. Bernhauer (1907) described *Atheta* (*Metaxya*) *angusticornis* from Mount Washington, New Hampshire, USA. Gusarov (2003) transferred it to the genus *Boreophilia*, and considered it closely related to *B. subplana*, from which he differentiated it by the "shape of aedeagus, particularly a narrower apex of the median lobe in parameral view". We have studied the median lobe of AK and NH specimens and found no differ-

ences warranting different species recognition. We therefore consider the two populations as belonging to the same species. The Mount Washington, NH, population represents a remnant, southernmost population of this species known only from higher elevations.

DNA Barcode data. Our data included two sequences of *B. subplana*, both from Finland but because they are < 500 bp in length, they were not assigned to a BIN on BOLD. Our calculations indicate these sequences have an average and within-species maximum distance of 0.0% and are 6.37% distant from their nearest neighbor.

12. *Boreophilia caseyi* Lohse, 1990

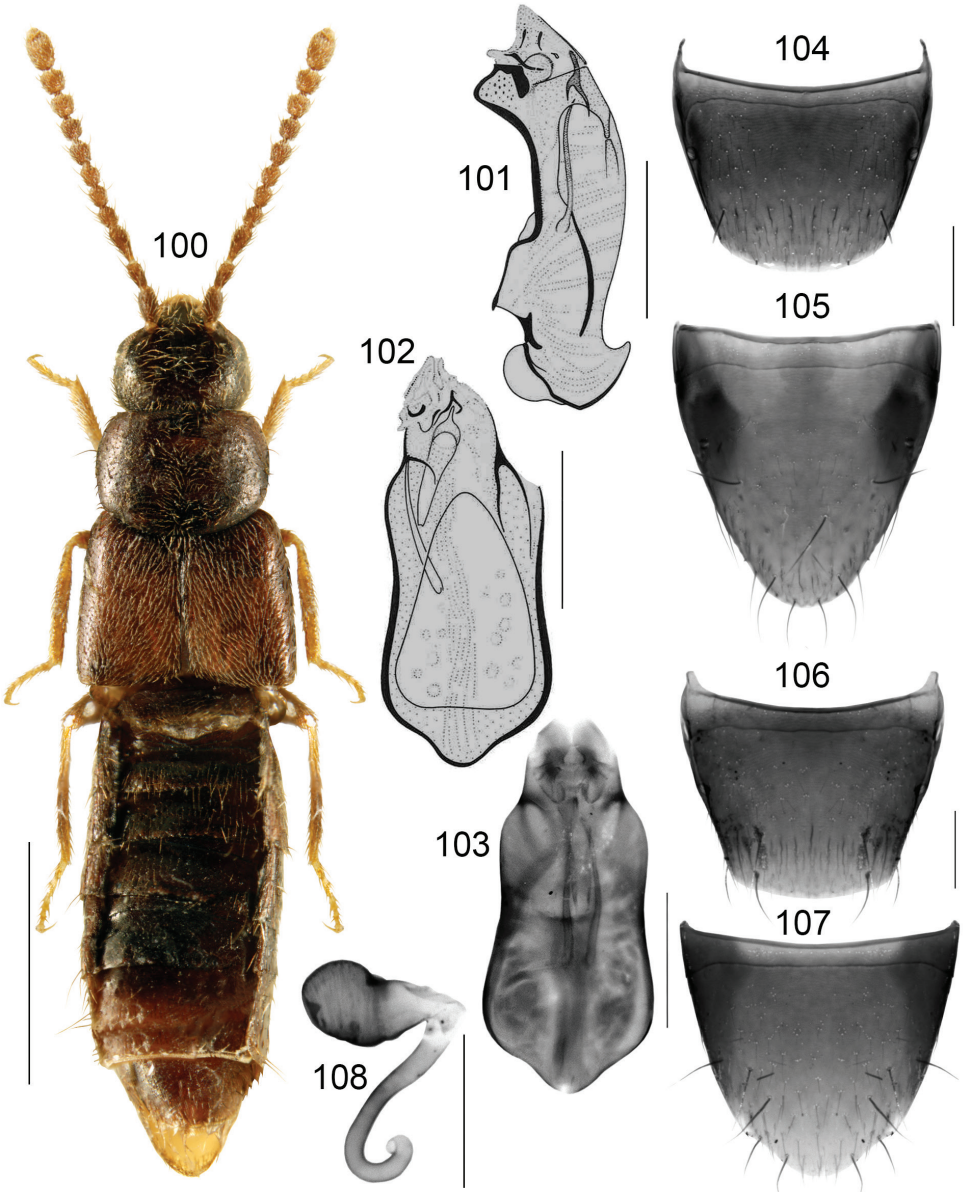
Figs 100–108

Boreophilia caseyi Lohse, in Lohse et al. 1990: 155. **Holotype** (male): USA, Alaska, Umiat, 5.VIII.1950, R Madge, *Boreophilia caseyi* Lohse, CNC No. 20309 (CNC) (fig. 0). **Paratypes**: USA, Alaska, Cape Thompson, 21.VII.1961, R Madge, CNC No. 20309 (CNC) 1 female; Canada, NWT, Wharton Lk., 63°52'N, 99°45'W, 18.VII.1966, JG Chillcott, CNC No. 20309 (CNC) 1 female; Canada, YT, North Fork Pass, Ogilvie Mts., 3500', 18.VI.1962, RE Leech, CNC No. 20309 (CNC) 1 female (figs 0).

Boreophilia manitobensis Lohse 1990, in Lohse et al. 1990. **New synonymy**. Canada: MB; USA: AK. **Holotype** (male): Canada, Manitoba, Churchill, 29.VI.1937, WJ Brown, CNC No. 20311 (CNC). **Paratypes**: USA, Alaska, Umiat, 12.VII.1959, JEH Martin (CNC) 1 sex undetermined; Manitoba, Churchill, 17.VI.1952, JG Chillcott (CNC) sex undetermined.

Diagnosis. Body narrow, subparallel, moderately glossy, abdomen slightly more so (Fig. 100); length 3.5–3.8 mm; head, pronotum and abdomen, except for its apex, dark brown, elytra reddish brown, legs yellowish red-brown, or body uniformly piceous with tarsi and tibiae reddish brown; antennomeres VIII-X subquadrate (females) to slightly elongate (males); pronotum as long as elytra at suture or slightly shorter, maximum width of pronotum slightly less than maximum width of elytra. **Male**. Tubus of median lobe of aedeagus straight basally and strongly projecting ventrad at apex, apex broad and angular in lateral view (Fig. 101), in dorsal view bulbus broad and angular apico-laterally, with two elongate narrow sclerites of internal sac (Figs 102, 103); tergite VIII arcuate apically (Fig. 104); sternite VIII elongate, parabolic apically (Fig. 105). **Female**. Spermatheca: capsule pitcher-shaped basally with subspherical apical part bearing small invagination, stem sinuate, narrow, looped posteriorly (Fig. 108); tergite VIII arcuate apically (Fig. 106); sternite VIII rounded apically, antecostal suture straight medially and sinuate laterally (Fig. 107).

The spermatheca of *B. caseyi* was illustrated in Lohse et al. (1990). It is slightly deformed and based on a female captured in a different locality than that of the male holotype. It may belong to *B. subplana,* a species with very similar spermatheca. The spermathecal stem of *B. caseyi* has a broad posterior loop and female sternite VIII is rounded apically (Fig. 108), and not triangularly produced and pointed medially as in *B. subplana* (Fig. 99).



Figures 100–108. *Boreophilia caseyi* Lohse: **100** habitus **101** median lobe of aedeagus in lateral view **102, 103** median lobe of aedeagus in dorsal view **104** male tergite VIII **105** male sternite VIII **106** female tergite VIII **107** female sternite VIII **108** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

Distribution. Nearctic species, recorded from Canada: MB, NT, YT; USA: AK.

Collection data. Habitat: arctic tundra. Collecting methods: pitfall traps. Collecting period: June and July.

Additional material examined. USA, Alaska, Toolik Lake Field Station, 724 m el., 68.6286N, 149.59772W, +/- 6m arctic tundra, 3 pitfalls, 2.VI–30.VII.2008, D Sikes UAM100044717, UAM100044680, UAM100044997 (UAM) 2 males, 1 female.

Comments. Lohse, in Lohse et al. (1990) described the new species *B. manitobensis* from MB and AK. The holotype from MB is represented by a male with a distorted median lobe of the aedeagus. We have studied the external and internal morphology of the two species, including the structures of internal sac, and found no significant differences between *B. manitobensis* and *B. caseyi*. Therefore, *B. manitobensis* is here synonymized with *B. caseyi*. The two species were published in the same paper, but *B. caseyi* has page priority and therefore was chosen as a valid species.

DNA Barcode data. Four specimens of *B. caseyi* from UAM were submitted for DNA barcoding and three did not produce DNA sequences. The one which was successfully sequenced was flagged on BOLD as possibly contaminated so we excluded it from our analyses.

13. *Boreophilia vega* (Fenyes, 1920)

Figs 109–116

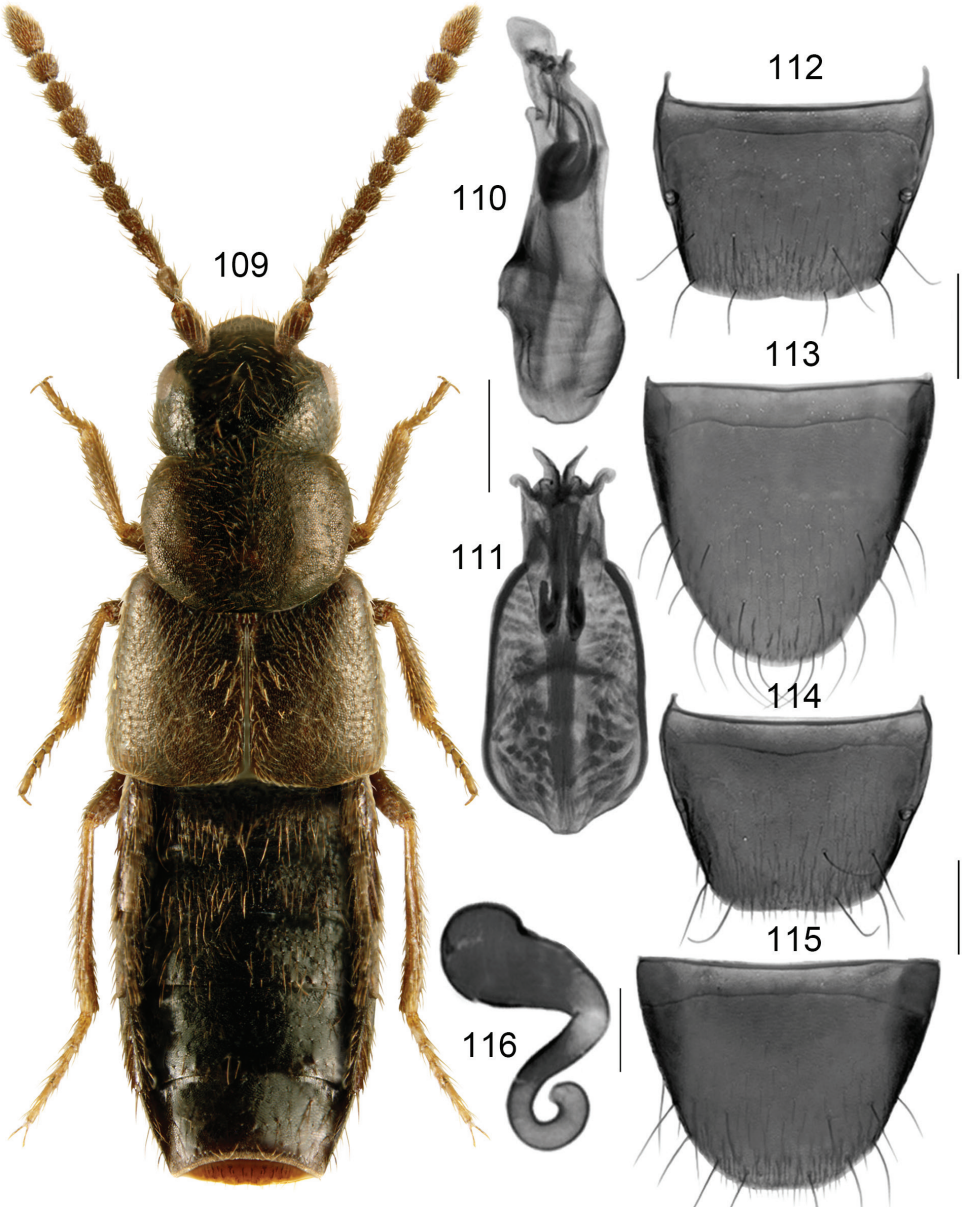
Atheta vega Fenyes, 1920: 198. Brundin 1954: 417, Smetana 2004: 396.
Boreophilia herschelensis Klimaszewski & Godin, in Klimaszewski et al. 2012: 232.
New synonymy.

Diagnosis. Body broad, narrowly oval, moderately glossy, abdomen slightly more so (Fig. 109); length 2.8–30 mm; uniformly dark brown to almost black, tarsi paler, yellowish-brown; antennomeres VIII-X subquadrate to slightly transverse; pronotum as long as elytra at suture, maximum width of pronotum distinctly shorter than maximum width of elytra. **Male**. Tubus of median lobe of aedeagus slightly arcuate in lateral view, apex narrowly rounded, bulbus broad and with two elongate narrow sclerites (Fig. 110), in dorsal view median lobe of aedeagus elongate oval (Fig. 111); tergite VIII truncate apically (Fig. 112); sternite VIII elongate, parabolic apically (Fig. 113). **Female**. Spermatheca: capsule broadly club-shaped, moderately long and rounded apically, with small apical invagination; stem short, sinuate and looped posteriad (Fig. 116); tergite VIII truncate apically (Fig. 114); sternite VIII rounded apically and truncate medially, antecostal suture arcuate and slightly sinuate laterally (Fig. 115).

Distribution. Holarctic species, known from West and East Siberia, Russian Far East, North Korea; and Canada: Herschel Island, YT. USA: not recorded.

Collection data. Habitat: Yukon specimens were collected in an alluvial fan in June and July (Klimaszewski et al. 2012).

Comments. *Boreophilia herschelensis* is conspecific with *B. vega* and is here synonymized. *Boreophilia vega* has a median lobe of aedeagus similar to that of *B. neoinsecuta* (Fig. 72) and *B. insecuta* (Fig. 82), but the spermatheca of *B. vega* compared to the latter two species is differently shaped (Figs 79, 80, 116). Externally, *B. vega* differs from *B. neoinsecuta* in elytra distinctly broader that the maximum width of



Figures 109–116. *Boreophilia vega* (Fenyes): **109** habitus **110** median lobe of aedeagus in lateral view **111** median lobe of aedeagus in dorsal view **112** male tergite VIII **113** male sternite VIII **114** female tergite VIII **115** female sternite VIII **116** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

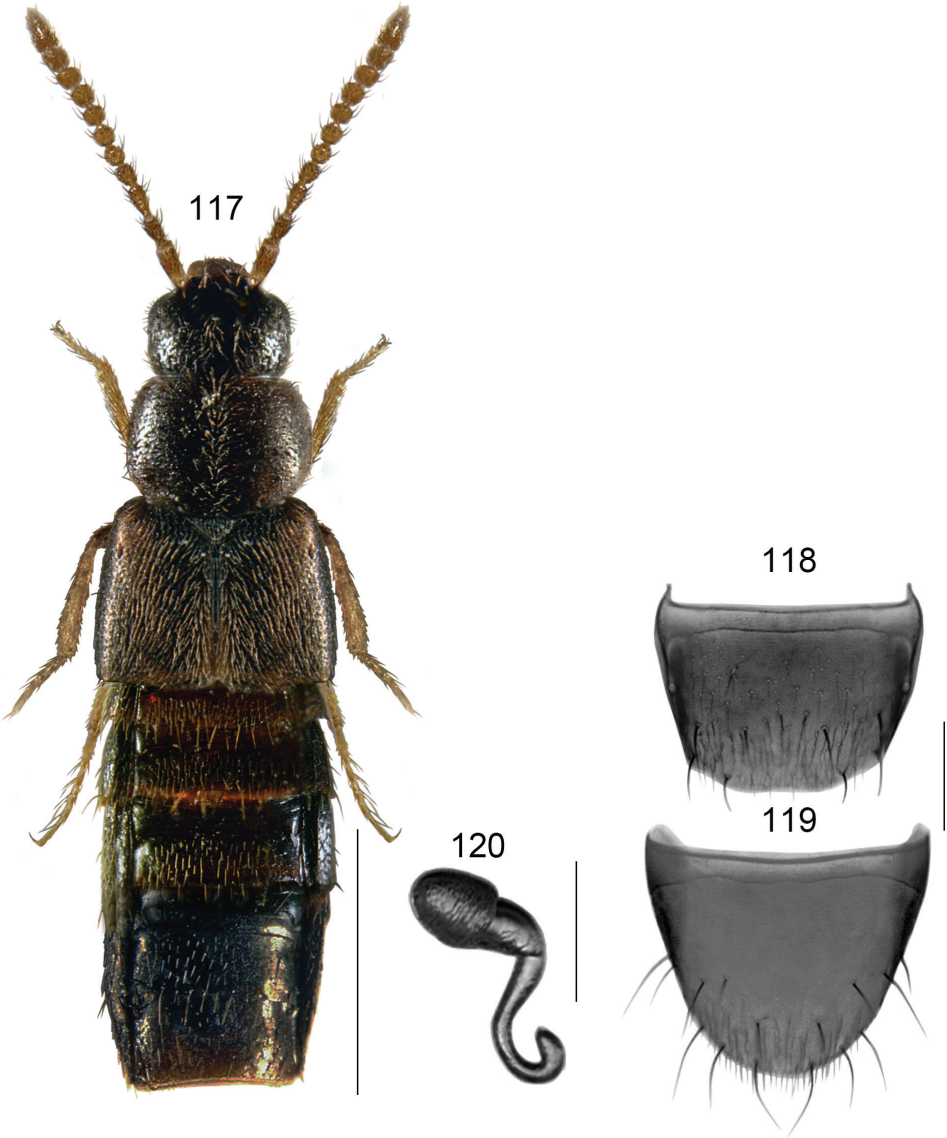
pronotum (Figs 71, 109). Female sternite VIII is differently shaped in the two species (Figs 78, 115).

DNA Barcode data. Our data included one sequence of *B. vega* from Yukon Territory, Canada, but because this sequence was < 500 bp long it was not assigned a BIN on BOLD. We calculate that this sequence is 6.5 % distant from its nearest neighbor.

14. *Boreophilia davidgei* Klimaszewski & Godin, 2012
Figs 117–120

Boreophilia davidgei Klimaszewski & Godin, in Klimaszewski et al. 2012: 232.

Diagnosis. Body moderately broad, subparallel, moderately glossy, abdomen slightly more so (Fig. 117); length 2.8–2.9 mm; uniformly dark brown to almost black, basal sternite slightly reddish-brown, legs paler, yellowish-brown; antennomeres VIII–



Figures 117–120. *Boreophilia davidgei* Klimaszewski and Godin: **117** habitus **118** female tergite VIII **119** female sternite VIII **120** spermatheca. Scale bars: 1 mm (for habitus); 0.2 mm (remaining).

X subquadrate to slightly transverse; pronotum slightly shorter than elytra at suture, maximum width of pronotum distinctly less than maximum width of elytra. **Male**. Unknown. **Female**. Spermatheca: capsule ovoid apically and pitcher-shaped basally, without apical invagination; stem short, narrow, sinuate and looped posteriad (Fig. 120); tergite VIII truncate apically (Fig. 118); sternite VIII rounded apically, antecostal suture arcuate and slightly sinuate laterally (Fig. 119).

This species may be distinguished by the unique shape of spermatheca.

Distribution. Nearctic species, known only from Canada, YT.

Collection data. Habitat: white spruce and feathermoss forest, mixed pine and willow forest, black spruce stand, mixed aspen and white spruce forest (Klimaszewski et al. 2012). Females from Yukon were collected in May to September using pitfall traps and litter sifting in mature forest.

Comments. This species is tentatively assigned to this group, because the male is unknown and morphology of median lobe of aedeagus could not be analysed.

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RESEARCH ARTICLE

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Designation of a neotype for *Enteromius pallidus* (Smith, 1841), an endemic cyprinid minnow from the Cape Fold Ecoregion, South Africa

Melissa B. Martin[1], Albert Chakona[2]

1 *School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu Darul Iman, 21030, Malaysia* **2** *South African Institute for Aquatic Biodiversity, Private Bag 1015, Grahamstown, 6140, South Africa*

Corresponding author: *Albert Chakona* (a.chakona@saiab.ac.za)

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Abstract

Enteromius pallidus was described by Smith in 1841 without a designated type specimen for the species. Herein, we designate a specimen from the Baakens River system as a neotype for *E. pallidus* and provide a thorough description for this species to facilitate ongoing taxonomic revisions of southern African *Enteromius*. *Enteromius pallidus* can be distinguished from the other minnows in the "goldie barb group" by having an incomplete lateral line, lack of distinct chevron or tubular markings around lateral line pores, absence of a distinct lateral stripe, absence of wavy parallel lines along scale rows and lack of black pigmentation around the borders of the scales. We provide mtDNA COI sequences for the neotype and an additional specimen from the Baakens River as DNA barcodes of types and topotypes are a fundamental requirement for further taxonomic studies.

Keywords

Enteromius, freshwater fish, Baakens River, Eastern Cape Province, southern Africa

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Introduction

The Cyprinidae is one of the most widespread and species-rich freshwater fish families, with 1685 valid species worldwide (Eschmeyer et al. 2018). The African continent currently contains at least 475 species in 24 genera, with the Congo River system being the centre of cyprinid diversity (Eschmeyer et al. 2018). The African cyprinids can be broadly divided into the small diploid species (e.g. *Caecobarbus*, *Barbopsis*, *Clypeobarbus*, *Barboides* and species that were previously referred to as *Barbus* or '*Barbus*'), small-to-medium sized tetraploid species (e.g. *Pseudobarbus*) and the large-sized hexaploid species (e.g. *Labeobarbus*) (Agnèse et al. 1990; Berrebi et al. 1996; Berrebi and Valiushok 1998; Ren and Mayden 2016; Van Ginneken et al. 2017). Recently, Yang et al. (2015) proposed that the small-sized African diploid minnows that were previously variously referred to as either *Barbus* or '*Barbus*' (Berrebi et al. 1996) should be preliminarily combined under the name *Enteromius* Cope, 1867 in the tribe Smiliogastrini. This suggestion has been provisionally accepted, pending a critical evaluation of the generic status of the African diploid minnows (e.g. Skelton 2016; Hayes and Armbruster 2017; Van Ginneken et al. 2017; Schmidt et al. 2017, 2018).

Enteromius is currently represented by 350 valid species, making it the most speciose and widely distributed cyprinid genus on the African continent (Hayes and Armbruster 2017), and new species have been recently described (e.g. Lederoun and Vreven 2016), revalidated (e.g. Schmidt et al. 2018) or await formal description (Van Ginneken et al. 2017). The genus *Enteromius* is distinguished from other small African diploid smiliogastrin genera (*Barboides*, *Barbopsis*, *Caecobarbus*, *Clypeobarbus*) based on differences in dorsal-fin placement in comparison to anal-fin origin, number of dorsal-fin rays, number of paired nostrils on either side of the snout, eye size, placement in the orbital rim and pigmentation pattern, shape and pattern of midlateral scale row (Hayes and Armbruster 2017). In southern Africa, this genus is represented by 38 species (Skelton 2001).

Despite *Enteromius* being the most common genus occurring in almost all river systems across the continent, these fishes are generally difficult to identify because of their very similar body morphology and colour pattern, coupled with the lack of revision within the group (Hayes and Armbruster 2017; Van Ginneken et al. 2017). As a result, a number of species within *Enteromius* are currently considered to have wide geographic ranges across multiple river systems (Skelton 2001). Such distribution patterns are unexpected for freshwater restricted taxa as their dispersal is limited by terrestrial and marine barriers, and they reflect the incomplete systematic and taxonomic knowledge of freshwater fishes in the region. This "taxonomic impediment" handicaps basic research in biological sciences and biodiversity conservation.

The present study forms part of an ongoing comprehensive taxonomic revision of the goldie barb group which comprises three cyprinid minnows endemic to southern Africa, *E. pallidus*, *E. brevipinnis* (Jubb, 1966) and *E. neefi* (Greenwood, 1962). There

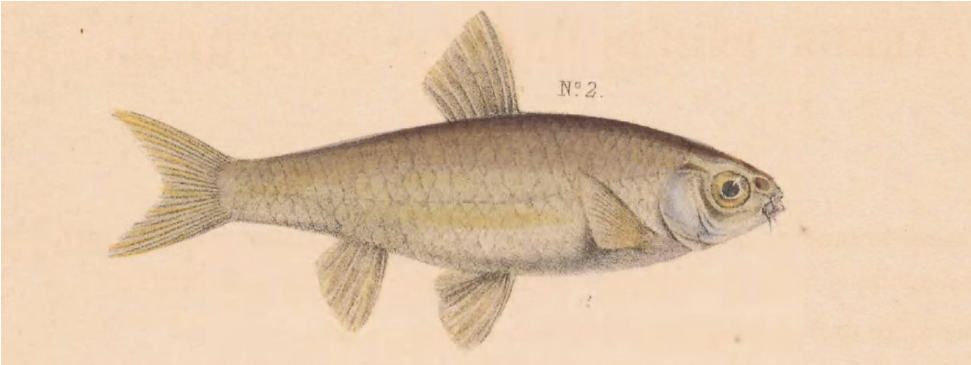

Figure 1. Illustration of *Enteromius pallidus* [formerly *Barbus* (*Pseudobarbus*) *pallidus*] from Smith (1841).

are however no existing type specimens for *E. pallidus* (Eschmeyer et al., 2018). *Enteromius pallidus* (Fig. 1) was described by Smith (1838–47) as *Barbus* (*Pseudobarbus*) *pallidus*, with the type locality listed as 'various parts of Cape Colony'. This potentially encompassed any of the southern coastal river systems in the present-day Eastern Cape Province of South Africa, from the Krom to the Great Fish, where *E. pallidus* is known to occur (Skelton 2001). The likely type locality is the Baakens River in Port Elizabeth (Fig. 2) as that is close to Fort Frederick, the former British Military establishment in the town, where Andrew Smith, who was a British army surgeon, might have been based between 1821–1825 when he was posted to the eastern frontier and at other times after he moved to Cape Town. Boulenger (1911) described *Barbus hemipleurogramma* from the Baakens River, but Barnard (1943) put this species into synonymy with *B. pallidus*.

The name *E. pallidus* (previously *B. pallidus*) has been applied for minnows with scattered spots on the lateral and dorsal side of the body from other river systems in South Africa, including tributaries of the Orange-Vaal, Tugela, Mfolozi, Pongolo, Incomati and Limpopo river systems. The species has, thus, for a long time been considered to have a distribution pattern divided into coastal and inland populations (Skelton 2001). Most recently, Chakona et al. (2015) revealed substantial genetic differentiation between the coastal and inland populations of *E. pallidus* and showed that the inland lineage is not closely related to *E. pallidus* s.s. As there are no types for *E. pallidus*, the aim of the present study was to designate a neotype and provide detailed description of this species based on the topotypic specimens collected from the Baakens River system in Port Elizabeth. The present study thus provides clarity on the likely type locality of *E. pallidus* and presents an accurate definition for this species in accordance with Article 75.3.1 of the International Code for Zoological Nomenclature, ICZN (International Commission on Zoological Nomenclature 1999). This is a fundamental requirement for future taxonomic comparisons and revision of spotted smiliogastrins in southern Africa whose taxonomic status is currently uncertain.

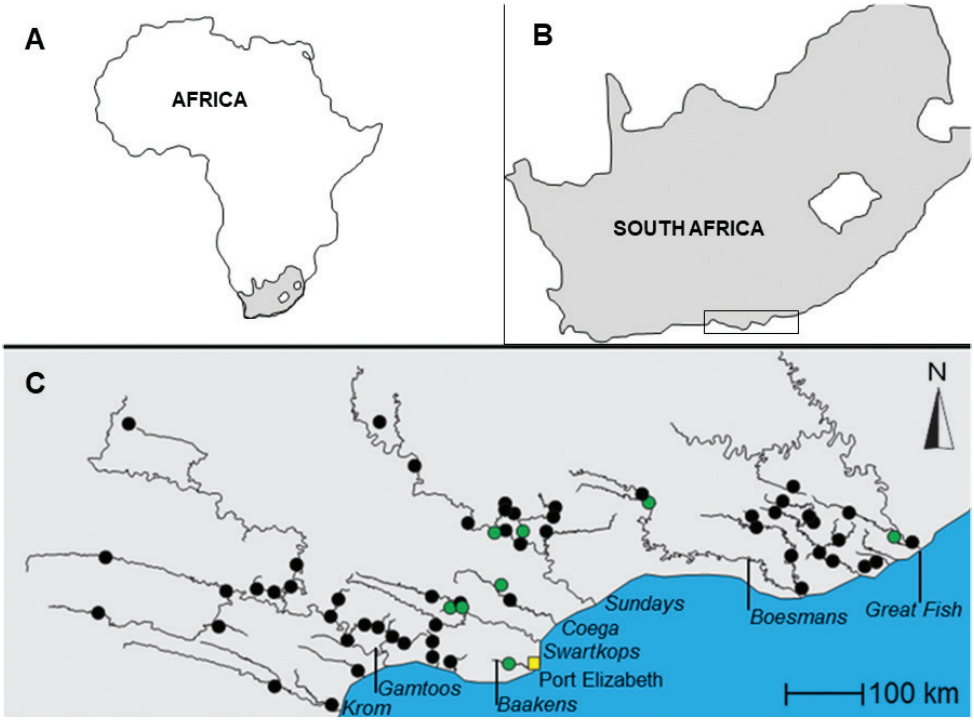

Figure 2. Map of the African continent (**A**) showing the position of South Africa (**B**), and the distribution of *Enteromius pallidus* in the eastern Cape Fold freshwater ecoregion (**C**). The Baakens River, which is the type locality of *E. pallidus*, is now entirely contained within the city of Port Elizabeth (Nelson Mandela Metropolitan). Green dots represent sampling localities for the tissue samples that were used for the genetic study of Chakona et al. (2015).

Materials and methods

Sample collection and deposition

Fishes were collected on the 3rd November 2018 using a seine net (3 m long, 3 mm mesh size). Captured fishes were anaesthetised with clove oil (0.2%) and digitally photographed using a Nikon D3100 7.4/9V camera on site to capture live colour pattern. For genetic analysis, a small piece of muscle tissue was dissected from the right side of each specimen in the field, preserved in 95% ethanol and later stored at -20°C in the molecular laboratory at the South African Institute for Aquatic Biodiversity (SAIAB). Voucher specimens were fixed in 10% formalin in the field. They were then put through 10% and 50% ethanol washes to rinse the formalin and eventually transferred to 70% ethanol for long-term storage. The neotype (SAIAB 207086) and additional topotypes (SAIAB 207084) were deposited into the fish collection facility at SAIAB as reference material. Permission for sampling was obtained from the Department of Economic Development, Environmental Affairs and Tourism (Eastern Cape Province) (permit number: CRO 44/18CR).

Morphological analyses

Meristic and morphological characters were selected as defined by Hubbs and Lagler (1958), Skelton (1988), Chakona and Swartz (2013) and Chakona et al. (2014). Morphometric measurements were taken point-to-point using an IP54 digital caliper to 0.1 mm precision. The characters considered for each specimen in the present study (22 morphometric measurements and 16 meristic counts) are presented in Chakona et al. (2014).

Molecular data

We provide mtDNA COI barcode sequences for the neotype (designated as neogenetype) and an additional specimen (designated as topogenetype) following definitions of Chakrabarty (2010) as these sequences will facilitate detailed phylogenetic analyses to determine the relationships of *E. pallidus* and other southern African congeners as more data become available through ongoing studies. These sequences were deposited in GenBank: neogenetype (MK900662) and topogenetype (MK900663). DNA extraction, PCR and sequencing methods follow Chakona et al. (2018).

Results

***Enteromius pallidus* (Smith, 1841)**
Figs 3, 4
Goldie barb (English), Goud-ghieliemientjie (Afrikaans)

Barbus (*Pseudobarbus*) *pallidus* Smith, 1841: no pagination, pl. 11 (fig. 2). Type locality: Defined in the original description as "various parts of the Cape Colony", but it is likely to be the Baakens River which is closest to the former British Army base, Fort Fredrick, where Andrew Smith, who was an army surgeon, would have been based at the time when he described this species.

Barbus hemipleurogramma Boulenger, 1911, fig. 126. Type locality: Baakens River, Port Elizabeth, Cape Province, South Africa; Bertin and Estève 1948.

Barbus pallidus: Barnard 1943; Lévêque et al. 1984; Skelton 1993; Engelbrecht and van der Bank 1996; Seegers 1996, Farm 2000; Skelton 2001; Muller et al. 2015; Chakona et al. 2015.

Enteromius pallidus: Hayes and Armbruster 2017.

Material examined. Neotype (Fig. 3A, B): In compliance with Article 75.3.7, the neotype was deposited at the South African Institute for Aquatic Biodiversity (catalogue no. SAIAB 207086) for future reference. The neotype is an adult female, 51.4 mm standard length (SL), collected on 3 November 2018 by Albert Chakona, Wilbert Kadye and Melissa Martin using seine netting, Baakens River system at Targetklooff

Figure 3. General body features and live (**A**) and alcohol preserved (**B**) coloration of the neotype of *Enteromius pallidus* (SAIAB 207086), a gravid adult female. Scale bar: 10 mm.

downstream of bridge on the road to Walmer, (33°58'12"S, 25°35'40"E), altitude 20 m, Port Elizabeth, South Africa.

Additional material. South Africa: Port Elizabeth: SAIAB 207084, (*n*= 6; 2 adult females, 1 adult male, 4 sub-adults), 17.1–36.1 mm standard length (SL), collection details similar to neotype (Fig. 4A–D).

South Africa: Eastern Cape: Port Elizabeth: SAIAB 200091; (n=38 juveniles and sub-adults, 17.1–36.1 mm standard length (SL), collected from the Baakens river on 4th April 2014 by Albert Chakona and Roger Bills downstream of low water bridge on the road to Green Acres, (33°57'28.1"S, 25°33'36.8"E).

South Africa: Eastern Cape: Port Elizabeth: SAIAB 127772; (n=2 gravid females, 46.8 and 47.6 mm standard length (SL), collected on 22 October 1981 by D. Heard from the Baakens River system, (33°58'S, 25°37'E).

Neotype designation for *Enteromius pallidus* (Smith, 1841). The generic status of the diploid Smiliogastrini minnows, currently placed in *Enteromius*, is the subject of ongoing investigation because this genus is polyphyletic (Yang et al. 2015, Ren and

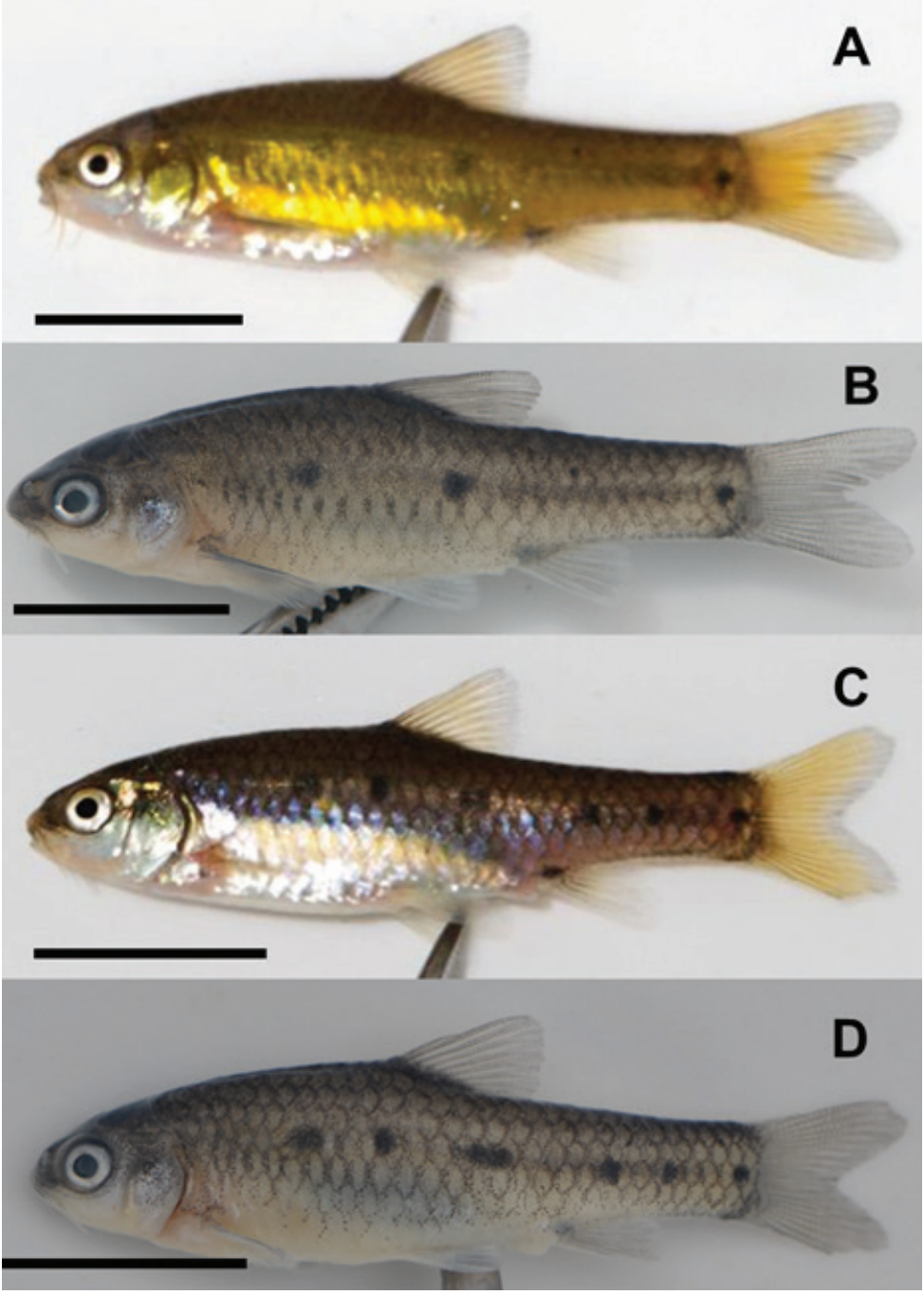

Figure 4. General body features and live (**A**) and alcohol preserved (**B**) coloration of a mature breeding male *E. pallidus* (**A, B** SAIAB 207084), and live (**C**) and alcohol preserved (**D**) coloration of an unsexed sub-adult (SAIAB 207084). Scale bar: 10 mm.

Mayden 2016, Hayes and Armbruster 2017). Detailed revision of the taxonomic statuses of the species belonging to this genus is plagued by a number of challenges, particularly similar body morphology, ambiguous type locality details and lack of extant type material for a number of species. This hinders accurate resolution of species identities, resulting in exaggerated geographic distribution ranges for many of the species (see Skelton, 2001). Without primary type specimens and better resolution of species identities and their distribution ranges, it would be difficult to resolve the bigger questions of the generic status and relationships of diploid smiliogastrins. Within southern Africa, a number of species in the genus *Enteromius* are in need of taxonomic revision as many are perceived to have broad geographic ranges, such as the case of *E. pallidus*. There are no extant types for this species, the illustration does not provide clear diagnostic characters to objectively associate it with *E. pallidus* or for comparisons with other species, and the species has a vague type locality description. The designation of a neotype is therefore essential to facilitate ongoing taxonomic revision of the "goldie barb" complex in southern Africa and for the broader evaluation of the phylogenetic relationships and generic placements of *Enteromius* species across the African continent. There is therefore an explicit need for the designation of a neotype (Art. 75.3 of ICZN).

All qualifying conditions (Art. 75.3 of ICZN) are met. The neotype is designated to clarify the taxonomic status of the species (Art. 75.3.1). *Enteromius pallidus* was described by Smith, who provided an illustration for a specimen with a brief description of the colour and form of the species, and a vague type locality defined as "clear streams in various parts of the Cape colony". Although Smith provided an illustration, there is no evidence within the text that he established a holotype or any expression of the equivalent. In compliance with Article 75.3.4 of the ICZN, the authors conducted a comprehensive search for the types, and it was established that extant types for *E. pallidus* are unlikely to be in existence. This was based on correspondences with Prof. Paul Skelton at the South African Institute for Aquatic Biodiversity (SAIAB), who confirmed that he examined and measured all the types of southern African freshwater fishes in 1981 at the British Museum of Natural History (BMNH). He searched for Smith's *Barbus pallidus* but found no trace of any record or specimen(s). The authors also contacted the curator at the British Museum, James Maclaine, who indicated that Andrew Smith's types of *E. pallidus* are not at the BMNH. While according to Article 73.1.4, Smith's (1841) illustration would be considered to represent the holotype of *E. pallidus*, unfortunately the illustration does not provide clear details to extract diagnostic features for the species.

In compliance with Articles 75.3.2 and 75.3.3, a diagnosis, redescription, and comparison of *E. pallidus* and the other congeners in southern Africa are presented below. Following Barnard (1943), the original specimens used for the description of *E. pallidus* could have come from a river system near Port Elizabeth, probably the Baakens River. We therefore chose a specimen from the Baakens River system for the neotype designation (in compliance with Article 75.3.6), because it is closest to the 1820's British army camp (Fort Frederick), where Andrew Smith is likely to have been based during the time when he made the description.

Diagnosis. *Enteromius pallidus* can be identified by the slightly convex dorsal surface; posterior barbel 2.0 to 3.0 times the length of anterior barbel; a slightly prominent snout; an incomplete lateral line; deep translucent light brown to golden sheen with the presence of irregular and scattered spots in mature adults; and the presence of 3–7 bold spots above the lateral line in juveniles and sub-adults.

Comparison with congeners in southern Africa. The species belongs to the group of *Enteromius* species in southern Africa that is characterised by a simple and flexible unbranched primary dorsal fin ray. Distinguished from *E. amatolicus* (Skelton, 1990), *E. anoplus* (Weber, 1897), *E. annectens* (Gilchrist & Thompson, 1917), *E. toppini* (Boulenger, 1916) and *E. radiatus* (Peters, 1853) by possession of two pairs of prominent and long barbels (*vs* single pair and/or minute oral barbels in other species). Distinguished from *E. lineomaculatus* (Boulenger, 1903), *E. viviparus* (Weber, 1897) and *E. unitaeniatus* (Günther, 1867) by absence of distinct chevron markings on the lateral line (*vs* presence of conspicuous chevron markings on the lateral line in the other three species), and from *E. bifrenatus* (Fowler, 1935) by absence of a distinct lateral stripe and absence of black tubular markings around lateral line pores (*vs* presence in *E. bifrenatus*). Distinguished from *E. anoplus*, *E. amatolicus*, *E. annectens*, *E. unitaeniatus*, *E. bifrenatus*, *E. gurneyi* (Günther, 1868), *E. motebensis* (Steindachner, 1894), *E. radiatus*, *E. toppini*, *E. treurensis* (Groenewald, 1958) and *E. viviparus* by the presence of scattered black spots on the body, particularly in juveniles (*vs* absence of scattered black spots in the other species). Lateral pigmentation pattern of *E. pallidus* is closely similar to that of *E. brevipinnis* and *E. neefi* (Greenwood, 1962), but it is distinguished from these two species by having an incomplete lateral line (*vs* complete lateral line in both *E. neefi* and *E. brevipinnis*). *Enteromius pallidus* is further separated from *E. neefi* by absence of wavy lines along the scale rows (*vs*. presence of conspicuous wavy lines along the scale rows in *E. neefi*), and from *E. brevipinnis* by lack of black pigmentation around the borders of the scales (*vs* presence of distinct black pigmentation around the scales in *E. brevipinnis*, giving a mesh-like pattern on the lateral side of the fish).

Figures 3, 4 show the general body features of *E. pallidus* as an adult female (neotype), adult male and juvenile. Morphometric and meristic data for the neotype and additional (topotypic) material are presented in Table 1.

Neotype description (Article 75.3.3.). (Fig. 3A, B). *Body* fusiform, moderately compressed laterally; with four visible, irregular spots above lateral line. *Dorsal profile* slightly convex from tip of snout to origin of dorsal fin; anterior-projection slightly pronounced; body depth greatest between dorsal fin and anal fin origin, tapering from posterior margin of dorsal fin base to base of caudal fin. *Ventral profile* slightly concave, curving downwards from operculum to origin of pelvic fin base, slightly tapering to posterior end of anal fin base, then slightly concave to caudal fin.

Head relatively small and slightly projected; 0.2 times standard length, head length sub-equal to body depth. *Eye* relatively large and round; located dorsolaterally, closer to tip of snout than distal margin of operculum, interorbital space slightly convex. *Snout* rounded, shorter than post-orbital length; sub-equal or less than eye diameter; nuptial tubercles absent.

Table 1. Morphometric measurements and meristic counts of *Enteromius pallidus* neotype and additional material from Baakens River. Ranges of characters are presented first, followed by the mean and standard deviation in parentheses. Meristic characters are given in the range first, with the mode in parentheses.

	Enteromius pallidus	
	Neotype	**Additional material**
No. of specimens	n=1	n=46
Morphometrics (mm)		
Standard length (SL) (mm)	51.4	17.1–49.3 (26.8; 8.1)
Head length (HL) (mm)	9.4	3.8–10.7 (5.7; 1.6)
Percentage of SL (%)		
Head length	18.3	17.9–25.1 (21.5; 1.6)
Predorsal length	54.1	46.9–56.2 (53.1; 1.9)
Dorsal fin base	10.5	4.7–20.3 (10.6; 2.8)
Dorsal fin height	20.8	16.5–27.0 (21.3; 2.3)
Body depth	29.9	20.5–30.9 (26.2; 1.9)
Body width	16.9	7.3–20.4 (11.4; 2.5)
Caudal peduncle length	20.4	19.9–32.8 (27.8; 2.9)
Preanal length	69.2	59.8–73.7 (68.7; 2.9)
Prepelvic length	47.7	42.7–54.3 (49.2; 2.5)
Pelvic fin length	13.0	12.6–21.2 (16.1; 1.5)
Pectoral to pelvic fin length	22.8	16.3–28.2 (21.2; 2.5)
Pelvic to anal fin length	17.9	12.2–21.6 (17.3; 1.9)
Anal fin base	7.59	2.9–9.0 (6.3; 1.3)
Percentage of HL (%)		
Head depth	105.3	75.5–109.0 (92.2; 7.5)
Snout length	31.9	20.0–44.4 (33.6; 5.5)
Orbit diameter	36.2	31.4–51.2 (40.5; 5.3)
Postorbital length	54.3	40.8–67.2 (55.2; 5.3)
Interorbital width	57.4	44.2–66.7 (55.7; 6.3)
Anterior barbel length	16.0	4.1–30.4 (15.7; 7.3)
Posterior barbel length	30.9	21.7–64.1 (37.5; 10.5)
Percentage of caudal peduncle length (%)		
Caudal peduncle depth	13.2	10.1–15.0 (12.6; 1.0)
Meristics		
Unbranched dorsal fin rays	3	3(3)
Branched dorsal fin rays	7	7 (7)
Unbranched anal fin rays	3	3 (3)
Branched anal fin rays	5	5 (5)
Unbranched pectoral fin rays	1	1 (1)
Branched pectoral fin rays	7	7 (7)
Unbranched pelvic fin rays	1	1 (1)
Branched pelvic fin rays	7	5–7 (5)
Unbranched caudal fin rays	2	2 (2)
Branched caudal fin rays	17	15–19 (17)
Lateral line scales	13	5–19 (9)
Number of scales in lateral series	31	23–30 (26)
Scales between lateral line and dorsal fin origin	4	3–5 (4)
Scales between lateral line and pelvic fin origin	2–3	2–5 (3)
Scales between lateral line and anal fin origin	2	2–3 (2)
Circumpeduncular scales	12	12 (12)
Predorsal scale rows	10	7–14 (10)

Mouth inferior; upper jaw sub-equal to lower jaw. *Lip* simple and thin; lower lip unretracted. *Two pairs of barbels;* rostral (anterior) barbels minute, reaching past posterior end of nostril, 0.3 times length of eye diameter; *maxillary* (posterior) *barbels* 3.0 times longer than rostral barbels, reaching beyond vertical through middle of eye.

Dorsal fin with 3 simple unbranched and 7 branched rays; distal margin almost straight; origin centered vertically with origin of pelvic fins. *Pectoral fin* with 1 simple unbranched and 7 branched rays; posterior edge gently rounded, not reaching pelvic fin origin. *Pelvic fin* with 1 simple unbranched and 5 branched rays; posterior edge gently rounded, almost reaching anus; origin midway between pectoral fin origin and anal fin origin. *Anal fin* with 3 unbranched and 5 branched rays; distal margin almost straight; origin inserted closer to origin of pelvic fin than base of caudal fin. *Caudal fin* bifurcate; with two pairs of 1 simple unbranched ray, 8 or 9 branched rays on each lobe.

Scales moderately large, radiately striated. *Lateral line* incomplete, with 4–13 (mode 9) perforated scales, 23–31 (mode 26) lateral scale series; 3–5 (mode 4) scale rows between dorsal fin origin and lateral line; 2–5 (mode 3) scale rows between pelvic fin origin and lateral line; 2–3 (mode 2) scale rows between lateral line and anal fin origin; 12 circumpeduncular scale rows; 7–14 (mode 10) predorsal scale rows, embedded in skin, smaller than flank scales. Scales between posterior base of pectoral fins and anterior base of pelvic fins smaller than flank scales and embedded.

Coloration. In life, the colour for both adult breeding males and females is deep greenish-brown with a golden sheen dorsally, golden-yellow laterally and silvery ventrally (Figs 3A, B; 4A, B). Fins are translucent-yellow. The neotype thus represents *E. pallidus* sensu Smith (1841) based on the consistent similarities in colour pattern as defined in the original description (Art. 75.3.5). Juveniles appear brown laterally and silvery ventrally. Black spots are present above the lateral line, with juveniles and sub-adults having bold or more prominent spots in comparison to adults which tend to have fewer and often less conspicuous spots or blotches. All the juveniles and sub-adults examined (46 in total) had at least 3 bold spots above the lateral line (4C and 4D) on both sides (range 3–7 bold lateral spots). At least one bold spot is consistently found within the pre-dorsal region, pre-anal and caudal regions, a dark spot is always present on vertical through dorsal fin insertion and at the base of the caudal peduncle. Alcohol preserved specimens appear either plain silvery, or dusky grey dorsally and laterally and cream-yellowish ventrally (Figs 3B; 4B, D). The black spots become more prominent in preserved specimens. Black pigmentation at the anterior base of the anal fin is more prominent in juveniles and sub-adults compared to adults.

Reproduction. There have been no dedicated studies on the breeding biology of *E. pallidus*, but spawning is likely to begin in summer (October – November) based on the general pattern of other congeners (Cambray and Bruton 1984; Skelton 2001), and other cyprinid minnows in the CFE (Cambray 1994). We have also observed presence of several gravid females and males with breeding coloration (prominent golden-yellowish sheen) during field surveys conducted during the summer period.

Distribution and habitat. *Enteromius pallidus* is endemic to the eastern Cape Fold Ecoregion (CFE) of South Africa where it is distributed from the Krom to the Great Fish river system (Fig. 2). Rivers in this region are characterized by variable flow regimes, with mountain tributaries generally flowing throughout the year, while some main-stem sections of the rivers recede into a series of disconnected pools during the dry season (O'Keeffe and de Moor 1988). The species inhabits pools within both perennial and seasonal streams with clear or moderately turbid water as well as rocky to fine (silt and mud) substrates. The species often favours river sections with emergent aquatic vegetation and woody riparian vegetation.

Discussion

Enteromius pallidus co-occurs with the chubby head barb, *E. anoplus*, across its distribution range in the CFE. *Enteromius pallidus* is readily distinguished from *E. anoplus* by possession of two pairs of barbels (*vs* single pair of barbels in *E. anoplus*), fewer lateral scale series (24–31 *vs* 33–37 in *E. anoplus*), presence of irregular scattered spots on the body (*vs* absence in *E. anoplus*). *Enteromius pallidus* is distinguished from the Amatola barb, *E. amatolicus*, another cyprind minnow that is endemic to the Eastern Cape Province of South Africa, by possession of two pairs of oral barbels (*vs* a single pair in *E. amatolicus*), fewer lateral scale series (24–31 scales *vs* 33–37), fewer scales around the caudal peduncle (12 *vs* 16 scales), and absence of tubercles in mature breeding males (*vs* development of nuptial tubercles in *E. amatolicus* during the breeding season).

Skelton (2001) grouped three southern African smiliogastrins, *E. pallidus*, *E. brevipinnis* and *E. neefi*, into a group which he referred to as the "goldie barb group" based on development of bright golden colour in breeding males. However, the taxonomy, phylogenetic relationships and historical biogeography of this group remain unclear (Engelbrecht and van der Bank, 1996). Studies are required to determine whether the goldie barb group forms a monophyletic unit and shed some light on the diversity and biogeographic patterns of species within this group. There is also need for phylogeographic and ecological studies to assess the mechanisms that shaped the contemporary distribution patterns of *E. pallidus* as it is one of the most widely distributed freshwater fishes in the eastern CFE.

Previous studies have identified sea-level regression, river capture events, interdrainage dispersal through intermittent freshwater connections and human mediated translocations through construction of inter-basin water transfers as the mechanisms that are likely to have played a role in shaping the distribution and phylogeographic patterns of a number of freshwater fishes in the CFE (Swartz et al. 2007; Chakona and Swartz 2013; Chakona et al. 2015; Cambray and Jubb 1977). However, the evolutionary history for several freshwater fishes in southern Africa, particularly for species within the genus *Enteromius*, remain poorly known. Future studies should aim to use a comparative phylogeographic approach to test whether the genetic structure of freshwater fishes with wide distribution ranges in the CFE, including *E. pallidus*, is congru-

ent with the boundaries of river basins, and determine whether co-distributed species experienced concerted, independent or multiple responses to evolutionary processes.

Recent surveys indicate that *E. pallidus* still persists in at least ten river systems in the eastern CFE including, the Krom, Gamtoos, Baakens, Coega, Swartkops, Sundays, Boesmans, Kariega, Kowie and Great Fish rivers. The species has, however, been affected by a number of human impacts, including hydrological modifications through inter-basin water transfers and excessive water abstraction, pollution, habitat degradation and widespread invasion of the rivers by non-native species (Muller et al. 2015), but its conservation status remains uncertain. Future studies should aim to provide fine scale geographic data and information on the ecology and biology of the species to facilitate effective biodiversity management in the CFE, one of the global endemic hotspots of freshwater fishes.

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Morphology, molecular genetics, and acoustics reveal two new species of the genus *Leptobrachella* from northwestern Guizhou Province, China (Anura, Megophryidae)

Jian Wang[1], Yu-Long Li[1], Yao Li[1], Hong-Hui Chen[1], Ya-Jun Zeng[2], Jing-Min Shen[2], Ying-Yong Wang[1]

1 *State Key Laboratory of Biocontrol / The Museum of Biology, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China* **2** *Guizhou Academy of Forestry, Guizhou 550005, China*

Corresponding author: *Ying-Yong Wang* (wangyy@mail.sysu.edu.cn)

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Abstract

Two new species of the genus *Leptobrachella* Smith, 1925, *L. bijie* J. Wang, Y.L. Li, Y. Li, H.H. Chen & Y.Y. Wang, **sp. nov.** and *L. purpuraventra* J. Wang, Y.L. Li, Y. Li, H.H. Chen & Y.Y. Wang, **sp. nov.**, were described from northwestern Guizhou Province, China based on a combination of acoustic, molecular, and morphological data. The new discoveries bring the total number of this genus to 73, with 16 congeners recorded in China, and represent the second and third species of the genus reported from Guizhou Province.

Keywords

Acoustics, *Leptobrachella bijie* sp. nov., *L. purpuraventra* sp. nov., molecular phylogeny, morphology, taxonomy

Introduction

The Asian leaf litter toad genus *Leptobrachella* Smith, 1925 currently contains seventy-one species, widely distributed from southern China west to northeastern India

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and Myanmar, through mainland Indochina to peninsular Malaysia and the island of Borneo (Eto et al. 2018; Frost 2017; Nguyen et al. 2018; Rowley et al. 2016, 2017; Yang et al. 2016; Yuan et al. 2017). Currently, 14 species of this genus are known from China, i.e., *L. alpinus* from Yunnan and Guangxi provinces, *L. laui* from southern Guangdong Province including Hong Kong, *L. liui* from Fujian, Jiangxi, Guangdong, Guangxi, Hunan and Guizhou provinces, *L. mangshanensis* from southern Hunan Province, *L. oshanensis* from Gansu, Sichuan, Chongqing, Guizhou and Hubei provinces, *L.* cf. *pelodytoides* (which may be a population of *L. eos* (Ohler et al. 2011)), *L. purpura*, *L. tengchongensis*, *L. ventripuntatus* and *L. yingjiangensis* from Yunnan Province, *L. wuhuangmontis* from southern Guangxi Province, *L. yunkaiensis* from western GuangdongProvince, and *L. sungi* and *L. maoershanensis* from Guangxi Province (Hou et al. 2018; Sung et al. 2014; Wang et al. 2018; Yang et al. 2016; Yuan et al. 2017, Yang et al. 2018).

During recent field surveys in northwestern Guizhou Province of China in 2018, a number of specimens were collected from Zhaozishan Nature Reserve and Wujing Nature Reserve in Qixingguan District of Bijie City, respectively (Figure 1), which can be morphologically assigned to the genus *Leptobrachella*, based on the following characters: (1) small or moderate size, snout-vent length not greater than 60.0 mm, (2) rounded finger tips, the presence of an elevated inner palmar tubercle

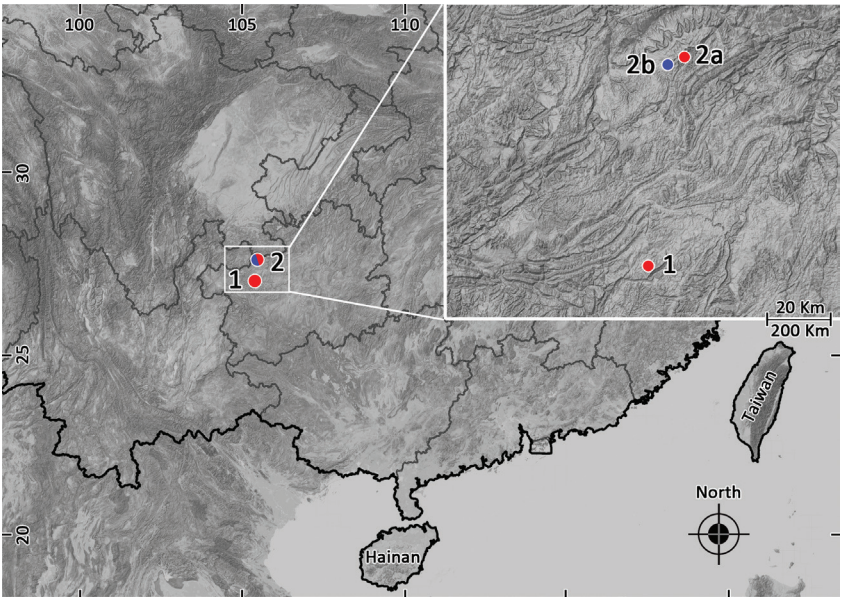

Figure 1. Collection localities of the two new *Leptobrachella* species: **1** Jinjiazhai Village in Wujing Nature Reserve, the type locality of *L. purpuraventra* sp. nov. **2a** Baimashan Forest Station in Zhaozishan Nature Reserve, the other collection site of *L. purpuraventra* sp. nov. **2b** Qingshan Village in Zhaozishan Nature Reserve, the type locality of *L. bijie* sp. nov.

not continuous to the thumb, (3) presence of macroglands on body including supra-axillary, pectoral, femoral and ventrolateral glands, (4) vomerine teeth absent, (5) tubercles on eyelids present, and (6) anterior tip of snout with whitish vertical bar (Dubois 1983; Matsui 1997, 2006; Lathrop et al. 1998; Delorme et al. 2006; Das et al. 2010). Subsequent 16S rRNA sequences from these specimens revealed that these collections represent two distinct evolving lineages. Combine of morphological characters, acoustic data, and molecular divergences; they are described herein as two new species.

Material and methods

Sampling

For molecular analyses, a total of 71 sequences (23 muscle tissues were sequenced and 48 sequences downloaded from GenBank) from 32 species of the genus *Leptobrachella* were used, including two undescribed species from China, i.e., the populations from Zhaozishan Nature Reserve and Wujing Nature Reserve of Guizhou Province. And four sequences were downloaded from GenBank as outgroups (see Table 1; *Pelobates syriacus*, *P. varaldii*, *Leptobrachium* cf. *chapaense*, and *Megophrys major*).

All specimens were fixed in 10 % buffered formalin and later transferred to 70 % ethanol for preservation, and deposited at the Museum of Biology, Sun Yat-sen University (**SYS**) and Chengdu Institute of Biology, the Chinese Academy of Sciences (**CIB**), China; tissue samples were preserved in 95% ethanol for molecular studies.

DNA Extraction, PCR and sequencing

DNA was extracted from muscle tissue using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. The mitochondrial gene 16S ribosomal RNA gene (16S rRNA) fragment from each sample was sequenced. Fragments were amplified using primer pairs L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCG-GTCTGAACTCAGATCACGT-3') (Simon et al. 1994). PCR amplifications were performed in a 20 μl reaction volume with the following cycling conditions: an initial denaturing step at 95 °C for five min; 35 cycles of denaturing at 95 °C for 40 s, annealing at 53 °C for 40 s and extending at 72 °C for one min; and a final extending step of 72 °C for 10 min. PCR products were purified with spin columns. The purified products were sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequencer in Shanghai Majorbio Bio-pharm Technology Co., Ltd.. All sequences have been deposited in GenBank (Table 1).

Table 1. Localities and voucher data for all specimens used in this study.

ID	Species	Locality	voucher No.	GenBank No. 16S rRNA
1	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007081	MK414517
2	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007277/CIB 110003	MK414518
3	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007278	MK414519
4	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007279	MK414520
5	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007280	MK414521
6	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007282	MK414522
7	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007283	MK414523
8	*Leptobrachella purpuraventra* sp. nov.	China: Wujing Nature Reserve, Bijie City, Guizhou	SYS a007284	MK414524
9	*Leptobrachella purpuraventra* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007300	MK414525
10	*Leptobrachella purpuraventra* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007301	MK414526
11	*Leptobrachella purpuraventra* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007302	MK414527
12	*Leptobrachella purpuraventra* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007303	MK414528
13	*Leptobrachella purpuraventra* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007304	MK414529
14	*Leptobrachella purpuraventra* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007305	MK414530
15	*Leptobrachella purpuraventra* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007306	MK414531
16	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007313/CIB 110002	MK414532
17	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007314	MK414533
18	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007315	MK414534
19	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007316	MK414535
20	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007317	MK414536
21	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007318	MK414537
22	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007319	MK414538
23	*Leptobrachella bijie* sp. nov.	China: Zhaozishan Nature Reserve, Bijie City, Guizhou	SYS a007320	MK414539
24	*Leptobrachella aerea*	Vietnam: Quang Binh	RH60165	JN848437
25	*Leptobrachella applebyi*	Vietnam: Kon Tum	AMS R 173778	KR018108
26	*Leptobrachella applebyi*	Vietnam: Kon Tum	AMS R 173635	KU530189
27	*Leptobrachella bidoupensis*	Vietnam: Lam Dong	AMS R 173133	HQ902880
28	*Leptobrachella bidoupensis*	Vietnam: Lam Dong	NCSM 77321	HQ902883
29	*Leptobrachella bourreti*	Vietnam: Lao Cai	AMS R 177673	KR018124
30	*Leptobrachella eos*	Lao: Phongsaly	MNHN: 2004.0278	JN848450
31	*Leptobrachella firthi*	Vietnam: Kon Tum	AMS R 176524	JQ739206
32	*Leptobrachella fritinniens*	Malaysia: Borneo	KUHE55371	AB847557
33	*Leptobrachella gracilis*	Malaysia: Borneo	KUHE55624	AB847560
34	*Leptobrachella hamidi*	Malaysia: Borneo	KUHE17545	AB969286
35	*Leptobrachella heteropus*	Malaysia: Peninsula	KUHE15487	AB530453
36	*Leptobrachella isos*	Vietnam: Gia Lai	VNMN A 2015.4 / AMS R 176480	KT824769
37	*Leptobrachella laui*	China: San zhoutian, Shenzhen	SYS a002540	MH055904
38	*Leptobrachella laui*	China: Mt. Wutong, Shenzhen	SYS a003477	MH605576
39	*Leptobrachella liui*	China: Mt. Wuyi, Fujian	SYS a002478	MH605573
40	*Leptobrachella liui*	China: Mt. Huanggang, Jiangxi	SYS a001620	KM014549
41	*Leptobrachella mangshanensis*	China: Mangshan, Hunan	MSZTC201702	MG132197
42	*Leptobrachella mangshanensis*	China: Mangshan, Hunan	MSZTC201703	MG132198
43	*Leptobrachella marmorata*	Malaysia: Borneo	KUHE53227	AB969289
44	*Leptobrachella maura*	Malaysia: Borneo	SP21450	AB847559
45	*Leptobrachella macrops*	Vietnam: Phu Yen Prov.	ZMMU-A5823	MG787993
46	*Leptobrachella maoershanensis*	China: Maoershan, Guangxi	KIZ019386	KY986931

ID	Species	Locality	voucher No.	GenBank No. 16S rRNA
47	*Leptobrachella melica*	Cambodia: Ratanakiri	MVZ258198	HM133600
48	*Leptobrachella minima*	Thailand: Chiangmai	/	JN848369
49	*Leptobrachella nyx*	/	ROM26828	MH055818
50	*Leptobrachella oshanensis*	China: Sichuan	SYSa001830	KM014810
51	*Leptobrachella pallida*	Vietnam: Lam Dong	UNS00511	KU530190
52	*Leptobrachella picta*	Malaysia: Borneo	UNIMAS 8705	KJ831295
53	*Leptobrachella pluvialis*	Vietnam: Lao Cai	MNHN:1999.5675	JN848391
54	*Leptobrachella pururus*	China: Yingjiang, Yunnan	SYS a006530	MG520354
55	*Leptobrachella pyrrhops*	Vietnam: Lam Dong	ZMMU A-5208	KP017575
56	*Leptobrachella pyrrhops*	Vietnam: Lam Dong	ZMMU A-4873 (ABV-00213)	KP017576
57	*Leptobrachella sabahmontana*	Malaysia: Borneo	BORNEENSIS 12632	AB847551
58	*Leptobrachella tengchongensis*	China: Tengchong County, Yunnan	SYS a004596	KU589208
59	*Leptobrachella tengchongensis*	China: Tengchong County, Yunnan	SYS a004598	KU589209
60	*Leptobrachella ventripunctata*	Laos: Phongsaly	MNHN 2005.0116	JN848410
61	*Leptobrachella ventripunctata*	China: Zhushihe, Xishuangbanna, Yunnan	SYS a001768	KM014811
62	*Leptobrachella yingjiangensis*	China: Yingjiang, Yunnan	SYS a006533	MG520350
63	*Leptobrachella yunkaiensis*	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004663	MH605584
64	*Leptobrachella yunkaiensis*	China: Dawuling Forest Station, Maoming City, Guangdong	SYS a004664 / CIB107272	MH605585
65	*Leptobrachella wuhuangmontis*	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003485	MH605577
66	*Leptobrachella wuhuangmontis*	China: Mt. Wuhuang, Pubei County, Guangxi	SYS a003486	MH605578
67	*Leptobrachella zhangyapingi*	Thailand: Chiang Mai	KJ-2013	JX069979
68	*Leptobrachium* cf. *chapaense*	Vietnam: Lao Cai	AMS R 171623	KR018126
69	*Pelobates syriacus*	/	MVZ234658	AY236807
70	*Pelobates varaldii*	/	/	AY236808
71	*Megophrys major*	Vietnam: Kon Tum	AMS R173870	KY476333

Phylogenetic analyses

Sequences were first aligned in Clustal X 2.0 (Thompson et al. 1997), with default. The alignment was then checked and manually revised, if necessary. Trimmed with the gaps were partially deleted in MEGA 6.06 (Tamura et al. 2013), while within high variable regions, all gaps were removed.We ran Jmodeltest v2.1.2 (Darriba et al. 2012) with Akaike and Bayesian information criteria on the alignment, resulting the best-fitting nucleotide substitution models of GTR + I + G. Phylogenetic trees were constructed using maximum likelihood (ML) implemented in RaxmlGUI 1.3 (Silvestro and Michalak 2012), and Bayesian inference (BI) using MrBayes 3.2.4 (Ronquist et al. 2012). For ML analysis, the maximum likelihood tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 60% of bootstrap replicates were collapsed. For BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1000 iterations. The first 25% of samples were discarded as burn-in. Convergence of

the Markov Chain Monte Carlo simulations was assessed with PSRF ≤0.01 and ESS (effective sample size) value > 200 using Tracer 1.4 (http://tree.bio.ed.ac.uk/software/tracer/). Pairwise distances were also calculated in MEGA 6.06 based on uncorrected *p*-distance (Tamura et al. 2013).

Morphometrics

Measurements followed Fei et al. (2009) and Rowley et al. (2013), and were taken with a digital caliper to the nearest 0.1 mm. These measurements were as follows:

SVL snout-vent length (from tip of snout to vent);
HDL head length (from tip of snout to rear of jaws);
HDW head width (head width at commissure of jaws);
SNT snout length (from tip of snout to anterior corner of eye);
EYE eye diameter (diameter of exposed portion of eyeball);
IOD interorbital distance (minimum distance between upper eyelids);
IND internasal distance (distance between nares);
TMP tympanum diameter (horizontal diameter of tympanum);
TEY tympanum-eye distance (distance from anterior edge of tympanum to posterior corner of eye);
TIB tibia length (distance from knee to heel);
ML manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
LAHL length of lower arm and hand (distance from tip of the third finger to elbow);
PL pes length (distance from tip of fourth toe to proximal edge of the inner metatarsal tubercle);
HLL hindlimb length (distance from tip of fourth toe to vent).

Sex was determined by direct observation of calling in life, the presence of internal vocal sac openings, and the presence of eggs in abdomen seen via external inspection. Comparative morphological data of *Leptobrachella* species were obtained from examination of museum specimens (see Appendix 1) and from the references listed in Table 2. Due to the high likelihood of undiagnosed diversity within the genus (Rowley et al. 2016; Yang et al. 2016), where available, we rely on examination of topotypic material and/or original species descriptions.

Acoustic analyses

We compared the advertisement calls from three localities. One was in Wujing Nature Reserve and two were in Zhaozishan Nature Reserve. Advertisement calls were recorded

Table 2. References for morphological characters for congeners of the genus *Leptobrachella*.

ID	*Leptobrachella* species	Literature obtained
1	*L. aereus* (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010)	Rowley et al. 2010c
2	*L. alpinus* (Fei, Ye & Li, 1990)	Fei et al. 2009
3	*L. applebyi* (Rowley and Cao, 2009)	Rowley and Cao 2009
4	*L. arayai* (Matsui, 1997)	Matsui 1997
5	*L. ardens* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
6	*L. baluensis* Smith, 1931	Dring 1983; Eto et al. 2016
7	*L. bidoupensis* (Rowley, Le, Tran & Hoang, 2011)	Rowley et al. 2011
8	*L. bondangensis* Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
9	*L. botsfordi* (Rowley, Dau & Nguyen, 2013)	Rowley et al. 2013
10	*L. bourreti* (Dubois, 1983)	Ohler et al. 2011
11	*L. brevicrus* Dring, 1983	Dring 1983; Eto et al. 2015
12	*L. crocea* (Rowley, Hoang, Le, Dau & Cao, 2010)	Rowley et al. 2010a
13	*L. dringi* (Dubois, 1987)	Inger et al. 1995; Matsui and Dehling 2012
14	*L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
15	*L. firthi* (Rowley, Hoang, Dau, Le & Cao, 2012)	Rowley et al. 2012
16	*L. fritinniens* (Dehling and Matsui, 2013)	Dehling and Matsui 2013
17	*L. fusca* Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
18	*L. fuliginosa* (Matsui, 2006)	Matsui 2006
19	*L. gracilis* (Günther, 1872)	Günther 1872; Dehling 2012b
20	*L. hamidi* (Matsui, 1997)	Matsui 1997
21	*L. heteropus* (Boulenger, 1900)	Boulenger 1900
22	*L. isos* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)	Rowley et al. 2015a
23	*L. itiokai* Eto, Matsui & Nishikawa, 2016	Eto et al. 2016
24	*L. juliandringi* Eto, Matsui & Nishikawa, 2015	Eto et al. 2015
25	*L. kajangensis* (Grismer, Grismer & Youmans, 2004)	Grismer et al. 2004
26	*L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
27	*L. kecil* (Matsui, Belabut, Ahmad & Yong, 2009)	Matsui et al. 2009
28	*L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010)	Das et al. 2010
29	*L. lateralis* (Anderson, 1871)	Anderson 1871; Humtsoe et al. 2008
30	*L. laui* (Sung, Yang & Wang, 2014)	Sung et al. 2014
31	*L. liui* (Fei and Ye, 1990)	Fei et al. 2009; Sung et al. 2014
32	*L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)	Duong et al. 2018
33	*L. maculosa* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
34	*L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018)	Hou et al. 2018
35	*L. maoershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017)	Yuan et al. 2017
36	*L. marmorata* (Matsui, Zainudin and Nishikawa, 2014)	Matsui et al. 2014b
37	*L. maura* (Inger, Lakim, Biun and Yambun, 1997)	Inger et al. 1997
38	*L. melanoleuca* (Matsui, 2006)	Matsui 2006
39	*L. melica* (Rowley, Stuart, Neang & Emmett, 2010)	Rowley et al. 2010b
40	*L. minima* (Taylor, 1962)	Taylor 1962; Ohler et al. 2011
41	*L. mjobergi* Smith, 1925	Eto et al. 2015
42	*L. nahangensis* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
43	*L. natunae* (Günther, 1895)	Günther 1895
44	*L. nokrekensis* (Mathew and Sen, 2010)	Mathew and Sen 2010

ID	*Leptobrachella* species	Literature obtained
45	*L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
46	*L. oshanensis* (Liu, 1950)	Fei et al. 2009
47	*L. pallida* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
48	*L. palmata* Inger and Stuebing, 1992	Inger and Stuebing 1992
49	*L. parva* Dring, 1983	Dring 1983
50	*L. pelodytoides* (Boulenger, 1893)	Boulenger 1893; Ohler et al. 2011
51	*L. petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)	Rowley et al. 2017
52	*L. pictua* (Malkmus, 1992)	Malkmus 1992
53	*L. platycephala* (Dehling, 2012)	Dehling 2012a
54	*L. pluvialis* (Ohler, Marquis, Swan & Grosjean, 2000)	Ohler et al. 2000, 2011
55	*L. puhoatensis* (Rowley, Dau & Cao, 2017)	Rowley et al. 2016
56	*L. purpura* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
57	*L. pyrrhops* (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)	Poyarkov et al. 2015
58	*L. rowleyae* (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)	Nguyen et al. 2018
59	*L. sabahmontana* (Matsui, Nishikawa & Yambun, 2014)	Matsui et al. 2014a
60	*L. serasanae* Dring, 1983	Dring, 1983
61	*L. sola* (Matsui, 2006)	Matsui 2006
62	*L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
63	*L. tadungensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
64	*L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das, 2010)	Sengupta et al. 2010
65	*L. tengchongensis* (Yang, Wang, Chen & Rao, 2016)	Yang et al. 2016
66	*L. tuberosa* (Inger, Orlov & Darevsky, 1999)	Inger et al.1999
67	*L. ventripunctata* (Fei, Ye & Li, 1990)	Fei et al. 2009
68	*L. wuhuangmontis* Wang, Yang and Wang, 2018	Wang et al. 2018
69	*L. yingjiangensis* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
70	*L. yunkaiensis* Wang, Li, Lyu and Wang, 2018	Wang et al. 2018
71	*L. zhangyapingi* (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)	Jiang et al. 2013

between 20:00–24:00 h on 2–6 July 2018, using a Sony PCM-D100 digital sound recorder held within 20 cm of the calling individuals. The ambient temperature of the type locality was obtained using a Volt TP-2200 Humidity & Temperature Logger. The sound files in wave format were sampled at 44.1 kHz with sampling depth 24 bits. Praat 6.0.27 (Boersma 2001) was used to obtain the oscillograms, sonograms and power spectrums (window length = 0.005s). Raven pro 1.5 software (Bioacoustics Research Program 2013) was used to quantify the acoustic properties (window size = 256 points, fast Fourier transform, Hanning window). The measurements taken were as follows:

Call Duration: the time between onset of the first pulse and offset of the last pulse in a call;

IQR (Inter-quartile Range): Duration of the difference between the 1st and 3rd quartile times which divides the selection into four time intervals containing equal energy in the selection;

Dominant Frequency: the frequency at which max power occurs within the selection;
IQR (Inter-Quartile Range): Bandwidth of the difference between the 1st and 3rd quartile frequencies which divides the selection into four frequency intervals containing equal energy in the selection;
fNote Pulses: the number of pulses for the first note in a call;
sNote Pulses: the number of pulses for the second note in a call;
Note Rise Time: the time between onset of the first pulse and pulse of max amplitude;
Note Interval: the interval between the first note and the second note in a call;
fNote Duration: the duration of the first note in a call;
sNote Duration: the duration of the second note in a call.

Mean and standard deviation (SD) were calculated. We used median and interquartile range instead of mean and SD when calculating the undivided properties, like fNote Pulses and sNote Pulses. To identify different groups on acoustic properties, a hierarchical clustering using Mahalanobis distance was conducted (Mahalanobis 1936). The dendrogram was constructed based on Ward's method (Ward Jr 1963). All statistical analyses were conducted in R 3.3.2 (R Core Team 2016).

Results

Molecular results

Bayesian inference (BI) and maximum likelihood (ML) phylogenetic trees were constructed based on DNA sequences of the mitochondrial 16S rRNA gene with a total length of 481-bp. The two analyses resulted in essentially identical topologies (Figure 2) which clustered the population of *Leptobrachella* from Jinjiazhai Village (JV) from Wujing Nature Reserve and those from Baimashan Forest Station (BFS) of Zhaozishan Nature Reserve together with very high node supporting values (0.97 in BI and 100% in ML) and represented a separately evolving lineage (Clade A). The population from Qingshan Village (QV) of Zhaozishan Nature Reserve (Clade B) was a sister taxon to Clade A with high node support values (0.99 in BI and 82% in ML). There was almost no genetic divergence between the two populations in Clade A even though the specimens were collected in two different sites with a straight-line distance at approximately 65 kilometers, and the smallest genetic divergence among individuals in Clade B was only 0.3%. The pairwise genetic divergence between Clade A and Clade B was 3.9–4.2%, and between Clade A and all other species of the genus *Leptobrachella* for which comparable sequences were included was 3.2% (between Clade A and *L. bourreti*), and between Clade B and all other species was 5.2–5.6% (between Clade B and *L. purpura*). However, these values were larger than or equal to observed pairwise genetic distances between recognized species (2.2% between *L. liui* and *L. mangshanensis*; 3.2% between *L. eos* and *L. purpura*) (Table 3).

Table 3. Uncorrected p-distances among *Leptobrachella* species based on 16S rRNA fragment (4 parts).

Species & ID No.	(1)–(15)	(16)–(23)	(24)	(25)–(26)	(27)–(28)	(29)	(30)	(31)	(32)
Leptobrachella purpuraventra sp. nov. **(1)–(15)**	0								
Leptobrachella bijie sp. nov. **(16)–(23)**	3.9–4.2	0.0–0.3							
Leptobrachella aerea **(24)**	9.7	10.5–10.9	-						
Leptobrachella applebyi **(25)–(26)**	13.5	14.7–15.1	14.7	0					
Leptobrachella bidoupensis **(27)–(28)**	17	17.8–18.2	15.9	10.6	0				
Leptobrachella bourreti **(29)**	3.2	5.6–5.9	10.9	14.6	17.4	-			
Leptobrachella eos **(30)**	5.6	7.3–7.7	12	14.7	15.4	4.2	-		
Leptobrachella firthi **(31)**	14.6	14.2–14.6	13.1	16.6	18.7	13.5	13.8	-	
Leptobrachella fritinniens **(32)**	18.9	19.3–19.7	16.1	18.9	16.5	18.5	17.2	18.5	-
Leptobrachella gracilis **(33)**	22	23.3–23.8	20.7	18.1	21.8	21.5	22.8	24.1	13.1
Leptobrachella hamidi **(34)**	18.6	20.8–21.2	17.4	14.9	18.1	19.4	16.5	19	8.7
Leptobrachella heteropus **(35)**	21	23.2–23.7	18.1	17	18.9	21.5	21.4	22.6	19.6
Leptobrachella isos **(36)**	13.5	15.1–15.5	13.5	16.2	14.6	12	13.5	12.8	18.9
Leptobrachella laui **(37)–(38)**	11.3	10.9–11.2	11.3	16.7	17.4	10.9	10.2	14.7	18.9
Leptobrachella liui **(39)–(40)**	8.3	9.0–9.4	9.4	15.4	14.6	8.7	8	13.1	17.7
Leptobrachella mangshanensis **(41)–(42)**	9.7	10.5–10.8	10.5	16.2	15.7	10.1	9.4	15.1	19.3
Leptobrachella marmorata **(43)**	15.7	17.7–18.1	15.7	13	17.7	16.1	14.9	17.3	9.4
Leptobrachella maura **(44)**	16.5	17.0–17.4	17	17	17.7	18.2	17.4	18.5	10.8
Leptobrachella macrops **(45)**	15.4	17.9–18.3	14.2	12	9.8	15.8	15	17.8	16.9
Leptobrachella maoershanensis **(46)**	9.8	12.8–13.2	8.7	16.2	14.6	10.9	10.9	17.1	18.9
Leptobrachella melica **(47)**	11.9	14.3–14.7	11.7	6.3	10.3	13.8	15.1	16.6	16.9
Leptobrachella minima **(48)**	11.2	11.2–11.6	6.2	15	17	11.2	12	14.2	18.5
Leptobrachella nyx **(49)**	9	10.8–11.2	5.9	13.5	15	9.4	9.7	11.6	18.1
Leptobrachella oshanensis **(50)**	4.9	5.6–5.9	10.5	14.2	18.6	3.9	5.9	13.4	18.1
Leptobrachella pallida **(51)**	16.1	17.3–17.8	14.7	11.2	6.6	17.7	14.9	19	16.1
Leptobrachella picta **(52)**	18.5	19.7–20.2	17.3	16.1	17.7	18.1	17.2	17.3	5.6
Leptobrachella pluvialis **(53)**	9.7	11.9–12.3	5.2	14.6	15.4	10.1	11.2	14.2	18.4
Leptobrachella purpura **(54)**	4.3	5.2–5.6	10.1	13.9	14.6	3.9	3.2	13	16
Leptobrachella ventripunctata **(60)–(61)**	10.4–10.8	10.8–12.3	5.6	16.2–16.6	17.9–18.3	11.6–12.7	11.9–13.1	11.6–11.9	16.9–17.6
Leptobrachella yingjiangensis **(62)**	10.9	12.0–12.4	12.4	15.6	13.9	10.9	9.4	16.2	18.1
Leptobrachella yunkaiensis **(63)–(64)**	10.5–10.8	12.0–12.7	11.7–12	17.5	16.5–16.9	10.1–10.5	10.1–10.5	16.2	20.2–20.6
Leptobrachella wuhuangmontis **(65)–(66)**	13.1	14.3–14.7	8	16	15.4	11.2	12	13.9	19
Leptobrachella zhangyapingi **(67)**	11.7	12.0–12.4	10.3	15.5	16.2	11.3	10.1	13.1	19.8

Part 2

Species & ID No.	(33)	(34)	(35)	(36)	(37)–(38)	(39)–(40)	(41)–(42)	(43)	(44)
Leptobrachella gracilis **(33)**	-								
Leptobrachella hamidi **(34)**	12.8	-							
Leptobrachella heteropus **(35)**	21.8	18.5	-						
Leptobrachella isos **(36)**	23.3	17.7	22.3	-					
Leptobrachella laui **(37)–(38)**	22.4	18.6	22.8	15.5	0				
Leptobrachella liui **(39)–(40)**	24.9	19.5	21.5	13.2	0.6	0			
Leptobrachella mangshanensis **(41)–(42)**	24.7	21.3	22.7	14.3	5.6	2.2	0		
Leptobrachella marmorata **(43)**	12.4	5.3	18.4	17.7	17.3	16.1	17.7	-	
Leptobrachella maura **(44)**	12	10.2	19.5	16.5	19.5	174	19.5	9.4	-
Leptobrachella macrops **(45)**	20.7	16.5	21.4	15.4	16.6	14.6	14.9	14.9	17

Leptobrachella maoershanensis **(46)**	24.3	20.4	21.9	15.1	7.7	6.3	6.2	17.7	19.5
Leptobrachella melica **(47)**	14.9	16.6	17.7	16.7	17.6	16.7	17.9	13.4	15
Leptobrachella minima **(48)**	21.5	19.5	19.4	14.3	9.8	9.4	9.7	16.9	17.8
Leptobrachella nyx **(49)**	23.7	17.3	18.1	13.1	9.8	8.3	9.7	15.7	17.3
Leptobrachella oshanensis **(50)**	20.2	19.4	22.8	12.7	8.7	8.3	9	17.3	17.7
Leptobrachella pallida **(51)**	19.7	16.9	20.5	18.3	15.4	15.3	15.7	15.3	16.5

Part 3

Species & ID No.	**(45)**	**(46)**	**(47)**	**(48)**	**(49)**	**(50)**	**(51)**	**(52)**	**(53)**
Leptobrachella macrops **(45)**	-								
Leptobrachella maoershanensis **(46)**	15	-							
Leptobrachella melica **(47)**	11.3	16.3	-						
Leptobrachella minima **(48)**	16.1	9.4	14.6	-					
Leptobrachella nyx **(49)**	16.1	8.1	12.8	8	-				
Leptobrachella oshanensis **(50)**	16.6	11.3	14.2	9.4	9.7	-			
Leptobrachella pallida **(51)**	9	15.4	12.1	15	15.8	16.1	-		
Leptobrachella picta **(52)**	16.5	19.3	16.1	18	18.1	18.4	16.9	-	
Leptobrachella pluvialis **(53)**	16.1	7.3	14.7	7.2	5.9	10.5	14.6	18.4	-
Leptobrachella purpura **(54)**	14.6	10.1	14.3	11.6	9.4	5.6	14.5	17.3	9.7
Leptobrachella pyrrhops **(55)–(56)**	8.3–8.7	14.9–15.3	12.4–12.8	16.1–16.5	15.7–16.1	16.5–16.9	7.6–8.0	16.9–17.3	15.7–16.1
Leptobrachella sabahmontana **(57)**	15.3	19.9	13.7	16.9	18.9	17.3	14.9	5.2	19.3
Leptobrachella tengchongensis **(58)–(59)**	15.8	10.9	13.9	9.4	9.7	8.7	15.8	16.4	10.8
Leptobrachella ventripunctata **(60)–(61)**	17.8–18.1	9.4–9.8	15.1–15.4	6.2–7.2	6.2–6.6	10.9–12.0	16.6–17.0	17.6–18.4	6.9–7.2

Part 4

Species & ID No.	**(54)**	**(55)–(56)**	**(57)**	**(58)–(59)**	**(60)–(61)**	**(62)**	**(63)–(64)**	**(65)–(66)**	**(67)**
Leptobrachella purpura **(54)**	-								
Leptobrachella pyrrhops **(55)–(56)**	15.7–16.5	0.3							
Leptobrachella sabahmontana **(57)**	16.9	16.1–16.4	-						
Leptobrachella tengchongensis **(58)–(59)**	8.7	16.1–16.5	16.5	0					
Leptobrachella ventripunctata **(60)–(61)**	10.8–11.9	16.1–16.2	16.5–16.6	16.5–17.3	9.4–10.5	0.9			
Leptobrachella yingjiangensis **(62)**	9.4	13.9–14.3	18.6	9.1	12.7	13.1	-		
Leptobrachella yunkaiensis **(63)–(64)**	10.1–10.5	16.2–16.5	16.6–17.0	21.1–21.5	12.4–12.7	11.6–11.9	0.3		
Leptobrachella wuhuangmontis **(65)–(66)**	12.4	16.2	16.6	19	13.9	9.8	10.9–15.6	0	
Leptobrachella zhangyapingi **(67)**	9.4	18.2	18.7	19	9.5	10.9	11.3	12.4	-

Acoustic results

Calling from nine male individuals were measured, respectively. They were recorded in Jinjiazhai Village (two males), Baimashan Forest Station (three males), and Qingshan Village (four males) at an ambient temperature approximately of 18.8 °C, 19.3 °C, and 18.6 °C, respectively. The result of hierarchical clustering analysis was consistent with the molecular result (Figure 3). Nine calling males were clustered into two clades based

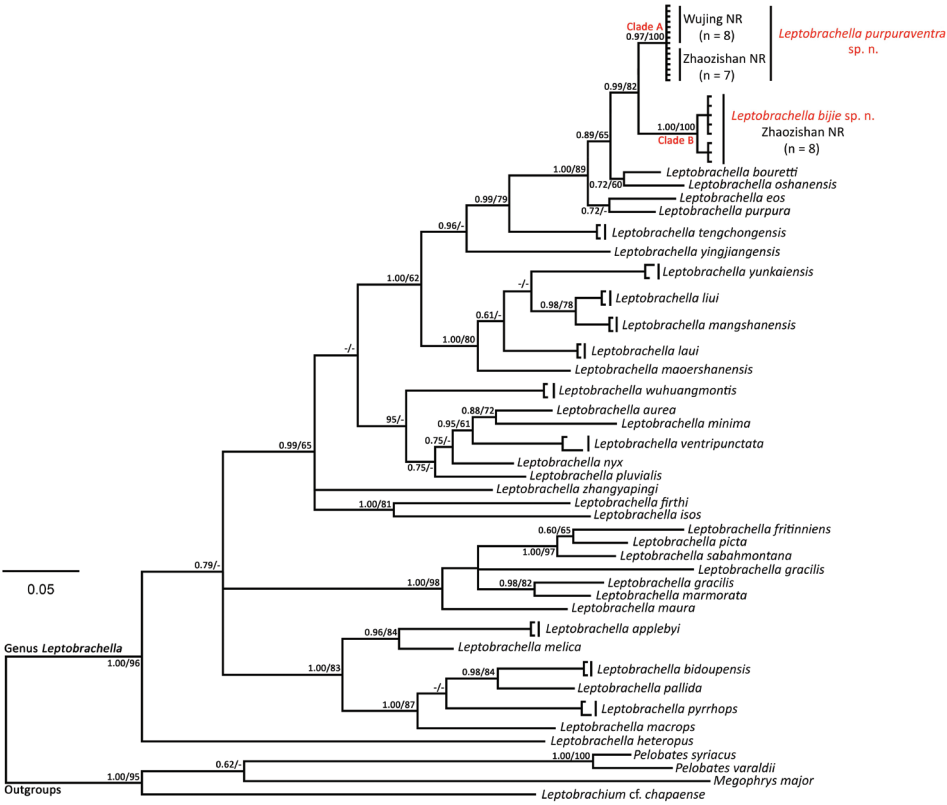

Figure 2. Bayesian inference tree of *Leptobrachella* species and out-groups derived from partial DNA sequences of the mitochondrial 16S r RNA gene. Numbers before slashes indicate Bayesian posterior probabilities (>0.6 retained) and numbers after slashes are bootstrap support for maximum likelihood (1000 replicates) analyses (>60 retained). The symbol "–" represents bootstrap value below 0.60/60%.

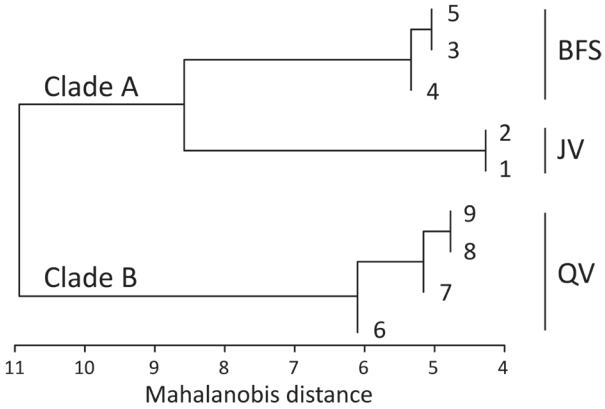

Figure 3. Hierarchical clustering of advertisement calls of *Leptobrachella purpuraventra* sp. nov. from **BFS** Baimashan Forest Station in Zhaozishan Nature Reserve and **JV** Jinjiazhai Village in Wujing Nature Reserve, respectively; and *L. bijie* sp. nov. from **QV** Qingshan Village in Zhaozishan Nature Reserve.

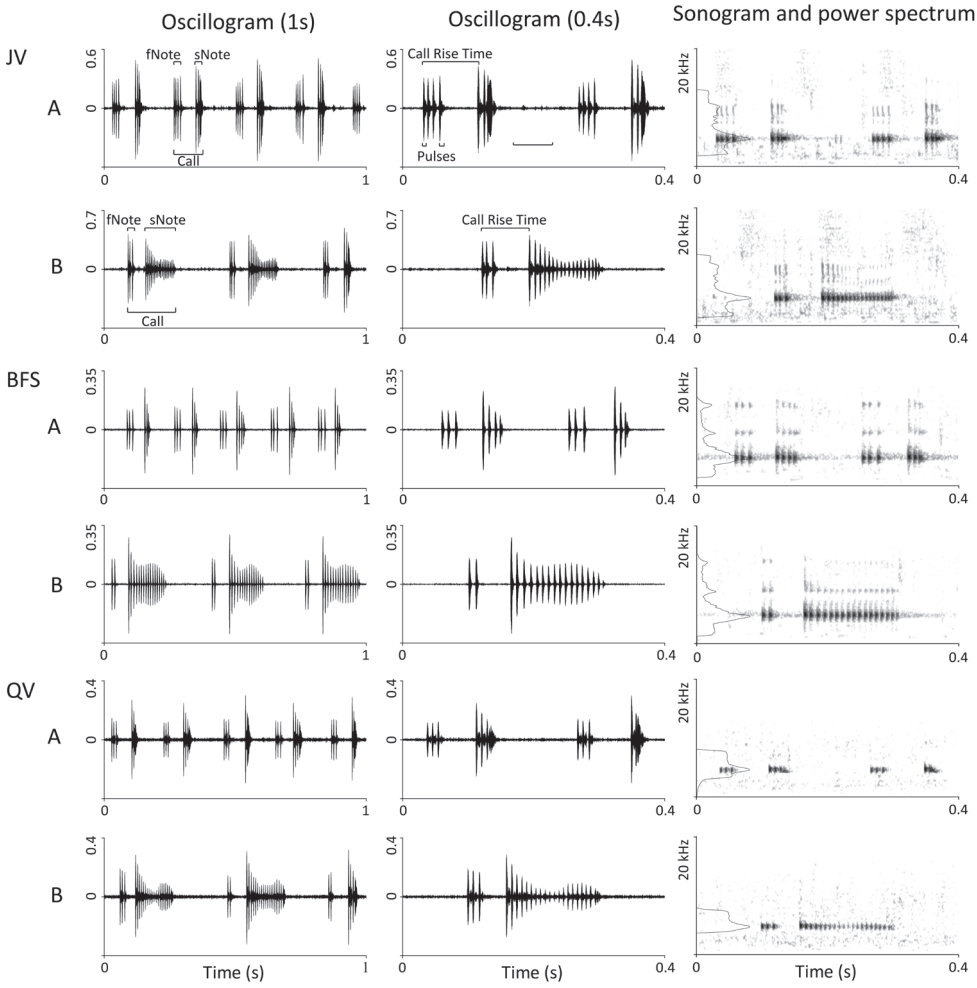

Figure 4. Different call types (**A** the first call type **B** the second call type.) of *Leptobrachella purpuraventra* sp. nov. from **BFS** Baimashan Forest Station in Zhaozishan Nature Reserve and **JV** Jinjiazhai Village in Wujing Nature Reserve, respectively; and different call types of *L. bijie* sp. nov. from **QV** Qingshan Village in Zhaozishan Nature Reserve. (Window length: 0.005 s).

on acoustic properties of advertisement calls. All JV males and BFS males were clustered into Clade A, and all the QV males were clustered into Clade B. In Clade A, there were some differences in the advertisement calls between JV and BFS in Clade B. Measurements of the advertisement calls of the three localities are listed in Table 4.

All advertisement calls contain two notes, each of which consists of repeated pulses (Figure 4). Clade A had more fNote pulses in second type of advertisement calls than those of Clade B (3 ± 1 vs. 2 ± 1), more sNote pulses in first type of advertisement calls (4 ± 1 vs. 3 ± 1), and less sNote pulses in the second type of advertisement calls (17 ± 3 vs. 21.5 ± 4). Accordingly, the sNote duration of Clade A was greater than those of

Table 4. Measurements (mean ± standard deviation) of 11 acoustic properties for *Leptobrachella* species in this study.

Locality	Individuals	Call Type	Call Duration (ms)	Call Interval (ms)	IQR Duration (ms)	Dominant Frequency (Hz)	IQR Bandwidth (Hz)	fNote Pulses*	sNote Pulses*	Note Rise Time (ms)	Note Interval (ms)	fNote Duration (ms)	sNote Duration (ms)
Leptobrachella purpuraventra sp. nov.													
JV	1	A (n=20)	111.6±3.0	119.1±8.0	30.8±27.3	4806.2±135.7	361.7±53.0	4±0	6±1	87.2±2.4	58.2±3.5	27.8±3.4	25.6±2.8
		B (n=14)	189.9±13.6	193.6±36.3	41.4±20.6	4835.7±196.6	319.9±148.9	4±1	20±2	69.3±9.6	42.6±11.2	24.7±5.6	122.5±11.4
	2	A (n=24)	110.7±5.0	128.5±13.7	57.8±23.1	4679.9±65.6	236.9±85.2	4±0	4±0	85.8±3.3	51.6±4.7	32.4±4.8	26.7±3.8
		B (n=18)	188±10.9	196.2±39.3	54.1±24.0	4679.9±66.0	306.2±73.7	3±0.75	19.5±1	57.3±30.1	43.1±9.1	25.3±5.9	119.7±7.4
BFS	3	A (n=20)	93.7±6.1	102.6±13.2	41.3±23.4	4823.4±0.0	180.9±38.5	3±0	4±1	70.5±3.1	45.0±4.2	24.5±2.8	24.2±6.0
		B (n=21)	192.2±13.0	174.5±51.1	50.4±20.1	4675.8±61.7	328.1±51.8	2±0	17±1	55.6±9.2	37.5±8.6	16.9±4.5	137.7±8.8
	4	A (n=20)	90.3±2.0	90.8±5.6	47.2±9.9	4823.4±0.0	344.5±0.0	3±0	4±0.25	69.4±2.0	39.4±2.2	28.2±1.1	22.8±2.4
		B (n=25)	191.8±11.9	174.5±51.1	60.6±16.6	4823.4±0.0	186.1±47.7	3±1	16±2	63.9±4.1	40.4±6.7	22.1±6.2	129.4±11.6
	5	A (n=20)	91.7±2.2	117.2±31.2	52.5±9.7	4720.1±86.6	198.1±63.1	3±0	4±0	67.4±3.4	40.3±4.7	25.2±3.7	26.2±3.4
		B (n=11)	144.8±31.5	217.8±64.9	27.2±7.1	4745.1±89.9	219.3±80.4	2±0.5	13±3.5	53.5±6.7	33.5±8.0	18.3±6.4	93.3±25.9
	Summary	A (n=104)	100.0±10.4	112.3±21.3	46.3±22.0	4767.1±97.3	263.4±92.8	3±1	4±1	76.4±9.1	47.1±8.1	27.8±4.4	25.2±4.1
		B (n=89)	185.0±21.7	182.7±47.9	49.7±21.4	4751.8±115.6	269.0±100.4	3±1	17±3	60.2±15.7	39.8±9.0	21.4±6.5	123.8±18.3
Leptobrachella bijie sp. nov.													
QV	6	A (n=26)	103.1±2.3	109.0±3.3	28.0±31.1	5068.6±86.8	344.5±0.0	2±0	3±0	87.8±2.1	70.1±7.8	16.4±7.2	16.6±0.8
		B (n=21)	221.0±14.0	235.6±45.1	38.1±20.5	5036.7±92.9	278.9±85.7	2±0	17±2	82.2±3.9	63.0±5.2	17.7±5.3	140.4±12.8
	7	A (n=25)	98.8±6.4	122.2±23.4	19.5±18.9	4823.4±0.0	172.3±0.0	3±1	4±1	76.4±3.7	55.9±4.9	19.3±4.6	23.5±6.5
		B (n=20)	206.8±10.1	245.2±79.4	56.6±20	4780.4±76.5	206.7±70.7	2±1	20±3	68.4±10.2	48.6±9.3	18.4±5.7	139.7±9.9
	8	A (n=22)	102.6±7.5	112.9±8.2	71.4±15.4	4909.6±88.2	172.3±0.0	3±0	4±1	85.3±5.2	61.3±5.9	22.5±1.6	18.9±3.2
		B (n=28)	253.0±19.0	225.6±61.1	71.5±34.9	4835.7±45.2	319.9±61.4	2±0.25	23±2.25	69.7±11.4	51.7±12.6	16.5±7.3	184.9±19.0
	9	A (n=33)	99.4±3.2	101.9±6.4	34.4±29.1	4823.4±0	302.8±74.9	2±1	2±1	83.0±2.9.0	60.1±6.6	21.7±6.6	17.7±1.9
		B (n=25)	264.0±12.5	166.5±44.5	64.6±23.5	4823.4±70.3	227.4±82.0	2±1	23±2	83.6±7.2	61.1±7.8	21.2±6.1	181.7±11.6
	Summary	A (n=106)	100.8±5.4	110.7±14.5	37.0±31.0	4901.4±116.8	255.2±86.4	3±1	3±2	83.1±5.4	61.8±8.2	20.0±6.0	19.0±4.5
		B (n=94)	239.0±27.0	216.3±65.4	59.0±28.7	4865.6±117.7	262.1±86.5	2±1	21.5±4	75.9±11.1	56.1±11.0	18.4±6.4	164.5±25.8

* Median instead of mean, inter-quartile range instead of SD. **JV**: Jinjiazhai Village; **BFS**: Baimashan Forest Station; **QV**: Qingshan Village.

Clade B (164.5 ± 25.8 vs. 123.8 ± 18.3 ms). Compared with individuals in Clade A, those from Clade B had little difference in the first type of advertisement calls, but had relatively short call duration (185.0 ± 21.7 vs. 239.0 ± 27.0 ms) and call interval (182.7 ± 47.9 vs. 216.3 ± 65.4 ms) in the second type. The dominant frequency of Clade A was higher than those of Clade B in both the first type of advertisement calls (4901.4 ± 116.8 vs. 4767.1 ± 97.3 Hz) and the second type (4865.6 ± 117.7 vs. 4751.8 ± 115.6 Hz).

Combining morphological, molecular genetics, and acoustic evidence, we herein describe these specimens as two new species.

Taxonomy accounts

Leptobrachella bijie **J. Wang, Y.L. Li, Y. Li, H.H. Chen & Y.Y. Wang, sp. nov.**
http://zoobank.org/550E8562-0EC9-40C4-A6B3-FFAC35B25444
Figure 5

Holotype. SYS a007316, adult male, collected by Jian Wang (JW hereafter) and Yulong Li (YLL hereafter) on 6 July 2018 from Qingshan Village (27°39'24"N, 105°23'14"E; 1670 m a.s.l.) in Zhaozishan Nature Reserve, Linkou Town, Qixingguan District, Bijie City, Guizhou Province, China.

Paratypes. Seven adult males, SYS a007313/CIB 110002, SYS a007314–7315, 7317–7320, collected by Honghiu Chen (HHC hereafter), Yongyou Zhao (YYZ hereafter) and Jiahe Li (JHL), the same collection data as the holotype.

Diagnosis. (1) small size (SVL 29.0–30.4 mm in eight adult males), (2) dorsal skin shagreened, some of the granules forming longitudinal short skin ridges, (3) iris bicolored, coppery orange on upper half and silver on lower half, (4) tympanum distinctly discernible, slightly concave, distinct black supratympanic line present, (5) internasal distance equal to interorbital distance, (6) supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible, (7) absence of webbing and lateral fringes on fingers, toes with rudimentary webbing and narrow lateral fringes, (8) longitudinal ridges under toes not interrupted at the articulations, (9) relative finger lengths I = II = IV < III, relative toe length I < II < V = III < IV, (10) heels just meeting, tibia-tarsal articulation reaches the region between middle of eye to anterior corner of eye, (11) dorsal surface shagreened and granular, lacking enlarge tubercles or warts, some of the granules forming short longitudinal folds, (12) dorsum greyish-brown grounding, with small light orange granules, distinct darker brown markings scattered with irregular light orange pigmentations, (13) flanks with several dark blotches, longitudinally in two rows, (14) ventral surface white, with distinct nebulous greyish speckling on chest and ventrolateral flanks, (15) dorsal limbs including fingers and toes with dark bars, and (16) dense tiny conical spines present on surface of chest in males during breeding season.

Comparisons. Comparative morphological data of *Leptobrachella bijie* sp. nov. and 45 recognized *Leptobrachella* species occurring north of the Isthmus of Kra were listed in Table 5.

Table 5. Selected diagnostic characters for species described herein and species in the genus *Leptobrachella* occurring north of the Isthmus of Kra (modified from Rowley et al. 2017; Yuan et al. 2017; Yang et al. 2018; Wang et al. 2018).

Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
L. bijie sp. nov.	29.0–30.4	Yes	Rudimentary	Narrow	White with distinct nebulous greyish speckling on chest and ventrolateral flanks	Shagreened and granular
L. purpuraventra sp. nov.	27.3–29.8	Yes	Rudimentary	Narrow	Grey purple with distinct nebulous greyish speckling on chest and ventrolateral flanks	Shagreened and granular
L. aerea	25.1–28.9	No	Rudimentary	Wide	Near immaculate creamy white, brown specking on margins	Finely tuberculate
L. alpinus	24.0–26.4	Yes	Rudimentary	Wide in males	Creamy-white with dark spots	Relatively smooth, some with small warts
L. applebyi	19.6–22.3	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
L. ardens	21.3–24.7	Yes	No	No	Reddish brown with white speckling	Smooth- finely shagreened
L. bidoupensis	18.5–25.4	Yes	Rudimentary	Weak	Reddish brown with white speckling	Smooth
L. botsfordi	29.1–32.6	No	Rudimentary	Narrow	Reddish brown with white speckling	Shagreened
L. bourreti	28.0–36.2	Yes	Rudimentary	Weak	Creamy white	Relatively smooth, some with small warts
L. crocea	22.2–27.3	No	Rudimentary	No	Bright orange	Highly tuberculate
L. eos	33.1–34.7	No	Rudimentary	Wide	Creamy white	Shagreened
L. firthi	26.4–29.2	No	Rudimentary	Wide in males	Creamy white	Shagreened with fine tubercles
L. fuliginosa	28.2–30.0	Yes	Rudimentary	Weak	White with brown dusting	Nearly smooth, few tubercles
L. isos	23.7–27.9	No	Rudimentary	Wide in males	Creamy white with white dusting on margins	Mostly smooth, females more tuberculate
L. kalonensis	25.8–30.6	Yes	No	No	Pale, speckled brown	Smooth
L. khasiorum	24.5–27.3	Yes	Rudimentary	Wide	Creamy white	Isolated, scattered tubercles
L. lateralis	26.9–28.3	Yes	Rudimentary	No	Creamy white	Roughly granular
L. laui	24.8–26.7	Yes	Rudimentary	Wide	Creamy white with dark brown dusting on margins	Round granular tubercles
L. liui	23.0-28.7	Yes	Rudimentary	Wide	Creamy white with dark brown spots on chest and margins	Round granular tubercles with glandular folds
L. macrops	28.0–29.3	Yes	Rudimentary	No	Greyish-violet with white speckling	Roughly granular with larger tubercles
L. maculosa	24.2–26.6	Yes	No	No	Brown, less white speckling	Mostly smooth
L. maoershanensis	25.2–30.4	Yes	Rudimentary	Narrow	Creamy white chest and belly with irregular black spots	Longitudinal folds
L. mangshanensis	22.22–27.76	Yes	Rudimentary	Weak	White speckles on throat and belly	Nearly smooth
L. melica	19.5–22.7	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
L. minima	25.7–31.4	Yes	Rudimentary	No	Creamy white	Smooth

Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
L. nahangensis	40.8	Yes	Rudimentary	No	Creamy white with light specking on throat and chest	Smooth
L. nokrekensis	26.0–33.0	Yes	Rudimentary	unknown	Creamy white	Tubercles and longitudinal folds
L. nyx	26.7–32.6	Yes	Rudimentary	No	Creamy white with white with brown margins	Rounded tubercles
L. oshanensis	26.6–30.7	Yes	No	No	Whitish with no markings or only small, light grey spots	Smooth with few glandular ridges
L. pallida	24.5–27.7	No	No	No	Reddish brown with white speckling	Tuberculate
L. pelodytoides	27.5–32.3	Yes	Wide	Narrow	Whitish	Small, smooth warts
L. petrops	23.6–27.6	No	No	Narrow	Immaculate creamy white	Highly tuberculate
L. pluvialis	21.3–22.3	Yes	Rudimentary	No	Dirty white with dark brown marbling	Smooth, flattened tubercles on flanks
L. puhoatensis	24.2–28.1	Yes	Rudimentary	Narrow	Reddish brown with white dusting	Longitudinal skin ridges
L. purpura	25.0–27.5	Yes	Rudimentary	Wide	Dull white with indistinct grey dusting	Shagreen with small tubercles
L. pyrrhops	30.8–34.3	Yes	Rudimentary	No	Reddish brown with white speckling	Slightly shagreened
L. rowleyae	23.4–25.4	Yes	No	No	Pinkish milk-white to light brown chest and belly with numerous white speckles	Smooth with numerous tiny tubercles
L. sungi	48.3–52.7	No or small	Wide	Weak	White	Granular
L. tadungensis	23.3–28.2	Yes	No	No	Reddish brown with white speckling	Smooth
L. tamdil	32.3	Yes	Wide	Wide	White	Weakly tuberculate
L. tengchongensis	23.9–26.0	Yes	Rudimentary	Narrow	White with dark brown blotches	Shagreened with small tubercles
L. tuberosa	24.4–29.5	No	Rudimentary	No	White with small grey spots/streaks	Highly tuberculate
L. ventripunctata	25.5–28.0	Yes	Rudimentary	No	Chest and belly with dark brown spots	Longitudinal skin ridges
L. wuhuangmontis	25.6–30.0	Yes	Rudimentary	Narrow	Greyish white mixed by tiny white and black dots	Rough, scattered with dense conical tubercles
L. yingjiangensis	25.7–27.6	Yes	Rudimentary	Wide	Creamy white with dark brown flecks on chest and margins	Shagreened with small tubercles
L. yunkaiensis	25.9–29.3	Yes	Rudimentary	Wide	Belly pink with distinct or indistinct speckling	Shagreened with short skin ridges and raised warts
L. zhangyapingi	45.8–52.5	No	Rudimentary	Wide	Creamy-white with white with brown margins	Mostly smooth with distinct tubercles

Compared with the 26 known congeners of the genus *Leptobrachella* occurring south of the Isthmus of Kra, by the presence of supra-axillary and ventrolateral glands, *L. bijie* sp. nov. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*,

L. maura, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola*, all of which lacking supra-axillary and ventrolateral glands; and by the significantly larger body size, SVL 29.0–30.4 mm in males, *L. bijie* sp. nov. differs from the smaller *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. bondangensis* (17.8 mm in male), *L. fusca* (16.3 mm in male), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males), *L. mjobergi* (15.7–19.0 mm in males), *L. natunae* (17.6 mm in one adult male), *L. parva* (15.0–16.9 mm in males), *L. palmata* (14.4–16.8 mm in males), *L. serasanae* (16.9 mm in female), and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" (15.0–16.0 mm in males).

For the remaining 45 members of the genus *Leptobrachella*, having SVL of 29.0–30.4 mm in males, *L. bijie* sp. nov. differs from the larger *L. eos* (33.1–34.7 mm in males), *L. nahangensis* (40.8 mm in male), *L. sungi* (48.3–52.7 mm in males), *L. tamdil* (32.3 mm in male), and *L. zhangyapingi* (45.8–52.5 mm in males); and from the smaller *L. alpinus* (24.0–26.4 mm in males), *L. applebyi* (19.6–22.3 mm in males), *L. ardens* (21.3–24.7 mm in males), *L. bidoupensis* (18.5–25.4 mm in males), *L. crocea* (22.2–27.3 mm in males), *L. isos* (23.7–27.9 mm in males), *L. khasiorum* (24.5–27.3 mm in males), *L. lateralis* (26.9–28.3 mm in males), *L. laui* (24.8–26.7 mm in males), *L. maculosa* (24.2–26.6 mm in males), *L. melica* (19.5–22.7 mm in males), *L. pallida* (24.5–27.7 mm in males), *L. petrops* (23.6–27.6 mm in males), *L. pluvialis* (21.3–22.3 mm in males), *L. puhoatensis* (24.2–28.1 mm in males), *L. purpura* (25.0–27.5 mm in males), *L. rowleyae* (23.4–25.4 mm in males), *L. tadungensis* (23.3–28.2 mm in males), *L. tengchongensis* (23.9–26.0 mm in males), *L. ventripunctata* (25.5–28.0 mm in males), and *L. yingjiangensis* (25.7–27.6 mm in males).

In having black spots on flanks, the new species differs from *L. aerea*, *L. botsfordi*, *L. firthi*, and *L. tuberosa*, all of which lacking distinct black spots on the flanks; by having rudimentary webbing on toes, the new species differs from *L. kalonensis* and *L. oshanensis*, both of which lacking webbing on toes, and from *L. pelodytoides*, which bears wide webbing on toes; by having narrow lateral fringes on toes, the new species differs from *L. aerea*, *L. firthi*, *L. liui*, and *L. yunkaiensis*, all of which having wide lateral fringes on toes, from *L. bourreti* and *L. fuliginosa*, both of which having weak lateral fringes on toes, and from *L. kalonensis*, *L. macrops*, *L. minima*, *L. nyx*, *L. oshanensis*, *L. pyrrhops*, and *L. tuberosa*, all of which lacking lateral fringes on toes; by having dorsal surface shagreened and granular, lacking enlarge tubercles or warts, the new species differs from *L. bourreti* (dorsum smooth with small warts), *L. fuliginosa* (dorsum smooth with fine tubercles), *L. liui* (dorsum with round tubercles), *L. macrops* (dorsum roughly granular with large tubercles), *L. maoershanensis* (dorsum shagreened with tubercles), *L. minima* (dorsum smooth), *L. nyx* (dorsum with round tubercles), *L. pelodytoides* (dorsum with small, smooth warts), *L. tuberosa* (dorsum hingly tuberculate), *L. yunkaiensis* (dorsum with raised warts), and *L. wuhuangmontis* (dorsum rough with conical tubercles); by having ventral surface white with distinct nebulous greyish speckling on chest and flanks, the new species differs from *L. botsfordi* and *L. pyrrhops*, (ventral reddish brown with white speckling), *L. maoershanensis* (belly with irregular black spots); by having tiny

spines on surface of chest in males during breeding season, the new species differs from all male specimens collected in breeding season of *L. liui*, *L. oshanensis*, *L. yunkaiensis*, and *L. wuhuangmontis*, all of which are lacking such spines.

Description of holotype. Adult male. Body size small, SVL in 29.3 mm. Head length slightly larger than head width, HDL/HDW 1.03; snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, internarial distance equal to interorbital distance, IND/IOD 1.00; pineal ocellus absent; vertical pupil; snout length larger than eye diameter, SNT/EYE 1.11; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye and larger than tympanum-eye distance, TMP/EYE 0.53 and TEY/TMP 0.47; upper margin of tympanum incontact with supratympanic ridge; distinct black supratympanic line present; vomerine teeth absent; vocal sac openings slit-like, paired, located posterolaterally on floor of mouth in close proximity to the margins of the mandible; tongue deeply notched behind; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths I = II = IV < III; nuptial pad absent; subarticular tubercles absent; a large, rounded inner palmar tubercle distinctly separated from small, round outer palmar tubercle; absence of webbing and lateral fringes on fingers. Tips of toes like fingers; relative toe length I < II < V = III < IV; subarticular tubercles absent; distinct dermal ridges present under the 3rd to 5th toes, not interrupted; large, oval inner metatarsal tubercle present, outer metatarsal tubercle absent; toes webbing rudimentary; narrow lateral fringes present on all toes. Tibia 47% of snout-vent length; tibiotarsal articulation reaches to middle of eye; heels just meeting each other when thighs are appressed at right angles with respect to body.

Dorsal surface shagreened and granular, lacking enlarge tubercles or warts, some of the granules forming short longitudinal folds; ventral skin smooth; dense tiny conical spines present on surface of chest; pectoral gland and femoral gland oval; pectoral glands greater than tips of fingers and femoral glands; femoral gland situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary gland raised. Ventrolateral gland distinctly visible, forming an incomplete line.

Measurements of holotype (in mm). SVL 29.2, HDL 10.0, HDW 9.7, SNT 4.0, EYE 3.6, IOD 3.0, IND 3.0, TMP 1.9, TEY 0.9, TIB 13. 8, ML 7.8, PL 13.2, LAHL 14.1, HLL 43.3.

Coloration of holotype in life. Dorsum greyish-brown grounding, with small reddish granules, distinct darker brown markings and rounded spots and scattered with irregular light orange pigmentation. A dark brown inverted triangular pattern between anterior corner of eyes, in connected to the dark brown W-shaped marking on interorbital region, and the W-shaped marking in connected to the other W-shaped marking between axillae. Tympanum brown. Small light orange granules present on dorsum of body and limb; a dark brown vertical bar under the eye; transverse dark brown bars on dorsal surface of limbs; distinct dark brown blotches on flanks from

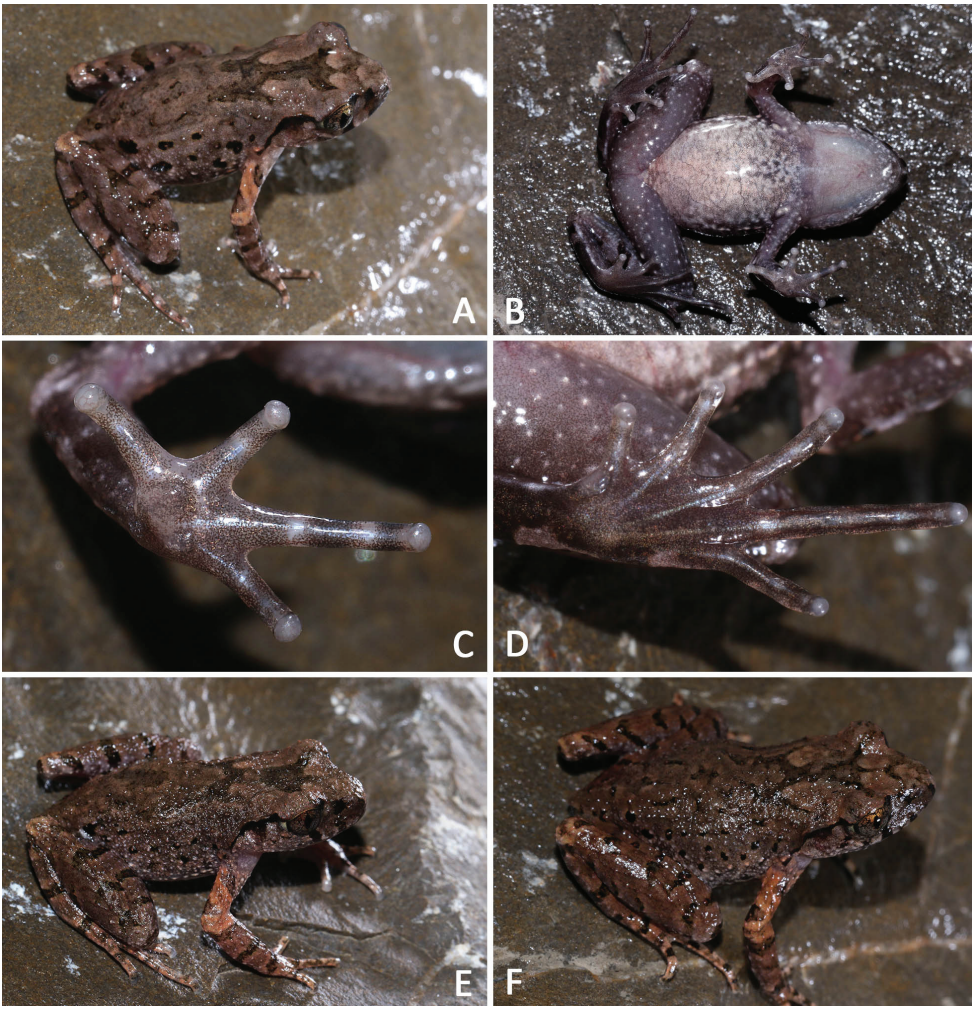

Figure 5. General aspect in life: **A–D** SYS a007316, the male holotype of *Leptobrachella bijie* sp. nov. **E** the male paratype SYS a007313 **F** the male paratype SYS a007317.

groin to axilla, longitudinally in two rows; elbow and upper arms with dark bars and distinct coppery orange coloration; fingers and toes with distinct dark bars.

Ventral surface of throat, chest, and belly white, presence of distinct nebulous greyish speckling on chest and ventrolateral flanks; ventral surface of limbs grey purple. Supra-axillary gland coppery orange; femoral, pectoral and ventrolateral glands greyish white. Iris bicolored, coppery orange on upper half and silver on lower half.

Coloration of holotype in preservative. Dorsum of body and limbs dark brown; transverse bars on limbs become more distinct; dark brown patterns, markings and spots on back become indistinct, orange pigmentations become greyish white. Ventral surface of body and limbs greyish white, nebulous speckling on chest and flanks balck brown. Supra-axillary, femoral, pectoral and ventrolateral glands greyish white.

Table 6. Measurements (minimum–maximum (mean ± SD); in mm), and body proportions of *Leptobrachella bijie* sp. nov. from Qingshan Village of Zhaozishan Nature Reserve.

SEX		Males (n = 8)	
SVL	29.0–30.4 (29.7 ± 0.6)	**HLL**	43.0–45.5 (43.7 ± 0.8)
HDL	10.0–10.6 (10.2 ± 0.2)	**HDL/HDW**	1.02–1.05 (1.04 ± 0.01)
HDW	9.5–10.2 (9.8 ± 0.3)	**HDL/SVL**	0.33–0.35 (0.34 ± 0.01)
SNT	4.0–4.7 (4.3 ± 0.3)	**SNT/HDL**	0.40–0.44 (0.42 ± 0.02)
EYE	3.6–4.1 (3.8 ± 0.2)	**SNT/EYE**	1.11–1.15 (1.13 ± 0.02)
IOD	2.8–3.4 (3.1 ± 0.2)	**EYE/TMP**	1.85–1.95 (1.89 ± 0.04)
IND	2.8–3.4 (3.1 ± 0.2)	**IND/IOD**	1
TMP	1.9–2.2 (2.0 ± 0.1)	**TMP/EYE**	0.51–0.54 (0.53 ± 0.01)
TEY	0.9–1.1 (1.0 ± 0.1)	**TEY/TMP**	0.45–0.53 (0.48 ± 0.02)
TIB	13.5–14.4 (13.8 ± 0.3)	**TIB/SVL**	0.45–0.47 (0.47 ± 0.01)
ML	7.4–8.3 (7.8 ± 0.3)	**LAHL/SVL**	0.47–0.49 (0.48 ± 0.01)
PL	13.0–13.8 (13.3 ± 0.2)	**HLL/SVL**	1.45–1.50 (1.47 ± 0.02)
LAHL	14.0–14.8 (14.3 ± 0.3)	**TIB/HLL**	0.31–0.32 (0.31 ± 0.01)

Variations. Measurements and body proportions were listed in Table 6. All paratypes match the overall characters of the holotype except that: coloration of tympanum brown in the holotype SYS a007316 (vs. black in paratypes SYS a007313/CIB 110002 (Figure 5E), SYS a007315, 7317 (Figure 5F)); heels just meeting, tibia-tarsal articulation reaching the middle of eye in the holotype (vs. heels slightly overlapping in paratypes SYS a007315, 7317, 7319–7320; tibia-tarsal articulation reaching the anterior corner of eye in paratypes SYS a007315, 7317, 7319); W-shaped marking on interorbital region in connected to the other W-shaped marking between axillae in the holotype (vs. such markings not in connected with each other in paratypes SYS a007313/CIB 110002, SYS a007320); a dark brown inverted triangular pattern between anterior corner of eyes in the holotype (vs. a V-shaped pattern between anterior corner of eyes instead in paratype SYS a007317, 7320); relatively larger black spots on flanks (vs. black spots distinctly small in paratypes SYS a007313/CIB 110002, SYS a007317).

Etymology. The specific epithet *bijie* is in reference to the type locality, Qingshan Village in Bijie City of Guizohu Province, China. For the common name, we suggest "Bijie Leaf Litter Toad", and for the Chinese name "Bi Jie Zhang Tu Chan (毕节掌突蟾)".

Distribution and habits. Currently, *Leptobrachella bijie* sp. nov. is known only from its type locality Qingshan Village in Zhaozishan Nature Reserve, Linkou County, Qixingguan District, Bijie City, Guizhou Province, China (Figure 1). The new species was found along a clear-water rocky stream (ca. 2 m in width and ca. 20–30 cm in depth; 1670–1750 m a.s.l.) in karst landforms. The stream was surrounded by broad-leaved forest at an altitude below 1700 m, and by coniferous forest at an altitude above 1700 m (Figure 6, 1700 m a.s.l.). On 6 July 2018 at 22:00–23:30 P.M., a large number of males were found calling on leaves of plants (Figure 10A), and some were found calling perching on the rocks or under rocks by the side of the stream.

Figure 6. The habitat of *Leptobrachella bijie* sp. nov. in Qingshan Village of Zhaozishan Nature Reserve in Guizhou Province.

Leptobrachella purpuraventra **J. Wang, Y.L. Li, Y. Li, H.H. Chen & Y.Y. Wang, sp. nov.**
http://zoobank.org/0B2C4A25-981B-4AE9-900D-60CAB4E7A560
Figure 7

Holotype. SYS a007284, adult male, collected by JW on 2 July 2018 from Jinjiazhai Village (27°7'5.92"N, 105°19'28.47"E; 1890 m a.s.l.) in Wujing Nature Reserve, Chahe Town, Qixingguan District, Bijie City, Guizhou Province, China.

Paratypes. A single adult female, SYS a007278 and seven adult males, SYS a007277/CIB 110003, 7279–7284, collected by JW, YLL, YYZ, HHC, JHL and Yingyong Wang (YYW hereafter), the same collection data as the holotype; besides, another three adult females, SYS a007304–7306, and four adult males, SYS a007300–7303, collected by JW, YLL, YYZ, HHC, JHL and YYW on 4 July 2018 from Baimashan Forest Station (27°41'25"N, 105°27'16"E; 1600 m a.s.l.) of Zhaozishan Nature Reserve, Shengji Town, Qixingguan District, Bijie City, Guizhou Province, China.

Diagnosis. (1) small size (SVL 27.3–29.8 mm in males, 33.0–35.3 mm in females), (2) dorsal skin shagreened, some of the granules forming longitudinal short skin ridges, (3) iris bicolored, coppery orange on upper half and silver on lower half, (4) tympanum distinctly discernible, slightly concave, distinct black supratympanic line present, (5) internasal distance smaller than interorbital distance, IND/IOD ratio 1.03–1.10, (6) supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible, (7) absence of webbing and lateral fringes on fingers, toes with rudimentary

Figure 7. General aspect in life: **A–D** SYS a007284, the male holotype of *Leptobrachella purpuraventra* sp. nov. **E** the male paratype SYS a007300 **F** the male paratype SYS a007283.

webbing and narrow lateral fringes, (8) longitudinal ridges under toes not interrupted at the articulations, (9) heels just meeting or slightly overlapping, tibia-tarsal articulation reaching to the middle of eye, (10) relative finger lengths I = II = IV < III, relative toe length I < II < V < III < IV, (11) dorsal surface shagreened and granular, lacking enlarge tubercles or warts, some of the granules forming short longitudinal folds, (12) dorsum purple brown to dark purple brown or grey purple grounding, with small light orange granules, distinct darker brown markings scattered with irregular light orange pigmentations, (13) flanks with several dark blotches, longitudinally in two rows, (14) ventral surface grey purple, with distinct or indistinct nebulous greyish speckling on chest and ventrolateral flanks, without black spots (seldom present), (15) dorsal limbs

including fingers and toes with dark bars, those on forearms indistinct, and (16) dense tiny conical spines present on surface of chest extending to anterior region of abdomen in males, and absent in females during breeding season.

Comparisons. Comparative morphological data of *Leptobrachella purpuraventra* sp. nov., *L. bijie* sp. nov., and 45 recognized *Leptobrachella* species occurring north of the Isthmus of Kra were listed in Table 5.

In the phylogenetic trees (Figure 2), *Leptobrachella purpuraventra* sp. nov. is a sister taxon to *L. bijie* sp. nov. with a high support value (99% in BI, 0.82 in ML), and it can be distinguished from the later by a genetic divergence (p=3.9–4.2%). Morphologically, it differs from the later by the coloration of dorsum and ventral, dorsum purple brown to dark purple brown or grey purple grounding, ventral grey purple grounding (vs. dorsum greyish-brown grounding, ventral white grounding); dark bars on dorsal limbs indistinct (vs. distinctly visible); dark bars on dorsal surface of tibia and tarsus much broader, especially those on dorsal skin of tarsus (vs. relatively narrow dark bars on dorsal surface of tibia and tarsus); internasal distance smaller than interorbital distance, IND/IOD ratio 1.03–1.10 (vs. internasal distance equal to interorbital distance, IND/IOD ratio 1.00); larger TEY value, TEY/TMP ratio 0.60–0.76 (vs. TEY/TMP ratio 0.45–0.53); dense tiny conical spines present on surface of chest extending to anterior region of abdomen (vs. such spines less developed, present on surface of chest, not extending to anterior region of abdomen); lateral fringes on toes narrow but more developed and distinct (vs. less developed); length of toe V < III (length of toe V = III).

Compared with the 26 known congeners of the genus *Leptobrachella* occurring south of the Isthmus of Kra, by the presence of supra-axillary and ventrolateral glands, *L. purpuraventra* sp. nov. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola*, all of which lacking supra-axillary and ventrolateral glands; and by the significantly larger body size, SVL 27.3–29.8 mm in males, 33.0–35.3 mm in females, *L. purpuraventra* sp. nov. differs from the smaller *L. baluensis* (14.9–15.9 mm in males), *L. bondangensis* (17.8 mm in male), *L. brevicrus* (17.1–17.8 mm in males), *L. fusca* (16.3 mm in male), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males and 18.9–19.1 mm in females), *L. mjobergi* (15.7–19.0 mm in males), *L. natunae* (17.6 mm in male), *L. parva* (15.0–16.9 mm in males and 17.8 mm in female), *L. palmata* (14.4–16.8 mm in males), *L. serasanae* (16.9 mm in female), and Dring's (1983) *Leptobrachella* sp. 3 "*baluensis*" (15.0–16.0 mm in males).

For the remaining 45 members of the genus *Leptobrachella*, in having SVL 27.3–29.8 mm in males and 33.0–35.3 mm in females, *L. purpuraventra* sp. nov. differs from the larger *L. bourreti* (42.0–45.0 mm in females), *L. eos* (33.1–34.7 mm in males and 40.7 in female), *L. lateralis* (36.6 mm in females), *L. nahangensis* (40.8 mm in male), *L. nyx* (37.0–41.0 mm in females), *L. sungi* (48.3–52.7 mm in males and 56.7–58.9 mm in females), *L. tamdil* (32.3 mm in male), and *L. zhangyapingi* (45.8–52.5 mm in males); and from the smaller *L. alpinus* (24.0–26.4 mm in males), *L. applebyi* (19.6–22.3 mm in males and 21.7–26.4 mm in females), *L. ardens*

(21.3–24.7 mm in males, 24.5 mm in female), *L. bidoupensis* (18.5–25.4 mm in males), *L. kalonensis* (28.9–30.6 mm in females), *L. maculosa* (27.0 mm in female), *L. maoershanensis* (29.1 mm in female), *L. mangshanensis* (30.2 mm in female), *L. melica* (19.5–22.7 mm in males), *L. pluvialis* (21.3–22.3 mm in males), *L. rowleyae* (23.4–25.4 mm in males), *L. tadungensis* (32.1 mm in female), and *L. tengchongensis* (23.9–26.0 mm in males).

In having black spots on flanks, the new species differs from *L. aerea*, *L. botsfordi*, *L. crorea*, *L. firthi*, *L. isos*, *L. pallida*, *L. petrops*, and *L. tuberosa*, all of which lacking black spots on flanks; by having rudimentary webbing on toes, the new species differs from *L. oshanensis*, *L. pallida* and *L. petrops*, all of which lacking webbing on toes, and from *L. pelodytoides*, which bears wide webbing on toes; by having narrow lateral fringes on toes, the new species differs from *L. aerea*, *L. firthi*, *L. isos*, *L. khasiorum*, *L. laui*, *L. liui*, *L. purpura*, *L. yunkaiensis*, and *L. yingjiangensis*, all of which having wide lateral frings on toes, from *L. fuliginosa*, which having weak lateral fringes on toes, and from *L. crocea*, *L. macrops*, *L. minima*, *L. oshanensis*, *L. pallida*, *L. pyrrhops*, *L. tuberosa*, and *L. ventripunctata*, all of which lacking lateral fringes on toes; by having dorsal surface shagreened and granular, lacking enlarge tubercles or warts, the new species differs from *L. fuliginosa* (dorsum smooth with fine tubercles), *L. laui* (dorsum with round granular tubercle, lacking skin ridges), *L. liui* (dorsum with round tubercles), *L. macrops* (dorsum roughly granular with large tubercles), *L. minima* (dorsum smooth), *L. pelodytoides* (dorsum with small, smooth warts), *L. tuberosa* (dorsum highly tuberculate), *L. yunkaiensis* (dorsum with raised warts), and *L. wuhuangmontis* (dorsum rough with conical tubercles); by having ventral surface grey purple with distinct nebulous greyish speckling on chest and ventrolateral flanks, the new species differs from *L. botsfordi* and *L. pyrrhops*, (ventral reddish brown with white speckling), *L. khasiorum* (ventral creamy white), *L. macrops* (ventral Greyish-violet with white speckling), *L. nokrekensis* (ventral creamy white), *L. puhoatensis* (ventral reddish brown with white dusting), *L. purpura* (ventral dull white with indistinct grey dusting), *L. tuberosa* (ventral white with small grey spots/streaks), *L. ventripunctata* (chest and belly with large dark brown spots), *L. wuhuangmontis* (ventral greyish white), *L. yunkaiensis* (belly pink with speckling), and *L. yingjiangensis* (ventral creamy white); by having tiny spines on surface of chest extending to anterior region of abdomen in males during breeding season, the new species differs from all male specimens collected in breeding season of *L. liui*, *L. oshanensis*, *L. yunkaiensis* and *L. wuhuangmontis*, all of which lacking such spines.

Description of holotype. Adult male. Body size small, SVL in 29.6 mm. Head length slightly larger than head width, HDL/HDW 1.05; snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, internarial distance larger than interorbital distance, IND/IOD 1.09; pineal ocellus absent; vertical pupil; snout length larger than eye diameter, SNT/EYE 1.14; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye

and larger than tympanum-eye distance, TMP/EYE 0.54 and TEY/TMP 0.68; upper margin of tympanum incontact with supratympanic ridge; distinct black supratympanic line present; vomerine teeth absent; vocal sac openings slit-like, paired, located posterolaterally on floor of mouth in close proximity to the margins of the mandible; tongue deeply notched behind; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths I = II = IV < III; nuptial pad absent; subarticular tubercles absent; a large, rounded inner palmar tubercle distinctly separated from small, round outer palmar tubercle; absence of webbing and lateral fringes on fingers. Tips of toes like fingers; relative toe length I < II < V < III < IV; subarticular tubercles absent; distinct dermal ridges present under the 3rd to 5th toes, not interrupted; large, oval inner metatarsal tubercle present, outer metatarsal tubercle absent; toes webbing rudimentary; narrow lateral fringes present on all toes. Tibia 45% of snout-vent length; tibiotarsal articulation reaches to middle of eye; heels just meeting each other when thighs are appressed at right angles with respect to body.

Dorsal surface shagreened and granular, lacking enlarge tubercles or warts, some of the granules forming short longitudinal folds; ventral skin smooth; dense tiny conical spines present on surface of chest and extending to anterior region of abdomen; pectoral gland and femoral gland oval; pectoral glands greater than tips of fingers and femoral glands; femoral gland situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary gland raised. Ventrolateral gland distinctly visible, forming an incomplete line.

Measurements of holotype (in mm). SVL 29.6, HDL 10.2, HDW 9.7, SNT 4.0, EYE 3.5, IOD 3.2, IND 3.5, TMP 1.9, TEY 1.3, TIB 13. 3, ML 7.7, PL 12.7, LAHL 13.8, HLL 42.7.

Coloration of holotype in life. Dorsum dark purple brown grounding, with small light orange granules, distinct darker brown markings and rounded spots and scattered with irregular light orange pigmentations. A dark brown V-shaped pattern between anterior corner of eyes, in connected to the dark brown W-shaped marking on interorbital region, and the W-shaped marking in connected to the other W-shaped marking between axillae. Tympanum brown. A dark brown vertical bar under the eye; transverse dark brown bars on dorsal surface of limbs; distinct dark brown blotches on flanks from groin to axilla, longitudinally in two rows; elbow and upper arms with dark bars and distinct coppery orange coloration; fingers and toes with distinct dark bars.

Ventral surface grey purple, with distinct nebulous greyish speckling scattered with white spots on chest and ventrolateral flanks. Supra-axillary gland coppery orange with dark brown speckling; femoral, pectoral and ventrolateral glands greyish white. Iris bicolored, coppery orange on upper half and silver on lower half.

Coloration of holotype in preservative. Dorsum of body and limbs dark brown; transverse bars on limbs become more distinct; dark brown patterns, markings and spots on back become indistinct, orange pigmentations become greyish

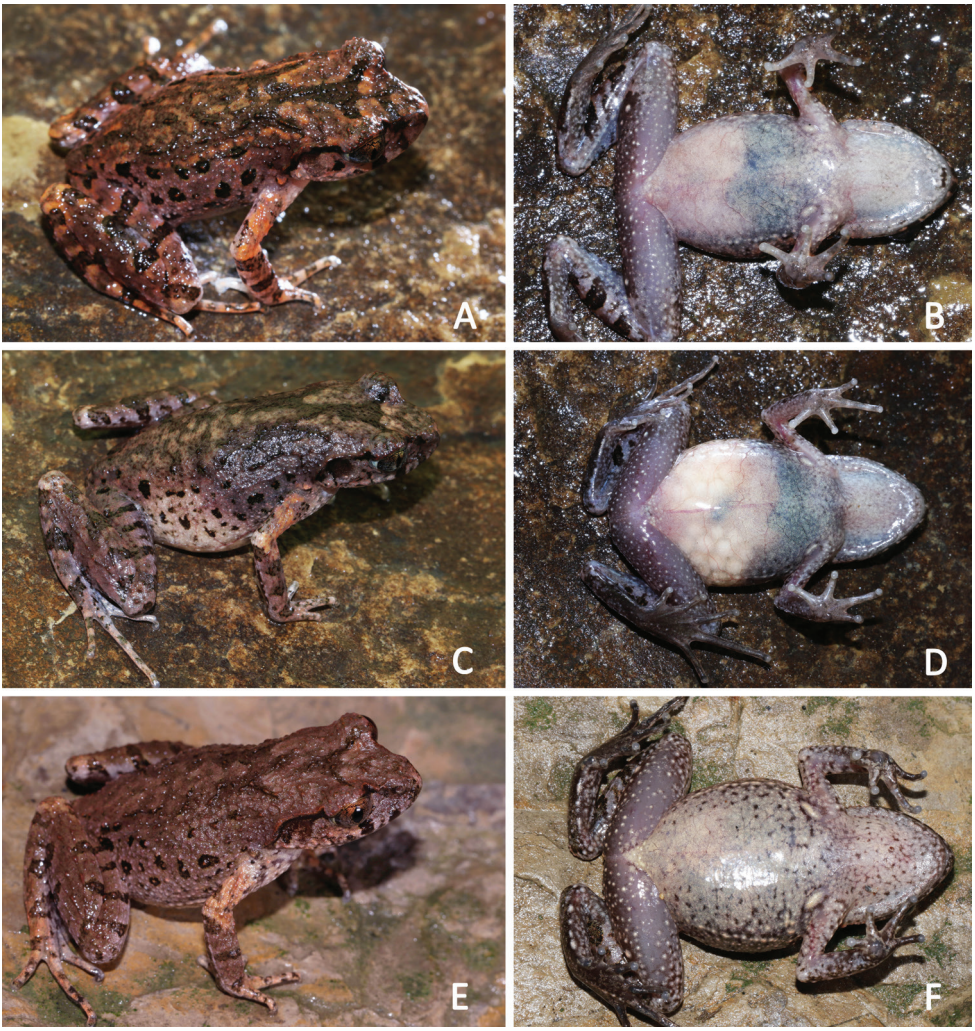

Figure 8. General aspect in life of the female paratypes of *Leptobrachella purpuraventra* sp. nov. **A, B** SYS a007304 **C, D** SYS a007305 **E, F** SYS a007278.

white. Ventral surface of body and limbs greyish white, nebulous speckling on chest and flanks balck brown. Supra-axillary, femoral, pectoral and ventrolateral glands greyish white.

Variations. Measurements and body proportions were listed in Table 7. All paratypes match the overall characters of the holotype except that: coloration of dorsum dark purple brown in the holotype SYS a007284 (vs. grey purple brown in paratypes SYS a007300 (Figure 7E), 7303, 7305 (Figure 8C), 7306; purple brown in paratypes SYS a007278 (Figure 8E), 7279, 7282, 7283 (Figure 7F), 7304 (Figure 8A)); heels just meeting (vs. heels slightly overlapping in paratypes SYS a007300, 7302); W-shaped

Table 7. Measurements (minimum–maximum (mean ± SD); in mm), and body proportions of *Leptobrachella purpuraventra* sp. nov.: population A from Wujing Nature Reserve in Bijie City, population B from Baimashan Forest Station of Zhaozishan Nature Reserve.

Population	A		B		A + B	
SEX	Males (n = 7)	Female (n = 1)	Males (n = 4)	Females (n = 3)	Males (n = 11)	Females (n = 4)
SVL	27.3–29.6 (28.6 ± 0.7)	35.3	28.3–29.8 (29.3 ± 0.6)	33.0–34.5 (33.5 ± 0.7)	**27.3–29.8 (28.9 ± 0.8)**	**33.0–35.3 (34.0 ± 1.0)**
HDL	9.6–10.2 (9.9 ± 0.2)	12	9.7–10.3 (10.1 ± 0.2)	11.0–11.7 (11.3 ± 0.3)	**9.6–10.3 (10.0 ± 0.2)**	**11.0–12.0 (11.5 ± 0.4)**
HDW	9.3–9.7 (9.5 ± 0.1)	11.5	9.6–9.8 (9.8 ± 0.1)	10.5–11.3 (10.9 ± 0.3)	**9.3–9.8 (9.6 ± 0.2)**	**10.5–11.5 (11.1 ± 0.4)**
SNT	3.5–4.0 (3.8 ± 0.1)	4.6	3.8–4.1 (4.0 ± 0.1)	4.2–4.4 (4.3 ± 0.1)	**3.5–4.1 (3.8 ± 0.2)**	**4.2–4.6 (4.4 ± 0.1)**
EYE	3.1–3.5 (3.3 ± 0.2)	3.8	3.3–3.6 (3.5 ± 0.1)	3.7–3.9 (3.8 ± 0.1)	**3.1–3.6 (3.4 ± 0.2)**	**3.7–3.9 (3.8 ± 0.1)**
IOD	2.6–3.2 (2.9 ± 0.2)	3.5	3.0–3.2 (3.1 ± 0.1)	3.2–3.3 (3.2 ± 0.1)	**2.6–3.2 (2.9 ± 0.2)**	**3.2–3.5 (3.3 ± 0.1)**
IND	2.7–3.5 (3.0 ± 0.3)	3.6	3.2–3.3 (3.0 ± 0.1)	3.3–3.5 (3.4 ± 0.1)	**2.7–3.5 (3.1 ± 0.2)**	**3.3–3.6 (3.5 ± 0.1)**
TMP	1.7–1.9 (1.8 ± 0.1)	2.1	1.8–1.9 (1.8 ± 0.1)	2.0–2.1 (2.0 ± 0.1)	**1.7–1.9 (1.8 ± 0.1)**	**2.0–2.1 (2.1 ± 0.1)**
TEY	1.2–1.3 (1.3 ± 0.1)	1.3	1.1–1.2 (1.1 ± 0.1)	1.2–1.3 (1.2 ± 0.1)	**1.1–1.3 (1.2 ± 0.1)**	**1.2–1.3 (1.3 ± 0.1)**
TIB	12.5–13.3 (12.8 ± 0.3)	15.5	13.2–14.0 (13.6 ± 0.4)	14.6–15.4 (15.0 ± 0.3)	**12.5–14.0 (13.1 ± 0.5)**	**14.6–15.5 (15.2 ± 0.4)**
ML	7.0–7.7 (7.3 ± 0.2)	7.8	7.5–7.6 (7.5 ± 0.1)	7.7–8.0 (7.9 ± 0.1)	**7.0–7.7 (7.4 ± 0.2)**	**7.7–8.0 (7.9 ± 0.1)**
PL	12.1–12.7 (12.4 ± 0.2)	14.8	12.6–13.2 (13.0 ± 0.2)	13.7–14.7 (14.2 ± 0.4)	**12.1–13.2 (12.6 ± 0.4)**	**13.7–14.8 (14.4 ± 0.4)**
LAHL	12.6–13.8 (13.2 ± 0.4)	15.5	13.4–14.0 (13.6 ± 0.2)	14.7–15.7 (15.1 ± 0.4)	**12.6–14.0 (13.3 ± 0.4)**	**14.7–15.7 (15.2 ± 0.4)**
HLL	39.0–42.7 (40.4 ± 1.4)	47.8	40.1–44.6 (43.2 ± 1.8)	46.0–47.3 (46.8 ± 0.6)	**39.0–44.6 (41.4 ± 2.1)**	**46.0–47.8 (47.0 ± 0.7)**
HDL/HDW	1.01–1.05 (1.04 ± 0.01)	1.04	1.01–1.06 (1.04 ± 0.02)	1.04–1.05 (1.04 ± 0.01)	**1.01–1.06 (1.04 ± 0.02)**	**1.04–1.05 (1.04 ± 0.01)**
HDL/SVL	0.33–0.35 (0.34 ± 0.02)	0.34	0.34–0.35 (0.34 ± 0.01)	0.33–0.34 (0.34 ± 0.01)	**0.33–0.35 (0.34 ± 0.01)**	**0.33–0.34 (0.34 ± 0.01)**
SNT/HDL	0.36–0.39 (0.38 ± 0.01)	0.38	0.39–0.40 (0.39 ± 0.01)	0.38	**0.36–0.40 (0.39 ± 0.01)**	**0.38**
SNT/EYE	1.12–1.19 (1.14 ± 0.02)	1.21	1.11–1.15 (1.14 ± 0.02)	1.11–1.16 (1.13 ± 0.02)	**1.11–1.19 (1.14 ± 0.02)**	**1.11–1.21 (1.15 ± 0.04)**
EYE/TMP	1.82–1.89 (1.86 ± 0.03)	1.81	1.83–1.94 (1.89 ± 0.04)	1.85–1.90 (1.87 ± 0.02)	**1.82–1.94 (1.87 ± 0.04)**	**1.81–1.90 (1.85 ± 0.03)**
TMP/EYE	0.53–0.55 (0.54 ± 0.01)	0.55	0.51–0.55 (0.53 ± 0.01)	0.53–0.54 (0.53 ± 0.01)	**0.51–0.55 (0.53 ± 0.01)**	**0.53–0.55 (0.54 ± 0.01)**
IND/IOD	1.03–1.09 (1.06 ± 0.02)	1.03	1.03–1.10 (1.07 ± 0.03)	1.03–1.06 (1.05 ± 0.01)	**1.03–1.10 (1.07 ± 0.03)**	**1.03–1.06 (1.05 ± 0.02)**
TEY/TMP	0.67–0.76 (0.71 ± 0.03)	0.62	0.61–0.67 (0.64 ± 0.02)	0.60–0.62 (0.61 ± 0.01)	**0.61–0.76 (0.68 ± 0.04)**	**0.60–0.62 (0.61 ± 0.01)**
TIB/SVL	0.44–0.46 (0.45 ± 0.01)	0.44	0.45–0.47 (0.47 ± 0.01)	0.44–0.46 (0.45 ± 0.01)	**0.44–0.47 (0.45 ± 0.01)**	**0.44–0.46 (0.45 ± 0.01)**
LAHL/SVL	0.45–0.47 (0.46 ± 0.01)	0.44	0.45–0.47 (0.46 ± 0.01)	0.44–0.46 (0.45 ± 0.01)	**0.45–0.47 (0.46 ± 0.01)**	**0.44–0.46 (0.45 ± 0.01)**
HLL/SVL	1.36–1.46 (1.41 ± 0.03)	1.35	1.42–1.51 (1.47 ± 0.04)	1.37–1.42 (1.39 ± 0.02)	**1.36–1.51 (1.43 ± 0.05)**	**1.35–1.42 (1.38 ± 0.02)**
TIB/HLL	0.31–0.32 (0.31 ± 0.01)	0.32	0.31–0.33 (0.32 ± 0.01)	0.32–0.33 (0.32 ± 0.01)	**0.31–0.33 (0.32 ± 0.01)**	**0.32–0.33 (0.32 ± 0.01)**

Figure 9. The habitat of *Leptobrachella purpuraventra* sp. nov. in Baimashan Forest Station of Zhaozishan Nature Reserve in Guizhou Province.

marking on interorbital region in connected to the other W-shaped marking between axillae in the holotype (vs. such markings not in connected with each other in paratypes SYS a007278, 7282); a V-shaped pattern between anterior corner of eyes in the holotype (vs. a dark brown inverted triangular pattern between anterior corner of eyes instead in the paratype SYS a007300); relatively smaller black spots on flanks (vs. black spots distinctly large in paratypes SYS a007300–7301, 7304, 7306); ventral surface without black spots in the holotype (vs. presence of irregular black spots in paratype SYS a007278 (Figure 8F)).

Etymology. The specific epithet *pupura* is given as a noun in apposition and means "purple color", and *ventra*, is given as a noun in apposition and means "ventral", in reference to the purple coloration of ventral of the new species. For the common name, we suggest "Purple-bellied Leaf Litter Toad", and for the Chinese name "Zi Fu Zhang Tu Chan (紫腹掌突蟾)".

Distribution and habits. Currently, *Leptobrachella purpuraventra* sp. nov. is known from its type locality Jinjiazhai Village in Wujing Nature Reserve, Chahe County, and Baimashan Forest Station in Zhaozishan Nature Reserve, both in Qixingguan District, Bijie City, Guizhou Province, China (Figure 1). The new species was found along a clear-water rocky stream (ca. 3 m in width and ca. 10–20 cm in depth) surrounded by a broad-leaved forest in karst landforms (Figure 9, 1600–1900 m a.s.l.). From 2 July to 4 July in 2018 at 21:00–23:50 P.M., a large number of males were found calling on leaves of plants (Figure 10B), and some were found calling perching on the rocks or under rocks by the side of the stream.

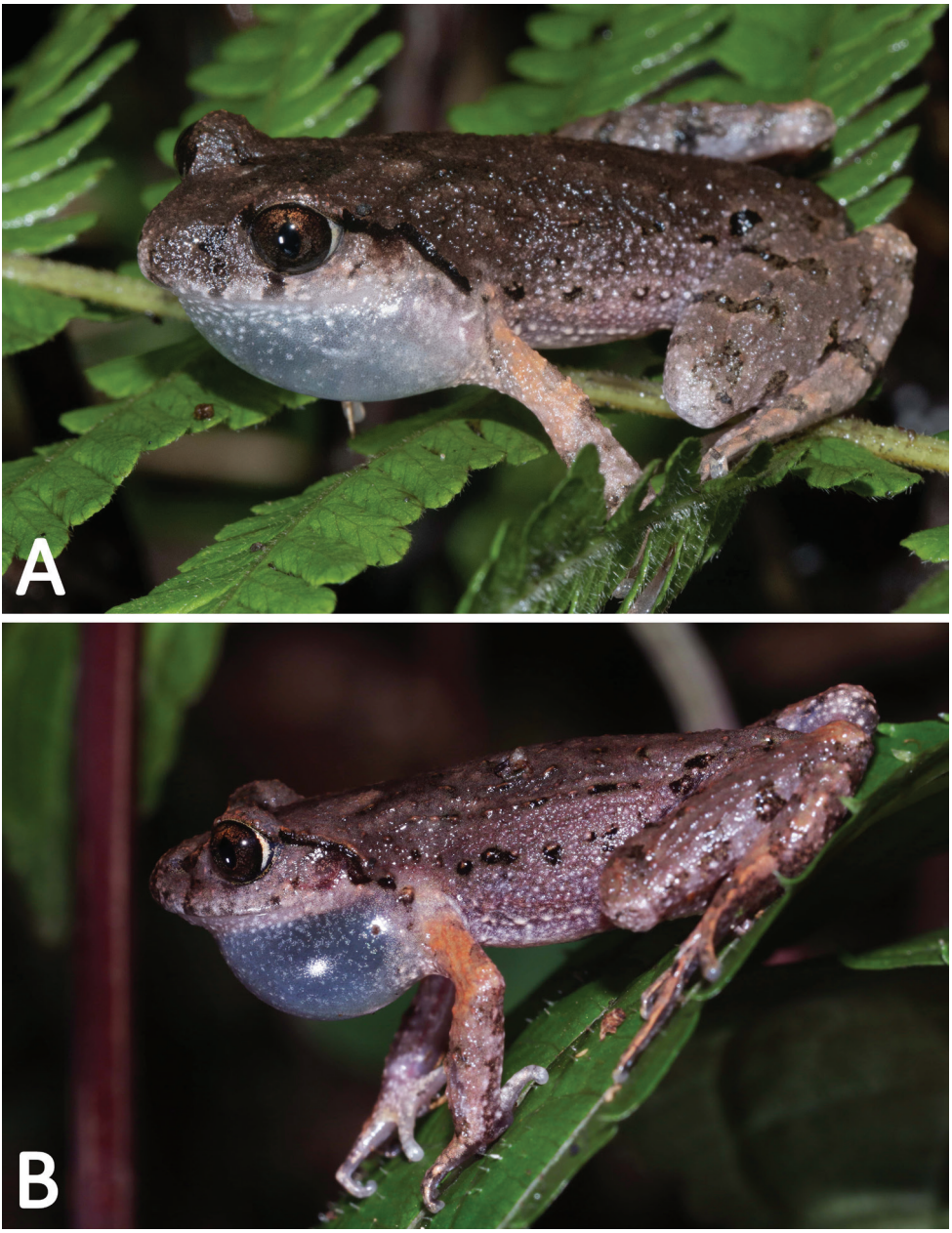

Figure 10. General aspect observed in the field of **A** *Leptobrachella bijie* sp. nov. and **B** *L. purpuraventra* sp. nov., showing a single vocal sac and different skin colors of the two new species.

Discussion

The discoveries of *Leptobrachella bijie* sp. nov. and *L. purpuraventra* sp. nov. bring the total number of this genus to 73, with 16 of them recorded in China (Fei et al. 2012; Frost 2017; Wang et al. 2018). Before the descriptions of the two new species from northwest-

ern Guizhou Province in this study, only *L. oshanensis* was recorded in northeastern and southern Guizhou Province, which further highlights the underestimated of the species diversity of the genus. Further investigation of the genus in adjacent regions is required.

Studies of the taxonomy and phylogeny of *Leptobrachella* were difficult to perform because of the morphological conservativeness of the species (for example, the two new species appeared very similar morphologically in the field (Figure 10)), which likely to hinder our understanding of these cryptic species (Ohler et al. 2010; Sung et al. 2014; Wang et al. 2018).

Leptobrachella bijie sp. nov. and *L. purpuraventra* sp. nov. were both found in Zhaozishan Nature Reserve, only approximately seven kilometers apart, straight-line distance, but they possessed a significant genetic divergence (p=3.9–4.2%). This compares to the two populations of *L. purpuraventra* sp. nov. from Zhaozishan Nature Reserve and Wujing Nature Reserve, which were approximately 65 kilometers apart, but displayed almost no genetic divergence. Without phylogenetic, morphological, and acoustic analyses, it would be difficult to determine the taxonomic status of these two species. Thus, specimen, acoustic data, and tissue sample collection play important roles in discovering the high species diversity of the genus *Leptobrachella*.

Leptobrachella bijie sp. nov. and *L. purpuraventra* sp. nov. were found along clear-water rocky streams, and such environments are very limited in the karst landforms. At present, little is known about the ecology and behavior of the two new species, however, the known habitat of the two new species is under threat of degradation, particularly as a result of grazing. Thus, further research on the true distribution, population size and trends, and conservation actions required, are urgently needed.

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Appendix 1

Specimens examined

Leptobrachella alpinus (n = 6): China: Yunnan Province: Jingdong County: Mt. Wuliang: CIB 24353 (Holotype), CIB 24354; SYS a 003927.

Leptobrachella laui (n = 26): China: Hong Kong: SYS a002057 (Holotype), SYS a002058; China: Guangdong Province: Shenzhen City: SYSa 001505–1507, 1515–1521, 3471–3472, 5644–5645.

Leptobrachella liui (n = 18): China: Fujian Province: Mt. Wuyi: CIB 24355 (Holotype), CIB 24356, SYS a001571–1578, 1595–1599, 2478–2479, 5925–5826.

Leptobrachella oshanensis (n = 2): China: Sichuan Province: Meishan City: Mt. Emei: SYS a001829–1830.

Leptobrachella tengchongensis (n = 6): China: Yunnan Province: Baoshan City: Mt. Gaoligong: SYS a004600 (Holotype), 4596–4599, 4601–4602.

Leptobrachella wuhuangmontis (n = 12): China: Guangxi Province: Pubei County: Mt. Wuhuang: SYS a003500/CIB107274, SYS a000578, 0580–0581, 3485–3489, 3499, 3504–3506.

Leptobrachella yunkaiensis (n = 8): China: Guangdong Province: Maoming City: Dawuling Forest Station: SYS a004664/CIB107272, SYS a004663, 4665–4669, 4690.