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RESEARCH ARTICLE

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A new species of *Arachnanthus* from the Red Sea (Cnidaria, Ceriantharia)

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Abstract

A new species of the genus *Arachnanthus* (Cnidaria: Ceriantharia), *Arachnanthus lilith* Stampar & El Didi, **sp. n.,** is described. This species is widely distributed in the Red Sea, and recorded from 2–30 m depths. *Arachnanthus lilith* Stampar & El Didi, **sp. n.** is the fifth species of the genus and the first recorded from the Red Sea. The number of labial tentacle pseudocycles, arrangement of mesenteries, and distribution of acontioids allow the differentiation of the new species from other species of the genus.

Keywords

Anthozoa, biodiversity, coral reefs, Indo-West Pacific, marine invertebrates, taxonomy

Introduction

While tube anemones are common objects for underwater photographers and are widely exhibited in aquaria, they remain undersampled in most regions of the world, and the diversity and distribution of species remains poorly documented (Stampar et al. 2016). This is especially true for species that are difficult to observe and collect, because of nocturnal habits, small body size, or deeply extended burrows. The small, nocturnal tube anemones of the family Arachnactidae are a case in point (den Hartog 1977; Stampar et al. 2012, 2015a). This family is comprised of two benthic genera, *Arachnanthus* Carlgren, 1912 and *Isarachnanthus* Carlgren, 1924 (Stampar et al. 2016), although other genera have been proposed based only on larval forms (Molodtsova 2004). However, larval genera are not currently linked to those of adults and therefore their status remains unclear (Stampar et al. 2015a). Carlgren (1912) established *Arachnanthus* for *A. sarsi* (which he described from the North Sea) together with *Cerianthus oligopodus* Cerfontaine, 1891 from the Mediterranean. Carlgren (1924, 1937) later described *A. bockii* Carlgren, 1924 from Fiji and *A. australiae* Carlgren, 1937 from Australia. Since these studies, the genus has received little attention, with Picton and Manuel's (1985) study and redescription of *A. sarsi* being the most substantive. Here a fifth species of *Arachnanthus* is described, the first known from Red Sea.

Materials and methods

Specimens were collected by hand at three sites across the Red Sea, from the Gulf of Aqaba to the Farasan Islands, in Saudi Arabia (Fig. 1). Collected polyps were preserved in 10 % buffered seawater formaldehyde solution, and later transferred to 75 % ethanol. The holotype and five paratypes are deposited in the Invertebrate Collections of the Florida Museum of Natural History, University of Florida (UF Cnidaria).

The anatomical study of polyps and cnidome were based on characters defined by previous authors (Carlgren 1912; den Hartog 1977; Stampar et al. 2012, 2015b). Six specimens were opened along the ventral side (opposite the siphonoglyph), using surgical scalpels, for anatomical study.

The classification of cnidae follows England (1991) and Stampar et al. (2015b). Thirty undischarged capsules were measured for each cnida type, sampled from each body region of two specimens (UF Cnidaria 9168 & 9229). The cnidome was studied with a Nikon Eclipse E200 microscope at 1000x magnification. Each part of the body was analyzed separately to avoid any contamination.

Systematics

Class Anthozoa Ehrenberg, 1834
Subclass Ceriantharia Perrier, 1883 (*sensu* Stampar et al. 2014)
Suborder Penicillaria den Hartog, 1977
Family Arachnactidae Carlgren, 1912

Genus *Arachnanthus* Carlgren, 1912

Diagnosis. Arachnactidae with sterile protomesenteries; metamesenteries in duplets (M and B), long ('M') metamesenteries with gonads and a double mesenteric filament,

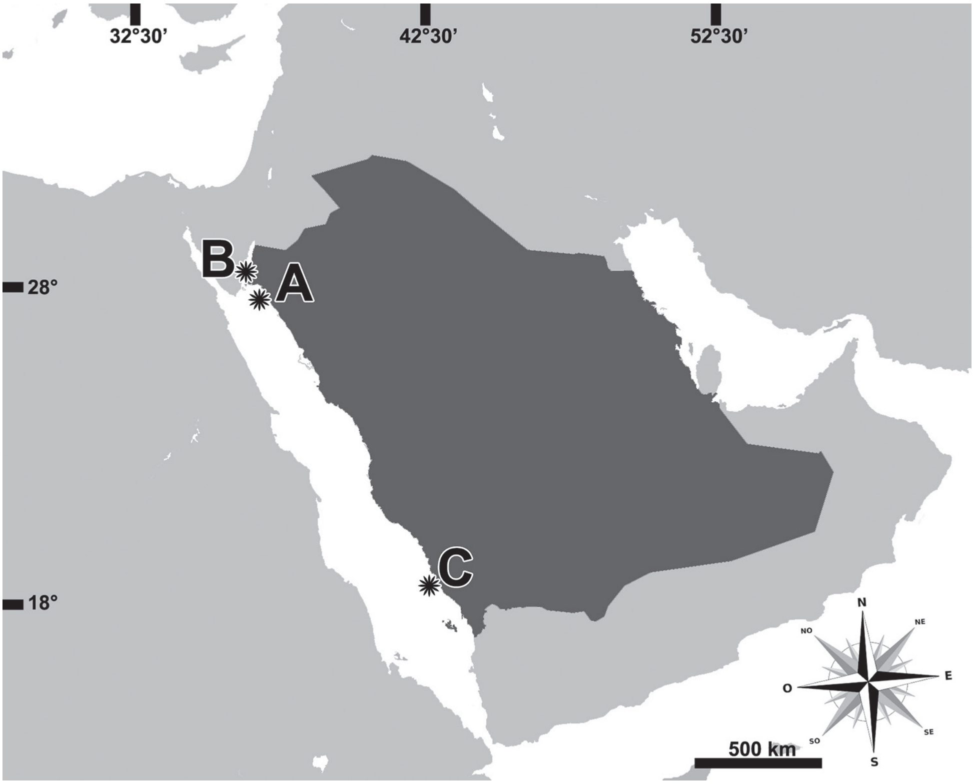

Figure 1. Records of *Arachnanthus lilith* sp. n. individuals studied, collected in Saudi Arabia (dark gray). A – UF Cnidaria 9168 (Holotype), B – UF Cnidaria 9167, UF Cnidaria 9227, UF Cnidaria 9229, UF Cnidaria 9230 (Paratype) and C – UF Cnidaria 9076.

short (B) betamesenteries sterile, with single, convoluted mesenteric filament; very long stomodeum; lacking a directive labial tentacle; cnidome with p-mastigophores and b-mastigophores (after Carlgren 1912, 1924, 1937 and den Hartog 1977).

Type species. *Arachnanthus oligopodus* (Cerfontaine, 1891)

Valid species

***Arachnanthus australiae* Carlgren, 1937**
***Arachnanthus bockii* Carlgren, 1924**
***Arachnanthus oligopodus* (Cerfontaine, 1891)**
***Arachnanthus sarsi* Carlgren, 1912**
***Arachnanthus lilith* sp. n.**

Distribution. North Sea, Mediterranean Sea, Red Sea, East Australia, and Melanesia.

Arachnanthus lilith Stampar & El Didi, sp. n.

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Figs 1–4, Tables 1–2

Material examined (six specimens). Holotype: UF Cnidaria 9168, adult individual (35 mm long), Saudi Arabia, island near Jaz'air Sila, (27.651°N, 35.2832°E) (Fig. 1A), 10–30 m depth, fore reef, under rocks, G. Paulay, Seabird McKeon, Daisuke Uyeno coll. (27/ix/2013). **Paratypes:** UF Cnidaria 9167, adult (31 mm long), same data as holotype. UF Cnidaria 9227, adult (35 mm long), UF Cnidaria 9229, adult (42 mm long), UF Cnidaria 9230, adult (26 mm long) all three from Saudi Arabia, Gulf of Aqaba, Joey's Shipwreck Bay, (28.1846°N, 34.6381°E) (Fig. 1B), 7–13 m depth, in sand and seagrass bed, collected at night, G. Paulay, Daisuke Uyeno, Casey Zakroff coll. (01/x/2013). UF Cnidaria 9076 (Fig. 2D), adult, Saudi Arabia, Farasan Banks, Atlantis Shoal (18.1917°N, 41.1138°E)

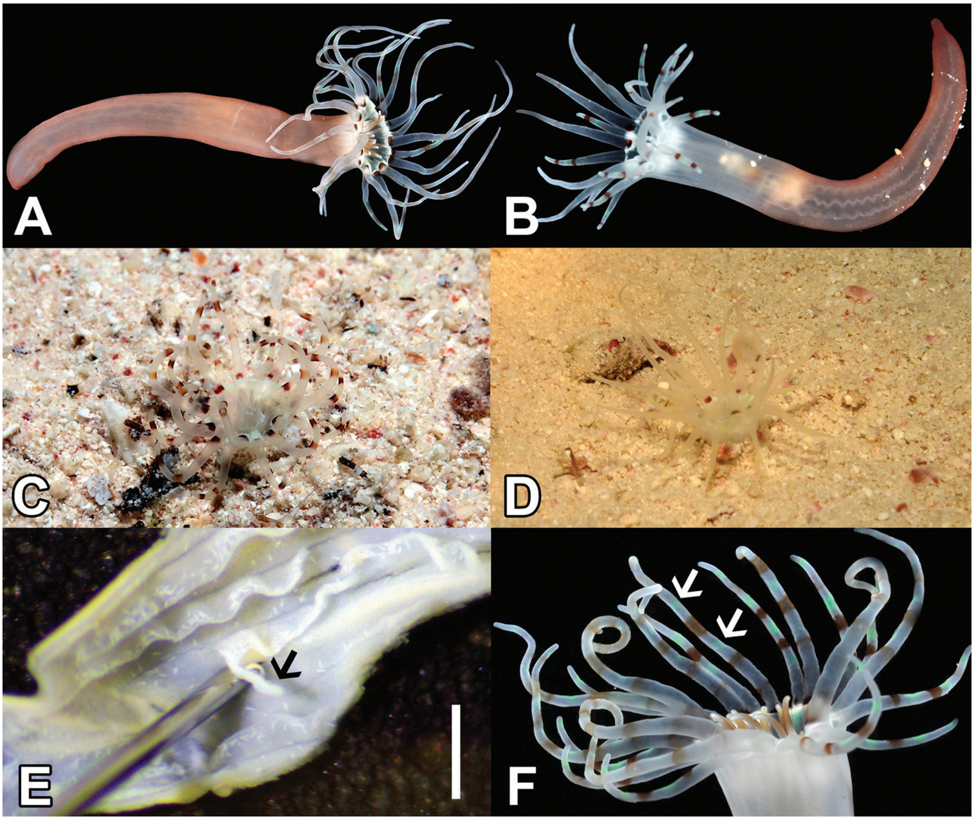

Figure 2. *Arachnanthus lilith* sp. n. **A** (Paratype UF Cnidaria 9227) (not to scale) **B** (Paratype UF Cnidaria 9168) (not to scale) **C–D** Live specimens in nature (not included as paratypes – ICZN 72.4.6) (not to scale) **E** Dissected specimen with detail of acontioids (arrows) (scale bar 2 mm) UF Cnidaria 9168 (Holotype) **F** Detail of oral disc UF Cnidaria 9229 (Paratype) with detail on tentacular pores with green fluorescent protein (GFP) (arrows) (not to scale).

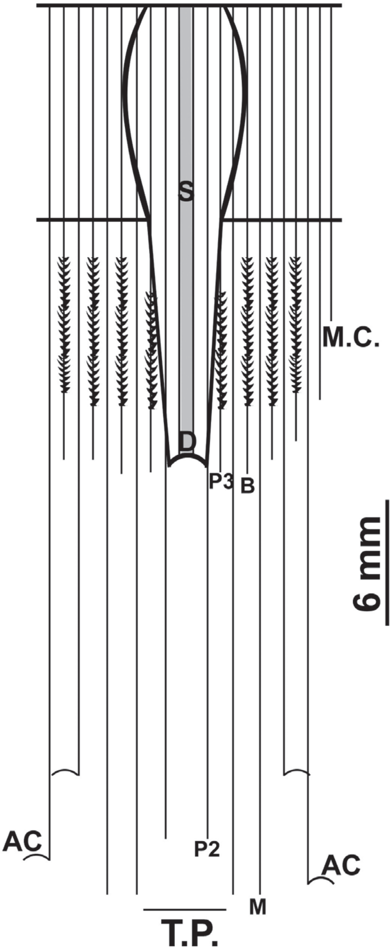

Figure 3. Graphical representation of the arrangement of mesenteries of *Arachnanthus lilith* sp. n. Abbreviations: M.C. multiplication chamber, D directives, T.P. terminal pore, S siphonoglyph, B betamesenteries (convoluted mesentery), M metamesenteries (double filament), P protomesenteries, AC acontioids

(Fig. 1–C), 9–11 m depth, sandy shoal with patch reefs, in sand, collected at night, Arthur Anker, Patrick Norby, Gustav Paulay coll. (07/iii/2013).

Diagnosis. Small ceriantharian, up to at least 42 mm long, 4–6 mm wide. With 19–24 translucent marginal tentacles (3–5 mm long in preserved specimens), each with 2–4 brown bands (Fig. 1); tentacle arrangement *(1)2.12.12.12.12…*; at least 5 pores per tentacle, pores marked by concentration of green fluorescent protein (GFP) (Fig 1–F); unpaired marginal tentacle present. With 11–15 pale labial tentacles (up to 2 mm long in preserved specimens), tentacle arrangement *(0)3.12.31.23.23.12…*; unpaired labial tentacle absent. Long actinopharynx extending over 1/3 of total body length, hyposulcus 3–4 mm long, hemisulci distinct; siphonoglyph wide, connected to eight mesenteries; directive mesenteries a little shorter than hyposulcus. Three pairs of protomesenteries (P), P2 and

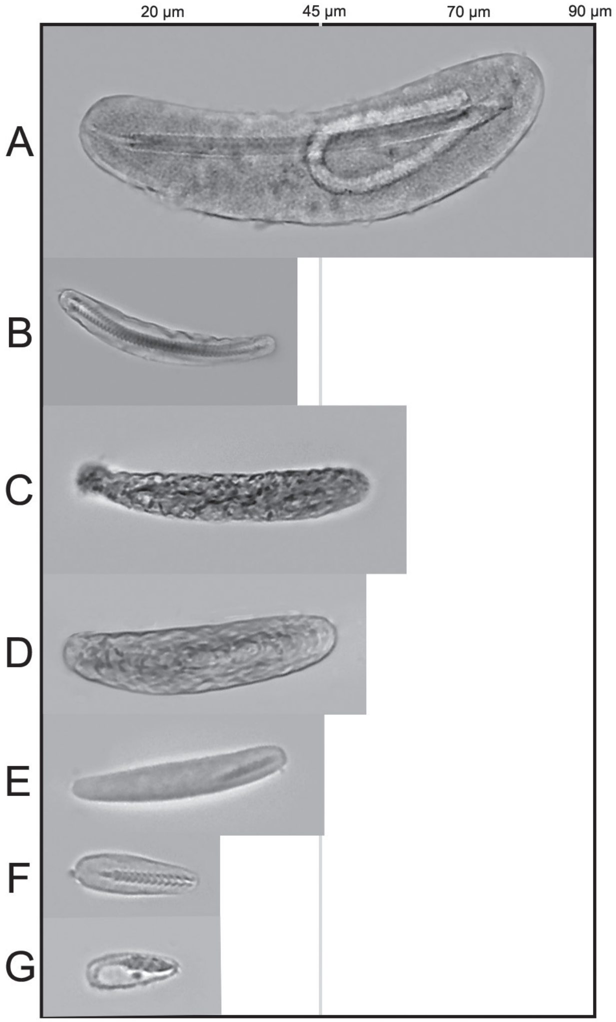

Figure 4. Cnidome of *Arachnanthus lilith* sp. n. **A** microbasic *p*-mastigophores I **B** microbasic *p*-mastigophores II **C** Atrich **D** Ptychocyst **E** microbasic *b*-mastigophores I **F** microbasic *b*-mastigophores II **G** microbasic *b*-mastigophores III.

P4 long and P3 short, metamesenteries (M), long, fertile with double mesenteric filament; betamesenteries (B) short, sterile with single mesenteric filament (double in a short part immediately below actinophrarynx) and rather convoluted; acontioids only in mesenteries M3 and M4; see Fig. 2 for schematic arrangement of mesenteries. Cnidome (Fig. 3) of spirocysts, atrichs, microbasic *b*-mastigophores (three types), microbasic *p*-mastigophores (two types), and ptychocysts; distributed as shown in Table 1.

Distribution. Presently known only from the Saudi Arabian Red Sea, from the Gulf of Aqaba to the Farasan Islands in the southern Red Sea. The species was found extended only at night.

Table 1. Cnidome of *Arachnanthus lilith* sp. n. based on two specimens (UF 9229; 9168). Mean and range given for each cnida.

		Length (in µm)	Width (in µm)
Column	Pytchocysts	**40.69** (37.7–44.2)	**9.18** (9.1–10.4)
	Atrichs	**48.57** (41.6–53.3)	**8.01** (6.5–10.4)
	b-mastigophores I	**32.32** (31.2–33.8)	**4.11** (3.9–5.2)
	p-mastigophores I	**86.45** (83.2–89.7)	**21.49** (19.5–23.4)
Marginal tentacles	p-mastigophores I	**84.15** (78.0–91.0)	**19.84** (18.2–20.8)
	p-mastigophores II	**33.75** (31.2–37.7)	**6.84** (6.5–7.8)
	b-mastigophores I	**32.63** (31.2–33.8)	**3.9** (3.8–4.0)
	b-mastigophores II	**21.06** (19.5–27.3)	**4.11** (3.9–5.2)
	Atrichs	**34.92** (31.2–39.0)	**6.58** (5.2–7.8)
Labial tentacles	p-mastigophores I	**64.87** (61.1–67.6)	**13.08** (11.7–14.3)
	b-mastigophores II	**25.3** (20.8–28.6)	**5.07** (3.9–7.8)
	Atrichs	**25.69** (24.7–28.6)	**6.02** (5.2–6.5)
Stomodeum	p-mastigophores I	**49.44** (45.5–54.6)	**9.83** (7.8–11.7)
	b-mastigophores II	**24.05** (20.8–26.0)	**5.76** (5.2–6.5)
	Atrichs	**33.28** (31.2–35.1)	**6.54** (5.2–7.8)
Betamesenteries	p-mastigophores I	**83.8** (80.6–89.7)	**23.14** (19.5–24.7)
	p-mastigophores II	**54.9** (52.0–58.5)	**15.34** (13.0–16.9)
	b-mastigophores II	**19.24** (15.6–23.4)	**4.03** (3.9–5.2)
Metamesenteries	b-mastigophores II	**25.04** (23.4–26.0)	**5.76** (5.2–6.5)
	b-mastigophores III	**17.76** (16.9–18.2)	**4.2** (3.9–5.2)

Etymology. The specific name *lilith* refers to the mythological figure of a female night demon in the vicinity of the Red Sea to ancient Mesopotamia (Saudi Arabia to Iraq).

Live color. Column pinkish tan at basal half or along most of its length, becoming clear toward base of tentacles. Marginal tentacles whitish/transparent, with brown and light green bands; extent of banding variable, with a basal brown band commonly developed. Labial tentacles clear to brown, with whitish base and tips. Oral disk with green and white colors.

Description of holotype (UF Cnidaria 9168). Small polyp, 35 mm long, 4 mm in diameter just below the marginal tentacles, 3 mm diameter near aboral end. With 19 marginal tentacles arranged in two pseudocycles, each 4 mm long and 0.5 mm in diameter near base, tentacle arrangement *(1)2.12.12.12.12….* With 12 labial tentacles, each ~1 mm long, brown with a white apical tip, directive labial tentacle absent, tentacle arrangement *(0)3.12.31.23.12….* Oral disc 0.7 mm wide, actinopharynx 17 mm long, light beige to light brown, siphonoglyph wide and elongate with eight mesenteries attached, hyposulcus 9 mm long. Directive mesenteries shorter than actinopharynx. Protomesenteries as in diagnosis, M-mesenteries (M), long, fertile with a double mesenteric filament; B-mesenteries (B) short, sterile with single mesenteric filament (double in a short part immediately below actinopharynx) and rather convoluted; acontioids only in mesenteries M3 and M4.

Comparison with other members of the genus. Although Fautin et al. (2007) suggested that morphology alone is insufficient to distinguish species of this genus,

Table 2. Comparison of anatomical features of species of *Arachnanthus* (after Carlgren 1912b; Carlgren 1924; Carlgren 1937; Picton and Manuel 1985; this study).

	A. australiae	***A. bockii***	***A. oligopodus***	***A. sarsi***	***A. lilith* sp. n.**
Marginal tentacles	Up to 40	Up to 30	~20	Up to 35	Up to 24
Arrangement of labial tentacles	(0)1.11.11.11.11	(0)1.11.11.11.11(?)	(0)1.11.11.11.11	(0)1.11.11.11.11	(0)3.12.31.23.23.12
Length of actinopharynx	~2/3 of gastric cavity	~1/2 of gastric cavity	~1/2 of gastric cavity	~1/2 of gastric cavity	>1/2 of gastric cavity
Hyposulcus	~1/2 size of stomodeum	~1/2 size of stomodeum	~2X size of stomodeum	< size of stomodeum	= size of stomodeum
Oral disc diameter	~0.7 cm	–	–	~1 cm	0.5 cm
Maximum n° of mesentery attached to siphonoglyph	12	12	4	6	8
Directive mesenteries	= length of Actinopharynx	< length of Actinopharynx	> length of Actinopharynx	< length of Actinopharynx	< length of Actinopharynx
P(C)2	Short, 1/2 of gastric cavity	Very short, 1/4 of gastric cavity	Short, 1/2 of gastric cavity	Long, 3/4 of gastric cavity	Long, 6/7 of gastric cavity, almost to aboral pole
P(C)3	Very short, <1/4 of gastric cavity	Very short, <1/4 of gastric cavity	Short, ~1/2 of gastric cavity	Short, ~1/3 of gastric cavity	Short, 1/3 of gastric cavity
M1	Almost to aboral pore	Almost to aboral pore	To aboral pore	Almost to aboral pore	To aboral pore
M3	4/5 of gastric cavity	Almost to aboral pore	1/5 of gastric cavity	Almost to aboral pore	3/4 of gastric cavity
Cnido-glandular tract of fertile mesenteries	Present (short?)	Present (short?)	Present	Present	Present
Cnido-glandular tract of B	Present (short?)	Present (short?)	Present (short?)	Present (short)	Present (short)
Acontioids	Only in M1, M2 and M3	Only in M1, M2 and M3	Only in M1	Only in M1, M2 and M3	Only in M3 and M4
Distribution	Northern Australia	Fiji	Mediterranean Sea	North Sea	Red Sea

internal anatomical characters do actually separate all known species (Table 2). While there are cases of cryptic species among tube-dwelling anemones (Stampar et al. 2012), none are yet documented for *Arachnanthus*.

Arachnanthus lilith has labial tentacles in three pseudocycles, unlike *A. australiae*, *A. oligopodus*, and *A. sarsi*, which all have them in one pseudocycle, while in *A. bockii* labial tentacles are not clearly organized and may be considered to fall into one or two pseudocycles. The actinopharynx is 2/3 as long as the gastric cavity in *A. australiae*, less than ½ as long in the other three described species, and a little over ½ as long in *A. lilith*. The maximum number of the mesenteries attached to the siphonoglyph is especially useful for distinguishing species: *A. australiae* and *A. bockii* have 12 each, *A. lilith* has eight, *A. sarsi* six, while *A. oligopodus* has four. The organization of mesenteries, particularly the mesentery P2 and M3, also provides useful characters to separate species (Table 2). Finally, the distribution of acontioids is also quite different in some species, especially in *A. lilith* where acontioids are present only on mesenteries M3 and M4. These mesenterial characters serve well to differentiate species of *Arachnanthus*, although how they vary over the ontogeny of each species remains to be studied.

Finally, the present study demonstrates the importance of more detailed investigations using non-standard collecting techniques. Small ceriantharians are rarely collected as they are frequently nocturnal and can be difficult to extract from the sediment as they retract quickly and rapidly. There are few described species of Ceriantharia with small body sizes; however, this may be the result of sampling limitations.

Acknowledgements

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Cladolasma ailaoshan, a new species of the genus *Cladolasma* Suzuki, 1963 from China (Opiliones, Nemastomatidae, Ortholasmatinae)

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Abstract

The fourth species of the Asian genus *Cladolasma*, *C. ailaoshan* **sp. n.** is described from specimens collected in Yunnan Province, China. The new species is distinct from *C. parvulum* Suzuki, 1963 and *C. angka* (Schwendinger & Gruber, 1992) in lacking enlarged, dorsally-directed tubercles on the abdominal scutum; and from *C. damingshan* Zhang & Zhang, 2013 in having keels around the eyes and in the position of the eyes. Differences in male genital structures between the Chinese species are small, while there are more differences with the Japanese species.

Keywords

Ailao Mountain, *Dendrolasma*, harvestmen, new species, taxonomy

Introduction

The genus *Cladolasma* Suzuki, 1963 was reinstated by Shear (2010), and is represented by three species restricted to Asia: China (*C. damingshan* Zhang & Zhang, 2013), Japan (*C. parvulum* Suzuki, 1963), and Thailand (*C. angka* Schwendinger & Gruber, 1992). The representatives of *Cladolasma* are tiny soil- and litter-dwelling harvestmen, usually found at high-altitude areas, e.g., *C. damingshan* at 1231 m, *C. parvulum* at 1200–1500 m, and *C. angka* at 2530 m (Zhang and Zhang 2013; Suzuki 1974; Schwendinger and Gruber 1992).

During biodiversity surveys, intensive collections were made at Ailaoshan National Natural Reserve in August 2011 by the personnel of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Among the collected specimens, a new species, *C. ailaoshan* sp. n. is recognized and described below. This constitutes the second species of the genus recorded from China.

Materials and methods

Specimens were extracted using Berlese funnels by Akihiro Nakamura at Ailaoshan, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, China, preserved in 75% ethanol, examined, and drawn under a Leica M205a stereomicroscope equipped with a drawing tube. Morphological terminology mostly follows Gruber (2007), Schwendinger and Gruber (1992), Shear and Gruber (1983), and Suzuki (1974). All measurements follow Shear (2010) and are given in millimeters (mm). Terminology for genital structures follows Shear and Gruber (1983), Martens (1986) and Macías-Ordóñez et al. (2010). Type specimens are deposited in the Museum of Hebei University, Baoding, China (MHBU).

Taxonomy

Nemastomatidae Simon, 1872
Ortholasmatinae Shear & Gruber, 1983

Cladolasma Suzuki, 1963

Cladolasma Suzuki, 1963: 40–41; Shear 2010: 17–18; Zhang and Zhang 2013: 444.
Dendrolasma: Suzuki 1974: 121–122; Shear and Gruber 1983: 51; Schwendinger and Gruber 1992: 57. [*Cladolasma* was placed in the synonymy of *Dendrolasma* by Suzuki (1974) and revalidated by Shear (2010)].

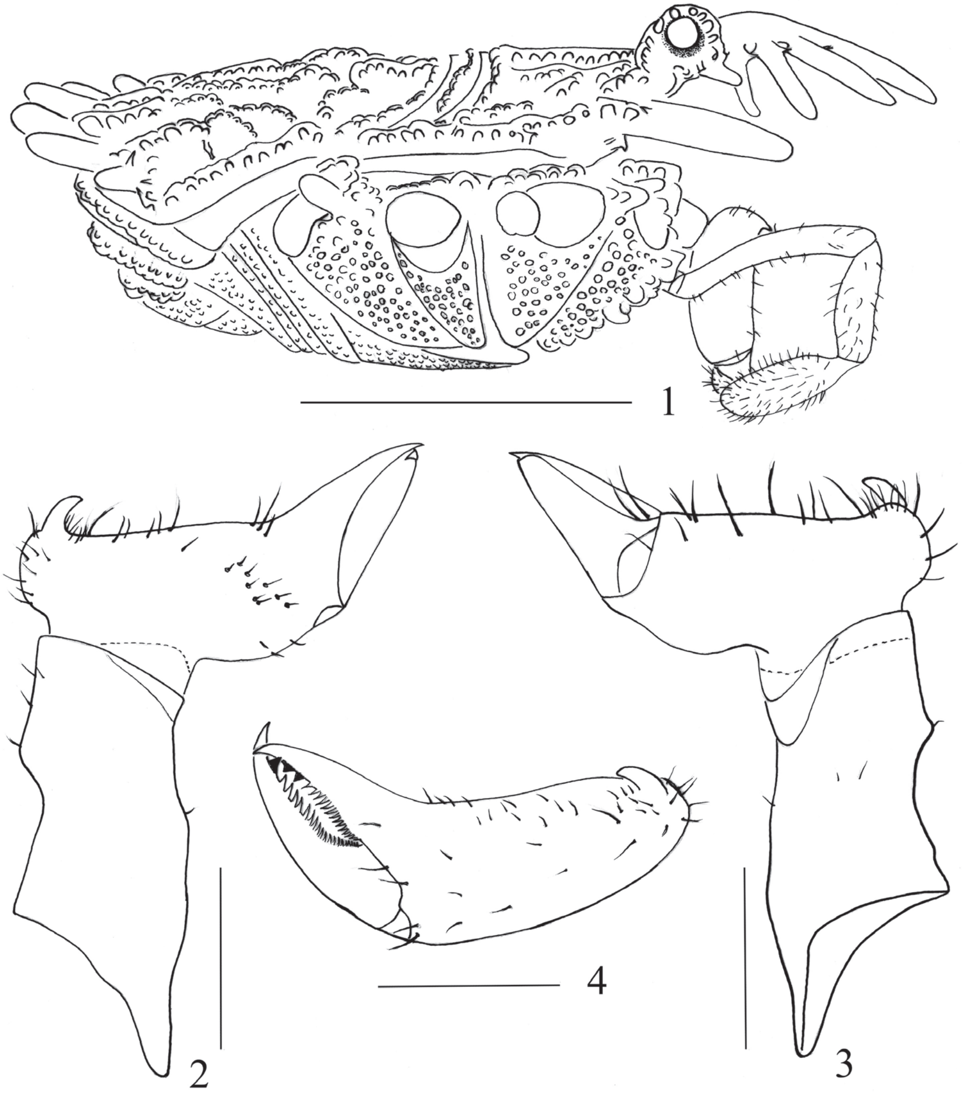
Type species. *Cladolasma parvula* Suzuki, 1963, by monotypy and original designation.

Distribution. China (Guangxi, Yunnan), Thailand (Doi Sutep), Japan (Kyushu).

Cladolasma ailaoshan sp. n.

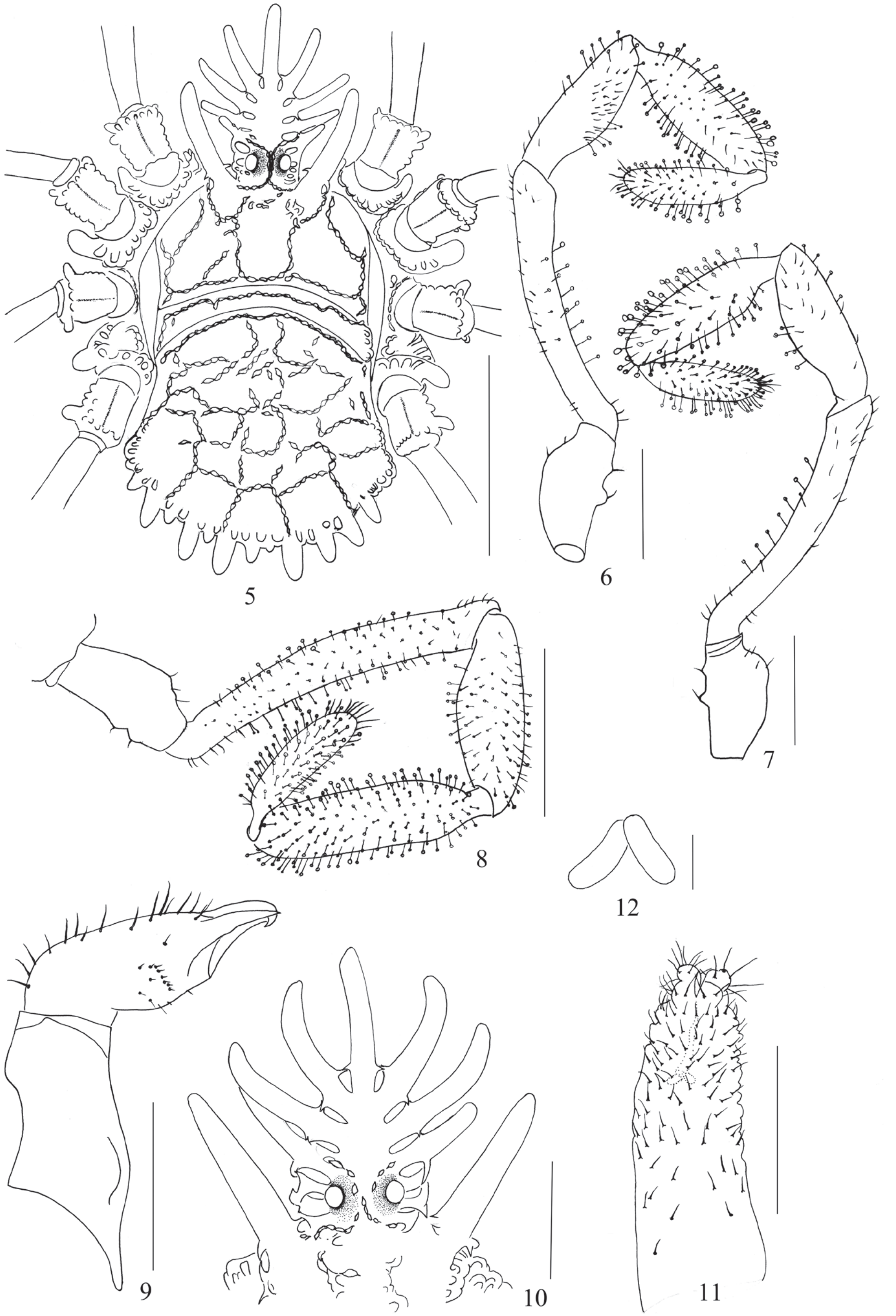
http://zoobank.org/66D046DE-CEE9-4378-BB85-6A914E82FE36
Figs 1–24

Diagnosis. Base of penis dorso-ventrally depressed, truncus bent at base (Fig. 14). Glans with 10 small spines and two large lateral spines: six small ones arranged around base of



Figures 1–4. *Cladolasma ailaoshan* sp. n. male (holotype) **1** Body, lateral view **2** Left chelicera, prolateral view **3** Left chelicera, retrolateral view **4** Second segment of chelicera, dorsal view. Scale bars: 1 mm (**1**); 0.25 mm (**2–4**).

stylus; two small ones situated on dorsal and ventral side separately (Figs 15–18). Ocularium (Figs 1, 5, 10, 19, 21–23) with circumocular keels (see Suzuki 1974: 123, fig. 1 for *C. parvulum*; Schwendinger and Gruber 1992: 58, fig. 2 for *C. angka*; in comparison to Zhang and Zhang 2013: 445, fig. 3, 447, figs 5, 6). Eyes placed at the base of the ocularium (Fig. 1; see Suzuki 1974: 123, fig. 1; Schwendinger and Gruber 1992: 58, fig. 3; in comparison to Zhang and Zhang 2013: 447, fig. 6). Abdominal scutum (Fig. 1) without enlarged, dorsad-directed tubercles (see Schwendinger and Gruber 1992: 58, fig. 3 for *C. angka*).



Figures 5–12. *Cladolasma ailaoshan* sp. n. **5** Body, male, dorsal view **6** Left pedipalp, male, prolateral view **7** Left pedipalp, retrolateral view **8** Left pedipalp, female, prolateral view **9** Left chelicera, female, prolateral view **10** Hood, female, dorsal view **11** Ovipositor **12** Receptacula seminis. Scale bars: 1 mm (**5**); 0.5 mm (**8–11**); 0.25 mm (**6–7**); 0.625 mm (**12**).

Type locality. CHINA,Yunnan Province: Zhenyuan County, Qianjiazai Town, Ailaoshan Natural Reserve, 24°16'12"N, 101°15'46"E, 2170 m, evergreen forest, extracted from leaf litter.

Type specimen. Holotype male (MHBU-Opi-20160422). Adult male preserved in 75% ethanol, with genitalia in a separate microvial. Original label: MHBU-Opi-20160422, CHINA: Yunnan Province, Zhenyuan County, Qianjiazai Town, Ailaoshan Natural Reserve, 24°16'12"N, 101°15'46"E, 2170 m of elevation, 18 August 2011, A. Nakamura leg.

Paratype. 1♀ (MHBU-Opi-20160423), same data as the holotype.

Etymology. The species epithet is a noun in apposition referring to the type locality.

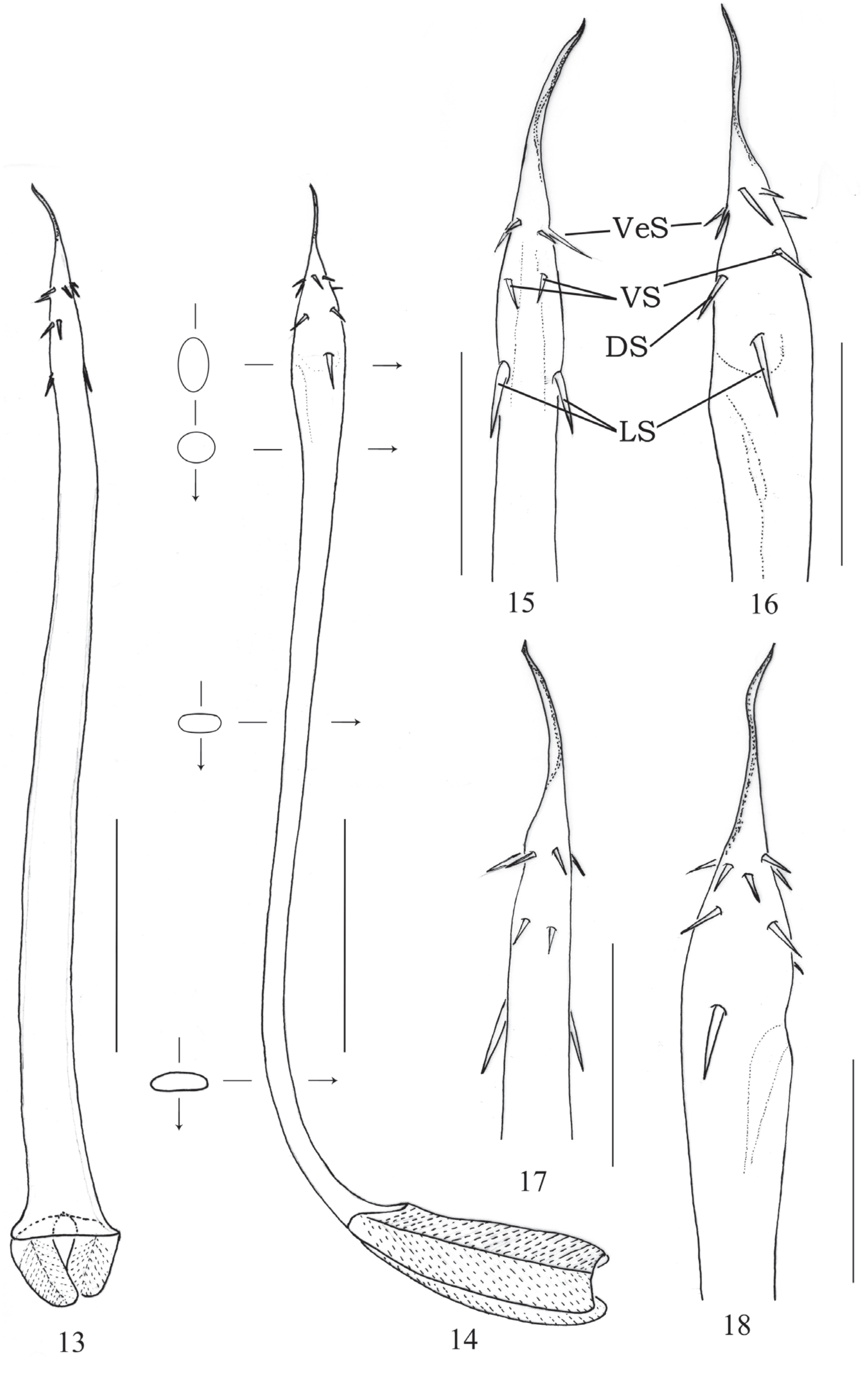
Description of the male holotype. Habitus as in Figs 1, 5, 19. Coloration in alcohol: dorsum yellowish brown (Fig. 19). Propeltidium with much darker brown areas. Eye rings black, hood pale tan (Fig. 21). Meso- and metapeltidium yellowish brown. Most of the opisthosomal scutum brown, only areas IV–V yellowish brown posteriorly. Venter dark brown, slightly lighter in ventral centre (Fig. 20). Chelicerae chestnut brown. Pedipalpi pale brown except for dark brown trochanters, tibiae and tarsi. Legs yellowish brown except for dark brown trochanters, metatarsi and tarsi.

Dorsum (Figs 5, 19). Entire body strongly sclerotized. Metapeltidium clearly separated from carapace and abdominal scutum (Figs 5, 19). Free tergites not visible from above. Surface covered with network of interconnected anvil-shaped tubercles. Anterior border of carapace with one lateral hood process on each side of ocularium. Metapeltidium with a transverse row of anvil-shaped tubercles (Figs 5, 19). Abdominal scutum with intricate lattice of interconnected anvil-shaped tubercles, its posterior margin with fence-like row of seven enlarged, posteriorly-directed digitiform tubercles. Free tergites on caudal surface of body with low keels in transverse rows (Figs 1, 20).

Hood (Figs 1, 5, 21) elevated above dorsal surface of body, arched, with one median, unpaired and 4 lateral, paired digitiform tubercles, diminishing in length toward base of hood; these digitiform tubercles usually with small basal cross-bars. Basal pair of digitiform tubercles connected at their base to circumocular keels touching each other above the eyes forming a short irregular median keel, the latter distally splitting into two branches.

Venter (Fig. 20). Coxae with dense wart-bearing setae on ventral surfaces and with dorso-distal rows of anvil-shaped tubercles; a row of anvil-shaped tubercles along anterior and posterior margins of coxae II, III and IV; coxae I and II with distal digitiform processes retro-laterally; coxa IV with similar process pro-laterally. Genital operculum short, almost tongue-shaped, surface with tubercles. Sternites with transverse rows of low keels, these reduced in the midline.

Chelicerae (Figs 2–4). Basal segment with a low dorso-medial tubercle, without glandular area, only ventrally and dorsally with a few setae. The basal end of second segment spherical, and with one basal pro-dorsal tooth (Fig. 2). Many long dorsal setae, and rows of short setae at base of fixed finger (Fig. 2). Fingers short, with diaphanous teeth and dark subapical teeth: one dark tooth on movable finger, two dark teeth on fixed finger (Fig. 4).



Figures 13–18. *Cladolasma ailaoshan* sp. n. male (holotype) **13** Penis, dorsal view **14** Penis, lateral view **15** Penis tip, ventral view **16, 18** Penis tip, lateral view **17** Penis tip, dorsal view. Abbreviations: **DS** dorsal spines **LS** lateral spines **VS** ventral spines **VeS** verticillate spines. Scale bars: 0.25 mm (**13–14**); 0.125 mm (**15–18**).

Pedipalpi (Figs 6–7). Trochanters with two ventral setiferous tubercles. Femora with few clavate hairs. Patellae medially with many clavate hairs and laterally with few clavate hairs. Tibiae and tarsi densely covered with clavate hairs.

Legs. All trochanters pro-dorsally and retro-dorsally with one enlarged tubercle. Femora, patellae and tibiae without pseudo-articulations, with distinctive microsculpture, composed of broad, thick, conical, slightly inclined denticles. Metatarsi and tarsi without annulations and microsculpture, only with setae. Tarsal segments I–II with two tarso-meres: 4 (2+2), 9 (7+2); the III–IV with three: 6 (2+2+2), 6 (2+2+2).

Penis (Figs 13–18) slender and lanceolate; no clear distinction between shaft, glans, and stylus. Shaft nearly parallel-sided, widened basally, then tapering distally (seen from ventral); in proximal portion dorso-ventrally depressed, in median portion elliptical and wider than long in cross-section, in distal portion close to glans almost circular in cross-section. Base of truncus dorsally bent almost at 90° together with two large lobe-like roots (seen from lateral). Glans bulged ventrally and dorsally (lateral view, Fig. 14); distal part of glans with six small spines at the base of the stylus and basal part with two small ventral and two small dorsal spines, and two large lateral spines (Figs 15–18). Stylus simple, slender, slightly torsion; tip of stylus bent.

Female (Figs 8–12, 22–24). Similar in appearance and coloration to male, but the body much larger, coloration lighter (Fig. 23). Free tergites partly visible from above (Fig. 23). Hood with three lateral, paired digitiform tubercles (Figs 10, 22). Genital operculum broadly rounded, with a medial triangular flat-topped projection on anterior margin (Fig. 24). Chelicerae unarmed, only with setae (Fig. 9). Femora and Patellae of pedipalpi with many clavate hairs (Fig. 8). Tarsal segments I–IV: 3 (2+1), 9 (7+2), 7 (3+2+2), 7 (3+2+2).

Ovipositor (Figs 11–12). Unsegmented, short, with nonglandular setae. The apical furca with two divisions. Two *receptacula seminis* long oval saclike (Fig. 12).

Measurements. Male holotype (female paratype): Total length (including hood and posterior tubercles) 2.80 (4.60). Prosoma 0.72 (0.94) long, 1.24 (1.88) wide. Opisthosoma 1.11 (1.92) long, 1.18 (2.06) wide. Median hood process 0.92 (1.19) long, 0.76 (1.13) wide. Basal segment of chelicerae 0.57 (0.77) long, 0.23 (0.32) deep; second segment of chelicerae 0.61 (0.74) long, 0.19 (0.26) deep. Penis 1.05 long (including glans), 0.10 wide at base, fork 0.26 long. Ovipositor 1.39 long. Measurements of left pedipalp and right legs as in Tables 1, 2.

Table 1. *Cladolasma ailaoshan* sp. n. Measurements of the pedipalp and legs of the male holotype, length/depth given for femora.

	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
Pedipalp	0.33	0.61/0.09	0.37	0.44		0.29	2.04
Leg I	0.31	1.22/0.19	0.50	0.92	0.38	0.53	3.86
Leg II	0.35	2.68/0.16	0.88	2.37	1.53	1.33	9.14
Leg III	0.33	1.30/0.20	0.49	1.03	0.37	0.59	4.11
Leg IV	0.33	1.77/0.19	0.58	1.67	0.51	0.61	5.47

Figures 19–24. *Cladolasma ailaoshan* sp. n. Photographs of holotype male and female paratype **19** Body and parts of appendages, male, dorsal view **20** Ditto, ventral view **21** Hood, male, dorsal view **22** Hood, female, dorsal view **23** Body and parts of appendages, female, dorsal view **24** Ditto, ventral view. Scale bars: 1 mm (**23–24**); 0.5 mm (**19–20**); 0.2 mm (**21–22**).

Habitat. This species was extracted from leaf litter of primeval evergreen forest using a Berlese funnel.

Distribution. Known only from the type locality, the Ailaoshan National Natural Reserve in Yunnan Province, China.

Remarks. After the genus *Cladolasma* was reinstated for the Asian species *C. parvulum* from Japan and *C. angka* from northern Thailand, one additional species was

Table 2. *Cladolasma ailaoshan* sp. n. Measurements of the pedipalp and legs of the female paratype, length/depth given for femora.

	Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
Pedipalp	0.42	1.02/0.12	0.59	0.76		0.49	3.28
Leg I	0.40	1.89/0.29	0.82	1.46	0.56	0.55	5.68
Leg II	0.40	4.49/0.24	1.21	4.09	1.94	1.58	13.71
Leg III	0.40	2.00/0.30	0.76	1.73	0.49	0.84	6.22
Leg IV	0.48	2.75/0.30	0.83	2.86	0.66	0.94	8.52

found, i.e., *C. damingshan* Zhang & Zhang, 2013 from subtropical southern China and in addition, the present *C. ailaoshan* sp. n., also from a subtropical environment. These specimens reinforce the distinctive characters between *Cladolasma* (Asiatic Ortholasmatinae) and *Dendrolasma* (American Ortholasmatinae) in morphological characters, e.g., metapeltidium in *Cladolasma* separated from abdominal scutum, while it is fused to it in *Dendrolasma*; *Cladolasma* with a relatively stout penis shaft, a compressed glans and a short, slender, pointed stylus, whereas *Dendrolasma* has a long, thinner shaft, a flattened glans and a contorted stylus.

According to the male genitalia of *Cladolasma* (penis unknown in *C. angka*), *C. ailaoshan* sp. n. and *C. damingshan* are clearly different from *C. parvulum*. The penial glans has a pair of large spines laterally in the new species and *C. damingshan*, while the glans has a lateral row of large spines in *C. parvulum*. Consequently, the penis of the new species shows closer relationship to *C. damingshan* than to *C. parvulum*.

The spination of glans penis follows the same pattern in the two Chinese species presently known (*C. ailaoshan* sp. n. and *C. damingshan*): the spines at the base of the stylus are arranged in a verticillate order (Figs 15–16; only small spines in *damingshan*; Zhang and Zhang 2013: 449, figs 22–24, larger ones in *C. ailaoshan* sp. n. sp.), the lateral spines are more distantly positioned from the base of stylus than in *C. damingshan*, and the two dorsal and two ventral spines are located between these two groups of spines. Additionally, the two Chinese species are different in the number of verticillate spines (six spines in *C. ailaoshan* sp. n., eight in *C. damingshan*) and by the size of the spines (small dorsal and ventral spines in *C. ailaoshan* sp. n., large ones in *C. damingshan*).

Moreover, *C. ailaoshan* sp. n. can be easily distinguished from *C. damingshan* by the slender and curved stylus, the shape of the dorso-basal tooth on the second segment of male chelicerae, the keels around the eyes, and the location of the eyes on the hood.

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RESEARCH ARTICLE

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Echinotermes biriba, a new genus and species of soldierless termite from the Colombian and Peruvian Amazon (Termitidae, Apicotermitinae)

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Abstract

A new Apicotermitinae genus and species *Echinotermes biriba* is described from workers collected on the Andean-Amazon Piedmont in Colombia and Peru. The enteric valve armature of *Echinotermes biriba* Castro & Scheffrahn, **gen. et sp. n.** is a remarkably diagnostic character. A Bayesian phylogenetic analysis using the COI gene and including all other Neotropical Apicotermitinae genera, supports the new genus as a distinct terminal.

Keywords

Anoplotermes-group, enteric valve, Neotropic, taxonomy

Introduction

The soldierless termites of Amazonia form a dominant group and comprise more than 30% of the termite diversity in neotropical assemblages (Davies 2002, Ackerman et al. 2009, Palin et al. 2011). Although the richness of soldierless taxa is recognized, most have not been described yet (Bourguignon et al. 2015). For example, Palin et al. (2011) list four undescribed *Anoplotermes* species and 18 undescribed species in 13 undescribed genera from Peru. Originally, all neotropical soldierless termites were placed in the genus *Anoplotermes* Müller, 1873. Recognition of much greater taxonomic diversity began with Mathews (1977) who described *Grigiotermes* and *Ruptitermes*, and Fontes (1986) who described *Aparatermes* and *Tetimatermes*. Fontes (1992) provided the first identification key for workers of these five genera. The descriptions of *Longustitermes* (Bourguignon et al. 2010), *Compositermes* (Scheffrahn 2013), *Amplucrutermes, Humutermes, Hydrecotermes, Patawatermes,* and *Rubeotermes* (Bourguignon et al. 2016), and *Disjunctitermes* (Scheffrahn et al. 2017) have advanced the classification of neotropical soldierless taxa but many more remain to be described.

Currently, 13 genera and 52 species of Apicotermitinae are known from the Neotropical region (Bourguignon et al. 2010; Krishna et al. 2013; Scheffrahn 2013; Carrijo et al. 2015; Bourguignon et al. 2016; Scheffrahn et al. 2017). For Colombia, *Anoplotermes ater, Anoplotermes parvus, Aparatermes silvestrii, Humutermes krishnai,* and *Patawatermes turricola* have been reported (Araujo 1977; Constantino 1998; Bourguignon et al. 2016; Pinzón et al. 2017), and Peru records include *Anoplotermes banksi, Anoplotermes pacificus, Disjunctitermes insularis, Rubeotermes jheringi*, and *Ruptitermes reconditus* (Constantino 1998, Bourguignon et al. 2010, Acioli and Constantino 2015, Bourguignon et al. 2016, Scheffrahn et al. 2017). Only 19% of the species of Apicotermitinae of the Neotropics are reported in these two countries.

In this paper *Echinotermes biriba* gen. n. et sp. n. is described based on the morphology of the worker caste and molecular data.

Materials and methods

The specimens were collected and preserved in 75% or 85% ethanol. The dissection of the enteric valve (EV) was done by removing the P2 tube from the worker's gut and then expelling all the food particles by means of controlled pressure. The tube was immersed in a PVA medium to completely detach the EV from surrounding muscle tissue and cut longitudinally to splay open the EV for mounting in the medium. The mandibles were also submerged in PVA medium. The terminology used for the worker gut follows Sands (1972) and Noirot (2001).

The COI sequence of *E. biriba* was obtained by DNA extraction and PCR performed by the Canadian Centre for DNA Barcoding following standard high-throughput protocols (deWaard et al. 2008). The PCR employed the primers LepF1 and LepR1 (Hebert et al. 2003) which generated 622 to 652bp of the barcode region of the mitochondrial gene cytochrome c oxidase subunit 1 (COI).

A gene tree was created under Bayesian Inference (BI) using the COI gene. In addition to the sequence of *E. biriba*, a total of 48 GenBank sequences were used: 34 sequences of neotropical Apicotermitinae (21 species, 13 genera), eight non neotropical Apicotermitinae genera, five non-apicotermitine Termitidae, and one Rhinotermitidae, (*Heterotermes crinitus*) as the outgroup. Sequences were aligned under MUSCLE algorithm implemented in Geneious v6.1.6 (Biomatters Ltd., Auckland, New Zealand). Substitution model used (GTR+I+G) was selected through the Akaike Information Criterion (AIC) with the software jModelTest2 (Darriba et al. 2012). The XML input files were generated with BEAUti 1.8.0, and the BI was performed with BEAST 1.8.0 (Drummond et al. 2012). A Yule speciation process, with a random starting tree, and relaxed molecular clock was used as tree priors. Four Markov chain Monte Carlo (MCMC) searches were conducted, each one for 15,000,000 generations, and they were combined to search the most probable tree. Convergence and stationarity were assessed with Tracer 1.5 (Rambaut et al. 2014) and the first 600 trees were discarded as burn-in with TreeAnnotator 1.8.0 and visualized using FigTree 1.3.1.

Systematics

Echinotermes Castro & Scheffrahn, gen. n.

http://zoobank.org/9872DC61-CA8C-42B5-9ABE-62160F532ECD

Type-species. *Echinotermes biriba* sp. n.

Imago. Unknown.

Description of worker. (Fig. 1). Monomorphic. *Head* capsule and antennae a light yellowish colour; pronotum pale yellow; legs hyaline. Head covered with approx. 30 longer setae (0.1 mm) and approx. 100 shorter setae (≤ 0.05 mm) (Fig. 1A). In lateral view, dorsal surface of the head capsule slightly convex; postclypeus is moderately inflated. Antennae with 14 articles. Pronotum with four or five long setae and numerous short hairs. Mandibles with apical teeth more prominent than first marginal teeth; left mandible with M1+2 equilateral, M3 forming right angle, molar prominence projecting in line with apical tooth; right mandible with concave margin between M1 and M2 (Fig. 1B).

Fore-tibia moderately inflated (Fig. 1C) and covered with approx. 60 longer setae and approx. 40 shorter setae; pilosity denser apically. Third (external) spur very small. Femur with approx. 20 sparse large setae. Tibial spurs 2:2:2.

Digestive tube (Fig. 1D) with very large crop, more voluminous than paunch (P3). Mesenteron forming complete 360° loop. Mesenteric tongue short, truncate. First proctodeal segment tubular, equal diameter throughout and visible its entire length in ventral view. Enteric valve seating trilobed, with smaller lobe not visible in intact gut. Enteric valve with six cushions, terminating at the opening to the P3 as spiny spheroids (Fig. 2).

Diagnosis. The crop of *E. biriba* is unusually large and the enteric valve armature, consisting of six spherical pectinate pads, is unique among all apicotermitine genera.

Remarks. Mandibles of *Rubeotermes jheringi* and *Humutermes krishnai* are very similar to *E. biriba*, but the first marginal teeth of *E. biriba* are less prominent that those two genera. The diagnostic character of *E. biriba* is the enteric valve armature which is also spiked in the *Humutermes* enteric valve (EV) but in *E. biriba* the EV

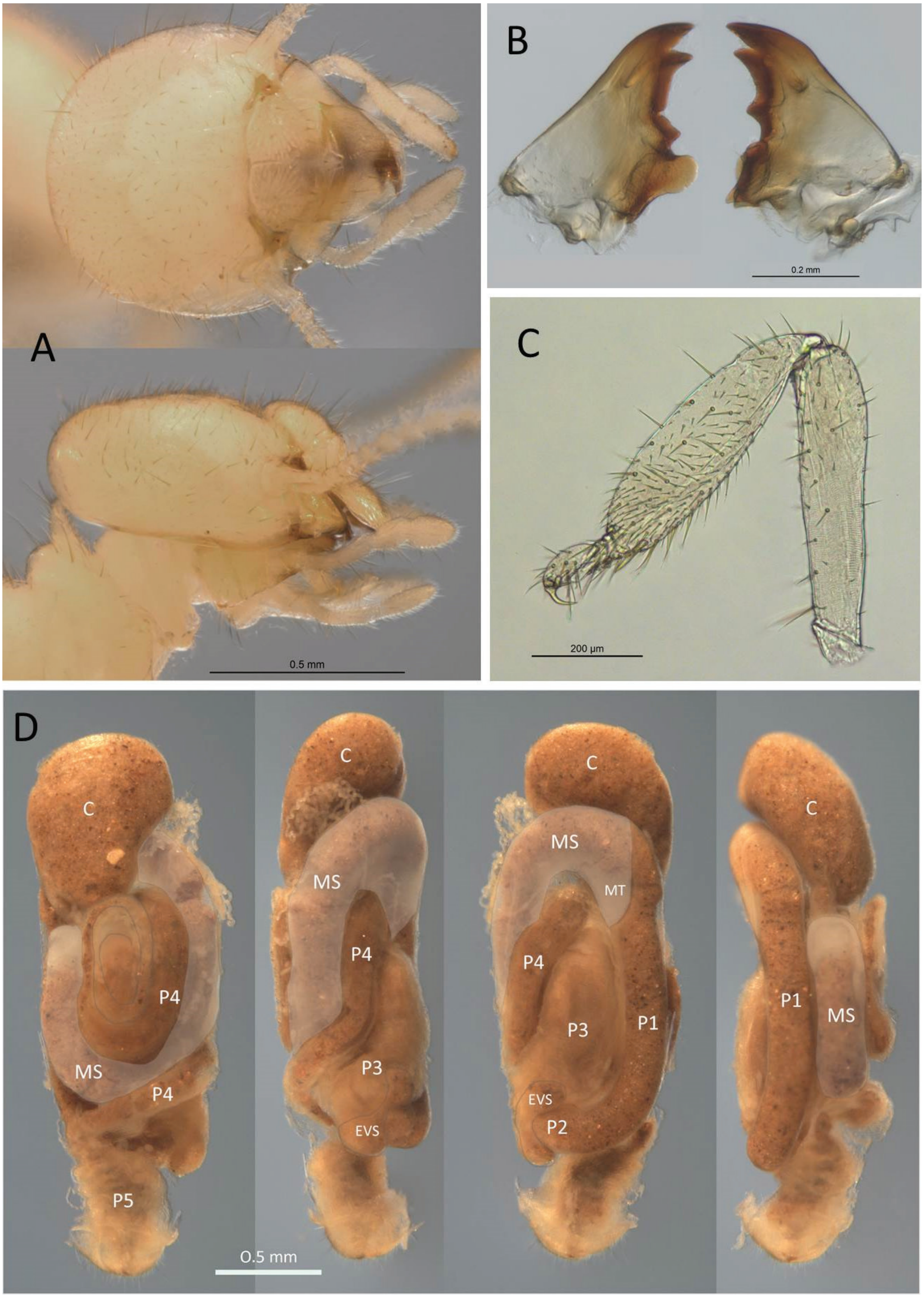

Figure 1. Worker of *Echinotermes biriba* sp. n.: **A** dorsal and lateral views of head and pronotum **B** mandibles **C** right fore-tibia **D** digestive tube from left to right: dorsal, right, ventral and left views. Abbreviations: C = crop, EVS = enteric valve seating, MS = mesenteron, MT = mesenteric tongue, P1–P5 = proctodeal segments.

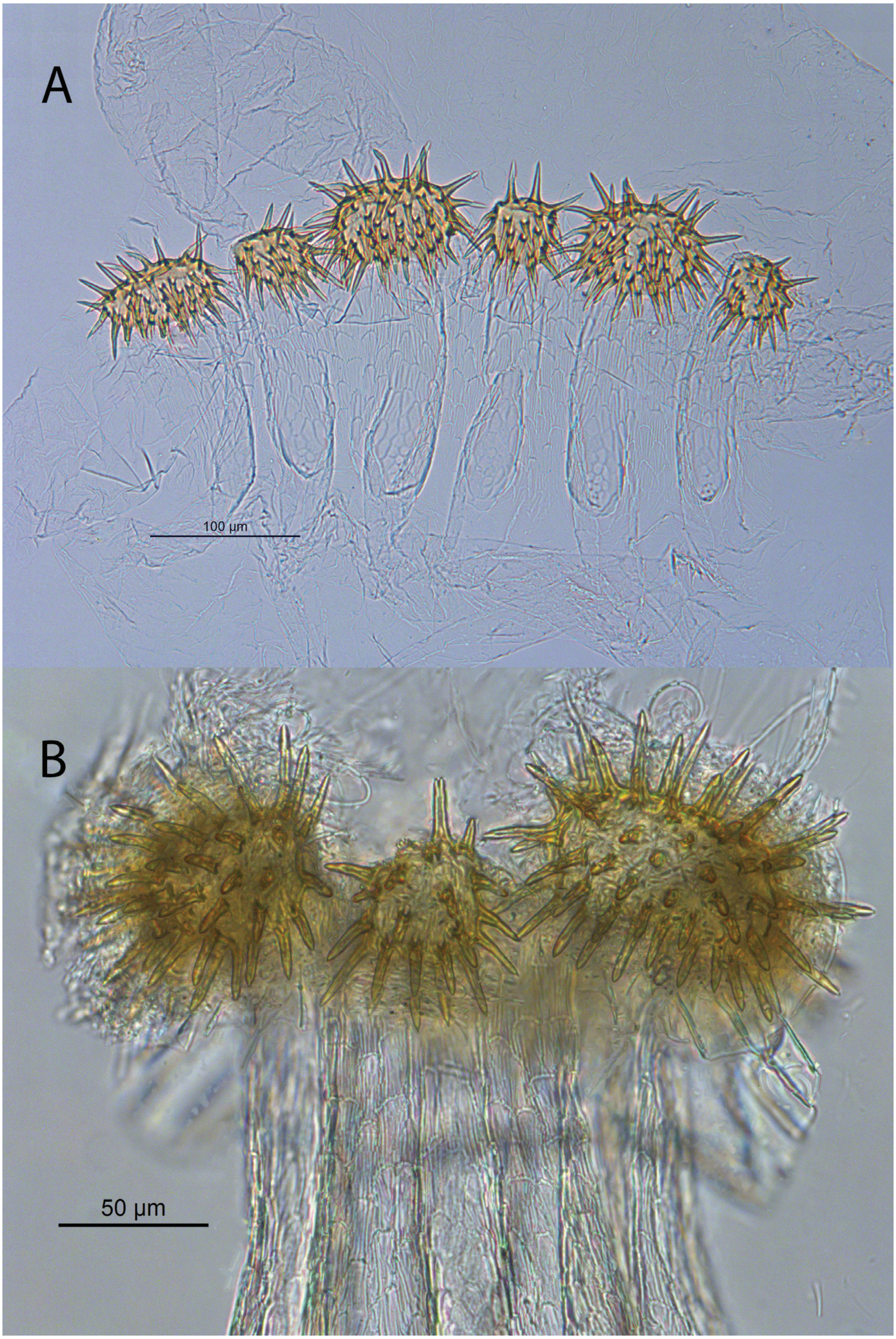

Figure 2. Worker enteric valve of *Echinotermes biriba* sp. n. **A** Spliced mount **B** whole mount, showing *in situ* position of armature. Note the filamentous bacteria attached to the spines. The trilobed seating anterior to the spines (removed in this preparation) is full of bacteria and devoid of food particles, referred by Noirot (2001) as the "bacterial pouch".

armature is spherical while in *Humutermes* it is rather flat. *Humutermes* species are smaller than *Echinotermes*. The enteric valve of *Grigiotermes* is composed of six uniform pectinate plates, while in *Patawatermes* the uniform plates are hemispherical.

Etymology. From the Latin *Echino*, meaning spiny, describing the EV armature.

Echinotermes biriba Castro & Scheffrahn, sp. n.

http://zoobank.org/9F9BC8F4-57E9-4608-BB48-FBE9E481940B

Holotype. Worker from colony CATAC 2736.

Type-locality. COLOMBIA: Caquetá, Belén de los Andaquíes (1.60794, -75.88683).

Paratypes. PERU: Pasco, Oxapampa, Chatarra forest, (-10.51303, -75.07276), 24/05/2014, 556 m, 14 workers (UF no. PU 144). Additional material: COLOMBIA: Caquetá, Belén de los Andaquíes, Camino Andaquí (1.60794, -75.88683), 31/01/2017, 625 m, 10 workers (CATAC 2736).

Description of worker. (Fig. 1, Table 1) EV armature consists of six prominent spheroids each covered with robust spiny armature; three larger (ca. 30–35 spines) and three smaller (15–20 spines) alternate inside the EV seating. Enteric valve with six unsclerotized cushions some four times longer than wide, each composed of approx. 10–20 ovoid scales.

Diagnosis. Unique armature of EV composed of alternating larger and smaller spheroids covered with robust spines.

Remarks. See genus remarks above.

Ecology and distribution. In Colombia, *E. biriba* foragers were collected in the same soil sample (0-10 cm depth) with *Longustitermes manni*. Gut contents confirm that *E. biriba* feeds on soil organic matter. This species is only known from the Chatarra forest in the southern Peruvian Amazon, and in a mature secondary forest in the northern Colombian Amazon (Fig. 4).

Molecular analysis. The gene tree recovered the Neotropical Apicotermitinae (NA) as monophyletic, however, the position of *Echinotermes biriba* inside this clade could not be established with this single gene. The low posterior probability of almost

Table 1. Measurements (mm) of ten workers from two colonies of *Echinotermes biriba* sp. n.

	Holotype	PU144		CATAC2736	
		Range	Mean	Range	Mean
Max Head Width	0.74	0.77–0.74	0.75	0.83–0.74	0.78
Pronotum Width	0.44	0.49–0.46	0.48	0.44–0.55	0.51
Hind Tibia Length	0.57	0.53–0.44	0.48	0.61–0.55	0.57
Fore Tibia Length	0.48	0.44–0.35	0.41	0.49–0.43	0.46
Fore Tibia Width	0.13	0.14–0.11	0.12	0.14–0.11	0.12
Fore Tibia Width: Length Ratio	0.27	0.36- 0.24	0.30	0.23–0.28	0.26

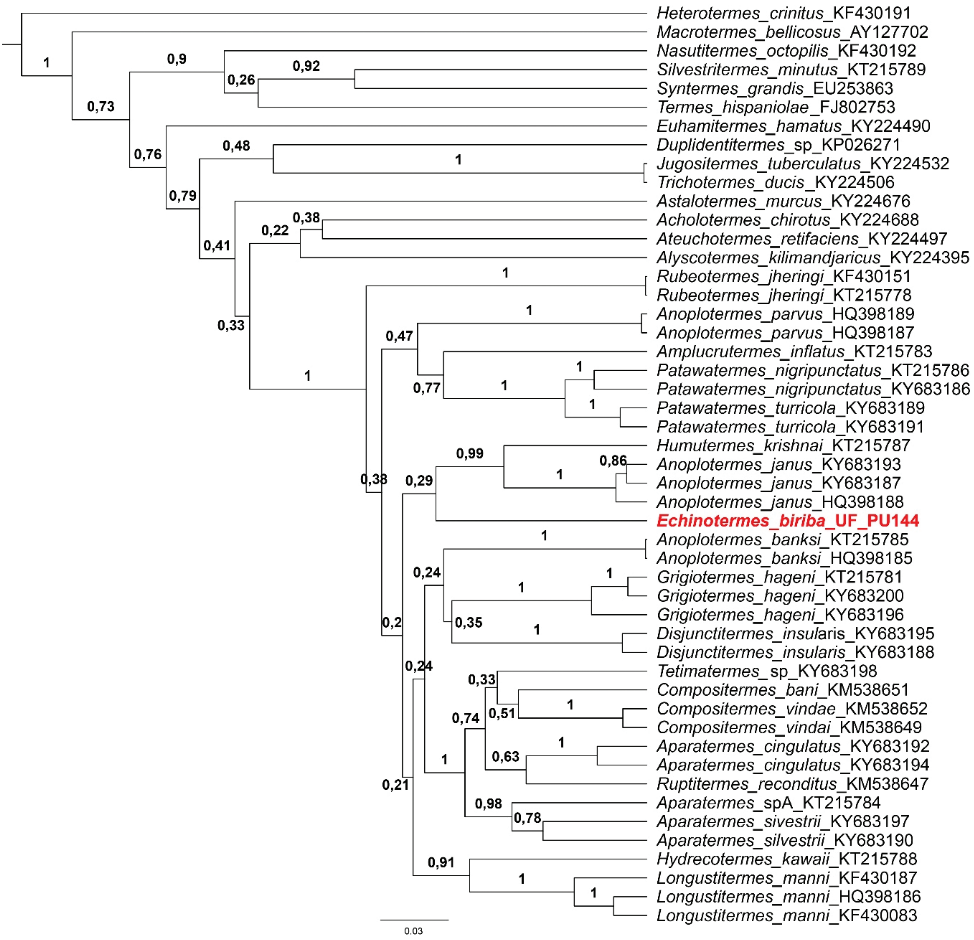

Figure 3. Bayesian gene tree of all described soldierless New World genera using the mitochondrial COI barcode gene showing posterior probabilities. Tree rooted on terminal *Heterotermes crinitus*.

every first branching clades in the NA group should be interpreted as a big polytomy, and the new genus as a branch in this polytomy, just as most of the other NA genera (Fig. 3).

Etymology. The species name is due to the resemblance of the EV armature with the Amazonian fruit *Rollinia mucosa* (Jacq.) Baill. which is known as "biriba" in the region.

Discussion

Neotropical soldierless termites have been a taxonomic problem to a large extent because enteric valve (EV) morphology was overlooked. Mathews (1977) showed it was possible to differentiate some New World Apicotermitidae using the EV as had already

Figure 4. Known localities of *Echinotermes biriba* sp. n.

been done in Africa (Grassé and Noirot 1954, Sands 1972), thus furthering the reclassification of the so-called *Anoplotermes*-group to this day. As with *D. insularis* (Scheffrahn et al. 2017), *E. biriba* is described only from the worker caste with the EV as its most robust diagnostic character.

The Amazon forest contains the greatest diversity of New World termites (Ackerman et al. 2009, Constantino and Cancello 1992), but currently the data show a low diversity of Apicotermitinae compared to other subfamilies such as Nasutitermitinae, Syntermitinae, and Termitinae (Constantino 1991, de Souza and Brown 1994). As new genera and species of neotropical Apicotermitinae are described, the richness of termites, especially in poorly studied countries such as Colombia and Peru will greatly increase.

Acknowledgments

We want to thank to the Colciencias, Instituto Amazónico de Investigaciones Científicas SINCHI and Expedición BIO project for the financial support in Colombian Amazon. We also thank to Dr. Clara P. Peña-Venegas for coordinating the soil component at SINCHI and for her logistic support on field trips. We heartily thank the reviewers for their help in improving the paper.

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RESEARCH ARTICLE

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Taxonomic notes on Babinskaiidae from the Cretaceous Burmese amber, with the description of a new species (Insecta, Neuroptera)

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Abstract

Babinskaiidae is an extinct lacewing family of the superfamily Myrmeleontoidea. Hitherto, nine species of seven genera are described from the Lower and mid-Cretaceous. Here a new species of Babinskaiidae is described from Cretaceous Burmese amber, namely *Parababinskaia makarkini* **sp. n.** The new species possesses an A2 vein in the hind wing, suggesting that the loss of this vein might not be an autapomorphy of Babinskaiidae. The female of *Electrobabinskaia burmana* Lu, Zhang & Liu, 2017 is also described for the first time based on two specimens with their abdomens perfectly preserved, exhibiting a specialised sternum VI with paired elongate projections. A brief discussion of female genital characters is provided, which may increase our understanding of the morphology and phylogenetic position of Babinskaiidae.

Keywords

Mesozoic, Myrmeleontoidea, Neuropterida, phylogeny, taxonomy

Introduction

The extinct lacewing family Babinskaiidae, belongs to the superfamily Myrmeleontoidea, and is recently considered to form an epifamily Nymphidoidae together with Nymphidae (Makarkin et al. 2017). Adults of Babinskaiidae can be characterised by long filiform antennae, narrowly elongated wings, with features such as trichosors, and presectorial cross veins present in both wings, and absence of forewing oblique vein (i.e., the base of MP2).

Hitherto, Babinskaiidae were only recorded in the Lower Cretaceous of Brazil (Crato Formation) and Russia (Zaza Formation), and the mid-Cretaceous of Myanmar (Martins-Neto and Vulcano 1989a, b; Ponomarenko 1992; Martins-Neto 1997; Lu et al. 2017; Makarkin et al. 2017). Currently, the family contains nine species assigned in seven genera, i.e., *Baisonelia* Ponomarenko, 1992 from the Lower Cretaceous of Russia; *Babinskaia* Martins-Neto & Vulcano, 1989, *Neliana* Martins-Neto, 1992, *Parababinskaia* Makarkin, Heads & Wedmann, 2017 from the Lower Cretaceous of Brazil; and *Burmobabinskaia* Lu, Zhang & Liu, 2017, *Electrobabinskaia* Lu, Zhang & Liu, 2017, and *Pseudobabinskaia* Makarkin, Heads & Wedmann, 2017 from the mid-Cretaceous of Myanmar. However, many of these species are from compression fossils, and some of them are known only from wing fragments (e.g., *Baisonelia vitimica* Ponomarenko, 1992 and *Neliana impolluta* Martins-Neto, 1997, each with only a hind wing preserved). Recent discovery of Babinskaiidae in Burmese amber provides important evidence to understand the morphology, taxonomy, and phylogenetic status of this family owing to the well-preserved specimens (Lu et al. 2017). Nevertheless, known Burmese amber specimens of Babinskaiidae are still scarce.

In this paper, with examination of more specimens of Babinskaiidae from the Burmese amber, a new species of *Parababinskaia* is reported based on two specimens with both the male and the female described, and the female of *Electrobabinskaia burmana* Lu, Zhang & Liu, 2017 is also described for the first time. A comparative study on the female genital morphology of Babinskaiidae is presented.

Materials and methods

The amber samples described are from the Hukwang Valley in Tanai Township, Myikyina District of Kachin State, Myanmar (Kania et al. 2015). The age of this deposit has been investigated and dated to be 98.8±0.6 million years by U-Pb dating of zircons from the volcanoclastic matrix of the amber (Shi et al. 2012).

The specimens are deposited in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing, China, while a paratype of the new species herein described is currently housed in the Entomological Museum, China Agricultural University (**CAU**), Beijing, and will eventually be deposited in the Collection of Xiao Jia in the Century Amber Museum (**CAM**), Shenzhen.

Photographs and drawing were taken and made using a Zeiss SteREO Discovery V12 microscope system. The figures were prepared with Adobe Photoshop CS6. Terminology of wing venation generally follows Aspöck et al. (1980) and Martins-Neto (2000). Breitkreuz et al. (2017) presented an alternative interpretation on the homology of wing venation in Neuropterida based on vein tracheation. The corresponding abbreviations of the veins based on the nomenclature in Breitkreuz et al. (2017) are given below in the parentheses for comparison. These two venation terminologies differ from each other mainly in homology interpretation and definition of MA, i.e., whether MA is considered to be fused with RP at the wing base. The venation terminology used for Babinskaiidae in Makarkin et al. (2017) is in generally similar to that of Breitkreuz et al. (2017). Terminology of genitalia follows Aspöck and Aspöck (2008).

Abbreviations used for wing veins are:

A (A)	anal vein;	**MP (MA+MP)**	media posterior;
C (C)	costa;	**R (R)**	radius;
Cu (Cu)	cubitus;	**RA (RA)**	radius anterior;
CuA (CuA)	cubitus anterior;	**RP (RP)**	radius posterior;
CuP (CuP)	cubitus posterior;	**ScP (Sc)**	subcosta posterior;
M (M)	media;	**ps**	presectorial crossveins (i.e., r-mp crossveins).
MA (RP1)	media anterior;		

Systematic palaeontology

Class Insecta Linnaeus, 1758
Order Neuroptera Linnaeus, 1758
Superfamily Myrmeleontoidea Latreille, 1802
Epifamily Nymphidoidae Rambur, 1842
Family Babinskaiidae Martins-Neto & Vulcano, 1989

Genus *Parababinskaia* Makarkin, Heads & Wedmann, 2017

Figs 1–4

Parababinskaia Makarkin, Heads & Wedmann, 2017: 153. Type species: *Parababinskaia elegans* Makarkin, Heads & Wedmann, 2017: 153 (original designation).

Revised diagnosis. Forewing: Narrowly elongate, slightly broadened distally, with four or five presectorial cross veins. RP+MA originating proximal of the termination of CuP, RP with five branches, most of which are simple. Six cross veins present between RA and RP. MP pectinately branched about at distal 1/5. CuA pectinately branched. A1 bifurcated. A2 and A3 present, and not fused with each other. A short outer gradate series of cross veins present. Hind wing: Slightly narrower than forewing. Three or four presectorial cross veins present. RP+MA originating almost at same level with termination of CuA. RP with four or five branches, posterior three branches of which are simple. Four to seven cross veins present between RA and RP. MP1 pectinately branched approx. at distal 1/5. MP2 pectinately branched nearly at its midpoint. CuP and A1 proximally fused. A2 present. Female abdominal segment VI without projections on sternum.

Parababinskaia makarkini **sp. n.**

http://zoobank.org/4B1E6677-C7E3-4ADC-968F-AE3988A8CC10
Figs 1–4

Diagnosis. Many CuA branches in forewing bearing small marginal fork. Hind wing with four or five cross veins between RA and RP, and with eight branches of MP2.

Description. ***Male*** (Fig. 1A). *Body* length 11.20 mm; head 0.90 mm long and 1.70 mm wide; antenna length 6.34 mm; forewing 11.11 mm long and 2.90 mm wide; hind wing 9.37 mm long and 2.52 mm wide. Abdomen length 7.64 mm.

Head with vertex with a pair of domed regions (Fig. 1C). Compound eyes large, semi-globular. Antenna filiform, with dense short setae; scape much wider and longer than pedicel; flagellum with 49 flagellomeres, each flagellomere much longer but narrower than pedicel.

Prothorax slightly longer but much narrower than head, laterally with some long hairs. Meso- and metathorax robust. Wings in general narrowly elongated, transparent, and immaculate.

Forewing with single trichosors between veins along distal margin; multiple trichosors (up to seven) between veins along costal and posterior margins. Costal space about three times as wide as subcostal space, but much narrower than radial space, with 18 simple veinlets on proximal 3/4 and 16 marginally forked, more inclined veinlets on distal 1/4; only one subcostal cross vein (1scp-r) present near the wing base. Four presectorial cross veins present. Origin of RP+MA slightly proximad termination of CuP. MA diverging from RP much distad separating point of RA and RP+MA; RP with five branches, and only anterior-most one bearing a small marginal fork. Six cross veins present in radial space. MA with a small marginal fork. MP long and straight, pectinately branched about at its distal 1/5, and all branches with a small marginal fork. A short outer gradate series cross veins present. Eleven crossveins present between MP and CuA. CuA and CuP diverging near wing base. CuA pectinately branched and slightly zig-zagged distally, with eight branches, most of which bear a small marginal fork. CuP pectinately branched, with six simple branches. Eight cua-cup cross veins present. A1 distally bifurcated. Two cup-a1 cross veins present. A2 and A3 short and simple, not fused with each other.

Hind wing: Slightly narrower than forewing. Trichosors as in forewing. Costal space nearly two times as wide as subcostal space, with 14 simple veinlets on proximal 3/4 while with 14 marginally forked veinlets on distal 1/4. Subcostal crossvein absent. Three or four presectorial crossveins present. RP+MA originating nearly at same level of termination of CuA. Four crossveins present in radial space. MP1 and MP2 diverg-

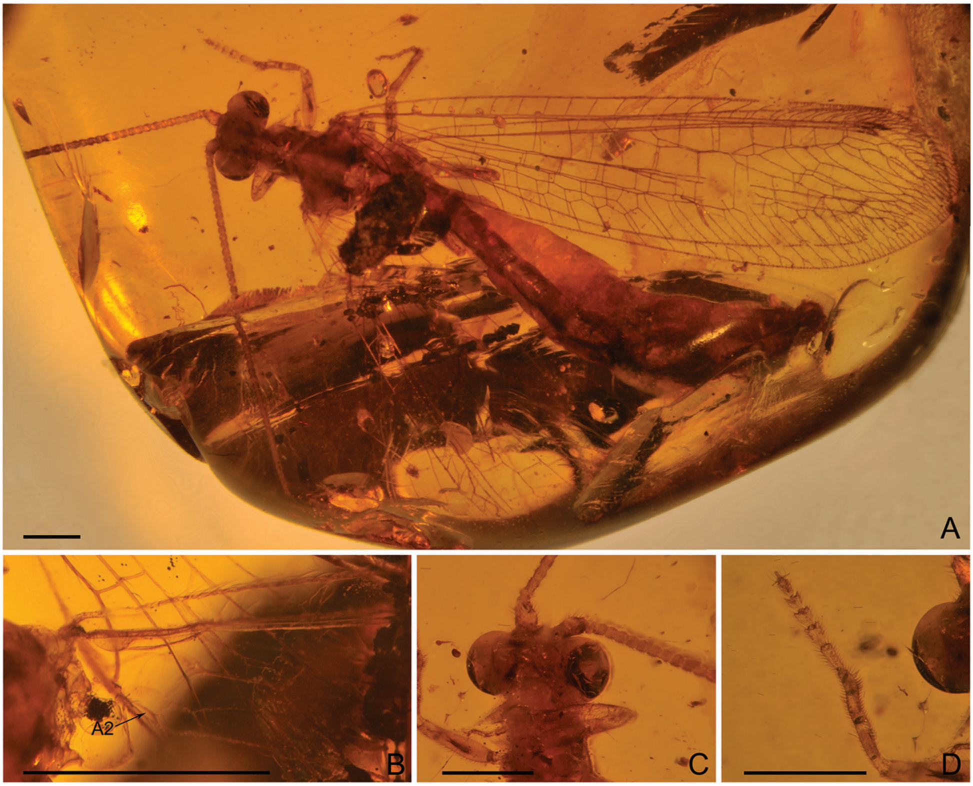

Figure 1. *Parabinskaia makarkini* sp. n., holotype male. **A** Habitus photograph, dorsal view **B** Photograph of left wing base **C** Photograph of head, ventral view **D** Photograph of tarsus. Scale bars: 1.0 mm.

ing near wing base; MP1 straight and long, pectinately branched approx. at its distal 1/5, and all branches bearing a small marginal fork; MP2 slightly zig-zagged distally, with eight pectinate branches (anterior three of them with a small marginal fork). Eight or nine intermedia cross veins present. CuA short, with five simple branches. CuP and A1 proximally fused, CuA distally strongly zig-zagged. A2 present, short and simple, slightly curved posteriad (Fig. 1B). An oblique a1-a2 crossvein present. Jugal lobe present.

Legs slender, with dense short setae; specialised setae absent (Figs 1D, 2C). Tarsus 5-segmented; tarsomere I slender, slightly longer than each of the rest tarsomeres; tarsomeres II-IV slightly wider than tarsomere I and feebly tapering on distal-lateral corners; tarsomere V ovoid. Pretarsal claws equal in length and shape, shorter than tarsomere V, without additional teeth. Arolium present, slightly shorter than pretarsal claw.

Abdomen slenderly elongate, with segments IV–VI slightly broadened.

Male genitalia (Fig. 4A–D): Tergum IX short; sternum IX invisible, probably rather small. Ectoprocts paired, broadly ovoid, with large callus cerci. A seemingly paired, darkly coloured (probably strongly sclerotised) sclerites (putative gonocoxite IX) present beneath ectoprocts and extending well beyond tergum IX.

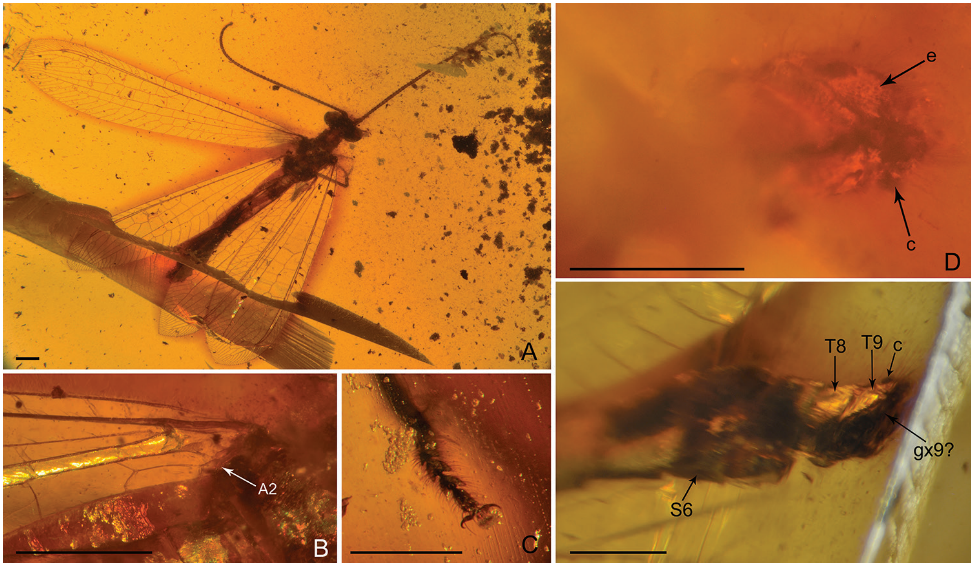

Figure 2. *Parababinskaia makarkini* sp. n., paratype female. **A** Habitus photograph, dorsal view **B** Photograph of left hind wing base, dorsal view **C** Photograph of tarsus **D** Photograph of female genitalia, dorsal view **E**. Photograph of female genitalia, ventral view. Abbreviations: T: tergum; S: sternum; c: callus cercus; e: ectoproct; gx: gonocoxite. Scale bars: 1.0 mm.

Female (Fig. 2A). *Body* length 10.68 mm; head 0.86 mm long and 1.32 mm wide; antenna length 8.55 mm; left forewing 13.05 mm long and 2.85 mm wide; left hind wing 10.37 mm long and 2.27 mm wide; right forewing (probably distorted) 10.09 mm long and 3.51 mm wide; right hind wing (probably distorted) 9.42 mm long and 2.75 mm wide; abdomen 7.14 mm long.

External morphology of female almost same as male. Antenna slightly longer, with 59 flagellomeres.

Forewing: Five presectorial cross veins present. MP with six pectinate branches, almost all bearing marginal fork. Fourteen cross veins present between MP and CuA. Six cua-cup crossveins present. Only one cup-a1 cross vein present.

Hind wing: Four presectorial cross veins present. Five cross veins present on radial space. RP with four branches. MP1 with ten pectinate branches; MP2 with nine simple branches; seven cross veins present between MP1 and MP2. CuA with six simple branches. A2 present (Fig. 2B).

Abdomen slender and elongated, with segments V–VII slightly broadened. Segment VI nearly rectangular, posteriorly without specialised projections.

Female genitalia (Fig. 2D–E): Tergum VIII broad, nearly rectangular in dorsal view, subtriangular in lateral view. Tergum IX arcuate in dorsal view, notably smaller than tergum VIII. A pair of putative gonocoxite IX present. Ectoprocts paired, cone-like, each with a short and slender projection posteriad. Callus cerci present, large.

Type material. Holotype: NIGP197965: Amber piece preserving a nearly complete male of *Parababinskaia makarkini* sp. n., it is polished in the form of arched

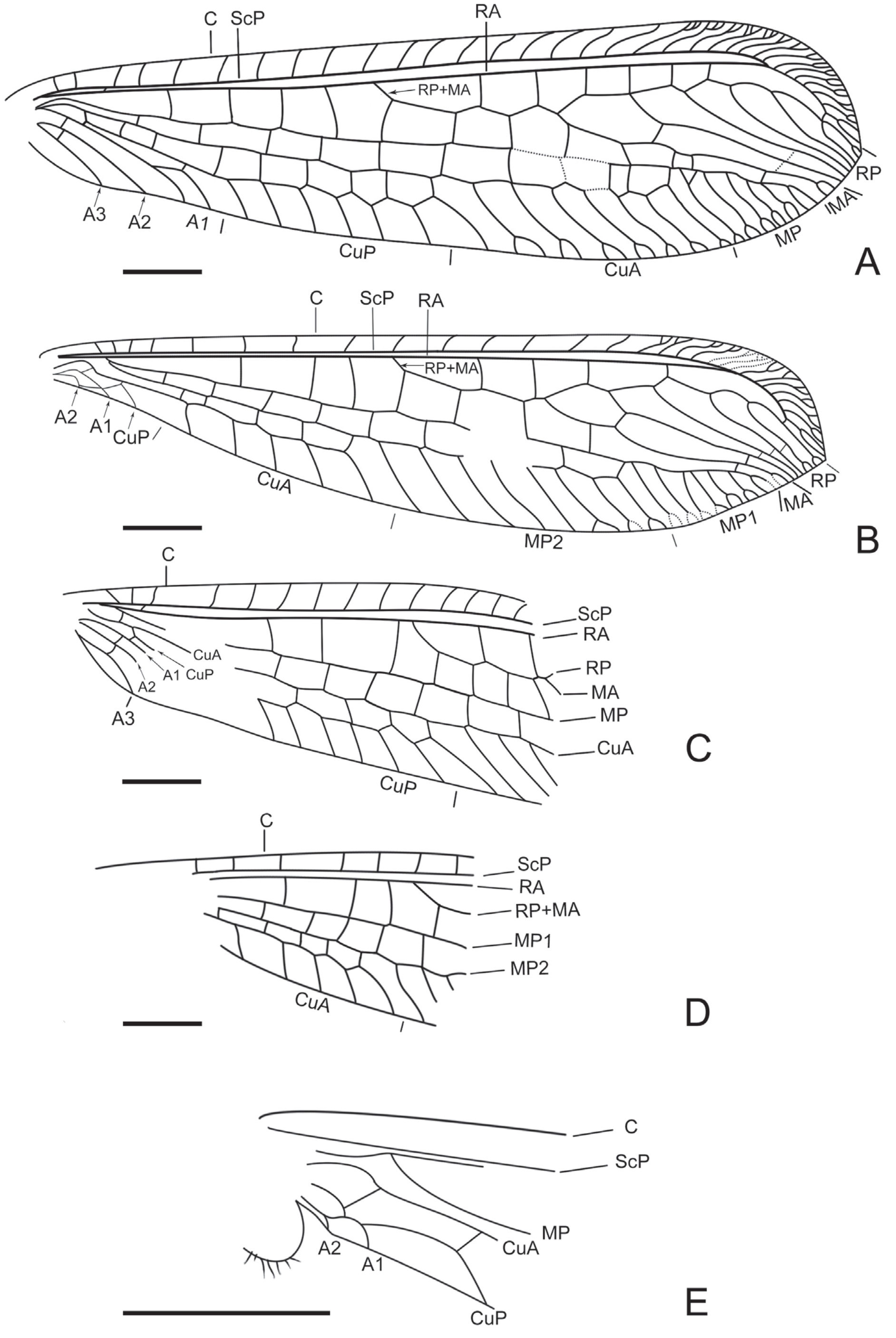

Figure 3. Wing venation of *Parababinskaia makarkini* sp. n., male. **A** Line drawing of right forewing **B** Line drawing of right hind wing **C** Line drawing of left forewing **D** Line drawing of left hind wing **E** Basal part of left hind wing. Scale bars: 1.0 mm.

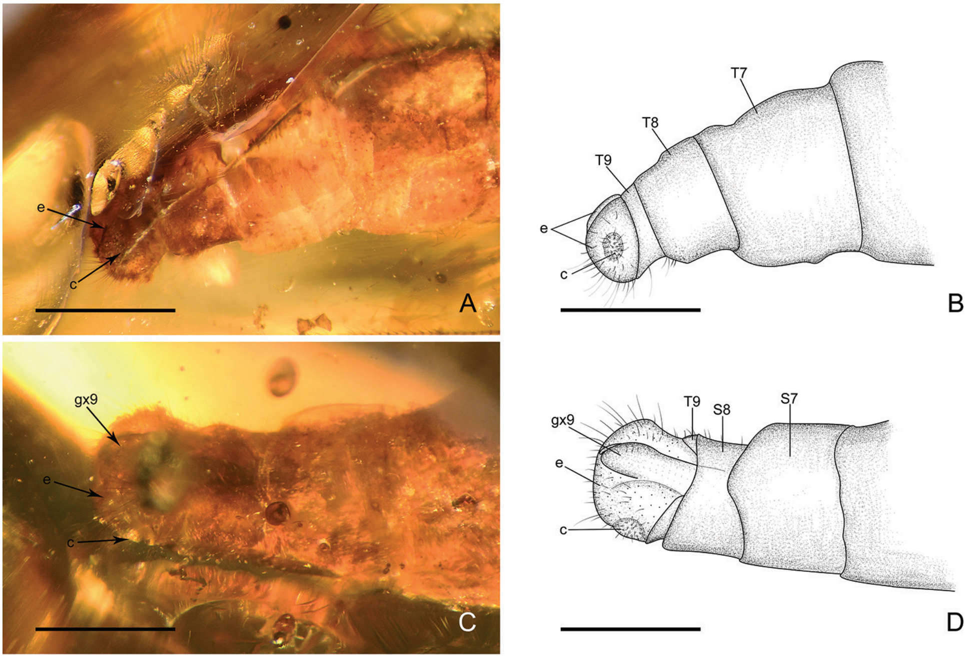

Figure 4. Genitalia of *Parababinskaia makarkini* sp. n., male. **A** Photograph of genitalia, lateral view **B** Drawing of genitalia, lateral view **C** Photograph of genitalia, ventral view **D** Drawing of genitalia, ventral view. Abbreviations: T: tergum; S: sternum; c: callus cercus; e: ectoproct; gx: gonocoxite. Scale bars: 1.0 mm.

pentagon cabochon, clear and transparent, with length × width about 24.18 × 21.44 mm, height 7.76 mm. Paratype: CAM BA-0012: amber piece preserving a complete female of *P. makarkini* sp. n. and a coleopteran larva, it is polished in the form of flattened rectangular cabochon, clear and transparent, with length × width about 3.66 × 23.92 mm, height 6.95 mm.

Etymology. The new species is dedicated to Dr. Vladimir N. Makarkin for his great contributions on the taxonomy of fossil lacewings.

Remarks. The new species is placed in *Parababinskaia* based on the similar number of presectorial crossveins (four or five in the forewing, and three or four in the hind wing), the presence of hind wing outer gradate series of crossveins, and the similar configuration of hind wing CuP, in comparison with the type species of *Parababinskaia*, i.e., *P. elegans*. However, the new species can be distinguished from *P. elegans* by the forewing CuA with most branches marginally forked (most branches of forewing CuA simple in *P. elegans*), the presence of four or five hind wing radial cross veins (six or seven in *P. elegans*), and the presence of eight branches of hind wing MP2 (11 or 12 in *P. elegans*). The new species apparently differs from the other Burmese amber babinskaiids by the bifurcated forewing A1.

The association between the male and female of the new species is based on the similar body size, the generally same wing venation, and the similar tarsi, with tarsomeres II–IV feebly tapering on distal-lateral corners.

Genus *Electrobabinskaia* Lu, Zhang & Liu, 2017
Figs 5–6

Electrobabinskaia Lu, Zhang & Liu, 2017: 20 Type species: *Electrobabinskaia burmana* Lu, Zhang & Liu, 2017: 20 (original designation).

Revised diagnosis. Forewing: RP+MA originated from R nearly at proximal 1/3 of wing. Five presectorial crossveins present. RP densely branched with 6–8 branches, most of which bears a marginal fork. CuA branched on distal half, with 9–10 branches, most of which bears a marginal fork; CuP distally zig-zagged, with 6–8 branches, most of which are simple. A1 simple, proximally approximating CuP stem; A2 and A3 simple. Hind wing: Slightly narrower than forewing, proximal part of wing distinctly narrowed, and wing apex acutely pointed and slightly bended posteriad. Three presectorial cross veins present. RP densely branched, most of which bears a marginal fork. MP1 pectinately branched into 5–6 branches; MP2 with 10 branches, most of them are simple. CuA short, with 5–6 simple branches; CuP and A1 possibly fused into CuP+?A1, short and simple. A2 present. Tarsomeres II–IV semilunar, and gradually shortened, tarsomere V ovoid; arolium present. Abdominal segment VI of female with a pair of long digitiform sternal projections.

Description. ***Female.*** *Body* length 9.83 mm; head 0.60 mm long and 1.32 mm wide; antenna length 6.60 mm; forewing 10.20 mm long and 3.13 mm wide; hind wing 9.51 mm long and 2.34 mm wide; prothorax 0.58 mm long and 0.62 mm wide; mesothorax 1.27 mm long and 1.59 mm wide; metathorax 0.67 mm long and 1.27 mm wide; abdomen length 6.71 mm.

External morphology similar to male. But a simple hind wing A2 present (Fig. 5B).

Abdominal segment VI with specialised sternum VI. Sternum VI subquadrate, posteriorly concaved, laterally with a pair of long digitiform projections, which are slightly longer than major part of sternum VI, slightly sinuated, bearing long setae.

Female genitalia (Figs 5D–F, 6B): Tergum VIII subquadrate; gonocoxite VIII paired, present as narrow ridges; putative gonapophysis VIII present, nearly semicircular. Tergum IX in dorsal view arcuate, distinctly enlarged ventrally; a pair of gonocoxite IX present, broadly valvate. Ectoprocts paired, in dorsal view subtriangular, with large callus cerci; a digitiform sclerite present ventral ectoprocts, putatively subanale.

Additional material. NIGP197966: Amber piece preserving a complete female of *E. burmana* and a midge; it is polished in the form of a flattened elliptical cabochon, clear and transparent, with length × width 18.85 × 21.44 mm, height 7.76 mm. NIGP197967: Amber piece preserving a complete female of *E. burmana*; it is polished in the form of a flattened rectangular cabochon, clear and transparent, with length × width 18.94 × 14.34 mm, height 3.31 mm.

Remarks. Association between male and female of *E. burmana* is based on the similar body length (approximately 10 mm), the nearly identical wing venations, and the similar tarsi with semilune tarsomeres II-IV.

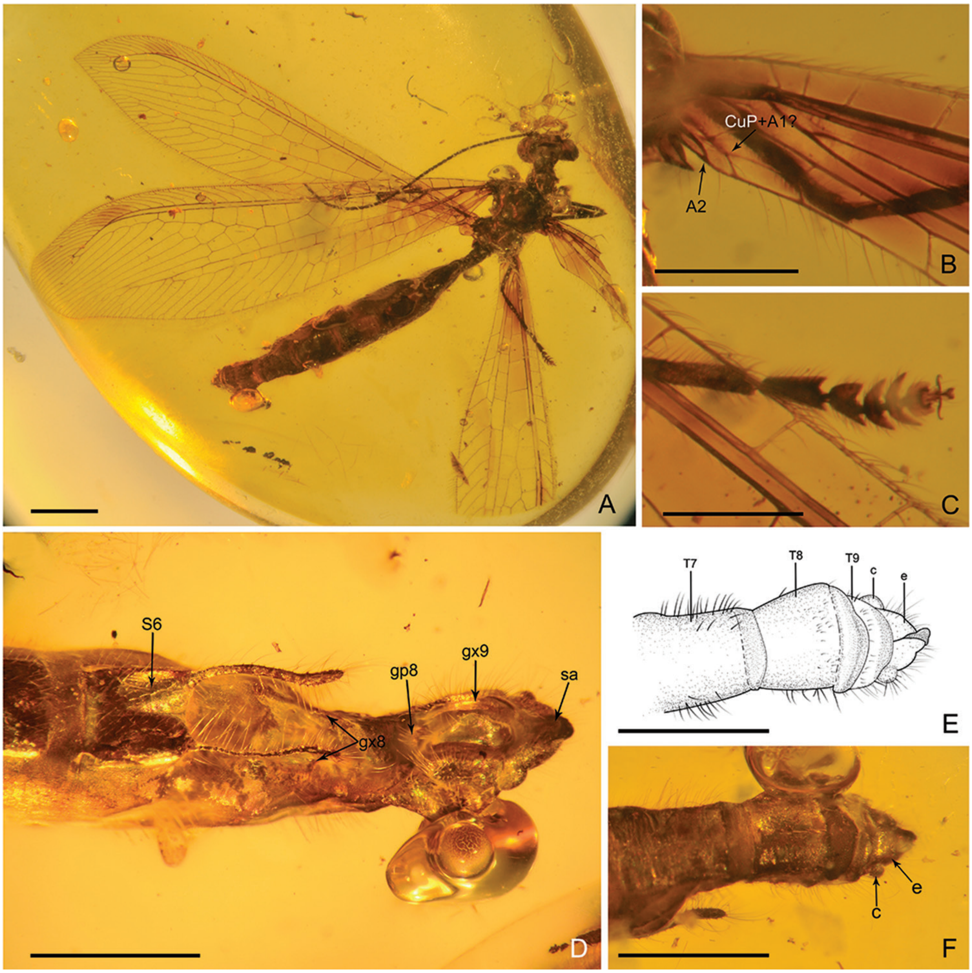

Figure 5. *Electrobabinskaia burmana* Lu, Zhang & Liu, 2017, female. **A** Habitus photograph, dorsal view **B** Photograph of right wing base **C** Photograph of tarsus **D** Photograph of female genitalia, ventral view **E** Line drawing of female genitalia, dorsal view **F** Photograph of female genitalia, dorsal view. Abbreviations: T: tergum; S: sternum; c: callus cercus; e: ectoproct; gp: gonapophysis; gx: gonocoxite; sa: subanale. Scale bars: 0.5 mm (**C**); 1.0 mm (**A–B, D–F**).

Discussion

The present new findings on the Burmese amber babinskaiids provide important information to further understand the morphology and systematics of Babinskaiidae. Makarkin et al. (2017) outlined four apomorphic wing characters to define Babinskaiidae, including the distal origin of RP+MA, the presence of presectorial cross veins in both wings, the single forewing MP, and the reduction of hind wing A2 and A3. The former three characters may be verified as the synapomorphies of Babinskaiidae although the distal origin of RP+MA and the presence of presectorial cross veins are also present

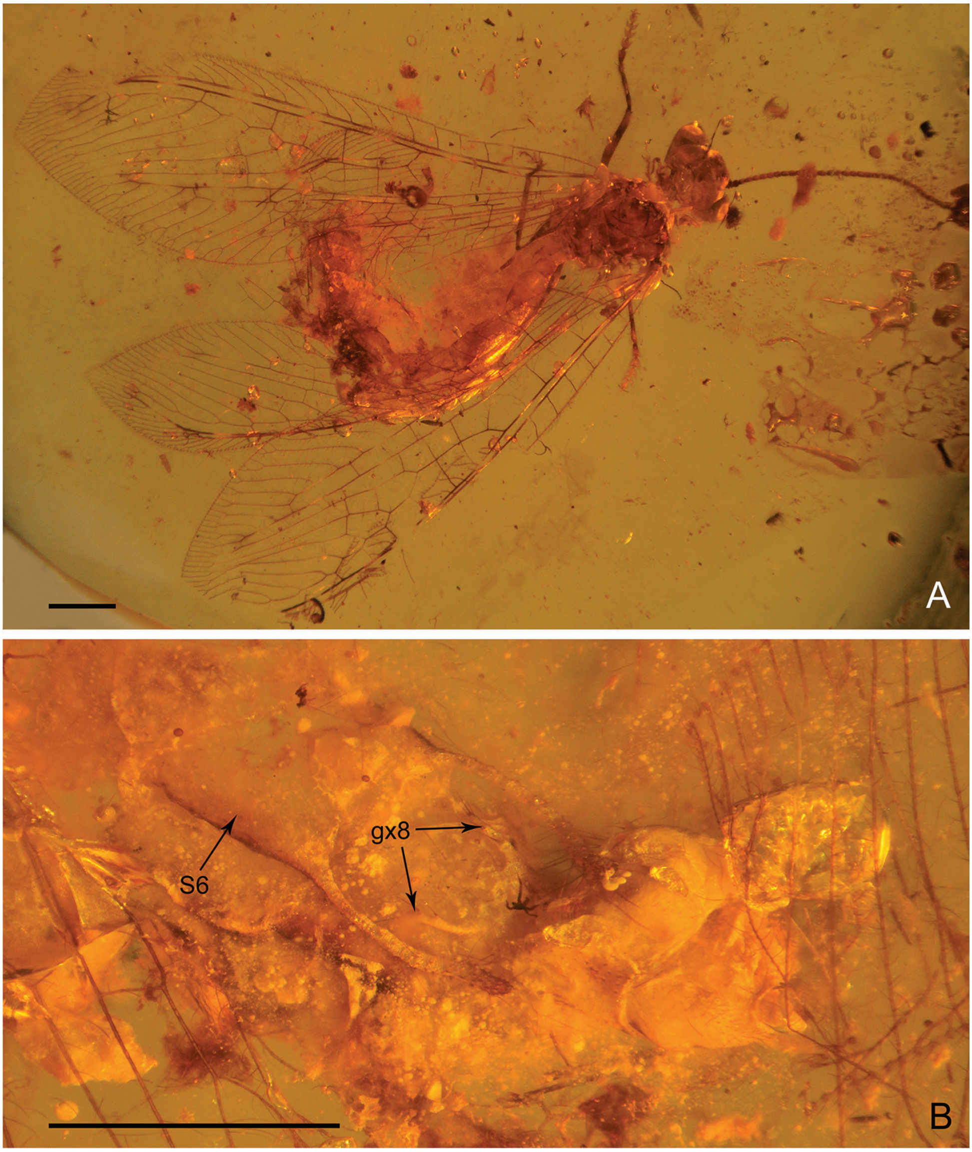

Figure 6. *Electrobabinskaia burmana* Lu, Zhang & Liu, 2017, female. **A** Habitus photograph, dorsal view **B** Photograph of abdomen, ventral view. Abbreviations: S: sternum; gx: gonocoxite. Scale bars: 1.0 mm.

in some lineages of Myrmeleontidae and Ascalaphidae. The long hypostigmal cell is another apomorphic character of Babinskaiidae mentioned in Lu et al. (2017), while it is also present in Nymphidae, Nemopteridae, Palaeoleontidae, and Myrmeleontidae. However, the hind wing A2, possible with A3 merged, is present in *P. makarkini* sp. n. and *E. burmana*, indicating that the reduction of A2 and A3 may not be the autapomorphy of Babinskaiidae as proposed by Makarkin et al. (2017).

The specialised sternum of abdominal segment VI in the female of *E. burmana* is remarkable. In the female of this species there is a pair of long digitiform projections, while such projections are not developed in the conspecific males. In light of the absence of these projections in males, this feature probably functions during courtship or mating although it does not belong to the genital segments. Notably, such modification of abdominal segment VI has never been found in Neuroptera. Previously reported sexually dimorphic features on pregenital segments of abdomen in Neuroptera are only known in males, such as the eversible sacs in some species of Nevrorthidae, Osmylidae and Mantispidae, and the hair pencils in some species of Myrmeleontidae, presumably being involved with chemical communication between sexes (New 1989). However, females of some species of Corydalidae (Megaloptera), e.g., *Protohermes differentialis* (Yang & Yang, 1986), display unusual features on ventral sclerites of abdomen, such as paired long projections on gonocoxites VIII (see Liu and Yang 2006: figs 16–17). In Neuroptera, females of Osmylidae have complex modifications of gonocoxites VIII and gonapophysis IX for grasping males during copulation (Martins et al. 2016). While the above female traits in Babinskaiidae and other groups of Neuropterida are clearly not homologous we cannot exclude that they may similarly function considering their morphological similarities.

Lu et al. (2017) described similar paired projections on sternum VII in the female of *Pseudobabinskaia martinsnetoi* (Lu, Zhang & Liu, 2017). The segmentation of abdomen cannot be clearly observed due to preservation condition in the holotype female of *P. martinsnetoi*. However, based on the position of these projections in respect of whole abdomen, we consider that these projections actually belong to the sternum VI in *P. martinsnetoi* and are homologous with that in *E. burmana*. Thus, *Electrobabinskaia* and *Pseudobabinskaia* might have close phylogenetic relationship by sharing this feature that is apparently apomorphic. In *P. makarkini* sp. n. a specialised female sternum VI is not present, suggesting that this female trait is not a diagnostic character of the whole family but only for some genera of Babinskaiidae.

The female genitalia of Babinskaiidae consist of paired gonocoxite VIII, gonapophysis VIII (at least present in *E. burmana*), paired gonocoxite IX, paired ectoprocts with well-developed callus cerci, and subanale (at least present in *E. burmana*) (see Fig. 7). The paired digitiform lobes in *Pseudobabinskaia*, interpreted as gonocoxites IX in Lu et al. (2017), are verified to be gonocoxites VIII. This feature appears to be similar to that in some antlion genera (e.g., *Nedroledon* Navás, 1914; see Aspöck and Aspöck 2008: fig. 149) although it is likely convergently derived in Babinskaiidae and Myrmeleontidae. In *Electrobabinskaia* and *Parababinskaia*, the female gonocoxite VIII are less modified.

The presence of subanale (or cataprocessus in New 1981) in Babinskaiidae is also noteworthy. The subanale is a small singular sclerite usually present beneath anus. In Myrmeleontoidea sensu Engel et al. (2018) it is previously reported only in Nymphidae (New 1981). Whether the presence of subanale is apomorphic or plesiomorphic is unknown as it is also present in Psychopsidae (New 1988; Bakkes et al. 2017), a phylogenetically basal family in Myrmeleontiformia that comprises Myrmeleotoidea and Psychopsoidea (Engel et al. 2018).

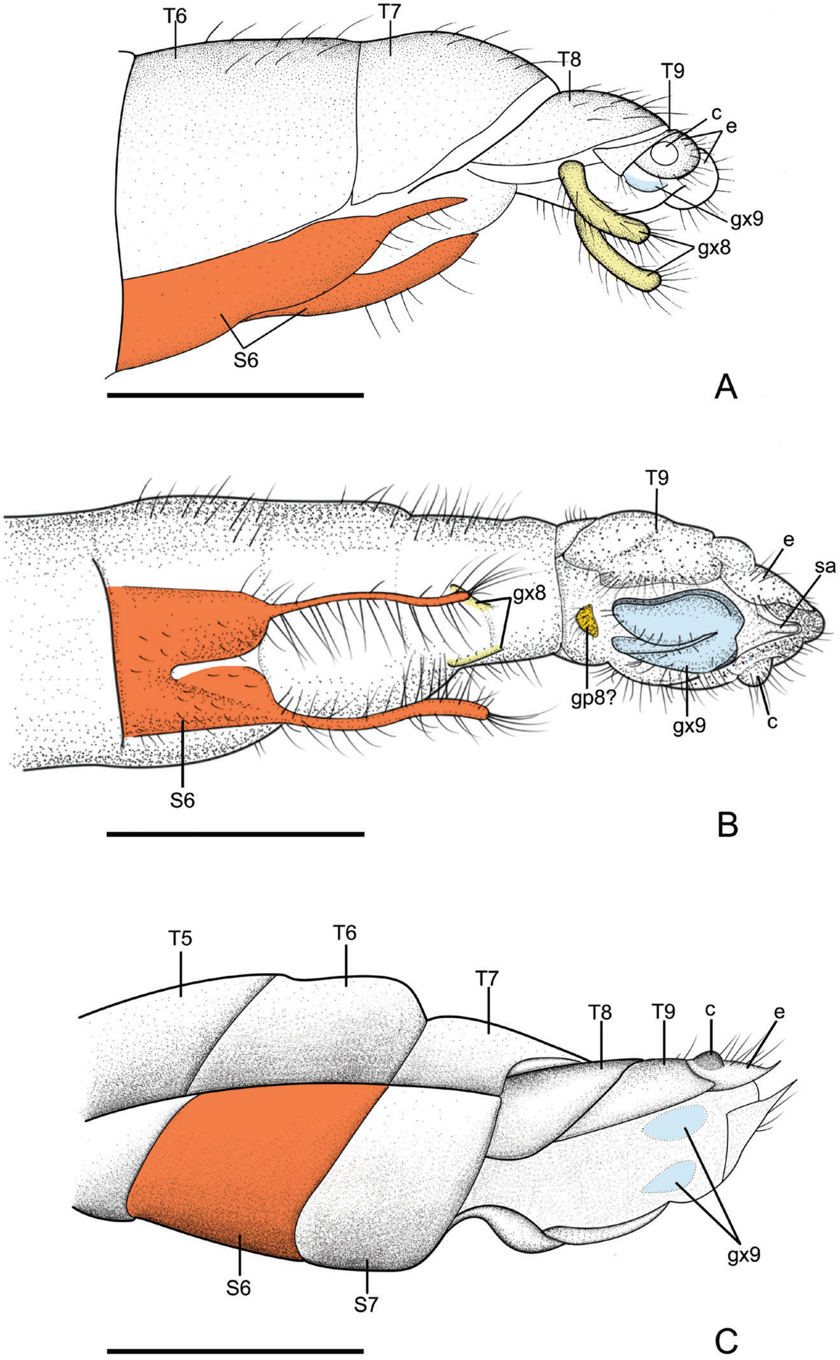

Figure 7. Female genitalia of babinskaiid species from the Burmese amber. **A** Female genitalia of *Pseudobabinskaia martinsnetoi* (Lu, Zhang & Liu) **B** Female genitalia of *Electrobabinskaia burmana* Lu, Zhang & Liu **C** Female genitalia of *Parababinskaia makarkini* sp. n. Abbreviations: T: tergum; S: sternum; c: callus cercus; e: ectoproct; gp: gonapophysis; gx: gonocoxite; sa: subanale. Scale bars: 1.0 mm.

The phylogenetic position of Babinskaiidae appears to be perplexing with mixture of character states that are shared with Nymphidae, Psychopsidae or Myrmeleontidae. Although Makarkin et al. (2017) deemed the sister group relationship between Babinskaiidae and Nymphidae, Engel et al. (2018) placed Babinskaiidae in a crown group within Myrmeleontoidea, together with Ascalaphidae and Mymeleontidae. In addition, it should be mentioned that the phylogenetic position of Nymphidae is still controversial. Many phylogenetic studies (Winterton et al. 2010; Wang et al. 2017) suggest the basal most position of Nymphidae in Myrmelontoidea sensu Engel et al. (2018). However, a phylogenetic study based on anchored hybrid enrichment data (Winterton et al. 2018) assigned Nymphidae to be the sister group of Ithonidae that is traditionally considered not to be the member of Myrmeleontiformia. Our new finding provides more knowledge on the morphology of Babinskaiidae. However, phylogenetic analysis combining fossil and extant families of Myrmeleontiformia is required in future studies to further elucidate the phylogenetic position of this enigmatic extinct lacewing family.

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We thank Mrs. Xiao Jia for kindly offering materials of Burmese amber lacewings for our study. This research was supported by the National Natural Science Foundation of China (Nos. 31672322, 41572010, 41622201, 41688103) and Beijing Natural Science Foundation (No. 5162016). We thank Dr. Shaun L. Winterton and four anonymous reviewers for their valuable comments which improved the manuscript.

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On a new species of *Micrambe* from Africa (Coleoptera, Cryptophagidae)

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Abstract

A new species of *Micrambe* Thomson, 1863 (Coleoptera, Cryptophagidae), *Micrambe camerunensis* **sp. n.** from Cameroon is described and illustrated. No other record of any Cryptophagidae of Cameroon is known. The differential diagnosis is established in relation to a group of other species of the genus.

Keywords

Cameroon, *Micrambe camerunensis* sp. n., new species, taxonomic key, taxonomy

Introduction

The African fauna of *Micrambe* is significantly rich in species, although it is scarcely known. Predictably, as the study of its fauna continues, the number of species will rise significantly. Coombs and Woodroffe (1962) suggest that according to the size of the aedeagus, the African species constitute a phylogenetic group different from the Palearctic ones. A large number of species in South Africa were examined and no significant differences were found in that character. On the contrary, more variability (in relation to the Palearctic and

Oriental species) was noticed in some morphological characteristics (pubescence, size, and shape of the eyes, etc.) and they present some exclusive characters such as setae in the last abdominal ventrite, protuberance in the margins of the aedeagus, etc. (Otero 2012).

The aim of this account is to contribute to the knowledge of Cryptophagidae from Africa. The study of abundant material of the genus *Micrambe* (Coleoptera, Cryptophagidae) from different museums suggests that the knowledge of this family in Africa needs to be updated. The study of numerous specimens borrowed from BMNH has allowed us to describe the new species, *Micrambe camerunensis* sp. n.

Methods

The terminology and the measurements of the new species follow Otero (2005, 2011, 2012, 2017). Structures were measured under a Leica M205C stereomicroscope equipped with an Application Suite analysis system. Acronyms: **L** – length; **WL** – width/length ratio; **E** – eccentricity of the eyes (width/half of the length). The width is measured across the widest part of a line joining the anterior and posterior limit of the eye. Length is the maximum length of the eye. **L** is used for length in dorsal view, **W** for width, and **Ø** for diameter.

Institutional abbreviations

BMNH	British Museum of Natural History, London, United Kingdom;
MHNG	Muséum d'Histoire Naturelle, Genève, Suisse (coll. Y. Gomy);
MNHN	Museum National d'Histoire Naturelle, Paris, France;
SMNS	Staatliches Museum fur Naturkunde, Stuttgart, Germany;
MSNF	Museo di Storia Naturale, Firenze, Italy (coll. Bartolozzi);
NHMW	Naturhistorisches Museum Wien, Vienna, Austria;
RMCA	Royal Museum Central Africa, Tervuren, Belgium;
TMSA	Transvaal Museum, Pretoria, South Africa.

Taxonomy

Micrambe camerunensis sp. n.

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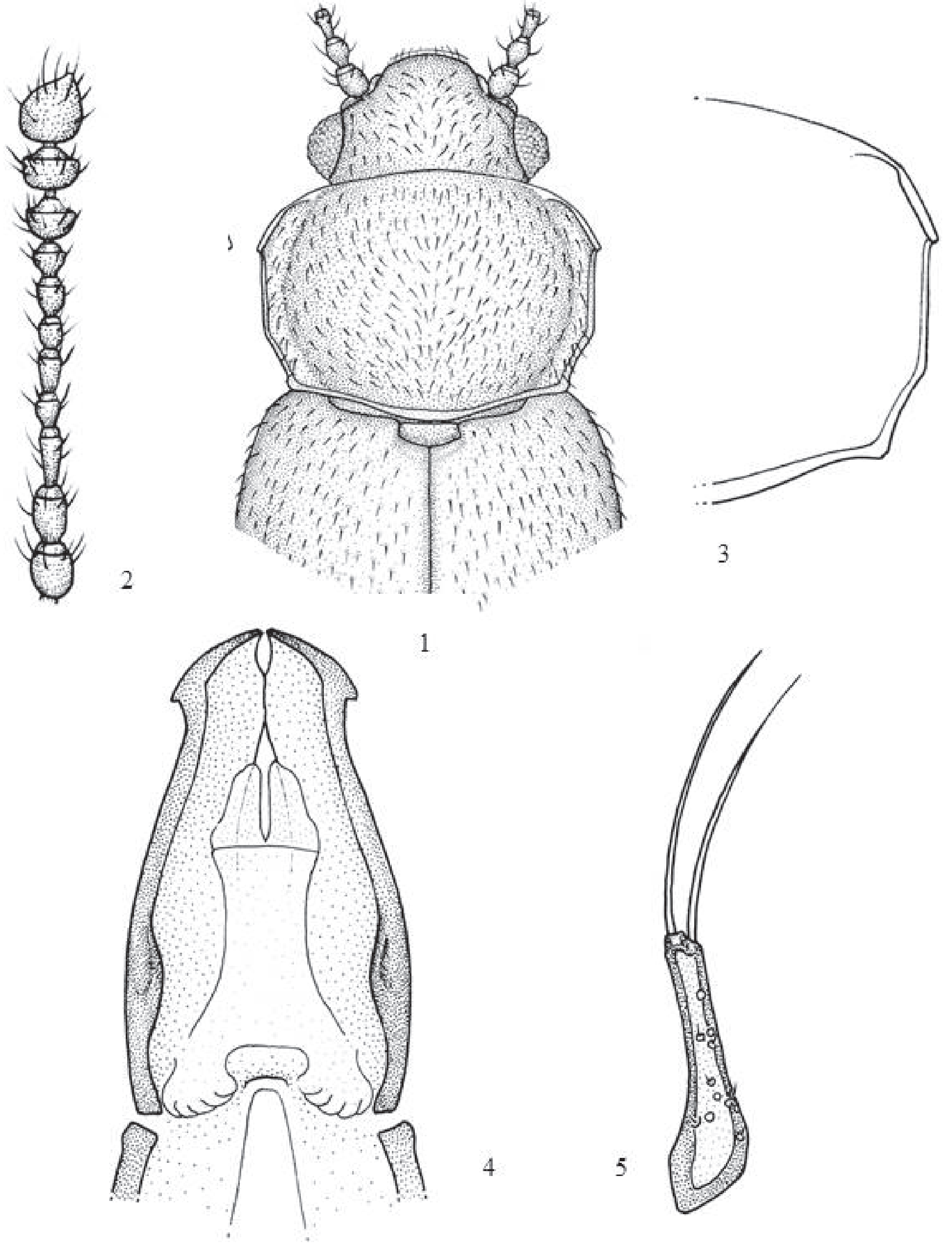
Figures 1–5

Material examined. "Holotype m*. **CAMEROON**. Mt Cameroon, Ist. Plateau, 12.I.1932 /10.000–12.000 ft (Leg. M. Steele)/B.M. 1934-240 (placed in BMNH)"//"Paratype, 5 m*m* and 3 f*f*, same locality, date and legtor as Holotype"// "1 f*, Mt Cameroon, Highest Point/13,360 ft (leg. M. Steele), B.M. 1934-240"//"1 f*, Mt Cameroon, Mann's Quelle/7,400 ft, 3.II.1932 (leg. M. Steele), B.M. 1934-240".



Figures 1–5. *Micrambe camerunensis*: **1** General view **2** antenna **3** pronotum **4** aedeagus **5** paramere.

Diagnosis. Morphologically, *Micrambe camerunensis* is very similar to other *Micrambe* in many external features, but can be distinguished by the configuration of the male genital apparatus.

Description. Length: 1.7–1.9 mm. Body oval, elongated and convex. Reddish grey-brown; appendages and first antennal articles yellowish grey-brown. Pubescence simple, short (L= 0.025–0.040 mm) and flattened. Metathoracic wings absent.

Transverse *head* (WL = 1.9–2.1). Punctation well -marked and dense; distance between punctures shorter than their diameter (Ø = 0.014–0.016 mm). Normal eyes (L = 0.127 mm), hemispherical or sub-hemispherical and protruding (E = 1.1–1.2). Eye facets smaller (Ø = 0.012 mm) than head punctures. Short antennae (Fig. 2) (L = 0.580 mm), not surpassing the base of the pronotum. 1st antennomere spherical; 2nd and 3rd as long as 1st but narrower; 4th and 6th 1.8 times shorter than 3rd; 5th 1.3 times longer than 4th; 7th sub-squared and 1.1 times as long as 6th; 8th transverse and as long as 7th; 9th and 10th equally long and strongly transverse; 11th elongated.

Pronotum (Figs 1, 3) slightly transverse (WL = 1.5). Callosity oval, elongated, large (1/4 times as long as side); not surpassing the lateral margin of the pronotum. Callosity margin strong. Callosity side not visible from above. Gland pore present but not visible. Callosity not angled rearwards but forming a 38.33°–39° angle with the body axis. Lateral margins parallel from the callosity to the basal quarter and converging from there to the base. Posterior angles obtuse. Basal groove reduced. Basal foveae not visible. Punctation well -marked and dense; distance between punctures shorter than their diameter (Ø = 0.016–0.018 mm).

Elytra three times as long and 1.2 times as wide as pronotum. Punctation more dispersed than on pronotum; distance between punctures greater than their diameter (Ø = 0.016–0.018 mm).

Mesosternum with a narrow medial area, strongly concave, with sides slightly lifted, curved and converging towards a weakly emarginate apex.

Tarsal formula 5-5-5 in males and 5-5-5 in females.

Aedeagus (Fig. 4) apically expanded and narrowed in anterior third. Ventrally, lateral margins showing strong protuberances in basal third. Endophallic orifice visible in the basal third of the aedeagus. Preputial sac comprising two membranous lobes. Endophallic armature made up of small spines. Long and narrow parameres (Fig. 5). Scarce pores with or without setae. Two apical setae as long as paramere.

Biology. On moss.

Etymology. Derived from Cameroon, where the type locality of this new species is found.

Key of the *Micrambe alluaudi* group from Africa

At the start of our investigation we were soon satisfied that a number of *M. johnstoni* (Scott) *M. helichrysi* Scott, and *M. alluaudi* (Scott) could be reliably recognised on external characters: body oval, elongated and moderately convex. Simple, short, recumbent, and whitish pubescence. Pronotum slightly transverse, sub-square, or moderately transverse. Callosities, oval, elongated (1/3 of the side length), visible from above, generally obliquely cut, not protruding from the lateral margin of the pronotum. Aedeagus apically expanded. Strong callosity in the basal third of the lateral margin. Very small, triangular parameres. Provided with three or four apical setae longer than the paramere. Paramere arms very dilated distally. Atypical forms occur, and cannot be distinguished except on parameres. Key is incorporated below:

1 Tarsal formula 5-5-5 in both sexes **2**

– Tarsal formula 5-5-4 in males and 5-5-5 in females. Dark grey-brown; many specimens reddish grey-brown along the suture and base of the pronotum; antennae and legs yellowish grey-brown. Pronotum (Fig. 12) little transverse or sub-squared (RD= 1.4). Lateral margins parallel from the callosity to shortly after the middle; next, converging towards the base. Aedeagus (Fig. 13). Parameres (Fig. 14). Length: 2.1–2.2 mm .... ***johnstoni* (Scott)**

2 Uniformly dark grey-brown; in some species the base of the elytra and the pronotum side are reddish; testaceous legs and antennae; dark antennal mace **3**

– Variable in colour, elytra usually dark grey-brown (sometimes with a more or less yellowish grey-brown spot along the suture; pronotum yellowish grey-brown; the head may be the same colour or dark although some specimens may be entirely yellowish grey-brown or dark grey-brown. Lateral margins parallel from the callosity to the basal third and from there converging towards the base (Fig. 9). Aedeagus (Fig. 10). Parameres (Fig. 11). Length: 1.9–2.3 mm... ***helichrysi* (Scott)**

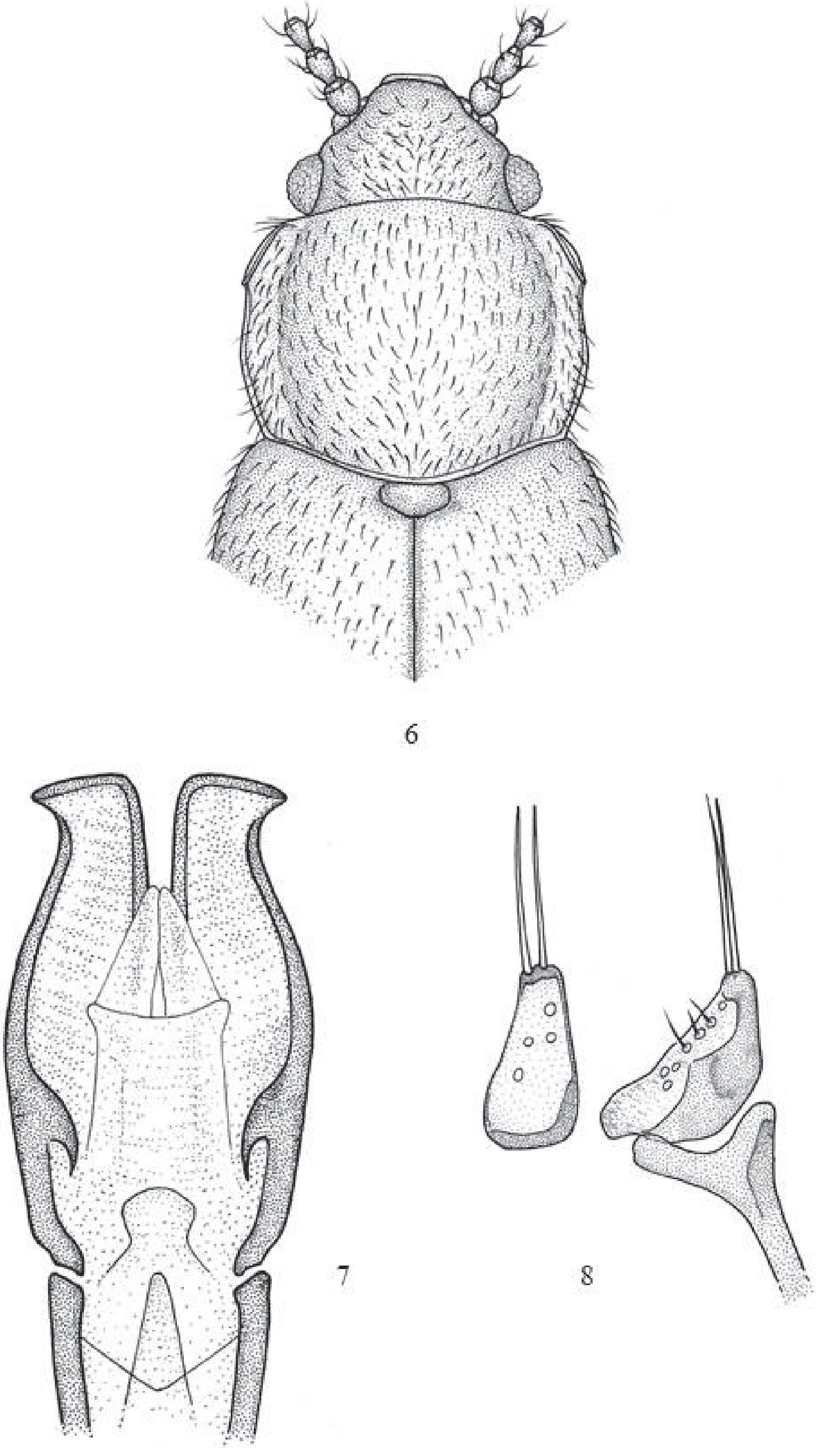
3 Pronotum (Fig. 6) little transverse, sub-squared (RD= 1.3). Large callosities (1/3 of side length). Lateral margins parallel from the callosity to shortly after the middle and from there converging towards the base. Aedeagus (Fig. 7). Parameres (Fig. 8). Length: 2.3–2.4 mm............................ ***alluaudi* (Scott)**

– Pronotum (Figs 1, 3) little or moderately transverse (RD= 1.5). Smaller callosities (1/4 of side length). Lateral margins parallel from the callosity to the basal quarter and from there converging towards the base. Aedeagus (Fig. 4). Parameres (Fig. 5). Length: 1.7–1.9 mm...................... ***camerunensis* sp. n.**

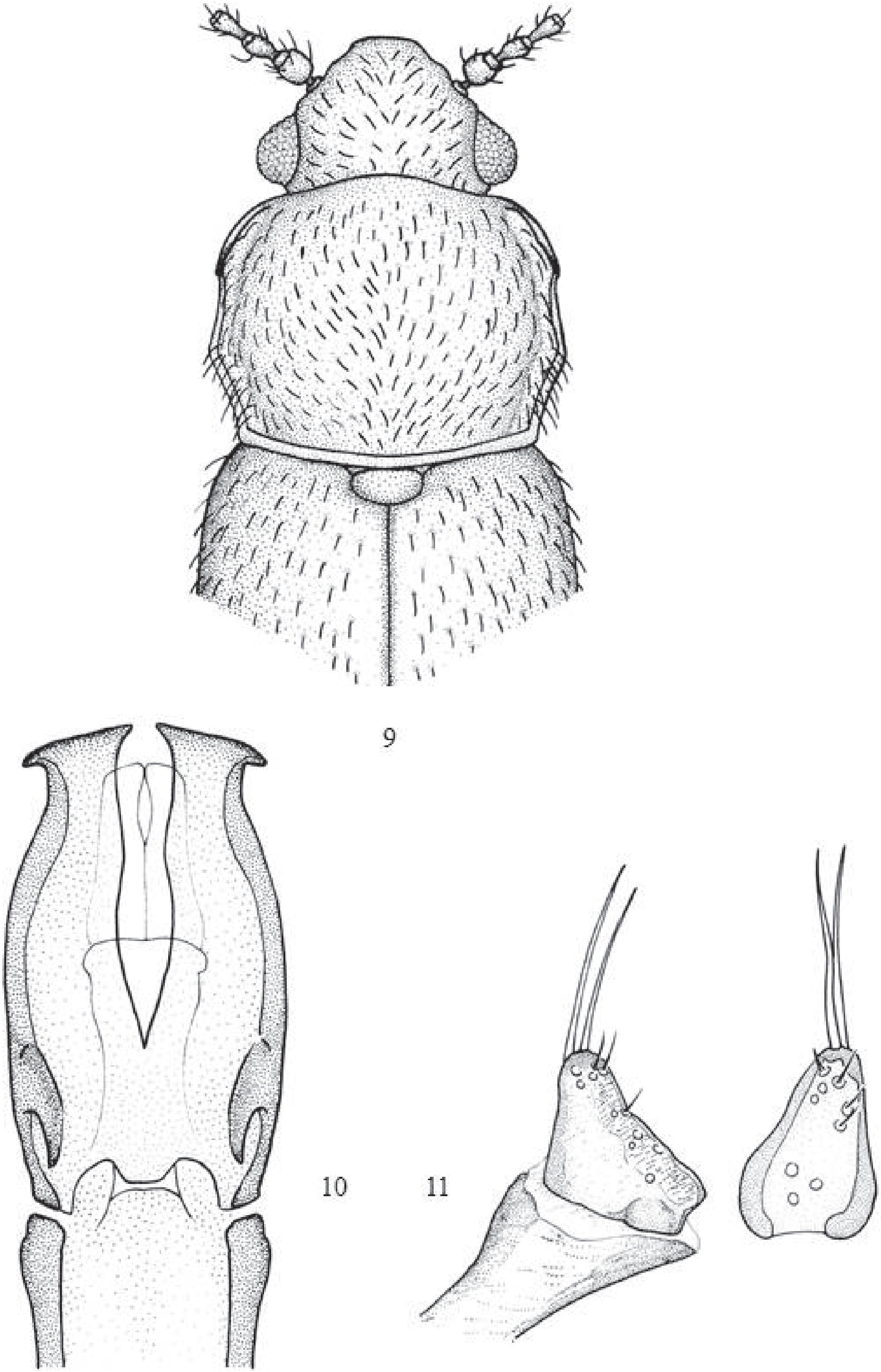
Discussion

Coombs and Woodroffe (1962) analyse the characteristics of the genus *Micrambe* from East Africa and designate the constant differences of their aedeagus with the species of paleartic dispersion. According to these authors, there is a “type of palaeartic aedeagus” that could be represented by that of *M. ulicis* (Stephens) and an African type that presents as characteristics: the apical expansion of the aedeagus present in few paleartic species and pronounced callosity in the basal third of the lateral margin. This type of aedeagus appears in the majority of the species that are distributed, throughout Eastern Africa, from Cap to Egypt. A third type, is present in a few species and could be represented by *M. alluaudi* (Scott).

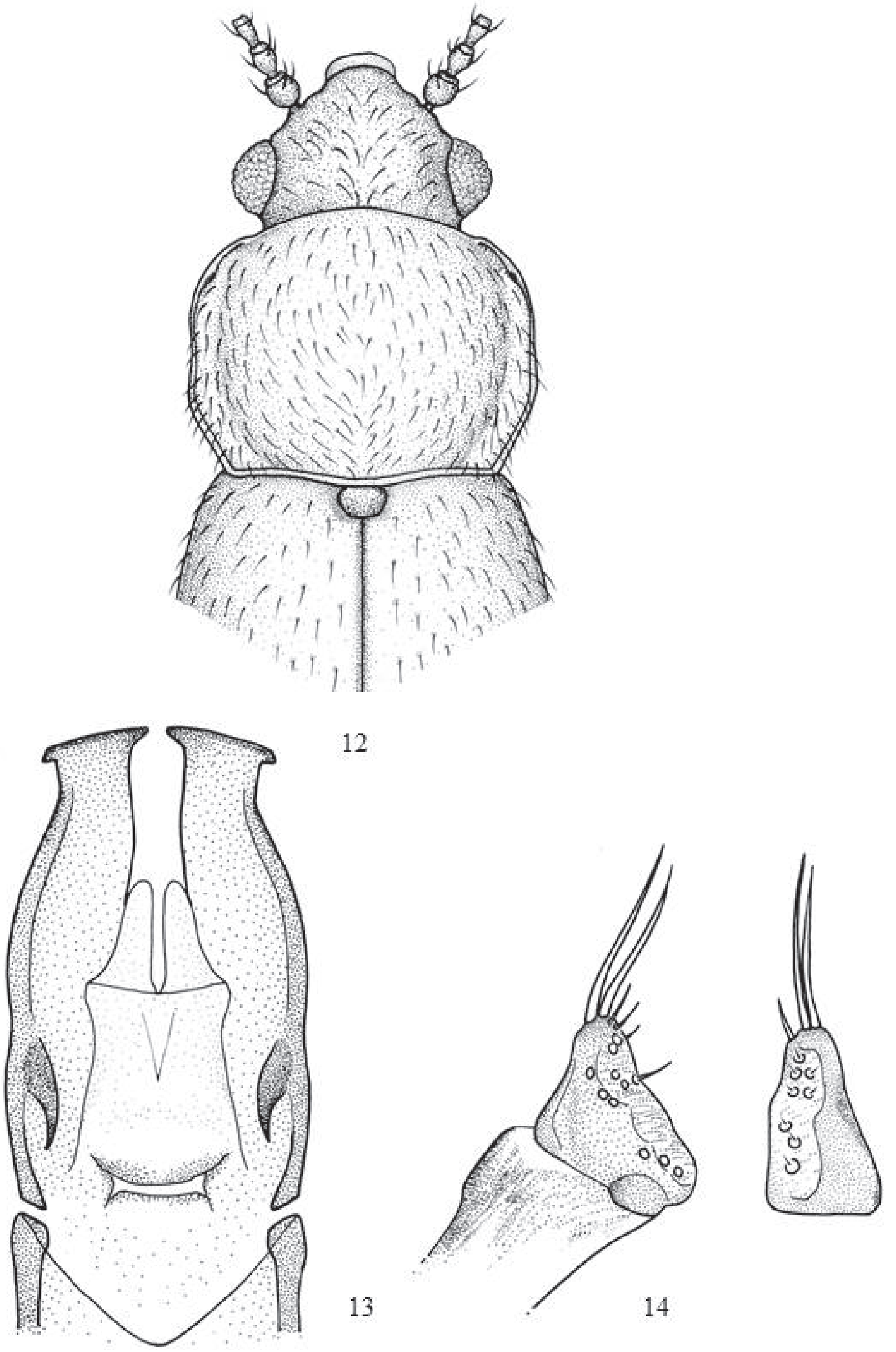
In Africa there is a significant fauna rich in species of *Micrambe*, that is distributed from Egypt to Cap (South Africa). This extensive mountainous region, throughout



Figures 6–8. *Micrambe alluaudi*: **6** General view **7** aedeagus **8** paramere dorsal and lateral view.



Figures 9–11. *Micrambe helichrysi*: **9** General view **10** aedeagus **11** paramere lateral and dorsal view.



Figures 12–14. *Micrambe johnstoni*: **12** General view **13** aedeagus **14** paramere lateral and dorsal view.

East Africa, and like other families of Coleoptera (Jeannel 1942), could be populated by native species, from the two extremes, austral and paleartic, and the orophiles. During the quaternary period the continuity of the mountain chains broke. After the decline of volcanic activity and erosion, the mountain ranges became true islands of alpine climate, isolated in the midst of the tropical climate oceans (Jeanel 1942). In these mountains, a small number of species from the south they developed in a cold climate and have therefore been relegated to the high mountains (Rwenzori, Mount Elgon, Kenya, and Kilimanjaro) in the equatorial zone, between 3,300 m and 4,400 m, thus showing a discontinuous distribution. Their habitat is restricted exclusively to different species of Lobelia sp., Senecio sp., and Helycrysum sp. (Bruce 1952, 1960; Grouvelle 1908; Scott 1935) in which they cohabit with other coleopteran species. The forms that populate it in general are brachythera forms, bicolor or uniformly dark. They have a very small parameres, not flattened (Fig. 5) and subject to the aedeagus by arms distally very dilated (Fig. 4).

The group of thermophilic species, possibly derived from eastern lines, are distributed by and from this mountain chain towards the west, by the great equatorial forests and, therefore, have a continuous distribution. Among them, *Micrambe camerunensis*, species that shows an external morphology similar to *M. alluaudi* and that it is only possible to differentiate it by the configuration of the male genital apparatus.

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RESEARCH ARTICLE

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A new species of *Diaphorocera* from Morocco with unclear relationships and a key to the species (Coleoptera, Meloidae, Cerocomini)

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Abstract

Diaphorocera neglecta **sp. n.** from Morocco is described. Photos of the new species are provided and male features are figured. The new species has intermediate characters between the groups of *D. hemprichi* and *D. promelaena* as defined in the literature. These groups are discussed and a new key to the species is presented.

Keywords

Coleoptera, *Diaphorocera neglecta* sp. n., key, relationships, Sahara

Introduction

Diaphorocera Heyden, 1863 is a Saharo-Sindian genus, most diverse in the western Sahara Desert and belonging to the tribe Cerocomini, family Meloidae. The phylogenetic relationships within the tribe were defined on the basis of both morphological and molecular evidence by Turco and Bologna (2008), who pointed out the affinities of

Diaphorocera with the eastern African genus *Somalarthrocera* Turco & Bologna, 2008. The genus was revised by Turco and Bologna (2007) who also summarised the distribution and bionomics of each species.

Diaphorocera is characterised by a metallic green-blue body, but with legs, antennae and mouthparts being yellow-orange in most species. Male antennae are extremely modified, as in other genera of the tribe and composed of eleven antennomeres. Turco and Bologna (2007) considered *Diaphorocera* and the genus *Anisarthrocera* as primitive elements within the tribe, both being strictly adapted to eremic habitats.

After the descriptions of some species in the XIX century, Kocher (1954) described *D. peyerimhoffi* from Morocco, while Kaszab (1983) described *D. johnsoni* and *D. hemprichi saudita*, both from the eastern part of the Arabian Peninsula. Subsequently, Batelka (2004) redescribed *D. carinicollis* Chobaut, 1921 from eastern Algeria and south Tunisia. In their revision, Turco and Bologna (2007), examined types of all taxa, synonymised *D. kerimii* Faimaire, 1875 with *D. chrysoprasis* Fairmaire, 1863, and redescribed the eighth species.

The aims of this paper are to describe a new species from eastern Morocco, *D. neglecta* sp. n., the ninth species in the genus, showing intermediate characters between the groups of *D. hemprichi* Heyden, 1863 and *D. promelaena* Fairmaire, 1876 , as defined in the literature and to design a new key to the species.

Materials and methods

Available specimens were examined using stereomicroscopes and measurements were taken with an ocular grid. The total length of the examined specimens is measured from the anterior apex of mandibles to the apex of elytra. Proportions of antennomeres were taken on holotype male measurements. Male genitalia were dissected and glued onto a paper label.

Photographs were takenby an Olympus OM-D E-M5 camera equipped with a macro lens Olympus M.Zuiko Digital ED 60 mm f/2.8 Macro.The type specimens are labelled with a printed red rectangular label: "HOLOTYPUS or PARATYPUS respectively, *Diaphorocera neglecta* sp. n., M. A. Bologna & L. Černý des. 2016". All specimens are glued onto paper labels.

Examined specimens are preserved in the following collections:

CB	M.A. Bologna collection, Museum of the Department of Sciences, University Roma Tre, Roma, Italy;
JMBC	The South Bohemian Museum in České Budějovice, Dukelská 1, CZ-370 51 České Budějovice, Czech Republic;
LCCC	L. Černý collection, České Budějovice, Czech Republic.

Taxonomic part

Diaphorocera neglecta sp. n.

http://zoobank.org/F63001C5-17E1-44C4-B39A-29C1E65D4EDA

Type material. Holotype ♂ (CB), Paratype ♂ (LCCC), Paratypes ♀♀ (CB, JMBC), all labelled Morocco SE, W of Erfoud, 31°30'53,2"N, 004°35'12"W, 26.–27.4.2012, L. Černý lgt. [printed].

Type locality. Eastern Morocco, W of Erfoud, 31°30'53,2"N, 004°35'12"W, 26.–27.4.2012.

Diagnosis. A *Diaphorocera* species characterized by male elongate last antennomere and simple fore tibiae, with head, pronotum and ventral parts dark shiny metallic blue.

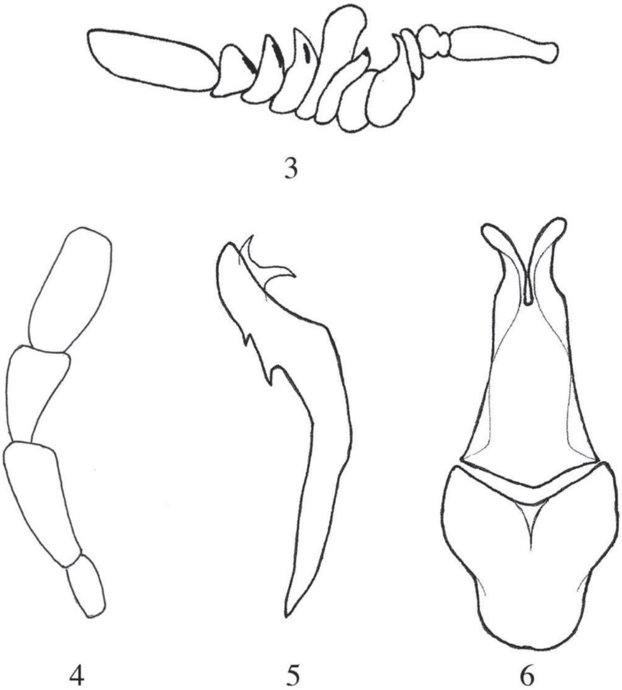
Description. *Male* (Fig. 1). *Body.* Head, pronotum, thorax, abdomen distinctly dark blue metallic, elytra green-blue metallic; coxae, trochanters, femurs, tibiae, and tarsi orange, but metatarsi darker and mesotarsi slightly darkened. Body and leg setation clear yellowish and in some parts almost white. Length: 9.8–9.9 mm. *Head* with punctures sparse, wide, and deep, surface among punctures smooth and shiny. Maximum width at level of eyes and temples, temple parallel, as long as the eye. Frontal callus smooth, neither keeled nor anteriorly protruding, in lateral view only few convex. Antennae as in Fig. 3; antennomeres I–XI orange, VIII–X with a single, linear and short black stripe on the apical portion of each antennomere, middle in position on VIII-IX, more basal on X; the extreme pointed apex of IV and VI black; XI very elongate, 2.8 times long as wide; X ca. 2/3 as long as XI. Mouthparts orange except the apical third of mandibles black; maxillary palpomere as in Fig. 4, last palpomere dark, as labial palpomeres; labrum ca. 3 times as long as clypeus and with a middle furrow dorsally. *Pronotum* narrow and parallel on the basal half, distinctly longer than wide, anteriorly restricted and with two lateral grooves on sides. Surface with punctures similar to those on the head, sparse but deep, surface among punctures smooth, shiny, and a longitudinal non-punctured area in the middle extending from the base to the anterior third of the length. Elytra with surface densely and coarsely punctuated, punctures merging together. Foretibiae simple and not modified; metatibial outer spur widened and spoon-like. *Male genitalia* as in Figs 5–6; aedeagus inclined on fore third, with two short and pointed hooks, hooks of the endophallic duct bent differently; lobes of parameres diverting and obtuse apically.

Female (Fig. 2). *Body* colouration similar to male. Antennae unmodified, length of antennomere XI equal to that of previous three together, antennomeres III–X transverse, wider than long (see Fig. 2). Lack lateral grooves of pronotum. Protibiae distinctly pointed and acuminate on external fore angle. Length: 12.5 mm.

Etymology. Following the recent revision of the genera (Turco and Bologna 2007) and due to the rarity of this *Diaphorocera* species, we named it *neglecta* (= neglected, ignored) according to the Latin adjective (feminine in the nominative case).

Figure 1–2. *Diphorocera neglecta* sp. n., **1** holotype male and **2** paratype female.

Systematic remarks. Turco and Bologna (2007) established three groups of species within the genus based on a cladistic analysis: (i) the *obscuritarsis* group (*carinicollis, johnsoni, obscuritarsis*) with the last male antennomere subquadrate and unmodified simple foretibiae; (ii) the *promelaena* group (*promelaena*) characterised by the last antennomere elongate, male foretibia modified only distally and by black body and pronotum; (iii) the *hemprichi* group (*chrysoprasis*, *hemprichi*, *sicardi*, *peyerimhoffi*) with the last antennomere elongate and foretibiae modified.



Figures 3–6. *Diphorocera neglecta* sp. n., **3** male antenna **4** male maxillary palpomeri **5** male aedeagus in lateral view **6** gonoforceps and gonocoxal piece in ventral view.

The new species differs from all described species due to the combination of characters mixed between those of the *promelaena* and *hemprichi* groups. *D. neglecta* has the last antennomere elongate as in both groups but the fore tibiae are simple, differently than in these groups; both these characters represent a plesiotypic condition. It is distinct from *D. promelaena* because its head, pronotum and ventral parts are dark metallic blue and not sub-opaque black. Moreover its pronotum is slender, longer than wide rather than as long as wide, and the black ornamentations on antennomeres VIII-X being lacking in *D. promelaena*.

Diaphorocera neglecta is more similar to the species of the *hemprichi* group because of the body colouration, the shape of pronotum and antennae, and probably represent a basal element without foretibial modification. It differs from all species of this heterogeneous group because of the shape of almost all antennomeres and of male genitalia; in particular the ventral shape of gonoforceps are slightly similar to that of *D. hemprichi.*The ornamentation on antennomeres is present only in *D. sicardi*, but differs greatly from that of this species which moreover is clearly distinguished by its foretibial shape.

Tentatively the new species is inserted in the group of *hemprichi*, as basal in position, although its relationship needs to be tested by molecular analysis and the possible relationships with the group of *D. promelaena* explored.

Figure 7. Habitat of the type locality of *Diaphorocera neglecta* sp. n.

Ecological remarks. All specimens were collected in a small area with many plants in bloom at the bottom of a dry river bed (wadi) at 872 m a.s.l., together with other species of Meloidae, namely *Diaphorocera promelaena*, *Mylabris* (*Ammabris*) *myrmidon* Marseul, 1870, *Hycleus saharicus* (Chobaut, 1901), *H. novemdecimpunctatus* (A.G. Olivier, 1811), *Actenodia suturifera* (Pic, 1896), *Croscherichia litigiosa* (Chevrolat, 1840), *Lyttolydulus nubeculosus* Kaszab, 1952, *Cabalia rufiventris* (Walker, 1871) and *Lydomophus* sp. (*chanzyi* (Fairmaire, 1876) ?), all typical Saharan elements.

A second attempt to collect further specimens of this species in the same locality (on 22 April 2017) failed, probably due to much drier conditions.

Distribution. The new species was collected only in the type locality (Fig. 7), but since the local desert environment is widely and homogeneously spread, its occurrence in other eremic habitats of the eastern Morocco is possible.

In E Morocco and SW Algeria, in localities close to Erfoud, six species of *Diaphorocera* are distributed (Turco and Bologna 2007); *D. promelaena* is syntopic with the new species. No differences in the ecological niche among these six species have been identified in the literature, all of them being synchronic and polyphagous.

Key to the *Diaphorocera* species (modified from Turco and Bologna 2007)

Male

1 Antennomere XI subquadrate. Foretibiae simple.. **2**

– Antennomere XI elongate. Foretibiae variously modified or simple in one species.. **4**

2 Two black and shiny lines on antennomere XI and one on antennomere X.. .... ***D. obscuritarsis***

– Antennomeres X-XI without lines **3**

3 Antennomere VII distinctly wider than VI and slightly narrower than VIII; antennomere I dark. Pronotum slender, anterior portion distinctly narrower than temples; anterior grooves only weakly developed. External margin of elytra only slightly sinuate .... ***D. johnsoni***

– Antennomere VII ca. 1/3 wider than VI and as wide as VIII; antennomere I yellow. Pronotum robust, anterior portion only slightly narrower than temples; anterior grooves deep. External margin of elytra posteriorly greatly sinuate .... ***D. carinicollis***

4 Head, pronotum, abdomen and antennomere I black .... ***D. promelaena***

– Head, pronotum, abdomen and antennomere I not black **5**

5 Frontal calli with a dorsal keel anteriorly protruded and pointed; fore tibiae with a basal inflated expansion **6**

– Frontal calli neither keeled nor anteriorly protruding; fore tibiae simple or with a laminar expansion, at least on the external side **7**

6 Antennomeres VIII-XI with black lines and spots, distal half of X wide, ca twice as wide as that of IX, VII obliquely truncate at apex, incision of distal portion of IV narrow .... ***D. sicardi***

– Antennomeres VIII-XI without black lines and spots, distal half of X narrow, as wide as that of IX, VII bilobed at apex, incision of distal portion of IV wide .... ***D. peyerimhoffi***

7 Fore tibiae simple. Antennomere X only 1/3 as wide as the length of XI .... ***D. neglecta***

– Fore tibiae greatly modified. Antennomere X wider than 1/3 the length of XI.... **8**

8 Antennomere X about as wide as the length of XI, anterior portion slender and pointed at apex; IX about as wide as VIII .... ***D. hemprichi***

– Antennomere X distinctly narrower than the length of XI. Anterior portion of antennomere IX wide and apically truncate; IX narrower than VIII .... ***D. chrysoprasis***

Female

1 Antennomere XI subquadrate **2**

– Antennomere XI elongate **4**

2 Fore and middle tarsomeres IV-V dark, basal segments yellow; trochanters only slightly dark .... ***D. carinicollis***

– Tarsomeres and trochanters dark, or fore tarsomere I light at base **3**

3 Labrum completely dark; temples slightly diverging posteriad, maximum width of head on temples. Femurs and tibiae orange-red .... ***D. obscuritarsis***

– Labrum dark with the anterior margin orange; temples parallel, maximum width of head on eyes. Femurs and tibiae yellow .... ***D. johnsoni***

4 Head and pronotum black .... ***D. promelaena***

– Head and pronotum metallic **5**

5 Coxae and trochanters black .... ***D. peyerimhoffi***

– Coxae metallic, green or bluish; trochanters yellow **6**

6 Body blue, but fore coxae yellow .... ***D. sicardi***

– Body and fore coxae green or green-blue metallic **7**

7 Head capsule transverse, about as wide as long; antennomere XI more than three times as long as the width of X .... ***D. neglecta***

– Head capsule slender, longer than wide; antennomere XI less than three times as long as the width of X **8**

8 Temples elongate, about as long as the eye length, with subparallel sides .... ***D. chrysoprasis***

– Temples shorter than the eye length, narrowing evenly posteriad **9**

9 Pronotum distinctly narrow. Head and pronotum punctures usually sparse and shallow, surface among punctures shiny, almost smooth. Temples in dorsal view squared and slightly longer; in lateral view the edge between vertex and occiput sharper. Antennomere VIII ca. as long as wide and more squared .... ***D. hemprichi saudita***

– Pronotum parallel but less narrow. Head and pronotum punctures usually dense and deep, surface among punctures shagreened. Temples in dorsal view more rounded and shorter; in lateral view the edge between vertex and occiput more rounded. Antennomere VIII wider than long and more trapezoidal .... ***D. hemprichi hemprichi***

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CHECKLIST

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Amphibians and reptiles of the state of Durango, Mexico, with comparisons with adjoining states

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Abstract

A summary of the species of amphibians and reptiles of Durango, as well as their geographic distributions, habitat, and conservation status have been compiled. The herpetofauna of Durango consists of 36 species of amphibians and 120 species of reptiles. Durango shares the most species with Chihuahua (74.0%), and shares fewer species with Sinaloa (48.0%), Nayarit (48.7%), and Coahuila (48.0%). Arid-semiarid and Sierras habitat types have the most species, with valleys and Quebradas habitat types having fewer species. In Durango, there are several taxa of particular conservation concern including eleutherodactylid frogs, eublepharid, iguanid, phrynosomatid, and xantusid lizards, boid, colubrid, and natricid snakes, and emydid and testudinid turtles.

Keywords

Checklist, Chihuahuan Desert, conservation status, herpetofauna, shared species, Sierra Madre Occidental

Introduction

Durango is located in central-northwestern Mexico, and covers 123,317 km^2 between 22°20'42"N, 26°50'42"N, and 102°28'22"W and 107°12'36"W (Fig. 1). It is the 4th largest state in Mexico, representing 6.3% of the country's territory. Durango is bordered by Chihuahua to the north, Coahuila to the northeast, Zacatecas to the southeast, Nayarit to the southwest, and Sinaloa to the west (Figs 2–4). Durango has great biodiversity, a consequence of the combination of its geographical location and complex topography. The Tropic of Cancer passes through the southern part of the state, and the Sierra Madre Occidental runs from north to south dividing Durango into three large climatic regions (warm, temperate, and arid-semiarid). Winds from the Pacific Ocean interact with the Sierra Madre Occidental, producing a rain shadow that results in a significant humidity gradient in the state. This gradient results in a great contrast in the

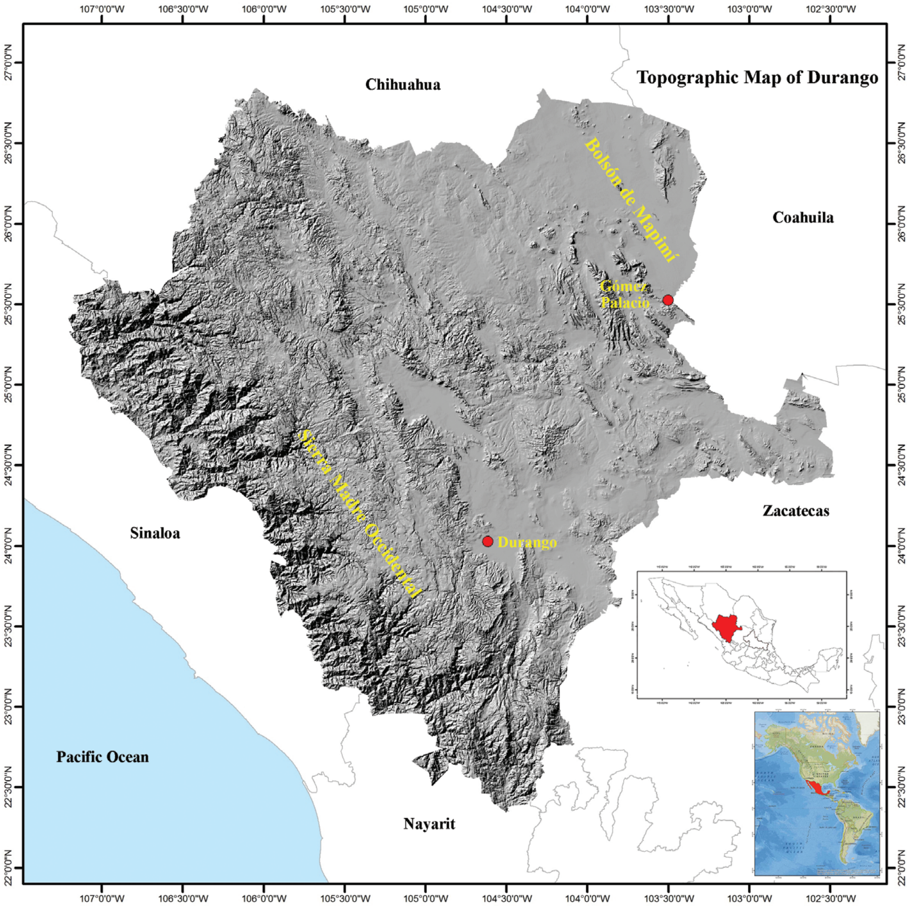

Figure 1. Topographical map of the state of Durango, Mexico (INEGI 2001). Map of America modified from http://www.gifex.com/fullsize/2009-09-17-3/Mapa-de-Amrica.html; Map of Mexico with the state of Durango in red modified from Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (2008).

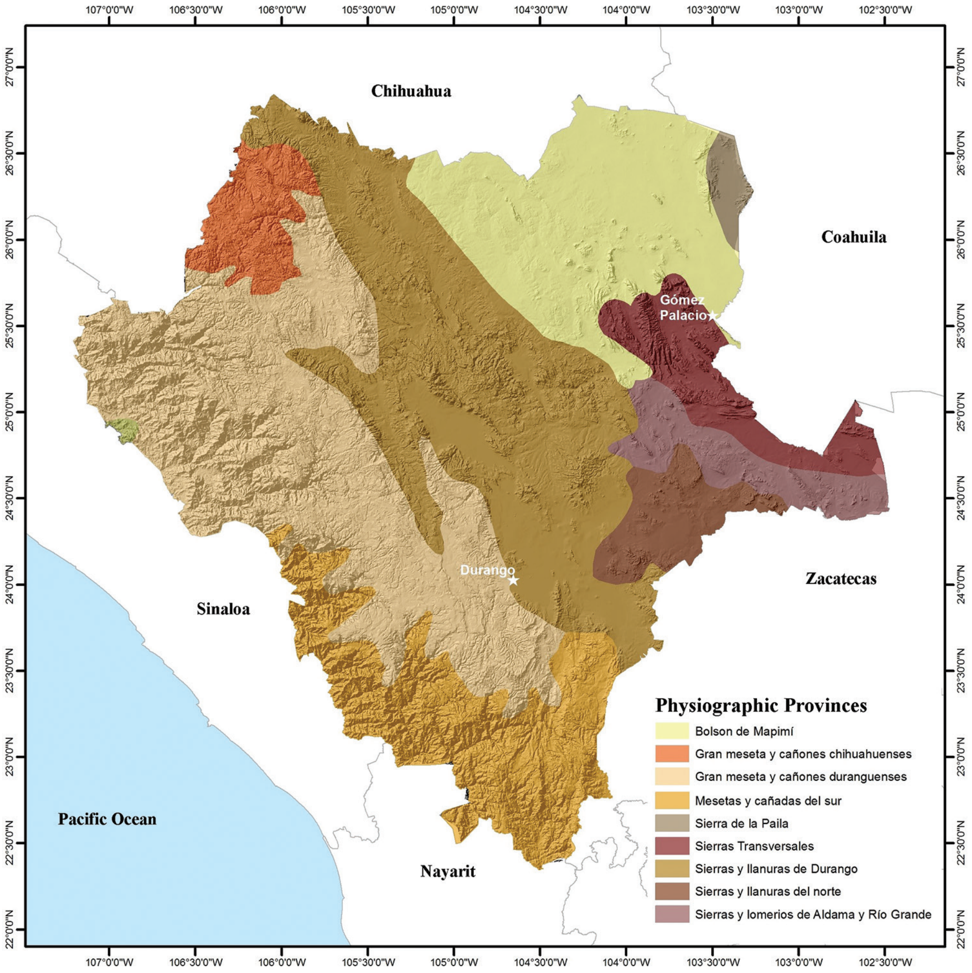

Figure 2. Physiographic provinces of the state of Durango, Mexico (modified from Cervantes-Zamora et al. 1990).

composition of species that inhabit the deep canyons of the western lowlands, the great elevations of the Sierra, the valleys of the foothills of the Sierra, and the arid-semiarid region of the eastern part of the state. The diversity of environmental conditions gives Durango a privileged place in terms of biodiversity. The state is home to dense forests of different timber species, such that, at the national level, Durango is the main producer of wood, contributing 28.5% of the total lumber production of the country (INEGI 2016). The Sierra Madre Occidental is considered a center of biodiversity in the North American continent, mainly due to its floral richness (Felger and Wilson 1994).

Topographically, Durango can be divided into four zones arranged (Fig. 1). In the westernmost zone adjacent to Sinaloa and Nayarit, ravines and canyons have formed through millions of years erosion by the rivers that run from the Sierra Madre Occidental to the Pacific Ocean. The southern part of this region is known as the Quebradas. To the east of the Quebradas is the Sierra region containing the mountainous massif of

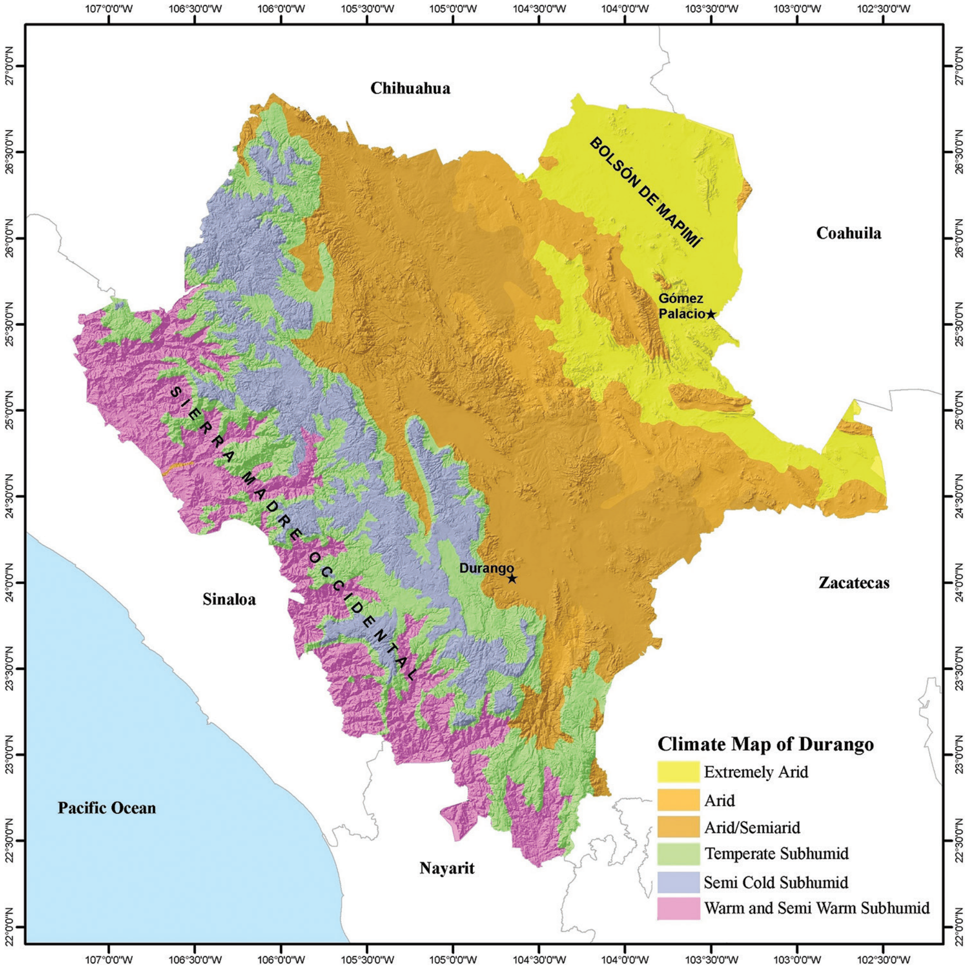

Figure 3. Climate map of the state of Durango, Mexico (modified from García – Comisión Nacional para el Conocimiento y Uso de la Biodiversidad 1998).

the Sierra Madre Occidental, running from the northwestern corner to the southern tip of Durango. The eastern foothills of the Sierra Madre Occidental are part of the Valley region. In the northeastern quarter of Durango is the arid-semiarid region, which includes the Bolsón de Mapimí. The Bolsón de Mapimí is a region that hosts a number of unique endemic species of lizards and turtles, such as *Uma paraphygas* (Chihuahua Fringe-toed Lizard), *Kinosternon durangoense* (Durango Mud Turtle), and *Gopherus flavomarginatus* (Bolson Tortoise), among other species. South of this region, the physiographic province of the Sierra Madre Oriental enters the eastern part of the state. It is in Durango that the Sierra Madre Occidental and Oriental come closest in Mexico, the western most branch of the Sierra Madre Oriental in eastern Durango is also home of a unique assemblage of lizard species such as *Sceloporus maculosus* (Northern Snub-nosed Spiny Lizard), *Xantusia bolsonae* (Bolson Night Lizard), and *X. extorris* (Durango Night Lizard) (Lemos-Espinal et al. 2017; Valdez-Lares et al. 2013).

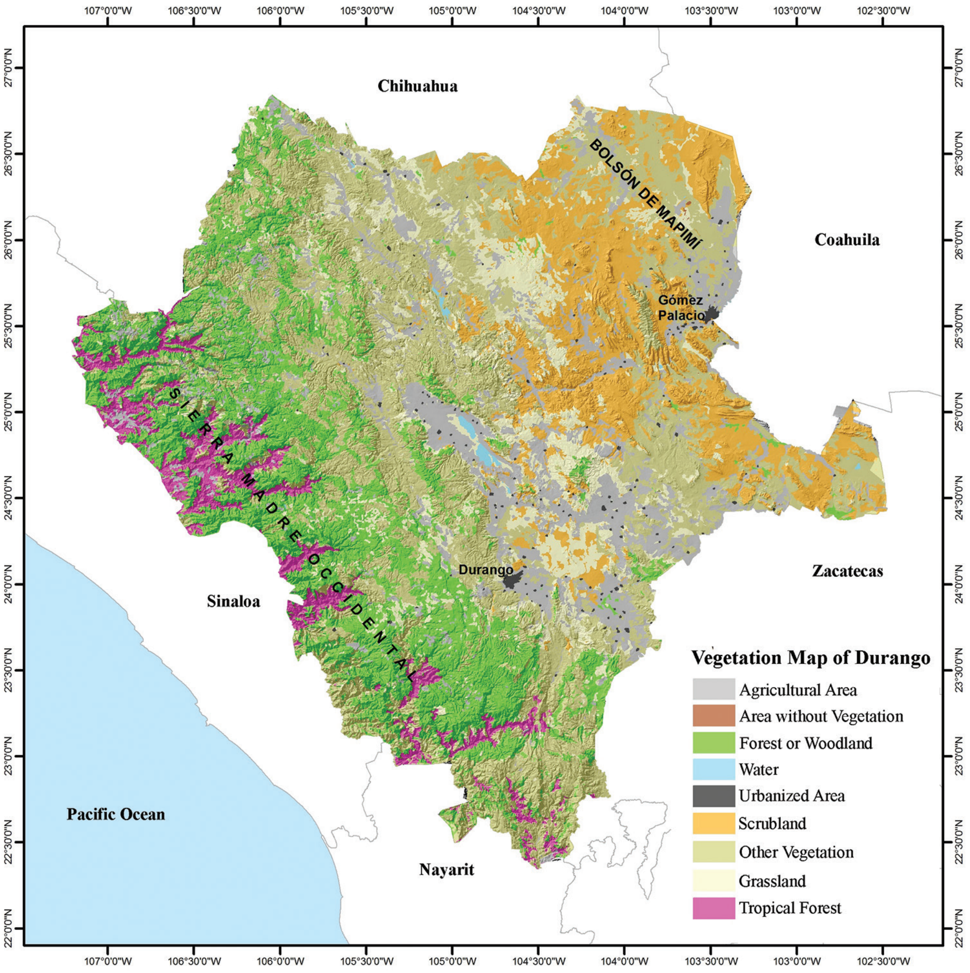

Figure 4. Vegetation map of the state of Durango, Mexico (modified from Dirección General de Geografía – INEGI 2005).

These characteristics of the state of Durango have contributed to the presence of a relatively high diversity of amphibian and reptile species, three of which are endemic to the state (*Xantusia bolsonae* [Bolson Night Lizard], *Adelophis foxi* [Fox's Mountain Meadow Snake], and *Thamnophis nigronuchalis* [Southern Durango Spotted Gartersnake]), or are limited to a small region including Durango and part of one or more of the adjacent states (*Incilius mccoyi* [McCoy's Toad], *Craugastor tarahumaraensis* [Tarahumara Barking Frog], *Eleutherodactylus pallidus* [Pale Chirping Frog], *E. saxatilis* [Marbled Peeping Frog], *Sceloporus lemosespinali* [Lemos-Espinal's Spiny Lizard], *S. maculosus* [Spotted Spiny Lizard], *S. shannonorum* [Shannons' Spiny Lizard], *Uma paraphygas* [Chihuahuan Fringe-toed Lizard], *Xantusia extorris* [Durango Night Lizard], *Lampropeltis webbi* [Webb's Kingsnake], *Thamnophis errans* [Mexican Wandering Gartersnake], *T. unilabialis* [Madrean Narrow-headed Gartersnake], *Crotalus stejnegeri* [Sinaloan Long-tailed

Rattlesnake], *Kinosternon durangoense* [Durango Mud Turtle], and *Gopherus flavomarginatus* [Bolson Tortoise]).

Here, the list of amphibians and reptiles that have been recorded in the state of Durango to date is reported upon. While checklists of the herpetofauna of Durango are available (e.g., Valdez-Lares et al. 2013, 2017a, b), these earlier efforts are expanded upon by collecting and by summarizing the conservation statuses and their distributions within the state as well as the global distribution for each documented species. The herpetofauna of Durango is compared to those of the four adjoining states for which recent checklists are available (Chihuahua, Sinaloa, Nayarit, and Coahuila). Our goal is to place this checklist into a regional and conservation context not available in previous publications.

Materials and methods

A list of amphibians and reptiles of the state of Durango was compiled from the following sources: (1) our own field work; (2) specimens from the Herpetological Collection of CIIDIR-IPN-Durango; (3) databases from the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (National Commission for the Understanding and Use of Biodiversity; CONABIO), including records from the following 22 collections Colección Herpetológica, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas (ENCB); Colección Herpetológica, Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias UNAM (MZFC-UNAM); Colección Nacional de Anfibios y Reptiles, Instituto de Biología UNAM (CNAR); Amphibians and Reptiles Collection, University of Arizona (UAZ); Collection of Herpetology, Amphibians and Reptiles Section, Carnegie Museum of Natural History, Pittsburgh; Collection of Herpetology, Biology Department, Tulane University, New Orleans (TU); Collection of Herpetology, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution (USNM); Collection of Herpetology, Herpetology Department, American Museum of Natural History (AMNH); Collection of Herpetology, Herpetology Department, California Academy of Sciences (CAS); Collection of Herpetology, Museum of Comparative Zoology, Harvard University Cambridge (MCZ); Collection of Herpetology, Museum of Vertebrate Zoology, Division of Biological Sciences, University of California Berkeley (MVZ); Collection of Herpetology, Museum of Zoology, University of Michigan Ann Arbor (UMMZ); Collection of Herpetology, Texas Cooperative Wildlife Collection, Texas A&M University (TCWC); Collection of Herpetology, Texas Natural History Collection, University of Texas Austin (TNHC); Collection of Herpetology, University of Colorado Museum (UCM); Collection of Herpetology, University of Illinois Museum of Natural History (UIMNH); Division of Amphibians and Reptiles, Field Museum of Natural History (FMNH); Fort Worth Museum of Sciences and History (FWMSH); Herpetology Section, Natural History Museum of Los Angeles County (LACM); Louisiana State University, Museum of Life Sciences; Merriam Museum, University of Texas Arlington (UTAMM); Museum of Natural History, Division of Herpetology, Kansas University

(MNHUK); and (4) a thorough examination of the available literature on amphibians and reptiles in the state.

Species were included in the checklist only if the record was confirmed, either by direct observation or through documented museum records or vouchers in the state. Scientific names used in this publication are based on the taxonomic list published in Lemos-Espinal (2015). The amphibian names follow Frost (2017) or AmphibiaWeb (2017, see paragraphs below) and the reptile names follow Uetz and Hošek (2016). In addition, the conservation status of each species was recorded based on three sources: 1) the IUCN Red List 2017; 2) Environmental Viability Scores from Wilson et al. (2013a,b); 3) listing in SEMARNAT (2010). The following state lists were used to compare the species composition between Durango and the adjoining states: Chihuahua, Lemos-Espinal et al. (2017); Sinaloa, Enderson et al. (2009); Nayarit, Woolrich-Piña et al. (2016); and Coahuila, Lemos-Espinal and Smith (2016). The lists were updated for Chihuahua (adding *P. ornatissimum* (Girard), Montanucci 2015); for Chihuahua and Coahuila (substituting *Sceloporus consobrinus* Baird & Girard for *S. cowlesi* Lowe & Norris, A. Leache, personal communication, April 2017); and for Sinaloa (adding *Gopherus evgoodei*, Edwards et al. 2016). The number of overlapping species between each of these states and Durango was determined, and a cluster analysis used to examine the similarities among the herpetofaunas of Durango and its neighboring states (e.g., Enderson et al. 2009; Smith and Lemos-Espinal 2015).

Recent taxonomic changes

Acevedo et al. (2016) used two mitochondrial genes and 23 morphometric landmarks to evaluate the taxonomic status of *Rhinella marina*. They demonstrated that there were two separate evolutionary lineages within *R. marina* represented by two distinct morphotypes, one eastern and one western Andean. The concordance between the observed geographic patterns in morphometric and genetic traits support the recognition of two distinct species. The eastern populations retained the name *R. marina*, and the name *R. horribilis* was revalidated for the western populations.

Duellman et al. (2016) treated two major clades as genera (*Hyla*, restricted to the Old World, and *Dryophytes* distributed primarily in the New World but with three species in Asia). *Dryophytes* is therefore used here. In addition, *Sarcohyla bistincta* was originally placed in the genus *Hyla* by Cope (1877), but was moved to the genus *Plectrohyla* by Faivovich et al. (2005). Duellman et al. (2016) performed a phylogenetic analysis of sequences from 503 species of hylid frogs and four outgroup taxa that resulted in a new phylogenetic tree of treefrogs. Among other results, a conservative new classification based on this tree has five new generic names, including *Sarcohyla*. This new genus contains 24 species, most of them from the *Hyla bistincta* and *Hyla arborescandens* groups of Duellman (2001), and includes the new combination *Sarcohyla bistincta*.

The six species of ranid frogs that occur in Durango were long considered to be in the genus *Rana*, however, Frost et al. (2006) recommended the use of the name

Lithobates, which was controversial. More recently, Yuan et al. (2016) retained all the species of these genera in the traditional genus *Rana*, based on a phylogenetic analysis of six nuclear and three mitochondrial loci sampled from most species of *Rana,* the lack of any diagnostic morphological characters for the genera recognized by Frost et al. (2006), and the clear monophyly of a larger group that include these genera. *Rana* is used here following Yuan et al. (2016) and AmphibiaWeb (2017).

Montanucci (2015) studied the comparative morphology and color pattern variation of short-horned lizards (*Phrynosoma douglasii* species complex) using multivariate analyses of 20 morphological and color-pattern characters, and univariate statistics were summarized for 52 samples. The results of the morphological data analyses supported the recognition of *P. douglasii* as a distinct species, and the resurrection of *P. brevirostris* and *P. ornatissimum* as species distinct from *P. hernandesi*. He recognized two subspecies of *P. ornatissimum*: *P. o. ornatissimum* from central and southern New Mexico and western Texas; and *P. o. brachycercum* from the lower eastern slopes of the Sierra Madre Occidental and the adjacent plains in the Mexican states of Chihuahua, Durango, and Zacatecas.

Tucker et al. (2016), based on Steyskal (1971), explained and justified why the genus name *Aspidoscelis* should be treated as masculine. Names used for species of *Aspidoscelis* occurring in Durango are thus *A. costatus*, *A. gularis*, *A. inornatus*, and *A. marmoratus*.

Card et al. (2016) analyzed the genetic structure and phylogenetic relationships of *Boa* populations using mitochondrial sequences and genome wide SNP data obtained from RADseq, finding evidence that supports three widely-distributed clades roughly corresponding with western North America (Pacific Coast of Mexico), eastern North America (Atlantic Coast of Mexico and Central America), and South America. One of those clades represented the populations of the Pacific slopes of Mexico, from northern Sonora to west of the Isthmus of Tehuantepec. They resurrected the name *sigma* from the population described by Smith (1943) as *Constrictor* (= *Boa*) *constrictor sigma* from the María Madre Island, Tres Marías Islands, Nayarit, Mexico, which was regarded as a junior synonym of *B. c. imperator* by Zweifel (1960). Card et al. (2016) recognized the *Boa* populations from the slopes of the Mexican Pacific as *Boa sigma*, and this is followed here.

Results and discussion

A total of 156 (three of them introduced) species of amphibians and reptiles is found in Durango. Thirty-six of these species are amphibians (33 anurans [one introduced]), three salamanders) and 120 are reptiles (five turtles, 54 lizards [one introduced], and 61 snakes [one introduced]) (Tables 1, 2). These represent 32 families: eight amphibian (seven anurans; one salamanders), and 23 reptile (12 of lizards [one introduced], eight of snakes [one introduced], and three of turtles). Durango has a total of 73 genera: 14 amphibian (one salamander, 13 anuran), and 59 reptile (22 lizard [one introduced], 34 snake [one introduced], and three turtle). The introduced species are the American Bullfrog (*Rana catesbeiana*), the Mediterranean House Gecko (*Hemidactylus turcicus*), and the Brahminy Blindsnake (*Indotyphlops braminus*).

Table 1. Ecoregion (1 = Arid-semiarid; 2 = Valleys; 3 = Sierra; 4 = Quebradas); IUCN Status (DD = Data Deficient; LC = Least Concern, V = Vulnerable, NT = Neat Threatened; E = Endangered; CE = Critically Endangered; NE = not Evaluated) according to the IUCN Red List (The IUCN Red List of Threatened Species, Version 2017-2; www.iucnredlist.org; accessed 9 November 2017), conservation status in Mexico according to SEMARNAT (2010) (P = in danger of extinction, A = threatened; Pr = subject to special protection, NL – not listed), and Environmental Vulnerability Score (EVS – the higher the score the greater the vulnerability: low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20) from Wilson et al. (2013a,b) and Johnson et al. (2015). Global Distribution: 0 = Endemic to Durango; 1 = Endemic to Mexico; 2 = Shared between the US and Mexico; 3 = widely distributed from Canada or the US to Central or South America; 4 = widely distributed from Mexico to Central America; IN = Introduced.

Taxon	Ecoregion	IUCN	SEMARNAT	EVS	Global
CLASS AMPHIBIA (36)					
ORDER ANURA (33)					
Family BUFONIDAE (11)					
Anaxyrus cognatus (Say, 1823)	1,2	LC	NL	L (9)	2
Anaxyrus compactilis (Wiegmann, 1833)	2,3	LC	NL	H (14)	1
Anaxyrus debilis (Girard, 1854)	1,2	LC	Pr	L (7)	2
Anaxyrus mexicanus (Brocchi, 1879)	3	NT	NL	M (13)	1
Anaxyrus punctatus (Baird & Girard, 1852)	1,2	LC	NL	L (5)	2
Anaxyrus woodhousii (Girard, 1854)	3	LC	NL	M (10)	2
Incilius marmoreus (Wiegmann, 1833)	2	LC	NL	M (11)	1
Incilius mazatlanensis (Taylor, 1940)	4	LC	NL	M (12)	1
Incilius mccoyi Santos-Barrera & Flores-Villela, 2011	2,3	NE	NL	H (14)	1
Incilius occidentalis (Camerano, 1879)	2,3,4	LC	NL	M (11)	1
Rhinella horribilis (Linnaeus, 1758)	4	NE	NL	L (3)	3
Family CRAUGASTORIDAE (4)					
Craugastor augusti (Dugès, 1879)	2,4	LC	NL	L (8)	2
Craugastor occidentalis (Taylor, 1941)	3	DD	NL	M (13)	1
Craugastor tarahumaraensis (Taylor, 1940)	3	V	Pr	H (17)	1
Craugastor vocalis (Taylor, 1940)	4	LC	NL	M (13)	1
Family ELEUTHERODACTYLIDAE (3)					
Eleutherodactylus nitidus (Peters, 1870)	3	LC	NL	M (12)	1
Eleutherodactylus pallidus (Duellman, 1958)	4	DD	Pr	H (17)	1
Eleutherodactylus saxatilis (Webb, 1962)	3	E	NL	H (17)	1
Family HYLIDAE (6)					
Agalychnis dacnicolor (Cope, 1864)	4	LC	NL	M (13)	1
Dryophytes arenicolor Cope, 1866	2,3,4	LC	NL	L (7)	2
Dryophytes eximius (Baird, 1854)	2,3	LC	NL	M (10)	1
Dryophytes wrightorum (Taylor, 1938)	3	LC	NL	L (9)	2
Sarcohyla bistincta (Cope, 1877)	3	LC	Pr	L (9)	1
Smilisca baudinii (Duméril & Bibron, 1841)	4	LC	NL	L (3)	3
Family MICROHYLIDAE (1)					
Gastrophryne olivacea (Hallowell, 1857)	1	LC	Pr	L (9)	2
Family RANIDAE (6)					
Rana berlandieri Baird, 1859	1,3	LC	Pr	L (7)	3
Rana catesbeiana Shaw, 1802	1	N/A	N/A	N/A	IN

Taxon	Ecoregion	IUCN	SEMARNAT	EVS	Global
Rana chiricahuensis Platz & Mecham, 1979	2,3	V	A	M (11)	2
Rana magnaocularis Frost & Bagnara, 1974	4	LC	NL	M (12)	1
Rana montezumae Baird, 1854	2	LC	Pr	M (13)	1
Rana pustulosa Boulenger, 1833	4	LC	Pr	L (9)	1
Family SCAPHIOPODIDAE (2)					
Scaphiopus couchii Baird, 1854	1,2	LC	NL	L (3)	2
Spea multiplicata (Cope, 1863)	1,2,3	LC	NL	L (6)	2
ORDER CAUDATA					
Family AMBYSTOMATIDAE (3)					
Ambystoma rosaceum Taylor, 1941	3	LC	Pr	H (14)	1
Ambystoma silvense Webb, 2004	3	DD	NL	H (14)	1
Ambystoma velasci (Dugès, 1888)	2,3	LC	Pr	M (10)	1
CLASS REPTILIA (120)					
ORDER SQUAMATA					
SUBORDER LACERTILIA (53)					
Family ANGUIDAE (4)					
Barisia ciliaris (Smith, 1942)	2,3	NE	NL	H (15)	1
Elgaria kingii Gray, 1838	3	LC	Pr	M (10)	2
Gerrhonotus infernalis Baird, 1859	1,3	LC	NL	M (13)	2
Gerrhonotus liocephalus Wiegmann, 1828	3	LC	Pr	L (6)	1
Family CROTAPHYTIDAE (2)					
Crotaphytus collaris (Say, 1823)	1	LC	A	M (13)	2
Gambelia wislizenii (Baird & Girard, 1852)	1	LC	Pr	M (13)	2
Family DACTYLOIDAE (1)					
Anolis nebulosus (Wiegmann, 1834)	3,4	LC	NL	M (13)	1
Family EUBLEPHARIDAE (2)					
Coleonyx brevis Stejneger, 1893	1	LC	Pr	H (14)	2
Coleonyx fasciatus (Boulenger, 1885)	4	LC	NL	H (17)	1
Family GEKKONIDAE (1)					
Hemidactylus turcicus (Linnaeus, 1758)	1	N/A	N/A	N/A	IN
Family HELODERMATIDAE (1)					
Heloderma horridum (Wiegmann, 1829)	3,4	LC	A	M (11)	4
Family IGUANIDAE (1)					
Ctenosaura pectinata (Wiegmann, 1834)	4	NE	NL	H (15)	1
Family PHRYNOSOMATIDAE (30)					
Cophosaurus texanus Troschel, 1852	1	LC	A	H (14)	2
Holbrookia approximans Baird, 1859	1,2	NE	NL	H (14)	1
Holbrookia elegans Bocourt, 1874	4	LC	NL	M (13)	2
Phrynosoma cornutum (Harlan, 1824)	1,2	LC	NL	M (11)	2
Phrynosoma modestum Girard, 1852	1	LC	NL	M (12)	2
Phrynosoma orbiculare (Linnaeus, 1758)	2,3	LC	A	M (12)	1
Phrynosoma ornatissimum (Girard, 1858)	2,3	NE	NL	NE	2
Sceloporus albiventris Smith, 1939	4	NE	NL	H (16)	1
Sceloporus bimaculosus Phelan & Brattstrom, 1955	1	NE	NL	NE	2
Sceloporus bulleri Boulenger, 1895	3	LC	NL	H (15)	1
Sceloporus clarkii Baird & Girard, 1852	4	LC	NL	M (10)	2
Sceloporus cowlesi Lowe & Norris, 1956	1	NE	NL	M (13)	2

Taxon	Ecoregion	IUCN	SEMARNAT	EVS	Global
Sceloporus grammicus Wiegmann, 1828	1,3	LC	Pr	L (9)	2
Sceloporus heterolepis Boulenger, 1895	3	LC	NL	H (14)	1
Sceloporus jarrovii Cope, 1875	1,3	LC	NL	M (11)	2
Sceloporus lemosespinali Lara-Góngora, 2004	3	DD	NL	H (16)	1
Sceloporus maculosus Smith, 1934	1	V	Pr	H (16)	1
Sceloporus melanorhinus Bocourt, 1876	3	LC	NL	L (9)	4
Sceloporus merriami Stejneger, 1904	1	LC	NL	M (13)	2
Sceloporus nelsoni Cochran, 1923	4	LC	NL	M (13)	1
Sceloporus poinsettii Baird & Girard, 1852	1,2,3	LC	NL	M (12)	2
Sceloporus scalaris Weigmann, 1828	2,3,4	LC	NL	M (12)	1
Sceloporus shannonorum Langebartel, 1959	3	NE	NL	H (15)	1
Sceloporus slevini Smith, 1937	3	LC	NL	M (11)	2
Sceloporus spinosus Weigmann, 1828	2	LC	NL	M (12)	1
Sceloporus torquatus Weigmann, 1828	1	LC	NL	M (11)	1
Uma paraphygas Williams, Chrapliwy & Smith, 1959	1	NT	P	H (17)	1
Urosaurus bicarinatus (Duméril, 1856)	4	LC	NL	M (12)	1
Urosaurus ornatus (Baird & Girard, 1852)	1	LC	NL	M (10)	2
Uta stansburiana Baird & Girard, 1852	1	LC	A	L (7)	2
Family PHYLLODACTYLIDAE (1)					
Phyllodactylus tuberculosus Wiegmann, 1834	4	LC	NL	L (8)	4
Family SCINCIDAE (5)					
Plestiodon bilineatus (Tanner, 1958)	3	NE	NL	M (13)	1
Plestiodon callicephalus (Bocourt, 1879)	4	LC	NL	M (12)	2
Plestiodon lynxe (Wiegmann, 1834)	3	LC	Pr	M (10)	1
Plestiodon obsoletus Baird & Girard, 1852	1	LC	NL	M (11)	2
Scincella lateralis (Say, 1823)	1	LC	Pr	M (13)	2
Family TEIIDAE (4)					
Aspidoscelis costatus (Cope, 1878)	4	NE	Pr	M (11)	1
Aspidoscelis gularis (Baird & Girard, 1852)	1	LC	NL	L (9)	2
Aspidoscelis inornatus (Baird, 1859)	1	LC	NL	H (14)	2
Aspidoscelis marmoratus (Baird & Girard, 1852)	1	NE	NL	H (14)	2
Family XANTUSIDAE (2)					
Xantusia bolsonae Webb, 1970	1	DD	P	H (17)	0
Xantusia extorris Webb, 1965	1	LC	NL	H (15)	1
ORDER SQUAMATA					
SUBORDER SERPENTES (61)					
Family BOIDAE (1)					
Boa sigma (Smith, 1943)	4	NE	NL	H (15)	1
Family COLUBRIDAE (31)					
Arizona elegans Kennicott, 1859	1,2	LC	NL	L (5)	2
Bogertophis subocularis (Brown, 1901)	1,2	LC	NL	H (14)	2
Conopsis nasus Günther, 1858	3	LC	NL	M (11)	1
Drymarchon melanurus (Duméril, Bibron & Duméril, 1854)	3,4	LC	NL	L (6)	3
Gyalopion canum (Cope, 1861)	1	LC	NL	L (9)	2
Lampropeltis alterna (Brown, 1901)	1,2,3	LC	A	H (14)	2
Lampropeltis mexicana (Garman, 1884)	3	LC	A	H (15)	1
Lampropeltis splendida (Baird & Girard, 1853)	1,2	NE	NL	M (12)	2

Taxon	Ecoregion	IUCN	SEMARNAT	EVS	Global
Lampropeltis webbi Bryson, Dixon & Lazcano, 2005	3	DD	NL	H (16)	1
Leptophis diplotropis (Günther, 1872)	4	LC	A	H (14)	1
Masticophis bilineatus Jan, 1863	4	LC	NL	M (11)	2
Masticophis flagellum (Shaw, 1802)	1,2,3	LC	A	L (8)	2
Masticophis mentovarius (Duméril, Bibron & Duméril, 1854)	2	LC	A	L (6)	4
Masticophis taeniatus (Hallowell, 1852)	1,2	LC	NL	M (10)	2
Mastigodryas cliftoni (Hardy, 1964)	3	NE	NL	H (14)	1
Oxybelis aeneus (Wagler, 1824)	4	NE	NL	L (5)	3
Pantherophis emoryi (Baird & Girard, 1853)	1	LC	NL	M (13)	2
Pituophis catenifer (Blainville, 1835)	1,2	LC	NL	L (9)	2
Pituophis deppei (Duméril, 1853)	2,3	LC	A	H (14)	1
Pseudoficimia frontalis (Cope, 1864)	4	LC	NL	M (13)	1
Rhinocheilus lecontei Baird & Girard, 1853	1	LC	NL	L (8)	2
Salvadora bairdi Jan, 1860	3	LC	Pr	H (15)	1
Salvadora deserticola Schmidt, 1940	1	NE	NL	H (14)	2
Salvadora grahamiae Baird & Girard, 1853	3	LC	NL	M (10)	2
Senticolis triaspis (Cope, 1866)	2,3	LC	NL	L (6)	3
Sonora semiannulata Baird & Girard, 1853	1	LC	NL	L (5)	2
Tantilla atriceps (Günther, 1895)	1	LC	A	M (11)	2
Tantilla bocourti (Günther, 1895)	3	LC	NL	L (9)	1
Tantilla nigriceps Kennicott, 1860	1	LC	NL	M (11)	2
Tantilla wilcoxi Stejneger, 1902	2,3	LC	NL	M (10)	2
Trimorphodon tau Cope, 1870	4	LC	NL	M (13)	1
Family DIPSADIDAE (7)					
Diadophis punctatus (Linnaeus, 1766)	1,3	LC	NL	L (4)	2
Geophis dugesii Bocourt, 1883	3	LC	NL	M (13)	1
Heterodon kennerlyi Kennicott, 1860	1,2	NE	NL	M (11)	2
Hypsiglena jani Dugès, 1865	1	NE	NL	L (6)	2
Hypsiglena torquata (Günther, 1860)	4	LC	Pr	L (8)	1
Leptodeira splendida Günther, 1895	4	LC	NL	H (14)	1
Rhadinaea laureata (Günther, 1868)	3	LC	NL	M (12)	1
Family ELAPIDAE (1)					
Micrurus tener Baird & Girard, 1853	1	LC	NL	M (11)	2
Family LEPTOTYPHLOPIDAE (1)					
Rena segrega (Klauber, 1939)	1	NE	NL	L (8)	2
Family NATRICIDAE (12)					
Adelophis foxi Rossman & Blaney, 1968	3	DD	Pr	H (16)	0
Nerodia erythrogaster (Forster, 1771)	1	LC	A	M (11)	2
Storeria storerioides (Cope, 1866)	3	LC	NL	M (11)	1
Thamnophis cyrtopsis (Kennicott, 1860)	1,2,3,4	LC	A	L (7)	3
Thamnophis eques (Reuss, 1834)	1,2,3	LC	A	L (8)	2
Thamnophis errans Smith, 1942	3	LC	NL	H (16)	1
Thamnophis marcianus (Baird & Girard, 1853)	1	LC	A	M (10)	3
Thamnophis melanogaster (Wiegmann, 1830)	1,2,3,4	E	A	H (15)	1
Thamnophis nigronuchalis Thompson, 1957	3	DD	Pr	M (12)	0
Thamnophis pulchrilatus (Cope, 1885)	3	LC	NL	H (15)	1
Thamnophis unilabialis Tanner, 1985	1,3	NE	NL	NE	1

Taxon	Ecoregion	IUCN	SEMARNAT	EVS	Global
Thamnophis validus (Kennicott, 1860)	2	LC	NL	M (12)	1
Family TYPHLOPIDAE (1)					
Indotyphlops braminus (Daudin, 1803)	1,2	N/A	N/A	N/A	IN
Family VIPERIDAE (7)					
Crotalus atrox Baird & Girard, 1853	1,2	LC	Pr	L (9)	2
Crotalus lepidus (Kennicott, 1861)	1,2,3	LC	Pr	M (12)	2
Crotalus molossus Baird & Girard, 1853	1,2,3	LC	Pr	L (8)	2
Crotalus pricei Van Denburgh, 1895	3	LC	Pr	H (14)	2
Crotalus scutulatus (Kennicott, 1861)	1,2	LC	Pr	M (11)	2
Crotalus stejnegeri Dunn, 1919	3,4	V	A	H (17)	1
Crotalus willardi Meek, 1905	2,3	LC	Pr	M (13)	2
ORDER TESTUDINES (5)					
Family EMYDIDAE (1)					
Trachemys gaigeae (Hartweg, 1939)	1	V	NL	H (18)	2
Family KINOSTERNIDAE (3)					
Kinosternon durangoense Iverson, 1979	1	DD	NL	H (16)	1
Kinosternon hirtipes (Wagler, 1830)	1,2,3,4	LC	Pr	M (10)	2
Kinosternon integrum LeConte, 1854	2,3	LC	Pr	M (11)	1
Family TESTUDINIDAE (1)					
Gopherus flavomarginatus Legler, 1959	1	V	P	H (19)	1

General distribution

Twenty-one of the 36 species of Amphibians that inhabit Durango are endemic to Mexico, 13 of them are limited to the Sierra Madre Occidental or to the Pacific Coast and the lowlands of the Sierra Madre Occidental (Table 1). Three more are species typical of the Mexican Plateau (Table 1). Another five have wide distributions that include parts of both Sierras Madre (Occidental and Oriental) and part of the Mexican Plateau (Table 1).

Of the 15 amphibian species of Durango that are not endemic to Mexico, one is an introduced species (*Rana catesbeiana*), and eleven more are found in the USA and Mexico (Table 1). The remaining three species have a wide distribution from southern USA to Central or South America (Table 1).

Twenty-four of the 54 species of lizards that occur in the state are endemic to Mexico, one of them to the state of Durango (*Xantusia bolsonae*), three more have narrow distributions in northeastern Durango: *Sceloporus maculosus* limited to the Río Nazas drainage in Durango and Coahuila; *Uma paraphygas* limited to the Bolsón de Mapimí of southeastern Chihuahua, southwestern Coahuila, and northeastern Durango; and *Xantusia extorris* limited to northeastern Durango and adjacent Coahuila. Two more are restricted to small areas in the Sierra Madre Occidental: *Sceloporus lemosespinali* to eastern Sonora, northern Chihuahua, and extreme northwestern Durango; and *S. shannonorum* in central Durango to extreme northern Jalisco. Another ten species that occur in Durango and are endemic to Mexico are typical to the Pacific Coast and/or the Sierra Madre Occidental: *Anolis nebulosus*, *Coleonyx fasciatus*, *Ctenosaura pectinata*, *Sceloporus*

Table 2. Summary of native species present in Durango by Family, Order or Suborder, and Class. Status summary indicates the number of species found in each IUCN conservation status in the order DD, LC, V, NT, E, CE (see Table 1 for abbreviations; in some cases species have not been assigned a status by the IUCN and therefore these may not add up to the total number of species in a taxon). Mean EVS is the mean Environmental Vulnerability Score, scores ≥ 14 are considered high vulnerability (Wilson et al. 2013a,b) and conservation status in Mexico according to SEMARNAT (2010) in the order NL, Pr, A, P (see Table 1 for abbreviations).

Taxon	Genera	Species	IUCN	EVS	SEMARNAT
Class Amphibia					
Order Anura	13	32	2,24,2,1,1,0	10.2	23,8,1,0
Bufonidae	3	11	0,8,0,1,0,0	9.9	10,1,0,0
Craugastoridae	1	4	1,2,1,0,0,0	12.8	3,1,0,0
Eleutherodactylidae	1	3	1,1,0,0,1,0	15.3	2,1,0,0
Hylidae	4	6	0,6,0,0,0,0	8.5	5,1,0,0
Microhylidae	1	1	0,1,0,0,0,0	9	0,1,0,0
Ranidae	1	5	0,4,1,0,0,0	10.4	1,3,1,0
Scaphiopodidae	2	2	0,2,0,0,0,0	4.5	2,0,0,0
Order Caudata	1	3	1,2,0,0,0,0	12.7	1,2,0,0
Ambystomatidae	1	3	1,2,0,0,0,0	12.7	1,2,0,0
SUBTOTAL	14	35	3,26,2,1,1,0	10.4	24,10,1,0
Class Reptilia					
Order Squamata					
Suborder Lacertilia	21	53	2,38,1,1,0,0	12.5	37,9,5,2
Anguidae	3	4	0,3,0,0,0,0	11	2,2,0,0
Crotaphytidae	2	2	0,2,0,0,0,0	13	0,1,1,0
Dactyloidae	1	1	0,1,0,0,0,0	13	1,0,0,0
Eublepharidae	1	2	0,2,0,0,0,0	15.5	1,1,0,0
Helodermatidae	1	1	0,1,0,0,0,0	11	0,0,1,0
Iguanidae	1	1	0,0,0,0,0,0	15	1,0,0,0
Phrynosomatidae	7	30	1,21,1,1,0,0	12.5	24,2,3,1
Phyllodactylidae	1	1	0,1,0,0,0,0	8	1,0,0,0
Scincidae	2	5	0,4,0,0,0,0	11.8	3,2,0,0
Teiidae	1	4	0,2,0,0,0,0	12	3,1,0,0
Xantusidae	1	2	1,1,0,0,0,0	16	1,0,0,1
Suborder Serpentes	33	60	3,46,1,0,1,0	11	39,10,11,0
Boidae	1	1	0,0,0,0,0,0	15	1,0,0,0
Colubridae	19	31	1,26,0,0,0,0	10.7	25,1,5,0
Dipsidae	6	7	0,5,0,0,0,0	9.7	6,1,0,0
Elapidae	1	1	0,1,0,0,0,0	11	1,0,0,0
Leptotyphlopidae	1	1	0,0,0,0,0,0	8	1,0,0,0
Natricidae	4	12	2,8,0,0,1,0	12.1	5,2,5.0
Viperidae	1	7	0,6,1,0,0,0	12	0,6,1,0
Order Testudines	3	5	1,2,2,0,0,0	14.8	2,2,0,1
Emydidae	1	1	0,0,1,0,0,0	18	1,0,0,0
Kinosternidae	1	3	1,2,0,0,0,0	12.3	1,2,0,0
Testudinidae	1	1	0,0,1,0,0,0	19	0,0,0,1
SUBTOTAL	57	118	6,86,4,1,1,0	11.8	78,21,16,3
TOTAL	68	153	9,112,6,2,2,0	11.5	102,31,17,3

albiventris, *S. bulleri*, *S. heterolepis*, *S. nelsoni*, *Urosaurus bicarinatus*, *Plestiodon bilineatus,* and *Aspidoscelis costatus*. One more is a species typical of the Chihuahuan Desert: *Holbrookia approximans*. Another species is typical of the Sierra Madre Oriental, with an isolated population occurring in southern Durango: *Plestiodon lynxe*. One more occurs in southern Mexico in the state of Puebla, Hidalgo, Oaxaca, and Chiapas, with isolated populations in Aguascalientes, Jalisco, and southwestern Durango: *Gerrhonotus liocephalus*. The remaining five lizard species endemic to Mexico have a wide distribution occurring in both Sierras Madres (Occidental and Oriental): *Barisia ciliaris*, and even in the Transvolcanic Belt of central Mexico (*Phrynosoma orbiculare*), or are species typical of the Mexican Plateau: *Sceloporus scalaris*, *S. spinosus*, and *S. torquatus*.

The remaining 30 species of lizards that inhabit Durango are not endemic to Mexico. Twenty-six of the non-endemics are species found in the USA and Mexico (Table 1). Three are found from northern Mexico to Central America (Table 1). The last one, *Hemidactylus turcicus*, is introduced to Durango.

Twenty-four of the 61 species of snakes that occur in Durango are endemic to Mexico. Two of them to Durango: *Adelophis foxi* and *Thamnophis nigronuchalis*. Four others have a narrow distribution in the Sierra Madre Occidental: *Lampropeltis webbi* (Pacific slope of the Sierra Madre Occidental near the Durango – Sinaloa border); *Thamnophis errans* (from central Chihuahua, Durango and adjacent Zacatecas); *Thamnophis unilabialis* (eastern Sonora and western Chihuahua to northern Durango); and *Crotalus stejnegeri* (western Durango and adjacent southern Sinaloa). Eight more are typical species of the Pacific slopes of the Sierra Madre Occidental: *Boa sigma, Leptophis diplotropis*, *Mastigodryas cliftoni*, *Pseudoficimia frontalis*, *Geophis dugesii*, *Hypsiglena torquata*, *Leptodeira splendida*, and *Thamnophis validus*. Another nine of the endemic snakes have a wide distribution in central Mexico that include the Mexican Plateau and/or the Transvolcanic Belt of central Mexico and the Sierra Madre Occidental and in some cases even the Sierra Madre Oriental: *Conopsis nasus*, *Lampropeltis mexicana*, *Pituophis deppei*, *Salvadora bairdi*, *Tantilla bocourti*, *Trimorphodon tau*, *Rhadinaea laureata*, *Storeria storerioides*, and *Thamnophis melanogaster*. The remaining endemic species, *Thamnophis pulchrilatus*, has a spotty distribution in highlands of the Sierra Madre Occidental and the Sierra Madre Oriental.

Thirty snake species that are found in Durango are distributed from the USA to Mexico (Table 1). Five more species are found from central or southern USA to Central or South America (Table 1). One species ranges from Mexico to northeastern South America: *Masticophis mentovarius*. The last species that inhabits Durango and is not endemic to Mexico is an introduced species to Mexico, *Indotyphlops braminus*.

Three of the five species of turtles that inhabit Durango are endemic to Mexico, two to the Bolsón de Mapimí in southeastern Chihuahua, southwestern Coahuila, and northeastern Durango: *Kinosternon durangoense* and *Gopherus flavomarginatus*. The other is widely distributed in the lowlands of western Mexico and throughout the central and southern portion of the Mexican Plateau: *Kinosternon integrum* (it is not native to the Valley of Mexico but has been introduced there). The two non-endemic species of turtles are found from southwestern USA to northern Mexico: *Trachemys gaigeae* and *Kinosternon hirtipes*.

Habitat types

The Sierra habitat type (46.1%) and the arid-semiarid habitat type (42.8%) had the highest percentages of the herpetofauna in Durango, whereas both the valley (29.9%) and Quebradas (24.0%) habitat types had a lower percentage (Table 3). For amphibians alone, the Sierra habitat type had slightly more than 50% of the species in Durango (52.8%) followed by the valley habitat type (41.7%) and Quebradas habitat type (30.6%). As might be expected, the arid-semi-arid habitat type had the fewest amphibian species (19.4%; Table 3). This distribution of species is also found when examining anuran species (Table 3). For salamanders, species are almost exclusively found in the Sierra habitat type, with one species found in the valley habitat type, and none in the arid-semi-arid and Quebradas habitat types (Table 3). Reptiles showed a different pattern, with the most species being found in the arid-semiarid habitat type (50%) and the Sierra habitat type (44.1%), with the valleys (26.3%) and the Quebradas (22.0%) having fewer species. This pattern is found in both lizards and snakes (Table 3), and is primarily driven by the most diverse families in these groups (e.g., Phrynosomatidae, Colubridae, and Natricidae). Turtles are found in the four habitat types with 80% of the species occurring in the arid-semiarid habitat type and less than half of the species found in the other three habitat types.

Comparisons with neighboring states

Overall, Durango shares the most species with Chihuahua (Table 4). This holds true for amphibians; however, Durango shares almost as many species of amphibians with Sinaloa and Nayarit as with Chihuahua. For reptiles, Durango and Chihuahua share the most species by a large margin over the other states (Table 4). The cluster analysis recovered the same tree structure for Durango and its neighboring states when the entire herpetofauna, reptiles, and amphibians are each considered (Figure 5). In each

Table 3. Summary of the number of native species (% of total number of species of taxonomic group in Durango in parentheses) in different taxonomic groups found in different habitat types in Durango, Mexico (see text for description of the habitat types).

Taxon	Arid-Semiarid	Valleys	Sierras	Quebradas
Amphibia	7 (19.4)	15 (41.7)	19 (52.8)	11 (30.6)
Caudata	0 (0)	1 (33.3)	3 (100)	0 (0)
Anura	7 (21.2)	14 (42.4)	16 (48.5)	11 (33.3)
Reptilia	59 (50)	31 (26.3)	52 (44.1)	26 (22.0)
Testudines	4 (80)	2 (40)	2 (40)	1 (20)
Squamata	55 (48.7)	29 (25.7)	50 (44.2)	25 (22.1)
Lacertilia	26 (49.0)	8 (15.1)	20 (37.7)	13 (24.5)
Serpentes	29 (48.3)	21 (35)	30 (50)	12 (20)
TOTAL	66 (42.8)	46 (29.9)	71 (46.1)	37 (24.0)

Table 4. Summary of the numbers of species shared between Durango and neighboring Mexican states (not including introduced species). The percent of species from Durango shared by a neighboring state are given in parentheses. – indicates either Durango or the neighboring state has no species in the taxonomic group, thus no value for shared species is provided.

Taxon	Durango	Chihuahua	Sinaloa	Nayarit	Coahuila
Class Amphibia	35	23 (65.7)	20 (57.1)	19 (54.2)	11 (30.6)
Order Caudata	3	2 (66.7)	1 (33.3)	1 (33.3)	0 (0)
Ambystomatidae	3	2 (66.7)	1 (33.3)	1 (33.3)	0 (0)
Order Anura	32	21 (65.6)	19 (59.4)	18 (56.2)	11 (33.3)
Bufonidae	11	8 (72.7)	7 (63.6)	4 (36.4)	4 (36.4)
Craugastoridae	4	2 (50)	3 (75)	3 (75)	1 (25)
Eleutherodactylidae	3	0 (0)	2 (66.7)	2 (66.7)	0 (0)
Hylidae	6	4 (66.7)	4 (66.7)	5 (83.3)	2 (33.3)
Microhylidae	1	1 (100)	0 (0)	0 (0)	1 (100)
Ranidae	5	4 (80)	2 (40)	2 (40)	1 (16.7)
Scaphiopodidae	2	2 (100)	1 (50)	2 (100)	2 (100)
Class Reptilia	118	90 (76.3)	53 (44.9)	55 (46.6)	63 (53.4)
Order Testudines	5	5 (100)	1 (20)	2 (40)	4 (80)
Emydidae	1	1 (100)	0 (0)	0 (0)	1 (100)
Kinosternidae	3	3 (100)	1 (33.3)	2 (66.7)	2 (66.7)
Testudinae	1	1 (100)	0 (0)	–	1 (100)
Order Squamata	113	85 (75.2)	52 (46.0)	53 (46.9)	59 (52.2)
Suborder Lacertilia	53	37 (69.8)	20 (37.7)	22 (41.5)	25 (47.2)
Anguidae	4	3 (75)	2 (50)	2 (50)	1 (25)
Crotaphytidae	2	2 (100)	–	–	2 (100)
Dactyloidae	1	1 (100)	1 (100)	1 (100)	0 (0)
Eublepharidae	2	1 (50)	1 (50)	0 (0)	1 (50)
Helodermatidae	1	1 (100)	1 (100)	1 (100)	0 (0)
Iguanidae	1	0 (0)	1 (100)	1 (100)	–
Phrynosomatidae	30	21 (70)	11 (36.7)	13 (43.3)	15 (50)
Phyllodactylidae	1	1 (100)	1 (100)	1 (100)	–
Scincidae	5	3 (60)	1 (20)	2 (40)	2 (40)
Teiidae	4	4 (100)	1 (25)	1 (25)	3 (75)
Xantusidae	2	–	–	–	1 (50)
Suborder Serpentes	60	48 (80)	32 (53.3)	31 (51.7)	34 (56.7)
Boidae	1	1 (100)	1 (100)	1 (100)	0 (0)
Colubridae	31	25 (80.6)	20 (64.5)	15 (48.4)	18 (58.1)
Dipsidadae	7	6 (85.7)	4 (57.1)	4 (57.1)	3 (42.8)
Elapidae	1	0 (0)	0 (0)	0 (0)	1 (100)
Leptotyphlopidae	1	1 (100)	0 (33.3)	0 (0)	1 (100)
Natricidae	12	9 (75)	3 (25)	8 (66.7)	6 (50)
Viperidae	7	6 (85.7)	4 (57.1)	3 (42.8)	5 (71.4)
TOTAL	153	113 (73.8)	73 (47.7)	74 (48.4)	74 (48.0)

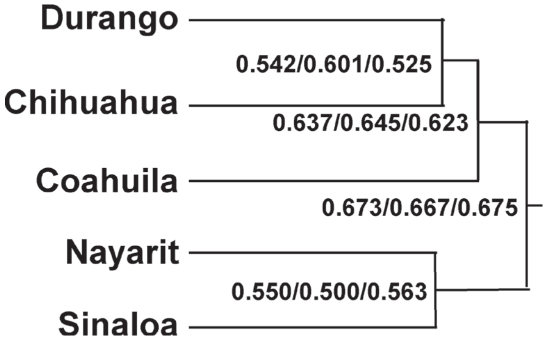

Figure 5. Results of cluster analysis of the herpetofaunas of Durango and its neighboring states (Chihuahua, Coahuila, Nayarit, and Sinaloa). The distances provided are Euclidean distances for the entire herpetofauna, reptiles only, and amphibians only, respectively.

case, Durango and Chihuahua made a cluster and Nayarit and Sinaloa made another cluster. In addition, Coahuila formed a cluster with the Durango-Chihuahua pairing. Such a pattern likely reflects the fact that Durango, Chihuahua, and Coahuila all have extensive tracts of Chihuahuan Desert habitats. Similarities and differences in species among Durango and its neighboring states likely is the result of the habitats and vegetation types found in each state (see also Smith and Lemos-Espinal 2015, Lemos-Espinal and Smith 2016, Lemos-Espinal et al. 2017). Such results suggest that the conservation of the herpetofauna of this region will need habitat specific conservation plans that cross state borders and will require integration of state, regional, and country-level efforts.

Conservation status

Overall, 7.6% of the amphibian and reptile species were IUCN listed (i.e., Vulnerable, Near Threatened, Endangered, or Critically Endangered), but 33.3% were placed in a protected category by SEMARNAT and 29.3% categorized at high risk by the EVS (Tables 1, 2). For amphibians, 12.1% were IUCN listed, 31.4% protected by SEMARNAT, and 20% at high risk according to the EVS (Tables 1, 2). For reptiles, 6.1% were listed in IUCN, 33.9% protected by SEMARNAT, and 32.2% at high risk by EVS. These results suggest that the herpetofauna of Durango is considered to be of relatively low conservation concern at a global scale, but at a national level, there is much greater conservation concern. There are several taxa that, based on their IUCN listing, SEMARNAT category, or their EVS, are of conservation concern. Families that include species of particular conservation concern include Eleutherodactylidae, Eublepharidae, Iguanidae, Phrynosomatidae, Xantusidae, Boidae, Colubridae, Natricidae, Emydidae, and Testudinidae (Tables 1, 2). The IUCN, SEMARNAT, and EVS categories are based on global or country-level assessments, and it is likely that there are amphibians and reptiles whose conservation status in Durango is not accurately assessed by the global level assessment. Additional assessments at the state level in Durango, and other Mexican states, are needed to establish conservation or management needs for states, or even regions.

To help determine which habitat types within Durango may house species of particular conservation concern, the conservation statuses of reptile and amphibian taxa in each habitat type found in Durango was summarized. None of the amphibians in the arid-semiarid habitats and Quebradas were in protected IUCN categories (VU, NT, EN, CE), 7.1% in the valleys, and 25% in the Sierra habitat. For SEMARNAT categories, 42.8% of amphibians in the arid-semiarid habitats, 26.7% in the valleys, 33.3% in the Sierra habitat, and 18.2% in the Quebradas were listed. For EVS, 100% of the amphibians in the arid-semiarid habitat were in the low category. Almost half (46.6%) of the amphibians in the valley habitat were in the low category, 40% in the medium category, and 13.3% in the high category. In the Sierra habitat type, 26.3% of amphibians were in the low category, 42.1% in the medium, and 31.6% in the high. For the Quebradas habitat, 45.4% were in the low and medium categories and 9.1% in the high. Based on this summary, it is clear that for amphibians, the Sierra habitat has the most at risk species and the arid-semiarid habitat has relatively fewer at risk species. For amphibians, therefore, the Sierra habitat would appear to be a priority target for conservation efforts.

For the IUCN listings, all habitat types had relatively few species of reptiles in the protected categories (arid-semiarid, 8.5%; valleys, 3.2%; Sierra, 3.8%; and Quebradas, 7.7%). However, 39% of reptiles in the arid-semiarid habitat, 41.9% from the valley habitat, 42.3% from the Sierra habitat, and 30.8% from the Quebradas habitat were in the protected SEMARNAT categories. For the arid-semiarid habitat type, 28.1% of reptiles were in the low EVS category, 43.8% in the medium, and 28.1% in the high. In valleys, 29% of the reptiles were in the low, 51.6% in the medium, and 19.4% in the high. Of the reptiles in the Sierra habitat type, 21.6% were in the low, 45.1% in the medium, and 33.3% in the high categories. For the Quebradas habitat type, 19.2% were in the low EVS category, 50% in the medium, and 30.8% in the high. In contrast to amphibians, at risk reptile species are more evenly distributed across the habitat types. Therefore, conservation efforts for reptiles should address all habitat types.

Three non-native species of amphibians and reptiles were documented in Durango: *R. catesbeiana*, *H. turcicus*, and *I. braminus*. Non-native species can negatively affect native herpetofaunal communities in Mexico (see Wilson and Townsend 2010). Of the three non-native species, *R. catesbeiana* is of particular concern. *Rana catesbeiana* is known to have many impacts on native communities as a competitor, predator, and disease vector on a global scale (reviewed in Moutou and Pastoret 2010; Kraus 2015), as well as in Mexico (e.g., Luja and Rodriguez-Estrella 2010; Becerra Lopez et al. 2017). The potential impacts of *H. turcicus* are less well documented, but its congener *H. frenatus* has affected native herpetofauna through competition (reviewed in Punzo 2005; Kraus 2015). The impacts of *I. braminus* are, to our knowledge, unstudied, even though it has been widely introduced around the world (see Borroto-Páez et al. 2015).

There is thus the potential for these non-native amphibians and reptiles to have negative impacts on the native herpetofauna, and other organisms, of Durango. The extent of these potential impacts need to be evaluated further.

Hopefully, this list of amphibian and reptile species in Durango with their global and country-level conservation statuses will prompt further investigations into the herpetofauna of this state, which could provide the needed information to allow for state- or regional-specific, or even habitat type, conservation measures to be undertaken.

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SHORT COMMUNICATION

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Occurrence of the large ostracod, *Chlamydotheca unispinosa* (Baird, 1862), in temporary waters of Montserrat, Lesser Antilles

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Abstract

Four populations of the large freshwater ostracod, *Chlamydotheca unispinosa* (Baird, 1862), were discovered on the Caribbean island of Montserrat. These are the first records of the species on Montserrat and extend its known distribution approximately 113 km northwest and 63 km southeast of the closest known populations on Îles des Saintes (Guadeloupe) and Nevis, respectively. We provide the first DNA barcode for *C. unispinosa*, a 686 bp fragment of the COI gene which may be used for future comparative studies of this widely distributed species.

Keywords

COI, Cyprididae, West Indies, Podocopida

Introduction

The New World genus *Chlamydotheca* Saussure, 1858 contains primarily tropical large freshwater ostracods. There are 36 species (Martens and Savatenalinton 2011) with the majority from continental waters. Two species are recorded from Caribbean Islands, *C. barbadensis* Sharpe, 1910 and *C. unispinosa* (Baird, 1862). *Chlamydotheca barbadensis* was described from Barbados, recorded from northern South America and several islands off the South American coast (Broodbakker 1984), and with a disjunct distribution in Antigua, Barbuda, St. Eustatius, and St. Martin in the northern Lesser Antilles (Broodbakker 1984). *C. unispinosa* was described from Jamaica (Baird 1862); recorded from the Greater Antilles and the Bahamas; Nevis (Bass 2006), Marie Galante, and Îles des Saintes (Broodbakker 1984) in the Lesser Antilles; and Illinois (Evenson 1942), Maryland (Tressler 1947), Yucatan (Furtos 1936), Colombia (Roessler 1986), and south to Brazil (Tressler 1949). It has also been reported from Hawaii (Baird 1862; Eldridge and Miller 1997). Montserrat is a small volcanic island in the northern end of the Lesser Antilles (Figure 1). Volcanic eruptions and subsequent lahar flows from 1995–2012 destroyed a substantial portion of the freshwater lentic and lotic environments on the island (Barclay et al. 2007) and perhaps caused local extinctions of some aquatic organisms. Surveys of the remaining freshwater habitats led to this note reporting the presence of *C. unispinosa* in temporary epigean fresh waters of Montserrat, Lesser Antilles.

Materials and methods

Living specimens of large ostracods were collected from shallow fresh water with fine mesh dip nets and fixed in 95 % ethanol. Empty valves located in dried temporary pools were collected by hand and stored dry. Three preserved specimens were deposited in the Academy of Natural Sciences, Philadelphia, two in 80% ethanol and one (DNA voucher) in 95 % ethanol (ANSP GI-19490). Empty valves were collected from a dry temporary pool dominated by the aroid, *Dieffenbachia seguine* (Jacq.) Schott, along the Blackwood Allen trail, Baker Hill, Montserrat (Fig. 1) on January 2, 2015 and January 10, 2017 (approximately 16°46'25.04"N, 62°12'27.17"W). Living specimens (Fig. 2) were collected from a shallow pool adjacent to the upstream edge of the road crossing over Bottomless Ghaut, Blake's Estate, Montserrat on January 10, 2016 (16°46'45"N, 62°10'32"W). On January 17, 2017, empty valves were collected from a muddy temporary puddle along the Jack Boy Hill trail (approximately 16°45’46"N, 62°10’46"W) Trant's Estate, Montserrat. Living specimens were collected from Dowdye Pond dominated by water lettuce, *Pistia stratiotes* L., along the road north of Gerald's, St. Peter, Montserrat on January 16, 2018 (approximately 16°48’19.60”N, 62°11’35.78”W). The ostracods collected were identified as *C. unispinosa* (Baird 1862) by comparing our specimens with descriptions and illustrations in Roessler (1986). Additionally, illustrations of *C. barbadensis* show valves of a different shape from our specimens and without a point on the posterolateral margin (Sharpe 1910).

Genomic DNA was extracted from one entire animal using a DNeasy Blood & Tissue Kit (QIAGEN) and a 710bp region of the mitochondrial COI gene was amplified using HCO2198 and LCO1490 (Folmer et al. 1994). PCR products were purified using a Qiaquick PCR Purification Kit (QIAGEN) and sequenced using the same primers as the PCR (DNA Analysis Facility on Science Hill, Yale University).

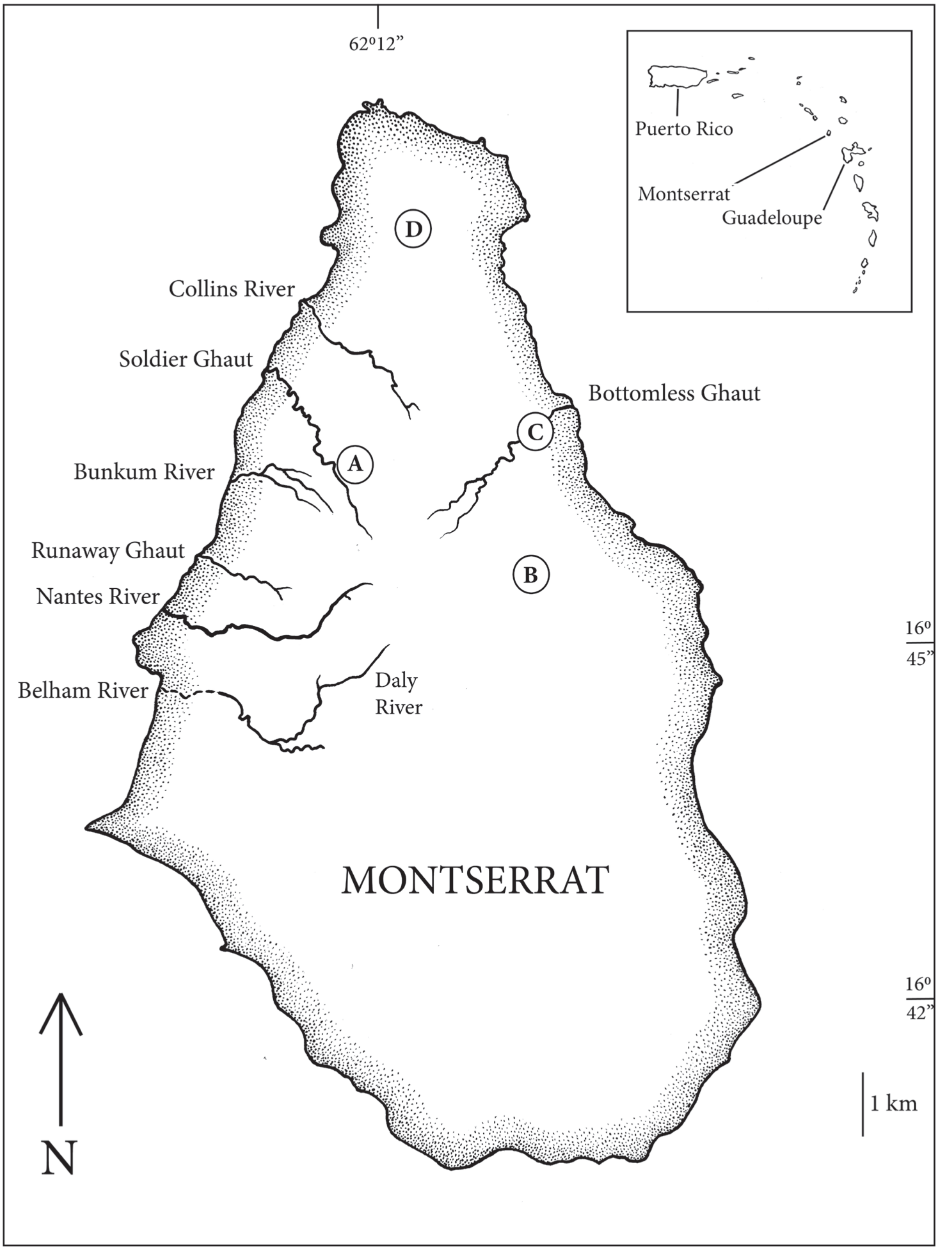

Figure 1. Map of Montserrat showing approximate collection localities of *Chlamydotheca unispinosa*. Empty valves were collected from a dry pool along Blackwood Allen Trail (**A**) and in a muddy puddle along Jack Boy Hill Trail (**B**). Living specimens were collected from Bottomless Ghaut (**C**) and Dowdye Pond (**D**).

Complimentary forward and reverse sequences were aligned and edited in BioEdit (Hall 1999) and the resulting sequence was used in a BLAST search of the GenBank nucleotide database (blastn). A selection of DNA sequences similar to the Montser-

Figure 2. Preserved specimen of *Chlamydotheca unispinosa* from Bottomless Ghaut, Montserrat, approximately 4.1 mm long. Living individuals were colored bluish-green.

rat ostracod were downloaded and aligned using ClustalW (MEGA, Tamura et al. 2013). Alignments were edited and poorly aligning flanking regions were removed. Aligned sequences were translated, using an invertebrate mitochondrial genetic code table, into amino acid sequences to check for alignment errors. A neighbor-joining tree (bootstrap, 1000 replications) was constructed of COI sequences representing the Montserrat ostracod and the four most similar species published in GenBank, as well as a sequence from one more distantly related ostracod, *Conchoecetta cuminata* Claus, 1890 (Podocopida, Cytherideidae) as an outgroup (MEGA). Pairwise distances (p-distance, complete deletion) were calculated between the nucleotide sequences of the Montserrat ostracod and the four most similar published sequences, as well as one with the outgroup sequence of *C. cuminata* (MEGA).

Results

The COI sequence generated for this Montserrat ostracod was deposited in GenBank (accession number KY678900). No COI, DNA, or amino acid sequence in GenBank was highly similar to the sequence obtained from the Montserrat ostracod. The most similar nucleotide and amino acid sequences included representatives from the genera *Bennelongia* De Decker & McKenzie, 1931; *Strandesia* Stuhlmann, 1888; *Eucypris* Vavra, 1891, and *Paracypria* Sars, 1910 (Fig. 3). The most similar DNA sequences had p-distances ranging from about 0.19 (*Bennelongia timmsi* Martens, Halse & Schön, 2013; #KF725009) to 0.22 (*Strandesia velhoi* Higuti, Schön, Aude-

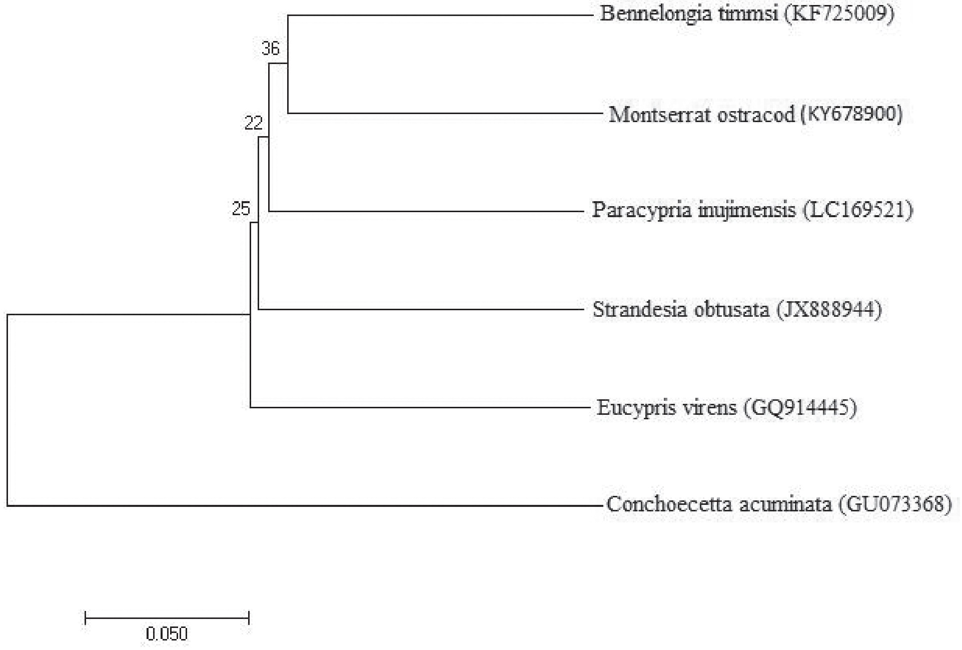

Figure 3. Neighbor-Joining tree of COI nucleotide sequences (codon positions 1, 2, and 3) from the Montserrat specimen of *Chlamydotheca unispinosa*, four of the most similar sequences and one outgroup. All positions containing gaps and missing data were eliminated. There was a total of 620 positions in the final dataset. Bootstrap values (1000 replicates) are shown at each node. Branch lengths represent evolutionary distances (p-distance) and are in the units of the number of base differences per site. All analyses were completed in MEGA 7 (Kumar et al. 2016).

naert, & Martens, 2013; #JX888939). The translated amino acid COI sequence of the Montserrat ostracod differed from its closest match, *S. velhoi*, by a p-distance of 0.02, and from *B. timmsi* by a p-distance of 0.04.

Discussion

The nearest populations of *C. unispinosa* are on Nevis (Bass 2006) and Îles des Saintes (Broodbakker 1984), 113 km northwest and 63 km southeast of Montserrat, respectively. However, nearby islands to the northeast and northwest of Montserrat are inhabited by *C. barbadensis* (Broodbakker 1984).

Members of the genus *Chlamydotheca* can be found in lotic and lentic, permanent and temporary waters (Diaz and Lopretto 2011). The Montserrat specimens were all collected from seasonally dry locations; three temporary pools and a stream, Bottomless Ghaut, which is usually dry during the dry season. Substrate in the three temporary pools was muddy. Substrate in Bottomless Ghaut was gravel and cobble. Specimens collected were all large adults. Populations in Bottomless Ghaut and the puddle along Jack

Boy Hill trail were small but there were probably thousands of empty valves in the dried pool along the Blackwood Allen trail and thousands of live animals in Dowdye Pond.

Chlamydotheca unispinosa belongs to the "*C. iheringi* group" (Roessler 1986). The center of diversity of this group of ostracods is in northern South America. *Chlamydotheca unispinosa* has the widest distribution of any member of the species group which may indicate that some or all of the populations outside of the South American continent are introduced. The records from Illinois, Maryland, and Hawaii are particularly suspect because the first two locations are temperate and all are vastly distant from northern South America. Some ostracods living in temporary freshwaters have desiccation-resistant eggs that can remain viable for >10 years (Boulton and Lloyd 1992) and could be transported great distances by wind, animals, or humans. Few studies have been done on this phenomenon in ostracods (Radzikowski 2013).

It is also possible that *C. unispinosa* is composed of several cryptic species. Studies comparing DNA sequences throughout the range of this species might determine whether cryptic species exist (Lara et al. 2010) or whether this species is particularly vagile. Unfortunately, these data do not currently exist. The COI sequence from this study (GenBank #KY678900) could be used to identify and compare similar sequences from other populations of *C. unispinosa*, particularly those reported from temperate regions.

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RESEARCH ARTICLE

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Molecular and morphological differentiation of Secret Toad-headed agama, *Phrynocephalus mystaceus*, with the description of a new subspecies from Iran (Reptilia, Agamidae)

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Abstract

The morphological and genetic variation of a wide-ranging Secret Toad-headed agama, *Phrynocephalus mystaceus* that inhabits sand deserts of south-eastern Europe, Middle East, Middle Asia, and western China is reviewed. Based on the morphological differences and high divergence in COI (mtDNA) gene sequences a new subspecies of *Ph. mystaceus* is described from Khorasan Razavi Province in Iran. Partial sequences of COI mtDNA gene of 31 specimens of *Ph. mystaceus* from 17 localities from all major parts of species range were analyzed. Genetic distances show a deep divergence between *Ph. mystaceus khorasanus* **ssp. n.** from Khorasan Razavi Province and all other populations of *Ph. mystaceus*. The new subspecies can be distinguished from other populations of *Ph. mystaceus* by a combination of several morphological features. Molecular and morphological analyses do not support the validity of other *Ph. mystaceus* subspecies described from Middle Asia and Caspian basin. Geographic variations in the *Ph. mystaceus* species complex and the status of previously described subspecies were discussed.

Keywords

Khorasan, molecular phylogenetics, morphology, phylogeography, *Phrynocephalus mystaceus khorasanus*, taxonomy

Introduction

Toad-headed agamas of the genus *Phrynocephalus* Kaup, 1825, are distributed from south-eastern Europe and southwest Asia (including the Middle East and Arabian Peninsula) through Middle Asia to Central Asia (northern and central China and Mongolia). This taxonomically complicated genus currently contains up to 32 species (Uetz and Hošek 2016). The secret toad-headed agama, or *Phrynocephalus mystaceus* (Pallas, 1776), is one of the largest representatives of the genus, and is easily distinguished from all other congeners by a pair of large fringed cutaneous folds at the mouth angles. It is a specialized psammophilous species that inhabits sand dunes from Caspian region of the south-eastern part of European Russia in the west to the Ili valley in eastern Kazakhstan and western China in the east, and from Kazakhstan in the north through Middle Asia to northeastern Iran in the south (Bannikov et al. 1977; Zhao and Adler 1993; Anderson 1999; Ananjeva et al. 2004; Molavi et al. 2014; Fig. 1).

Phrynocephalus mystaceus was shown to have a high level of anatomical variability (Ananjeva “1986” 1987), which, together with its unique karyotype (Zeng et al. 1997), has led to its uncertain taxonomic classification at the generic level. Eichwald (1831) proposed the new generic name *Megalochilus* Eichwald, 1831 for *Ph. mystaceus*, which was synonymized with the genus *Saccostoma* by Fitzinger (1843). Ananjeva (“1986” 1987) restored the monotypic genus *Megalochilus*, but such taxonomic change was contradicted by Golubev and Sattorov (1992), as they argued that the differences proposed by Ananjeva were too slight to warrant a separate genus status. Molecular phylogenetic analyses based on mtDNA markers failed to resolve the phylogenetic position of *Ph. mystaceus* (Pang et al. 2003), which led Barabanov and Ananjeva (2007) to consider *Megalochilus* as a junior synonym of *Phrynocephalus*. However, a recent phylogeny based on the analysis of *RAG1* nuDNA gene indicated *Ph. mystaceus* as a sister lineage with respect to all other examined *Phrynocephalus* species (Melville et al. 2009). Further study with better taxon sampling based on mtDNA data suggested that *Ph. mystaceus* is a member of the “core” *Phrynocephalus* clade and is associated with *Ph. axillaris* (Solovyeva et al. 2014). The most recent study proposed to consider *Megalochilus* as a subgenus of the genus *Phrynocephalus* (Solovyeva et al. 2014).

There was little consensus in the understanding of intraspecific taxonomy of *Ph. mystaceus*. Krassowky (1932) was the first to split *Ph. mystaceus* into two subspecies: European nominative subspecies *Ph. m. mystaceus* (Pallas, 1776) and Middle-Asian subspecies *Ph. m. galli* Krassowsky, 1932. This taxonomic classification was supported by subsequent studies of Soviet herpetologists (Shibanov 1941; Terentjev and Chernov 1949; Khonyakina 1961). However, morphometric studies by Vel’dre (1964a, 1964b) suggested that it is impossible to distinguish geographical races within *Ph. mystaceus* due to its high morphological variability among populations. Consequently, Ananjeva (1987 “1986”) suggested to upgrade the Middle-Asian subspecies *Ph. m. galli* to full species status and recognized a distinct subspeciesin Daghestan (*Megalochilus mystaceus dagestanica* in Ananjeva et al. 1987 “1986”). Semenov and Shenbrot (1990) analyzed morphological and chromatic differentiation of *Ph. mystaceus* from “Semirechye” (an area east of lake Balkhash in Eastern Kazakhstan), and suggested that this area is inhabited by a distinct subspecies, *Ph. mystaceus aurantiacocaudatus* Semenov et Shenbrot, 1990, which differs from the Middle Asian subspecies *Ph. m. galli* by its bright orange-red coloration of the ventral surface of the tail in young specimens (*versus* lemon-yellow coloration in other subspecies). However, *Ph. mystaceus aurantiacocaudatus* was synonymized with *Ph. m. galli* by Barabanov and Ananjeva (2007) without any discussion.

In summary, three subspecies of *Phrynocephalus mystaceus* are recognized in recent literature (see Barabanov and Ananjeva 2007):

1. *Ph. m. mystaceus* (Pallas, 1776), that inhabits eastern Ciscaucasia (eastern part of Chechen Republic, Daghestan, Kalmykia), Caspian region (southern part of Astrakhan Region, east of the Volga-Ural Sands; introduced to the Apsheron Peninsula, Azerbaijan) and northwestern Kazakhstan (Ananjeva et al. 2004). **Terra typica restricta:** Ryn-Peski (Ryn Sands), Ural Region, northwestern Kazakhstan (Barabanov and Ananjeva 2007). This form includes *Megalochilus mystaceus dagestanica* Ananjeva, “1986” 1987, described from Kumtorkala, Daghestan, Russia, as a junior synonym.
2. *Ph. mystaceus galli* Krassowsky, 1932, that inhabits Transcaspian Region and Middle Asia from Turkmenistan, Uzbekistan, Kazakhstan, to northeastern and eastern Iran and adjacent areas of Afghanistan (Anderson 1999; Ananjeva et al. 2004). **Terra typica:** Repetek station, Lebapsky District, Turkmenistan (Barabanov and Ananjeva 2007). Based on its distribution, this subspecies is supposed to inhabit north-eastern Iran (Anderson 1999).
3. *Ph. mystaceus aurantiacocaudatus* Semenov & Shenbrot, 1990, known from eastern Kazakhstan and western China (Ili River Valley in Xinjiang). **Terra typica:** 70 km north northwest of Ushtobe, Eastern Kazakhstan. Regarded as a junior synonym of *Ph. mystaceus galli* by Barabanov and Ananjeva (2007), however, without any justification.

It is notable that all previous works on geographic variations of *Ph. mystaceus* omitted populations from the southernmost edge of its range, Iran and Afghanistan, from the analyses. Morphological characterization and analysis of distribution of *Ph. mystaceus* in Iran was carried out by Anderson (1999) and Molavi et al. (2014). Anderson (1999) examined specimens from Iran and Uzbekistan, and proposed that Iranian populations demonstrate intermediate morphology between *Ph. m. galli* and *Ph. mystaceus*. Molavi et al. (2014), based on a study of seven specimens from Semnan Province, repeated earlier conclusions by Anderson (1999) and suggested that further investigation of both morphological and molecular characters are required to clarify the taxonomic status of Iranian *Ph. mystaceus* populations.

The recent analysis of phylogenetic relationships within the genus *Phrynocephalus* based on four mitochondrial genes revealed a remarkable divergence between *Ph. mystaceus* samples from Iran and Middle Asia (Solovyeva et al. 2014). Based on these results the Iranian population was tentatively indicated as a putative new subspecies *Ph. mystaceus* ssp. In the present study, we provide a detailed analysis of both morphological and genetic variation of *Ph. mystaceus* across its range and confirm deep differentiation between the population from Khorasan Province of Iran and other populations in the species range. The currently recognized subspecies of *Ph. mystaceus* are reviewed and a new subspecies from Khorasan Province is described, based on both molecular and morphological features.

Materials and methods

Sampling. Historical collections of the Zoological Museum of Lomonosov Moscow State University (**ZMMU**) were examined, in total, 70 adult and subadult specimens of all currently recognized subspecies (Appendix 1). In addition, type specimens of *Ph. mystaceus galli* (lectotype, ZMMU R-6413) and *Ph. mystaceus aurantiacocaudatus* (holotype, ZMMU R-6412) were also examined. Sampling was carried out in the Khorasan Province of Iran in April of 2005, April of 2006, May and June of 2009, and May of 2010. Specimens from Iran were obtained through the collaboration with the Zoological Museum of International Center for Science, High Technology and Environmental Sciences (**ICSTZM**; Kerman, Iran; MOU no. 158/2010). Tissue samples from 31 *Ph. mystaceus* specimens were used in molecular analyses, and their geographic distribution is shown in Fig. 1. Details on museum IDs and localities of origin for each sample are summarized in Table 1.

Molecular analyses. Mitochondrial DNA COI gene (cytochrome oxidase *c* subunit I) fragment, 654 b. p. in length was analyzed. Muscle and skin tissues were disintegrated with Proteinase K and total genomic DNA was extracted using a standard phenol-chloroform extraction protocol followed by ethanol precipitation of DNA (Sambrook et al. 1989). PCR amplification was performed using MyCycler BioRad under conditions described by Ivanova et al. (2006). Standard pair of primers was used: VF1d (5'-TTCTCAACCAACCACAARGAYATYGG-3') and VR1d (5'-TAGACTTCTGGGTGGCCRAARAAYCA-3') or Rep-COI-F (5'-TNTTMTCAACNAACCACAAAGA-3') and Rep-COI-R (5'-ACTTCTGGRTGKCCAAARAATCA-3'). PCR reaction volume was 20 µl and it contained ca. 100 ng of template DNA, 0.3 pM/µl of each PCR primer, 1xTaq-buffer with 25 mM of $MgCl_2$ (Silex, Moscow Russia), 0.2 mM dNTPs, and 1 unit of Taq-polymerase (Silex, Moscow Russia; 5 units/µl). The results of the amplification were examined using electrophoresis in 1% agarose gel in presence of ethidium bromide. The length of the obtained fragments was 680 bp. We included two sequences of *Ph. mystaceus* from western China available from Genbank (NC022131 and KC578685; see Chen et al. 2014) in the analyses. Samples of *Ph. melanurus* (ZMMU R-12328, GenBank AN MF567976) and *Trapelus sanguinolentus* (ZMMU R-12709, GenBank AN KF691668) were used as outgroups.

Sequences were aligned using Seqman 5.06 and checked using BioEdit Sequence Alignment Editor 7.1.3.0 (Hall 1999). All sequences were deposited in GenBank (see Table 1 for all voucher information, with corresponding GenBank accession numbers).

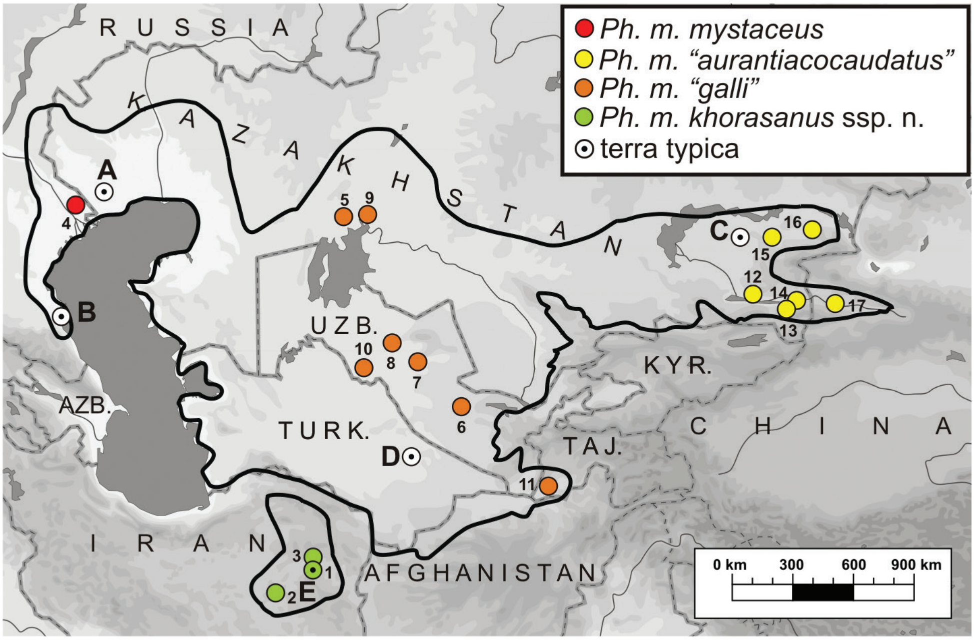

Figure 1. Geographical distribution of *Phrynocephalus mystaceus* and locations of the sites where the samples that were examined in the molecular analyses of the present study were obtained. Locality numbers correspond to those given in Table 1. Dot in the center of a circle indicates the type locality; type localities for taxa are shown as follows: **A** *Lacerta mystacea* Pallas, 1776 **B** *Megalochilus mystaceus dagestanica* Ananjeva, "1986" 1987 **C** *Phrynocephalus mystaceus aurantiacocaudatus* Semenov & Shenbrot, 1990 **D** *Phrynocephalus mystaceus galli* Krassowsky, 1932; and **E** *Ph. mystaceus khorasanus* ssp. n.

Mean uncorrected *p*-distances and sequences characteristics were calculated using MEGA 6 (Tamura et al. 2011). Phylogenetic analyses were conducted using Treefinder (Jobb et al. 2011) and MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) software.

PartitionFinder v1.0.1 (Lanfear et al. 2012) was used to estimate the optimal evolutionary models for Bayesian inference analysis. The preferred model for *COI* alignment was HKY + G for two partitions (codon position 1 and 2 vs. codon position 3) as suggested by the Akaike information criterion (AIC). Bayesian phylogenetic analysis was performed using MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) with two simultaneous runs, each with four chains, for 20 million generations, 2 million generations were cut as burn in. The convergence of the runs was checked to make sure that the effective sample sizes (ESS) were all above 200 by examining the likelihood plots using TRACER v.1.5 (Rambaut and Drummond 2007).

The Maximum Likelihood (ML) analysis was conducted using Treefinder (Jobb et al. 2011). Each dataset was divided into three partitions according to codon positions; for each partition the best fitting substitution model was selected using the AIC in Treefinder. For ML-analysis we used 1000 pseudoreplics (BS) and Expected Likelihood Weights (ELW).

Table 1. List of the samples used in molecular analyses. Locality numbers correspond to those in Figure 1.

Voucher Nº	Subspecies	Locality	GenBank Nº
ZMMU R-12202	*Ph. mystaceus khorasanus* ssp. n.	Iran, Khorasan Razavi Prov., Gonabad **(1)**	MF567983
ZMMU R-13009-1	*Ph. mystaceus khorasanus* ssp. n.	Iran, Khorasan Razavi Prov., Boshrue **(2)**	MF567989
ZMMU R-13009-2	*Ph. mystaceus khorasanus* ssp. n.	Iran, Khorasan Razavi Prov., Boshrue **(2)**	KF691714
ZMMU R-13011-1	*Ph. mystaceus khorasanus* ssp. n.	Iran, Khorasan Razavi Prov., Gonabad **(1)**	MF567987
ZMMU R-13011-2	*Ph. mystaceus khorasanus* ssp. n.	Iran, Khorasan Razavi Prov., Gonabad **(1)**	MF567988
ZMMU R-11913	*Ph. mystaceus khorasanus* ssp. n.	Iran, Khorasan Razavi Prov., Gonabad **(1)**	MF567975
ZMMU R-13169	*Ph. mystaceus khorasanus* ssp. n.	Iran, Khorasan Razavi Prov., 30 km N Gonabad **(3)**	MF567974
RuHF-072-1	*Ph. mystaceus mystaceus*	Russia, Astrakhan Prov., Dosang **(4)**	MF567968
RuHF-072-2	*Ph. mystaceus mystaceus*	Russia, Astrakhan Prov., Dosang **(4)**	MF567969
ZMMU-R-12457-2	*Ph. mystaceus mystaceus*	Russia, Astrakhan Prov., Dosang **(4)**	MF567990
ZMMU-R-12457-3	*Ph. mystaceus mystaceus*	Russia, Astrakhan Prov., Dosang **(4)**	MF567986
RuHF-079-1	*Ph. mystaceus galli*	Kazakhstan, N Priaralye, S border of Malye Barsuki sands **(5)**	MF567971
RuHF-079-2	*Ph. mystaceus galli*	Kazakhstan, N Priaralye, S border of Malye Barsuki sands **(5)**	MF567970
ZMMU-R-12517-2	*Ph. mystaceus galli*	Kazakhstan, N Priaralye, S border of Malye Barsuki sands **(5)**	MF567985
ZMMU R-12772	*Ph. mystaceus galli*	Kazakhstan, Aralsk **(6)**	MF567982
ZMMU R-12775	*Ph. mystaceus galli*	Uzbekistan, Qarakalpaqiston Republic **(7)**	MF567981
ZMMU-R-12266	*Ph. mystaceus galli*	Uzbekistan, Qarakalpaqiston Republic, Chukurkak **(8)**	MF567978
ZMMU-R-12252-1	*Ph. mystaceus galli*	Uzbekistan, Navoi Prov., Terankuduk **(9)**	MF567977
ZMMU-R-12261-1	*Ph. mystaceus galli*	Uzbekistan, Navoi Prov., Yamankum desert **(10)**	KF691713
ZMMU R-12799	*Ph. mystaceus galli*	Tajikistan, Shaartuz **(11)**	MF567979
RuHF-077-1	*Ph. mystaceus aurantiacocaudatus*	E Kazakhstan, N Kapchagai Reservoir **(12)**	MF567972
RuHF-077-2	*Ph. mystaceus aurantiacocaudatus*	E Kazakhstan, N Kapchagai Reservoir **(12)**	MF567973
ZMMU R-12518	*Ph. mystaceus aurantiacocaudatus*	SE Kazakhstan, left bank of Ili River,125 km of the road Almaty-Bakanas **(13)**	MF567984
ZMMU R-12778	*Ph. mystaceus aurantiacocaudatus*	Kazakhstan, Pidzhim env. **(14)**	MF567980
ZMMU R-14715-1	*Ph. mystaceus aurantiacocaudatus*	Kazakhstan, S Balkhash lake, N of Matay **(15)**	MF567991
ZMMU R-14715-2	*Ph. mystaceus aurantiacocaudatus*	Kazakhstan, S Balkhash lake, N of Matay **(15)**	MF567992
ZMMU R-14715-3	*Ph. mystaceus aurantiacocaudatus*	Kazakhstan, S Balkhash lake, N of Matay **(15)**	MF567993
ZMMU R-14715-4	*Ph. mystaceus aurantiacocaudatus*	Kazakhstan, S Balkhash lake, N of Matay **(15)**	MF567994
ZMMU NAP-05510	*Ph. mystaceus aurantiacocaudatus*	Kazakhstan, E Balkhash lake, environs of Kabanbay **(16)**	MF567995
No voucher number	*Ph. mystaceus aurantiacocaudatus*	China, Ili River valley, Huocheng **(17)**	NC021131

Confidence in tree topology was tested by using non-parametric bootstrap analysis (Felsenstein 1985) with 1000 replicates and posterior probability (PP) for Bayesian inference (BA) in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Branches with bootstrap values of 70% or higher and posterior probabilities values over 0.95 were regarded as sufficiently resolved (Huelsenbeck and Hillis 1993).

Morphological analyses. Pholidosis was examined and morphometrics acquired for 79 individuals in four groups of *Ph. mystaceus*, including 20 specimens of nominative subspecies *Ph. m. mystaceus*, seven specimens from Khorasan Province of Iran, 32 specimens of *Ph. m. aurantiacocaudatus* from Eastern Kazakhstan, and 20 specimens of *Ph. m. galli* from Middle Asia (Appendix 1). In order to take into account sexual dimorphism, males (n = 26) and females (n = 44) were analyzed separately.

Morphological characteristics and the methods for their measurement are generally the same as in the study by Solovyeva et al. (2012). The following measurements and scalation counts were used: (1) snout-vent length (SVL); (2) tail length (TL); (3) SVL/TL ratio; (4) number of flat supralabials anterior to angular enlarged spine-like supralabial scales (SLbA); (5) total number of flat supralabials from tip of snout to insertion of cutaneous fold at mouth angle (SL); (6) relative length of the dark distal part of the tail to the total tail length (in ventral aspect, calculated as TL-black/TL ratio); (7) number of scales surrounding subnasal from below (SSbNb); (8) subnasal in contact with medial side of supranasal (*vs.* subnasal not in contact with medial side of supranasal) (SbN-SpN); (9) supranasal edges nostril dorsally along the full length of nostril (*vs.* supranasal edges nostril dorsally along only half of nostril length) (SpN); (10) height of supranasal is less than or equal to height of subnasal (*vs.* height of supranasal exceeds height of subnasal) (hSpN SbN); (11) number of scale rows that separate subnasal and labial scales (SbN-L); (12) longitudinal row of white scales in supraorbital area outlined by continuous black lines (or intermitted) (WS&BL); (13) number of small rows of scales between anterior (2d and 3d) inframandibulars and large rows of scales under infralabial scales – 1-2 or 2-3 (aIMd-IL); (14) number of scales that underlay enlarged spiny scales on edge of cutaneous fold at mouth angle (SuSSCF); (15) number of small granular scales between posteriormost supralabial and insertion of cutaneous fold at mouth angle (pSL-CF); (16) number of flat infralabials anterior to angular enlarged spine-like infralabial scales (ILbA); (17) total number of infralabials from tip of snout to insertion of cutaneous fold at mouth angle (IL); (18) number of subdigital lamellae under toe III (SLIII); (19) number of enlarged triangular scales on lateral fringes of toe III (FrIII); (20) number of subdigital lamellae under toe IV (SLIV); (21) number of enlarged triangular scales on lateral fringes of toe IV (FrIV). Characteristics 18-21 (SLIII, FrIII, SLIV, FrIV) were registered with no regard to the sex of the individual. Following standard measurements were additionally taken for holotype and paratypes: head height (HH); head length (HL, measured on ventral side from snout tip to gular fold); head width (HW, measured at broadest part of head excluding cutaneous folds); pileus width (PW). Measurements were taken using a digital caliper and rounded to the nearest 0.1 mm.

Box-and-whiskers-plots and values of descriptive statistics were calculated using R (R Core Team, 2013). The Mann-Whitney test of independent series was used to determine the differences between the pairs of subspecies (with confidence level of $p \leq$

0.05). Principal Components Analysis (PCA) was performed using R (R Core Team, 2013) to visualize morphological variation between Khorasan specimens of *Ph. mystaceus* and specimens from other populations.

Results

Sequence characteristics. The sequenced fragments from 31 *Ph. mystaceus* specimens were up to 654 b.p. in length, among which 577 sites were identified as conservative, 74 as variable and 59 as potentially parsimony-informative. Nucleotide frequencies were equal to: 30.2% (A), 27.5% (T/U), 27.9% (C), and 14.4% (G). The transition-transversion bias (R) was estimated to be 6.574 (all data given for in-group only).

Phylogenetic analysis. The results of phylogenetic analysis are presented in Fig. 2. BI and ML yielded trees that show essentially similar topologies. All analyses reveal the presence of two reciprocally monophyletic clades within *Ph. mystaceus.* The first clade consists of Irainan *Ph. mystaceus* ssp. from Khorasan Province (node support values are 1.0/86; hereafter given for BI PP/ ML BS; clade I on Fig. 2). The second clade includes all other *Ph. mystaceus* populations from Middle and Central Asia and Caspian Region (1.0/99; clade II on Fig. 2). Further phylogenetic structure within the second clade of non-Iranian *Ph. mystaceus* is poorly resolved. Populations from the eastern part of the range including Eastern Kazakhstan and Xinjiang (China) that correspond to the *Ph. m.* "*aurantiacocaudatus*" occupy basal position in the clade II, but are not monophyletic and fall into three poorly differentiated subclades: from the environs of Kapchagai (subclade A; 0.90/82), Ili River Valley (subclade B; from Zharkent to Xinjiang; 1.0/95), and the environs of Lake Balkhash (subclade C; 0.98/-) (see Fig. 2). Phylogenetic positions of two samples from Eastern Kazakhstan (ZMMU NAP-05510 and ZMMU R-12518-2) are not resolved. All other populations from Middle Asia (Kazakhstan and Uzbekistan – Fig. 2D) and Caspian Region (Astrakhan Province, Russia – Fig. 2E) form a significantly monophyletic clade (1.0/95), which is deeply nested within the basal differentiation of East Kazakhstan *Ph. m.* "*aurantiacocaudatus*" clades (see Fig. 2), rendering the latter taxon paraphyletic. The Middle Asian – Caspian clade (D + E) corresponds to the nominative subspecies *Ph. mystaceus mystaceus* and also includes populations previously classified as *Ph. mystaceus galli* (Aral Sea Region, Uzbekistan and Tajikistan – Fig. 2D). Populations of *Ph. mystaceus mystaceus* and *Ph. mystaceus* "*galli*" are mixed with each other without any clear structure (see Fig. 2 D1, D2, E1, E2).

Genetic distances. Uncorrected genetic *p*-distances between and within clades of *Ph. mystaceus* are shown in Table 2. The *p*-distances within the Middle Asian – Caspian clade of *Ph. mystaceus*, including comparisons between different lineages of *Ph. m.* "*aurantiacocaudatus*" and between *Ph. m.* "*aurantiacocaudatus*" and *Ph. m. mystaceus* are quite low (0.55–0.88% and 1.56–1.87%, respectively), which is less than intraspecific genetic distances for *COI* for some other species of *Phrynocephalus* (e.g. see Solovyeva et al. 2011 for *Ph. helioscopus*). However, *p*-distances between *Ph. mystaceus* ssp. from Khorasan Province and all other groups of *Ph. mystaceus* are very high (6.84–7.28%), they even exceed interspecific

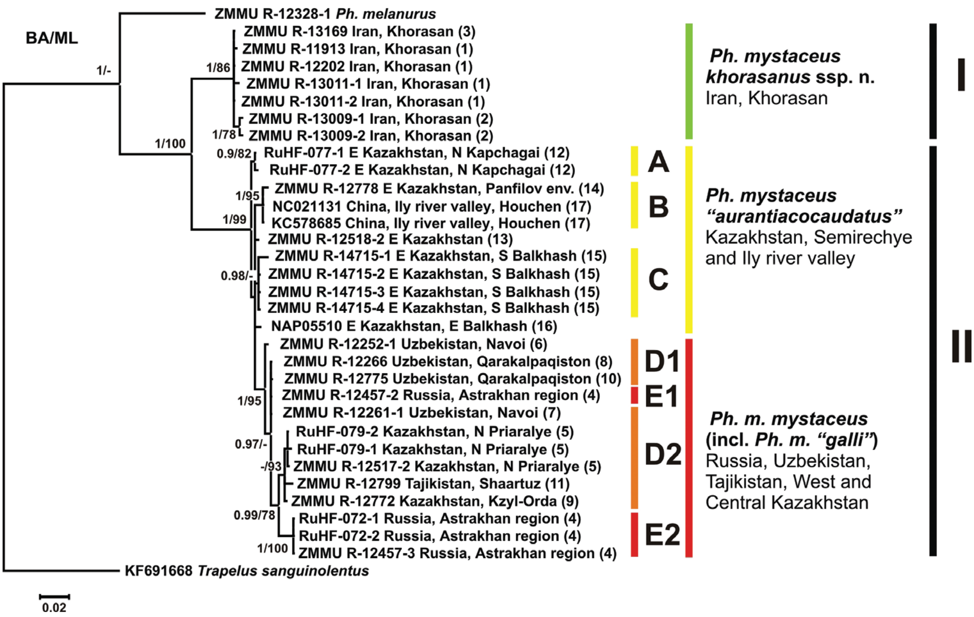

Figure 2. BI-inferred dendrogram that illustrates the phylogenetic relationships of the *Phrynocephalus mystaceus* species complex based on the analysis of 654 b. p. fragment of *COI* gene (mtDNA). Numbers at the tree nodes show Bayesian Posterior Probabilities/ Maximum Likelihood Bootstrap Support. Only PP values higher than 0.90 and BS values higher than 75% are shown. *COI* sequence of *Trapelus sanguinolentus* is used as an outgroup.

Table 2. Uncorrected *p*-distances (percentage) between and within the groups of *Ph. mystaceus* complex. Distances are shown under the diagonal row; standard error values are given above the diagonal row. *Ph. m. aurantiacocaudatus* A corresponds to the population from N Kapchagai (RuHF-077); a – all specimens of *Ph. m. aurantiacocaudatus*, except for *Ph. m. aurantiacocaudatus* A, b – *Ph. m. aurantiacocaudatus* from southeast Pribalkhashye (Matay) (ZMMU R-14715), c – *Ph. m. aurantiacocaudatus* from Ili river valley, except for R-12518-2.

Group		1	2			3	4
			2-a	2-b	2-c		
1. *Ph. m. mystaceus* [Including “*Ph. m. galli*”)		**0.97**	0.4	0.47	0.43	0.41	0.96
2. *Ph. m.* “*aurantiacocaudatus*”	**2-a**	1.7	**0.63**	–	–	0.22	0.94
	2-b	1.87	–	**0.16**	0.34	0.32	1.02
	2-c	1.65	–	0.88	**0.27**	0.25	1.03
3. *Ph. m.* “*aurantiacocaudatus*” A		1.56	0.66	0.88	0.55	**0.15**	0.97
4. *Ph. m. khorasanus* ssp. n.		7.28	7.18	7.24	7.17	6.84	**0.37**

genetic distances for *COI* gene reported for certain species of *Phrynocephalus* (Solovyeva et al. 2014). This data clearly suggest a deep divergence between *Ph. mystaceus* populations from Khorasan Province and populations from the rest of the range of the species.

Morphology. Our study supports the results of previous researchers that indicated very high morphological variation in the absence of consistent morphological variation patterns that could delimit recognized subspecies in Middle Asian populations of *Ph. mystaceus* (Vel'dre 1964a, 1964b; Semenov and Shenbrot 1990). Most characteristics, including body size, were uninformative for distinguishing subspecies and local populations of *Ph. mystaceus*. Only four morphological characteristics showed consistent differences between Iranian and Middle Asian/Caspian populations of *Ph. mystaceus*, including SLIV, FrIII, SL, and TL-black/TL. Specifically, SLIV was lower in the population from Khorasan Razavi Province (N = 7) than in other subspecies of *Ph. mystaceus* (differences are significant; p = 0.000 for comparison with *Ph. h.* "*aurantiacocaudatus*", N = 32; p = 0.000 for comparison with *Ph. h.* "*galli*", N = 20; p = 0.087 for comparison with *Ph. m. mystaceus* sensu stricto, N = 20; for measurement ranges see Table 3). FrIII was also significantly lower in Khorasan population (N = 7) than in *Ph. m. mystaceus* sensu stricto (p = 0.000 N = 20). SL was also lower in Khorasan population (N = 7) than in other subspecies (p = 0.007 for comparison with *Ph. m.* "*aurantiacocaudatus*", N = 32; p = 0.001 for comparison with *Ph. m. mystaceu*s sensu stricto, N = 20; p = 0.050 for comparison with *Ph. m.* "*galli*", N = 20). Finally, the dark distal part of the tail (TL-black/TL) was relatively longer in the Khorasan population (differences are significant; p = 0.023, for comparison with *Ph. m.* "*aurantiacocaudatus*", N = 32; p = 0.000 for comparison with *Ph. m. mystaceu*s sensu stricto, N = 20; p = 0.001 for comparison with *Ph. m.* "*galli*", N = 20). Morphological comparison of four geographical population groups that correspond to the subspecies "*mystaceus* sensu stricto", "*galli*", "*aurantiacocaudatus*" and the "Khorasan population" for the diagnostic morphological characteristics mentioned above is given in Fig. 3. Other characteristics with p-values for pairwise comparisons <0.05 showed significant overlap of values between subspecies and cannot be reliably used in diagnostics; p-values for morphological characteristics for pairwise comparisons are summarized in Appendix 2. Standard measurements of *Ph. mystaceus* ssp. from Khorasan Province are presented in Table 4.

Comparison of Khorasan *Ph. mystaceus* ssp. population with other populations of *Ph. mystaceus* from Middle Asia and Caspian region (data for "*mystaceus* sensu stricto", "*galli*", "*aurantiacocaudatus*" combined together) also demonstrated significant differences for many traits with p<0.05 (Appendix 2), however, values for most of them were overlapping. The six of the characters with the minimal overlap were the following: SVL/TL, TL-black/TL, SL, ILbA, SLIII, SLIV (see Fig. 4 for details).

PCA showed differences between Khorasan population and other *Ph. mystaceus* populations, although these two groups are slightly overlapping with two Khorasan specimens falling into the *Ph. m. mystaceus* sensu lato area (Fig. 5). PCA failed to reveal any clear structuring within the Middle Asian / Caspian populations of *Ph. mystaceus*.

Taxonomy

MtDNA data strongly indicates the presence of two deeply divergent clades within *Ph. mystaceus*: one from northeastern Iran, the other occupying the rest of the species range in Middle Asia (see Fig. 1). MtDNA divergence in *COI* gene fragments between these

Table 3. Mean, standard deviation, and range of measurements (mm) of adult *Ph. mystaceus* ssp. For abbreviations, see Materials and methods. In SbN-SpN, SpN, hSpN-SbN, and WS&BL: 0 equals "no", 1 equals "yes".

Subspecies	**A. *mystaceus* s. str.**		**B. "*galli*"**		**C. "*aurantiacocaudatus*"**		**D. *mystaceus* s. l. (A+B+C)**		**E. *khorasanus* ssp. n.**	
Measure-ments	**m N = 9**	**f N = 11**	**m N = 7**	**f N = 13**	**m N = 8**	**f N = 15**	**m N = 26**	**f N = 44**	**m N = 2**	**f N = 5**
SVL	10.13±1.06 (7.9–11.2)	9.26±0.89 (6.3–10.5)	8.41±1.00 (6.4–9.5)	8.18±1.02 (6.3–10)	8.10±1.23 (6.5–10.5)	6.97±1.31 (5.5–10)	9.01±1.42 (6.4–11.2)	8.19±1.41 (6.3–10.5)	8.55±0.07 (8.5–8.6)	5.96±0.65 (5.4–7)
TL	11.12±0.97 (9.3–11.2)	10.14±0.87 (7.3–11.2)	8.53±0.99 (6.4–9.7)	7.28±1.03 (6.1–10.6)	8.30±1.50 (7–11.5)	7.27±1.28 (6–10.5)	9.50±1.74 (6.4–11.5)	8.49±1.63 (6–11.2)	7.60±0.00 (7.6)	5.8±0.61 (5.1–6.7)
SVL/TL	0.92±0.05 (0.85–1.00)	0.91±0.04 (0.85–0.97	0.97±0.05 0.93–1.06	1.02±0.05 (0.83–1.10	0.98±0.04 (0.91–1.03)	0.96±0.04 (0.88–1.03)	0.96±0.05 (0.85–1.06)	0.97±0.07 (0.83–1.10)	1.13±0,01 (1.12–1.13)	1.03±0.03 (1.00–1.06)
SLbA	12.11±0.60 (11–13)	11.6±3.03 (7–17)	10.14±1.35 (8–12)	11.25±1.95 (7–15)	10.5±1.77 (8–14)	11±1.63 (7–13)	10.93±1.55 (8–14)	11.06±2.27 (7–17)	8.50±3.53 (6–11)	9.00±1.73 (8–11)
SL	15.78±1.39 (14–18)	16.11±1.90 (13–19)	13±1.63 (10–15)	14.75±1.88 (12–19)	14.75±1.49 (13–17)	14.69±1.54 (12–18)	14.83±1.76 (10–18)	14.94±1.77 (12–19)	11.5±2.12 (10–13)	12.67±1.15 (12–14)
TL-black/TL	0.42±0.02 (0.38–0.45)	0.44±0.03 (0.39–0.48)	0.44±0.02 (0.41–0.47)	0.41±0.04 (0.33–0.47)	0.39±0.02 (0.35–0.41)	0.39±0.03 (0.32–0.45)	0.42±0.03 (0.35–0.47)	0.41±0.04 (0.32–0.48)	0.36±0.00 (0.36)	0.39±0.01 (0.37–0.40)
SSbNb	5±0.71 (4–6)	4.8±0.42 (4–5)	6.14±0.69 (5–7)	5.81±1.38 (3–8)	5.75±1.(8 4–8)	5.13±1.09 (4–7)	5.57±0.94 (4–8)	5.38±1.16 (3–8)	5±1.41 (4–6)	4.33±1.53 (3–6)
SbN-SpN	0.44±0.53 (0–1)	0.40±0.52 (0–1)	0.71±0.49 (0–1)	0.56±0.51 (0–1)	0.38±0.52 (0–1)	0.13±0.34 (0–1)	0.43±0.50 (0–1)	0.38±0.49 (0–1)	0.50±0.71 (0–1)	0±0.00 (0)
SpN	0.33±0.50 (0–1)	0.50±0.53 (0–1)	0.71±0.49 (0–1)	0.88±0.34 (0–1)	0.25±0.46 (0–1)	0.56±0.51 (0–1)	0.50±0.50 (0–1)	0.63±0.49 (0–1)	0±0.00 (0)	0.67±0.58 (0–1)
hSpN-SbN	0.78±0.44 (0–1)	0.90±0.32 (0–1)	0.57±0.53 (0–1)	0.44±0.51 (0–1)	0.88±0.35 (0–1)	0.56±0.51 (0–1)	0.70±0.47 (0–1)	0.65±0.48 (0–1)	0±0.00 (0)	0±0.00 (0)
SbN-L	5.22±1.39 (3–8)	5.30±0.79 (4.5–7)	4.14±1.07 (3–6)	4.78±1.22 (3–7	3.88±0.64 3–5)	4.34±0.60 (3–5	4.50±1.14 (3–8)	4.76±1.03 (3–7)	5.00±0.00 (5)	5±0.00 (5)
WS&BL	1±0.00 (1)	1±0.00 (1)	1±0.00 (1)	0.94±0.25 (0–1	0.75±0.46 0–1)	0.75±0.41 (0–1)	0.90±0.31 (0–1)	0.88±0.32 (0–1)	0.5±0.71 (0–1)	0.33±0.58 (0–1)
aIMd-IL	2±0.87 (1–3)	1.95±1.30 (0–4)	2±0.58 (1–3)	1.59±0.84 (0–3	1.88±0.64 1–3	1.31±0.48 (1–2)	1.80±0.71 (1–3)	1.57±0.83 (0–4)	1.5±0.71 (1–2)	2.33±0.29 (2–2.5)

Subspecies	**A. *mystaceus* s. str.**		**B. “*galli*”**		**C. “*aurantiacocaudatus*”**		**D. *mystaceus* s. l. (A+B+C)**		**E. *khorasanus* ssp. n.**	
SuSSCF	2.4±0.53 (2–3)	2.60±0.52 (2–3)	1.79±0.49 (1–2.5)	1.72±0.55 (1–3)	2.31±0.70 (1–3)	2.41±0.46 (2–3)	1.98±0.79 (1–3)	2.14±0.63 (1–3)	2±0.71 (1.5–2.5)	1.67±0.58 (1–2)
pSL-CF	1.56±0.53 (1–2)	1.60±0.70 (0–2)	2.14±0.90 (1–3)	2.19±0.91 (1–4)	1.25±0.71 (0–2)	1.36±0.5 (1–2)	1.77±0.77 (0–2)	1.83±0.75 (0–4)	1.2±0.71 (1–2)	0.67±0.58 (0–1)
ILbA	4.17±1.03 (3–6)	4±1.38 (1–6)	5.10±1.20 (3–7)	5.29±1.07 (2.5–7)	4.5±0.53 (4–5)	5.07±1.62 (2–7)	4.57±1.04 (3–7)	4.70±1.50 (1–7)	2.50±0.71 (2–3)	3.4±0.55 (3–4)
IL	6.5±1.45 (3–9)	7.15±1.04 (5–9)	7.50±1.51 (5–10)	7.47±0.92 (6–9)	6.75±0.71 (6–7)	6.6±1.24 (3–8)	6.9±1.35 (3–10)	7.08±1.13 (3–9)	6±1.41 (5–7)	5.2±2.05 (3–7)
SLIII	21.77±1.61 (19–25)		20.27±1.68 (17–29)		20.44±2.05 (16–24)		20.79±1.89 (16–29)		18.14±1.45 (16–20)	
FrIII	9.97±0.85 (9–12)		9.87±0.97 (8–12)		8.31±0.86 (7–11)		9.35±1.18 (7–12)		8.86±1.35 (7–11)	
SLIV	30.93±1.95 (27–35)		29.67±2.11 (25–35)		30.03±1.93 (25–33)		30.15±1.99 (25–35)		25.71±1.25 (24–27)	
FrIV	19.97±1.87 (16–25)		18.87±1.70 (16–23)		16±1.87 (13–21)		18.22±2.48 (13–25)		18.86±1.21 (18–21)	

Table 4. Measurements (mm) of adult *Ph. mystaceus khorasanus* ssp. n. For abbreviations, see Materials and methods. In SbN-SpN, SpN, hSpN-SbN and WS&BL: 0 equals "no", 1 equals "yes".

Measurements	ZMMU Specimen ID	SVL	TL	SVL/TL	HL	HH	HW	PW	SLbA	SL	TL-black/ TL	SSbNb	SbN-SpN
Males (N = 2)	R-13011-1	85.0	76.0	1.12	19.8	11.0	21.7	13.8	6	10	0.355	6	0
	R-13009-1	86.0	76.0	1.13	19.3	11.8	18.3	14.3	11	13	0.355	4	1
	Range	**85.0–86.0**	**76.0**	**1.12–1.13**	**19.3–19.8**	**11.0–11.8**	**18.3–21.7**	**13.8–14.3**					
Females (N = 5)	R-13169	54.0	54.0	1.00	13	9	13.5	10.4	11	14	0.370	6	0
	R-11913	70.0	67.0	1.05	18.2	10.9	17.4	13.0	9	12	0.358	5	1
	R-13011-2	60.0	60.0	1.00	16.6	9.6	14.8	10.7	8	12	0.397	4	0
	R-13009-2	60.0	60.0	1.00	15.5	9.3	15.0	13.3	10	15	0.333	5	0
	R-12202	54.0	51.0	1.06	13.7	8.9	13.3	11.0	8	12	0.392	3	0
	Range	**54.0–70.0**	**51.0–67.0**	**1.00–1.06**	**13.7–18.2**	**8.9–10.9**	**13.3–17.4**	**10.7–13.3**	**6–11**	**10–15**	**0.33-0.39**	**3–6**	-
	Mean ± S.D.	**59.6±6.5**	**58.4±6.2**	**1.02±0.03**	**16.0±1.9**	**9.7±0.9**	**15.1±1.7**	**12.0±1.3**	**9.0±1.8**	**12.6±1.6**	**0.4±0.0**	**4.7±1.1**	-

Table 4. Continued.

Measurements	ZMMU Specimen ID	SpN	hSpN- SbN	SbN-L	WS&BL	aIMd-IL	SuSSCF	pSL-CF	ILbA	IL	SLIII	FrIII	SLIV	FrIV
Males (N = 2)	R-13011-1	0	0	5	1	2	1.5	2	3	7	18	10	26	21
	R-13009-1	0	0	5	0	1	2.5	1	2	5	20	11	26	18
	Range													
Females (N = 5)	R-13169	1	0	5	1	2	2	0	4	6	20	9	27	19
	R-11913	0	0	6	1	3	2	1	3	3	16	9	24	20
	R-13011-2	0	0	5	0	2.5	1	1	3	7	17	8	25	18
	R-13009-2	1	0	4.5	0	2.5	1	1	3	3	18	8	25	18
	R-12202	1	0	5	0	2.5	2	1	4	7	18	7	26	18
	Range	-	-	**4.5–6**	–	**1–3**	**1–2.5**	**0–2**	**2–4**	**3–7**	**16–20**	**7–11**	**24–27**	**18–21**
	Mean ± S.D.	-	-	**5.2±0.4**	-	**2.0±0.8**	**1.6±0.5**	**1.0±0.6**	**3.1±0.7**	**5.4±1.8**	**18.1±1.5**	**8.7±1.3**	**25.7±1.3**	**18.9±1.2**

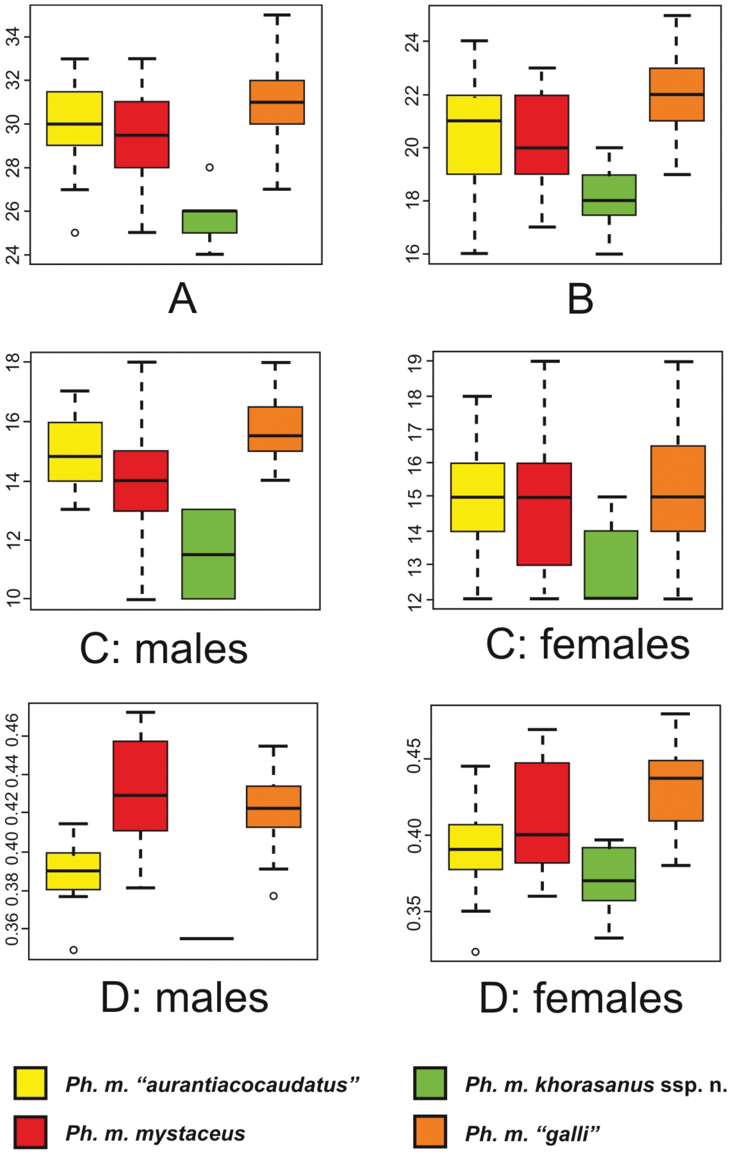

Figure 3. Statistically significant morphological differences between *Ph. mystaceus khorasanus* ssp. from Iran and other subspecies of *Ph. mystaceus*: **A** the number of subdigital lamellae on the toe IV (SLIV) **B** the number of enlarged triangular scales on the lateral fringe of the toe III (FrIII) **C** the total number of supralabial scales (SL) **D** the relative length of the dark distal part of the tail to the total tail length (TL-black/TL).

lineages is significant, 6.84–7.28% of substitutions, what corresponds to species-level divergence values in lizards, including the genus *Phrynocephalus* (Nagy et al. 2012; Nazarov et al. 2012, 2014; Solovyeva et al. 2012, 2014; Hartmann et al. 2013; Nazarov & Poyarkov 2013; Amarasinghe et al. 2017; Orlova et al. 2017). According to

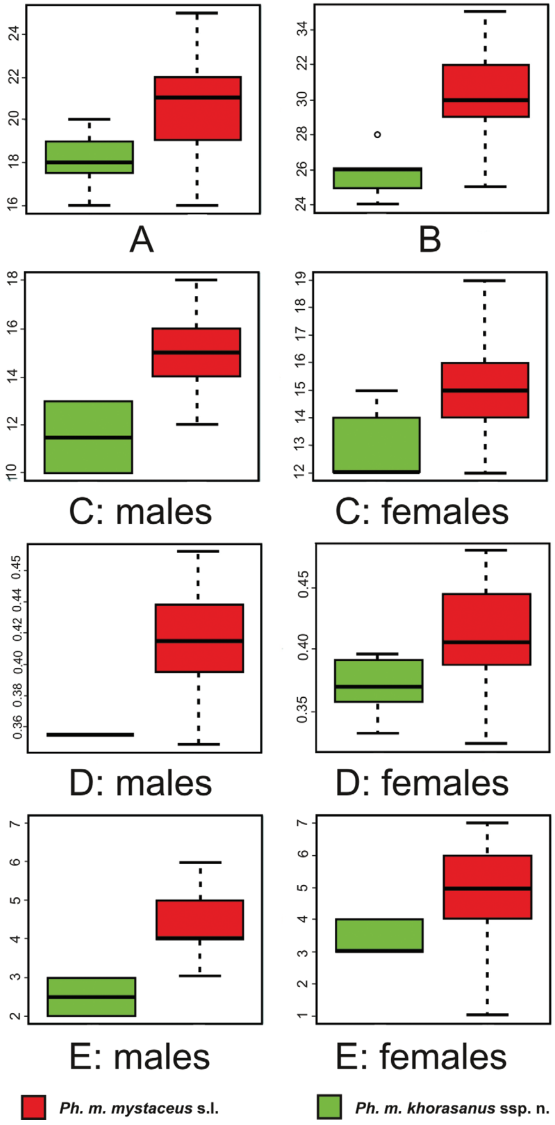

Figure 4. Statistically significant morphological differences between *Ph. mystaceus khorasanus* ssp. from Iran and other *Ph. mystaceus*: **A** the number of subdigital lamellae on the toe III (SLIII) **B** the number of subdigital lamellae on the toe IV (SLIV) **C** the total number of supralabial scales (SL) **D** the relative length of the dark distal part of the tail to the total tail length (TL-black/TL) **E** number of flat infralabials anterior to the angular enlarged spine-like infralabial scales (IlbA).

the data of Solovyeva et al. (2014), sequences of three other mtDNA genes of Iranian and Middle-Asian lineages of *Ph. mystaceus* are also deeply divergent: ND4 (6.6%), ND2 (8.0%) and cyt b (6.6%). Divergence time estimates (Solovyeva et al., 2018) suggest that the split between Iranian and Middle Asian *Ph. mystaceus* happened in the

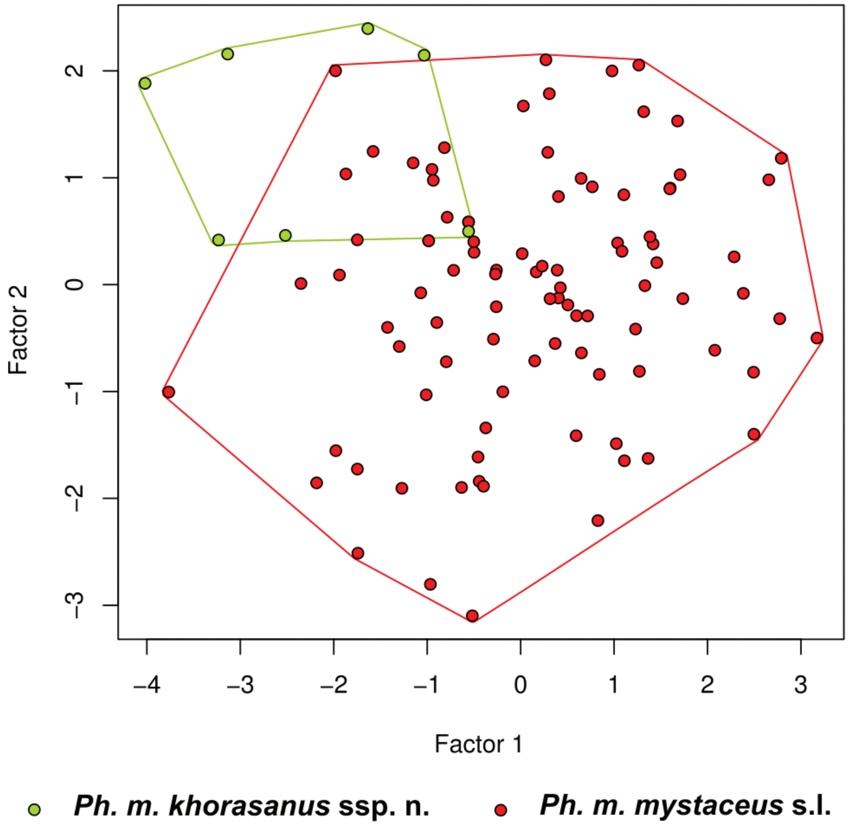

Figure 5. Principal Components Analysis (PCA) of 19 morphological traits (excluding SVL and TL).

Pliocene, ca. 3.7 Ma (6.0–2.0 Ma). Thus, our data strongly indicate the presence of a deep-divergent mtDNA lineage of *Ph. mystaceus* in northeastern Iran which deserves taxonomic recognition.

The question of the taxonomic status proposed for the Khorasan *Ph. mystaceus* populations, is, however, a matter of taste. On one hand, biogeographically the Khorasan *Ph. mystaceus* populations appear to be isolated from the main part of the species range in Middle Asia. The sands of the northeastern Iranian Plateau are located on much higher elevations (700-1000 m a.s.l.) than in the Middle Asia where *Ph. m. mystaceus* sensu lato occur (usually, 0-400 m a.s.l.), and are separated from the Caspian Basin by the Kopet-Dagh mountains, which has an estimated geologic uplift time of 5 Ma (Smit et al. 2013). The formation of Kopet-Dagh might be responsible for the initial split between the populations of *Ph. mystaceus*. The montane area of Kopet-Dagh lacking habitats suitable for *Ph. mystaceus*, such as sand dunes, serves as a barrier preventing gene flow between the Middle Asian and the Khorasan populations. Geographic isolation resulted in deep molecular divergence might suggest that the full species status should be proposed for the Khorasan populations of *Ph. mystaceus*.

However, despite the significant molecular divergence, morphological differentiation between the Khorasan and Middle Asian linages of *Ph. mystaceus* is weak with only few morphological characteristics separating them. At the same time, individuals of *Ph. mystaceus* from the vast range in the Caspian Region and Middle Asia are poorly differentiated both by morphometric characteristics (see Vel'dre 1964a, 1964b; Semenov and Shenbrot 1990; Golubev and Sattorov 1992) and by mtDNA (this paper). High morphological plasticity and variability are often recorded in specialized psammophilous groups of lizards (see Semenov and Shenbrot 1990). Both mtDNA and morphological data fail to resolve differentiation between the currently recognized non-Iranian subspecies of *Ph. mystaceus*: *mystaceus* sensu stricto, "*galli*" and "*aurantiacocaudatus*". These subspecies are not supported as respective monophyletic groups in mtDNA analysis: the variation pattern is more likely clinal along the range from Xinjiang of China and Eastern Kazakhstan westwards to Middle Asia and Caspian Region. This suggests a recent dispersal of the non-Iranian *Ph. mystaceus* ancestor from a refugium in Eastern Kazakhstan westwards towards Caspian Basin.

There is no morphological or mtDNA evidence for recognizing *Ph. m. galli* as a distinct subspecies; we therefore confirm the conclusions of Semenov and Shenbrot (1990) who regarded *Ph. m. galli* as a junior synonym of the nominative subspecies. The East Kazakhstan *Ph. m. aurantiacocaudatus* is paraphyletic with respect to *Ph. m. mystaceus* and is not supported as a valid taxon according to our mtDNA data. The only existing character distinguishing *Ph. m. aurantiacocaudatus* from the representatives of other populations from Caspian Region and Middle Asia is the bright orange-red coloration of the tail ventral surface in juvenile specimens. Unfortunately, this character cannot be verified on museum collections since orange tail coloration fades quickly after preservation. Analysis of morphometric and meristic characters could separate *Ph. m. aurantiacocaudatus* from the nominative form *Ph. m. mystaceus*. We conclude that the subspecific status of *Ph. m.* "*aurantiacocaudatus*" requires further justification.

Our data show that the significant genetic differentiation of Khorasan *Ph. mystaceus* and presence of a number of stable diagnostic morphological characters warrants its recognition as a separate taxon. As noted above, genetic divergence between *Ph. mystaceus* from Khorasan and individuals from the rest of the species range is high, comparable or even exceeds the species-level genetic distances in *Phrynocephalus* (Solovyeva et al. 2014). However, we tentatively refrain from proposing the full species status for this lineage, and suggest that, at least at the current stage of research, it should be recognized as a subspecies, for the following three reasons:

(1) Due to matrilineal way of mtDNA inheritance and absence of recombination, even deep genetic divergence in mtDNA markers, does not guarantee reproductive isolation and should not serve as a sole reason for suggesting the full species status.

(2) Morphologically, the Khorasan population is still quite similar to other *Ph. mystaceus* populations and the revealed morphological differences are mostly quantitative, further morphological evidence is needed.

(3) Our sampling from Khorasan Province of Iran is limited, further studies in northeastern Iran are needed to uncover new populations in the area between the

Ph. m. mystaceus range in Turkmenistan and the Khorasan population, genetic and morphological characterization of these populations is required.

A recent analysis had shown that subspecies are getting more rarely proposed for the extant reptiles in the last 50 years (Uetz and Stylianou 2018), what is connected with a growing tendency to elevation of many subspecies to species and also with growing prevalence of the phylogenetic species concept (Cracraft 1983), which does not recognize subspecies. However, we still consider subspecies to be a useful taxonomic category for reflecting geographic variation and evolutionary specificity in wide-ranged complexes of reptiles. Though taxonomic status of Middle Asian subspecies "*galli*" and "*aurantiacocaudatus*" is questionable, both mtDNA sequences and external morphology of the Khorasan population of *Ph. mystaceus* significantly differ from all other populations of this species. This allows us to describe it herein as a new subspecies:

Phrynocephalus mystaceus khorasanus **ssp. n.**

http://zoobank.org/6E926506-3D7A-4C99-BF64-A02C48157B5C
Figs 6A, B; 7; 8; Table 4

Holotype. ZMMU R-11913 (adult female; field number NR-1191).

Type locality. Iran, Khorasan historical area, Khorasan Razavi Province (ostan), environs of Gonabad, the right bank of the Kale-Shur River; sand dunes (see Fig. 9); N34°39', E58°43'; elevation 850 m a. s. l. Collected by Roman A. Nazarov and Mehdi Radjabizadeh on April 25, 2005.

Paratypes. ZMMU R-13009 (one adult male with everted hemipenial structures, field number RAN 1723; and one adult female, field number RAN 1724) was collected in Iran, Khorasan historical area, Khorasan Razavi Province, 20 km east of the town of Boshrouyeh (N33°54', E57°30'; elevation 864 m a. s. l.) by Dmitriy A. Bondarenko, Roman A. Nazarov, and Mehdi Radjabizadeh on May 05, 2009. The rest of paratypes were collected in the area close to the type locality. ZMMU R-13011 (one adult male with hemipenial structures, field number RAN 1947; and one subadult female, field number RAN 1948) was collected in Iran, Khorasan Razavi Province, 60 km north of the town of Gonabad, stabilized or semi-stabilized sands (N34°36', E58°14'; elevation 867 m a. s. l.) by Roman A. Nazarov, Rustam K. Berdiev, Vlad G. Starkov, and Mehdi Radjabizadeh on June 02, 2009. ZMMU R-13169 (subadult female) was collected in Iran, Khorasan Razavi Province, 30 km north of the town of Gonabad, on sandy massif on the right bank of the Kale-Shur river (N34°35', E58°43'; elevation 888 m a. s. l.) by Roman A. Nazarov, Dmitriy A. Bondarenko, and Mehdi Radjabizadeh on May 10, 2010. ZMMU R-12202 (juvenile female with slightly orange lower surface of the tail, field number N-093) was collected in Iran, Khorasan Razavi Province, 60 km north of the town of Gonabad, on sands (N34°36', E58°44'; elevation 881 m a.s.l.) by Dmitriy A. Bondarenko on April 20, 2006.

Diagnosis. A member of *Ph. mystaceus* species complex based on the following combination of morphological attributes: (1) a large-sized *Phrynocephalus* species with

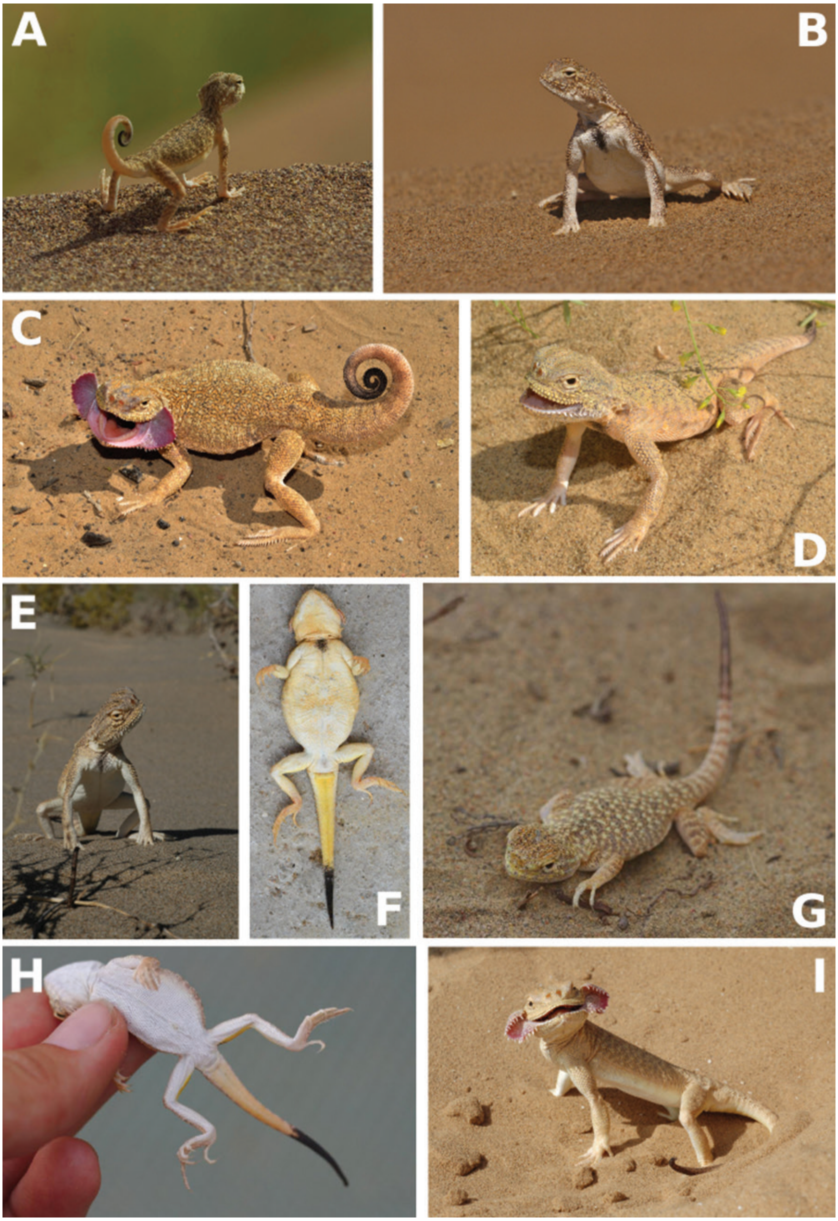

Figure 6. *Ph. mystaceus* in life: **A** subadult *Ph. mystaceus khorasanus* ssp. n., orange lower surface of the tail is shown, Iran (photograph by R. A. Nazarov) **B** *Ph. mystaceus khorasanus* ssp. n., female, Iran (photo by R. A. Nazarov) **C** *Ph. m. mystaceus*, Russia, Astrakhan region, Dosang (photograph by E. A. Dunayev) **D** *Ph. m. mystaceus*, Dagestan, Sarykum sands (photograph by E. A. Dunayev) **E** *Ph. m. mystaceus*, Uzbekistan, Qarakalpaqiston (corresponds to the previously recognized subspecies "*galli*"; photograph by E. A. Dunayev) **F** *Ph. m. mystaceus*, Dagestan, Sarykum sands (corresponds to the previously recognized subspecies "*dagestanica*"; photograph by E. A. Dunayev) **G** *Ph. m. aurantiacocaudatus*, E Kazakhstan, SE Balkash Lake (photograph by E. N. Solovyeva) **H** *Ph. m. aurantiacocaudatus*, E Kazakhstan, SE Balkash lake (photograph by E. N. Solovyeva) **I** *Ph. m. mystaceus*, Russia, Astrakhan region, Dosang (photograph by E. A. Dunayev).

SVL up to 97.5 mm, tail shorter than SVL; (2) pair of cutaneous flaps present at mouth corners with numerous spiny scales along flap edges; (3) distinctly flattened body and tail; (4) toes with fringes formed by triangular scales; subdigital lamellae on toes III and IV with ridges. *Phrynocephalus mystaceus khorasanus* ssp. n. can be distinguished from the nominative subspecies of *Ph. mystaceus* by the following combination of two diagnostic morphological characteristics: (1) 24–27 lamellae on toe IV; (2) few supralabial scales (less than 14). In life, the new subspecies can be further distinguished from the nominative subspecies by the orange color of the lower surface of tail in young specimens (lemon to yellowish in *Ph. m. mystaceus* except the populations from Eastern Kazakhstan and western China, formerly described as *Ph. m. aurantiacocaudatus*). MtDNA sequences of *Phrynocephalus mystaceus khorasanus* ssp. n. are markedly distinct from those in all other populations of *Ph. mystaceus* with sequence divergence in the range of 6.84–7.28% between them. The new subspecies is notably smaller than the representatives of southern populations of *Ph. m. mystaceus* from Uzbekistan and Turkmenistan, formerly described as *Ph. m. galli*, which can reach SVL up to 122.7 mm (Anderson 1999), whereas for Iranian population Anderson (1999) reported the largest specimen of *Ph. mystaceus* to have SVL up to 77.7 mm. SVL in the largest specimen in our sampling reached 86.0 mm, while Molavi et al. (2014) recorded a specimen with SVL of 97.5 from Semnan Province.

Etymology. The name of the new subspecies *khorasanus* is a Latinized toponymic adjective, derived from Khorasan, the name of the historic area and a Khorasan Razavi Province in the northeast Iran, where the new subspecies was found. We suggest the “Khorasan Secret Toad-headed Agama” as a common name in English.

Description of holotype. Medium-sized agamid lizard, adult female, specimen in good state of preservation; body dissected on ventral side along the midline of belly (dissection ca. 20 cm in length). Measurements and counts of the holotype are presented in Table 4.

Head large, rounded, distinctly wider than neck region (see Fig. 7A); body and tail notably flattened. Snout abruptly blunt, head almost vertical in profile view (see Fig. 7E), nostrils invisible dorsally (see Fig. 7C). Nasals separated from each other by single scale (see Fig. 7D). Dorsal surface of head with distinct pileus consisting of small slightly keeled scales; ca. 30 scales across the pileus. Pineal scale separated from nasals by 13 smaller scales; scales covering orbital area somewhat smaller than those on frontal surface of head; occipital scales not enlarged. Five scales contacting subnasal ventrally (see Fig. 7D). Subnasal scale not in contact with inner (medial) side of supranasal. Supralabials separated from subnasal scale by 6 rows of small granular scales (see Fig. 7D). Pair of skin-folds form characteristic ear-shaped flaps in mouth corners, edges of each flap with enlarged conical scales, two groups of similarly enlarged conical scales on each side of head posterior to the mouth angle at tympanal area (see Fig. 7E). Supralabial scales anterior to cutaneous fold at mouth angle 11/12 (hereafter data for symmetrical characteristics is given in Right/Left order); 9/9 of anterior supralabials notably flattened, 2/3 posterior supralabials conical-shaped; supralabials separated from small granular scales of lower eyelid by 3/4 rows of scales, ventral row of these

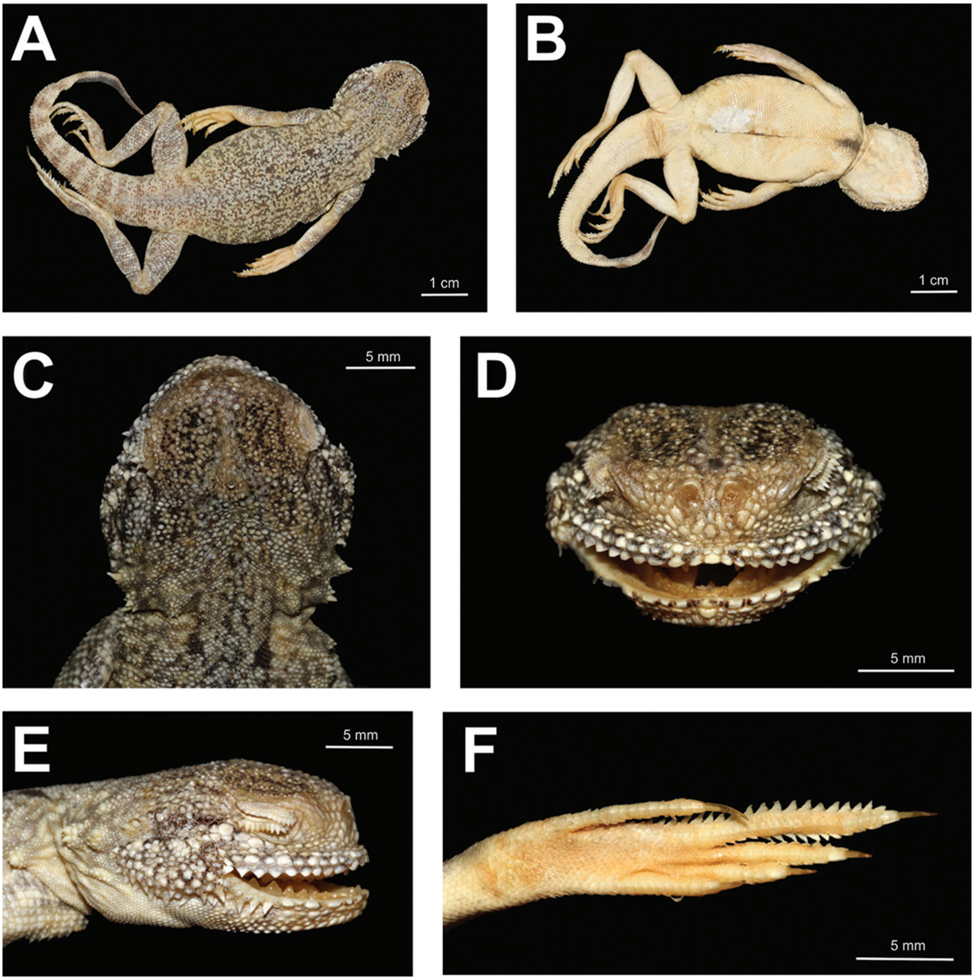

Figure 7. Holotype of *Ph. mystaceus khorasanus* ssp. n. in preservative: **A** dorsal view **B** ventral view **C** head in dorsal view **D** head in frontal view **E** head in lateral view; **F** right foot in thenar view (photographs by E. N. Solovyeva).

scales almost the same size as supralabials (see Fig. 7E). Single small scale between the posteriormost supralabial and insertion of cutaneous fold at mouth angle. Infralabial scales anterior to cutaneous fold — 6/6, 3/3 of anterior infralabials notably flattened, posterior infralabials cone-shaped. Posterior corner of eye and insertion of cutaneous fold at mouth angle separated by row of three enlarged flat scales (see Fig. 7E). Vertebral scales not enlarged. Scales at middle of dorsum slightly bigger than scales on dorsolateral and lateral surfaces of body. Dorsal scales with weak keels, becoming cone-shaped laterally, forming almost triangular spines on the flanks. Notably enlarged spiny scale (about four times the size of adjacent scales) on each side of thorax behind maxilla, two groups of enlarged spiny scales on each lateral surface of neck region (see Fig. 7E). Tail notably flattened along its whole length. Scales on dorsal surface of tail and on ventral surface of tail posterior half notably keeled; scales on lateral sides of tail with well-pronounced spines. Limbs comparatively long: hindlimb length greater than distance from cloaca to gular fold. Toe IV bearing a single row of subdigital lamellae, each with a well-pronounced ridge on its volar surface; lateral sides of toe IV with two rows of enlarged triangular scales that form distinct serrated fringe (see Fig. 7F). Similar crests present on lateral surfaces of toe III, triangular scales on toe III notably smaller compared to those on toe IV (see Fig. 7F). Number of lamellae on toe IV 24/24, on toe III 16/16; number of enlarged triangular scales on toe IV 20/20, on toe III 9/9.

Color of holotype in life. In life dorsum sandy-beige; with numerous small black and white dots and reticulations; row of three pairs of irregular-shaped larger dark blotches on each side of vertebral line; ventral surfaces of body, limbs and proximal part of tail white; ventral surface of tail tip black, chin and throat with gray reticulations, chest with blackish longitudinal blotch. Ten brownish transverse bars (wider than interspaces) on dorsal surface of tail, faint at tail basis, get more distinct towards tail tip. Internal surfaces of mouth angle cutaneous flaps in life are pinkish, and may become red when animal displays a threatening posture.

Color of holotype in preservative. In preservative, numerous dark spots and mottling are distinct on dull sandy-gray background color of dorsum. They form vermiculate patterns ca. 1–2 scales wide. On lateral parts of dorsum these lines form 6–7 indistinct dark transverse bands. Ten dark transverse bars on dorsal side of tail are well-distinct (Fig. 7A). Three posterior dark bars have a distinct light-beige longitudinal line between them along midline of tail. Tail ventral surface light yellowish-white. Ventral surface of head with distinctive dark greyish marbling (Fig. 7A). Distinct triangular longitudinal black spot in the middle of chest area resembling a "necktie", ca. 8.8 mm in length. Black coloration of distal part of ventral surface of tail 24 mm in length.

Paratype variation. Variations of morphological characteristics in the type series are shown in Table 4 and in Fig. 8. In general, morphology of paratypes corresponds well to morphology of the holotype. SVL of new subspecies varies in range of 85.0–86.0 mm in two males, and in range of 54.0–70.0 in five females; tail length 76.0 mm in males, 51.0–67.0 mm in females; tail comparatively shorter in male specimens (SVL/TL ratio 1.12–1.13) than in females (SVL/TL ratio 1.00–1.06); however, the sample size is too small to detect significant differences. Length of dark distal part of ventral surface of tail varies from 20 to 27 mm. Number of subdigital lamellae on toe III varies from 17 to 20, from 25 to 28 on toe IV. Number of enlarged triangular scales of lateral fringes on toe III from 7 to 11, on toe IV from 18 to 21. Number of flattened anterior supralabials 6–11, total number of supralabials (to insertion of cutaneous fold at mouth angle) varies from 10 to 15. Number of small scales ventrally in contact with subnasal scale 3–6. Subnasal scale in all paratypes (except one specimen ZMMU R-13009) touches supranasal along medial edge of latter. In nearly all paratypes supralabials are separated from subnasal by five rows of small scales (only in ZMMU R-13009 by 4/5 rows of small scales). In most specimens, there is one small scale between last supralabial and insertion of cutaneous fold at mouth angle (specimen ZMMU R-13011 has two scales, ZMMU R-13169 lacks such scales). Number

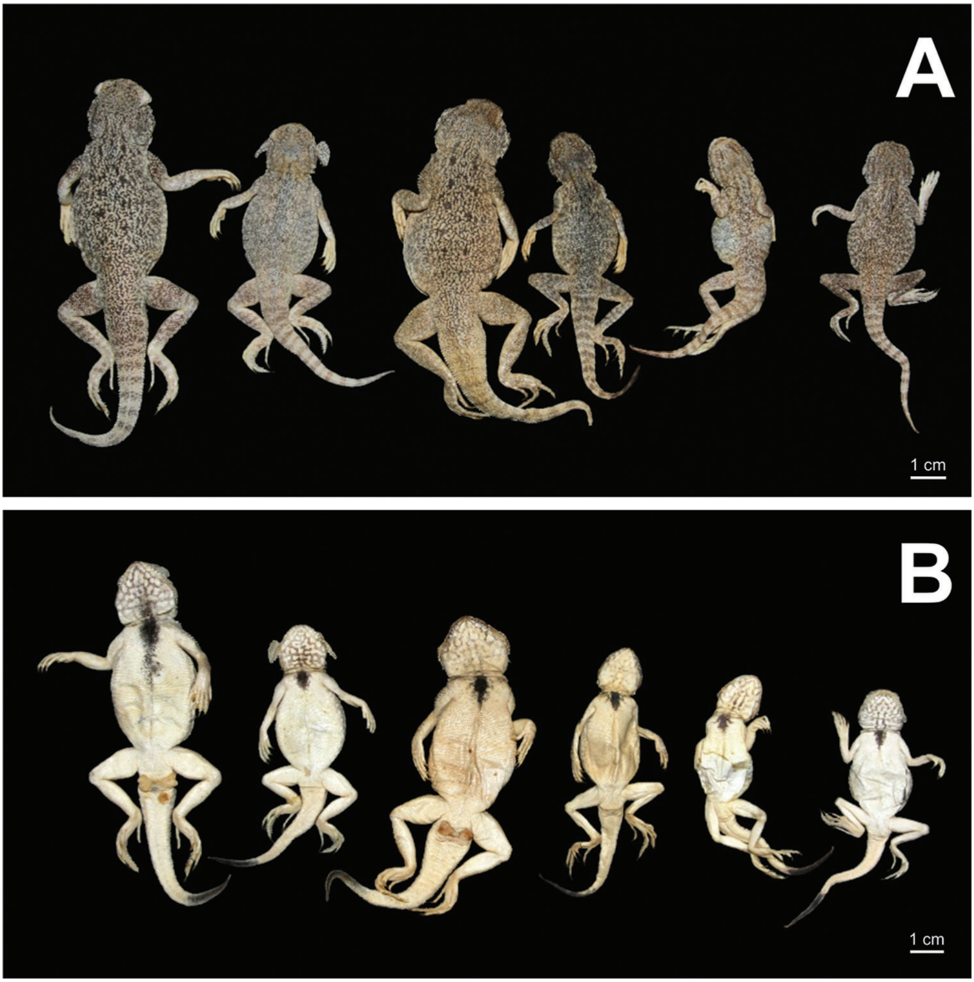

Figure 8. Paratypes of *Ph. mystaceus khorasanus* ssp. n. in preservative: **A** in dorsal view **B** in ventral view (photographs by E. N. Solovyeva).

of flat anterior infralabials varies from 2 to 4, total number of infralabials to insertion of cutaneous fold at mouth angle varies from 5 to 7 (only ZMMU R-13009 has 3/3 infralabials). Number of black irregularly shaped spots on dorsum also may vary: from 4 to 6 pairs of black spots on each side of vertebral line (see Fig. 8A).

We were unable to detect sexual dimorphism in morphometric and meristic characteristics of *Ph. mystaceus khorasanus* ssp. n., however our sample size (N = 7) was too small. Molavi et al. (2014), who also examined seven specimens of both sexes from Semnan Province, was also unable to detect sexual dimorphism in morphological features in their sample.

Distribution. To date, the new subspecies is known from two major localities in southwestern part of Khorasan Razavi Province (environs of the towns of Gonabad and Boshrouyeh, this study) and from a single locality in the easternmost part of Semnan

Province of Iran (Ahmad Abad village, Molavi 2014). The record from the environs of the town of Boshrouyeh appears to be the southernmost known locality for *Ph. mystaceus* complex known to date. The three records of *Ph. mystaceus* by Anderson (1999) from the northern part of Khorasan Razavi Province, North Khorasan and Golestan provinces are all located along the border with Turkmenistan. These populations most likely correspond to *Ph. m. mystaceus* rather than to *Ph. mystaceus khorasanus* ssp. n. as they are close to the range of the nominative form and there are no biogeographic barriers that separate these populations. On the contrary, localities in Khorasan Razavi and Semnan provinces are situated on different elevations and sand massifs are isolated from the range of *Ph. m. mystaceus* by at least 200 km of habitats unsuitable for *Ph. mystaceus*. We anticipate new records of the new subspecies in sandy areas of Khorasan Razavi, Semnan and, possibly, northern part of Yazd and South Khorasan provinces.

Habitat. *Ph. mystaceus khorasanus* ssp. n. inhabits sandy areas with sparse vegetation in northeast Iran at comparatively higher altitudes, than other *Ph. mystaceus* subspecies. The usual habitat is represented by dunes of loose sands and semi-stabilized dunes with rare grass, occasional bushes of *Haloxylon* sp. and *Tamarix* sp. and large open sandy areas (Fig. 9). The areas inhabited by the new subspecies receive almost no rainfall during the year. In the town of Gonabad the average annual temperature is 17.3 °C, the average temperature in July reaches 29.2 °C, the average temperature in January is 4.8 °C; In Boshrouyeh the average annual temperature is 19.7 °C, the average temperature in July is 31.9 °C, the average temperature in January is 6.6 °C. (http://www.climate-data.org).

Lizards burrow in sand, digging short tunnels and chambers; they can quickly dig into sand by rapid lateral movements of the body (Anderson, 1999).

Comparisons with other subspecies. Comparisons of the new subspecies from Khorasan Razavi and Semnan provinces of Iran with the nominative subspecies *Ph. m. mystaceus* sensu lato from Middle Asia, Caspian basin, and westernmost Xinjiang (China) are summarized below. In preservative, the new subspecies can be differentiated from specimens of *Ph. m. mystaceus* by the following combination of morphological attributes: lower number of subdigital lamellae on the IVth toe (SLIV 25.7 (24–27; N = 7) in *vs.* 30.2 (25–35; N = 70) in *Ph. m. mystaceus* sensu lato); comparatively lower number of supralabials (SL 12.1 (10–14; N = 7) *vs.* 14.9 (10-19; N = 70) in *Ph. m. mystaceus* sensu lato) and by the comparatively shorter black distal part on the tail ventral surface (TL-black/TL 0.38 (0.36–0.40; N = 7) *vs.* 0.42 (0.32–0.48; N = 70) in *Ph. m. mystaceus* sensu lato). In life, juvenile and young specimens of the new subspecies can be further distinguished from Middle Asian / Caspian Basin populations of *Ph. mystaceus* by is rusty orange color of the proximal part of tail ventral surface (vs. lemon-yellow in *Ph. m. mystaceus* sensu stricto), but is similar to orange tail coloration in juveniles of East Kazakhstan – western China populations described as *Ph. m. aurantiacocaudatus*.

We do not recognize *Ph. m. galli* as a separate subspecies due to the absence of stable genetic and morphological differences of this subspecies from *Ph. m. mystaceus* (see above). The *Phrynocephalus mystaceus dagestanica* form from Daghestan (Ananjeva, “1986” 1987) is very close to the populations from the Volga River basin and was

Figure 9. Typical habitat of *Ph. mystaceus khorasanus* ssp. n. at the type locality in the vicinity of Gonabad, Khorasan Razavi Province, Iran (photo by R. A. Nazarov).

considered a synonym of *Ph. m. mystaceus* by several authors (Semenov and Shenbrot 1990; Barabanov and Ananjeva 2007). Our molecular and morphometric data do not support monophyly or significant differentiation of *Ph. m. aurantiacocaudatus* from Eastern Kazakhstan and western China. The only stable difference between this population and *Ph. m. mystaceus* sensu stricto is the tail coloration in juveniles. We consider that additional genetic and morphological data is needed to clarify taxonomic status of East Kazakhstan *Ph. mystaceus* populations.

Discussion. Our study indicates deep genetic divergence between Iranian populations of *Ph. m. khorasanus* ssp. n. and the rest of the populations within the range of the species. However, morphological differentiation within *Ph. mystaceus* complex is less clear with only a few morphological characteristics that reliably separate these two lineages. Differentiation pattern for the mtDNA *COI* gene within the Middle Asian and Caspian populations of *Ph. mystaceus* complex suggests that East Kazakhstan was populated by *Ph. mystaceus* earlier than the rest of Middle Asia. After that, a dispersal process from the east to the west likely took place. Morphologically different populations of *Ph. mystaceus* across Middle Asia present considerable amount of variation both in body size and in such morphological features as the relative size of cutaneous flaps in the mouth angles, relative tail length, etc. This high morphological plasticity may be connected with psammophilous biology of this species, as it was suggested by previous researchers (Vel'dre 1964a, 1964b; Semenov and Shenbrot 1990; Golubev and Sattorov 1992).

The data of phylogenetic analyses in the present paper clearly indicates that the whole territory of Middle Asia, including westernmost China and Caspian region, is inhabited by a single poorly differentiated mtDNA lineage. Golubev and Sattorov (1992) argued that coloration of the ventral tail surface in juveniles of *Ph. mystaceus* is also subject to high variation, and "orange-" and "yellow-tailed" specimens can be occasionally recorded within the same population, thus suggesting that subspecies within *Ph. mystaceus* should not be recognized. Our mtDNA genealogy indicates that both *Ph. m.* "*galli*" and *Ph. m.* "*aurantiacocaudatus*" do not form a respective monophyletic units and are genetically indistinguishable or very close the nominative subspecies *P. m. mystaceus* sensu stricto (p-distance 1.65–1.87% in case of East Kazakhstan populations).

On the contrary, the Khorasan population described herein as *Ph. m. khorasanus* shows very deep genetic divergence in mtDNA which is comparable to the species-level divergence in *Phrynocephalus*, but is only moderately differentiated morphologically. Indeed, previous research on four mtDNA genes also showed significant differentiation between *Ph. mystaceus* from Khorasan and *Ph. m. mystaceus* (*p*-distances: *COI* – 7.18%; *ND4* – 6.6%; *ND2* – 8.0%; and *cyt b* – 6.6%) (see Solovyeva et al. 2014). According to our unpublished data on molecular dating of 4 mtDNA genes these two forms diverged during Pliocene about 3.7 Ma (Solovyeva et al., 2018). Further studies are required to verify the taxonomic status of *Ph. m. khorasanus* ssp. n., including morphological examination of larger samples and molecular analysis of the nuclear DNA markers in order to check the presence of possible isolation between the Iranian and Middle Asian forms of *Ph. mystaceus*. The new subspecies inhabits sand dunes in the northeastern Iran; this desert area is separated from the range of *Ph. m. mystaceus* by Kopet-Dagh Mountain Ridge making the possibility of gene flow between these populations quite low. However, the taxonomic status of *Ph. mystaceus* populations reported by Anderson (1999) from northern Iran (northern parts of Golestan, North Khorasan and Khorasan Razavi provinces) is unclear and require verification. Additional fieldwork in northern Iran, western Afghanistan, and southern Middle Asia is required to recover new populations of *Ph. mystaceus* complex. Further progress in understanding of the phylogenetic relationships within *Ph. mystaceus* complex might lead to reconsideration of the taxonomic status of the Khorasan population as a full species.

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Appendix 1

Material examined in morphological analysis.

***Phrynocephalus mystaceus mystaceus*:** Kalmykia (ZMMU R-3455 [N = 8: 4 females, 4 males]); Russia, Astrakhan, Dosang (ZMMU R-8696 [N = 12: 9 females, 3 males]). Additionally, we examined the holotype ZMMU R-6412.

Phrynocephalus mystaceus galli: Turkmenistan, Repetek (ZMMU R-2043 [N =11: 6 females, 5 males], ZMMU R-2045 [N =9: 5 females, 4 males]). Additionally, we examined the lectotype ZMMU R-6413; **lectotype** of *Phrynocepahlus mystaceus galli* Krassowsky, 1932 (ZMMU R-6413, previously part of ZMMU R-2047; male, Turkmenistan, Repetek; coll. on 07-09.08.1929 by S.S. Turov, L.G. Turova; see Fig. 10).

Phrynocephalus mystaceus aurantiacocaudatus: Kazakystan, Muyunkum sands (ZMMU R-6858 [N = 4: 2 females, 2 males], ZMMU R-6566 [N = 1 female]); Kazakhstan, Ili River (ZMMU R-3794 [N = 7: 4 females, 3 males]); Kazakhstan, Alma-Aty (ZMMU R-10906 [N = 1 juvenile]); Kazakhstan, left bank of Ili River

(ZMMU R-12518 [N = 1 female]); Kazakhstan, Kapchagay (ZMMU R-12140 [N = 2 juveniles]); Kazakhstan (ZMMU R-2051 [N = 3: 1 female, 1 male, 1 juvenile)]; Kazakhstan, Bakanas (ZMMU R-7470 [N = 2 females]); Kazakhstan, right bank of Ili river (ZMMU R-2828 [N = 4: 2 females, 1 male, 1 juvenile]); Kazakhstan, Djarkent env. (ZMMU R-2049 [N = 5: 2 females, 1 male, 2 juveniles]); Kazakhstan, SE Balkhash env. (ZMMU R-557 [N = 2: 1 female, 1 subadult]); **holotype** of *Phrynocepahlus mystaceus aurantiacocaudatus* Semenov & Shenbrot, 1990 (ZMMU R-6412; male, East Kazakhstan, 70 km N-N-W from Ushtobe, 45°50'N; 77°40'E; coll. on 12-13.06.1987 by D.V. Semenov, G.I. Shenbrot; see Fig. 11).

***Phrynocephalus mystaceus khorasanus* ssp. n.**: Iran, Khorasan (ZMMU R-11913 [N = 1 female], ZMMU R-13011 [N = 2: 1 female, 1 male], ZMMU R-12202 [N = 1 female], ZMMU R-13169 [N = 1 female], ZMMU R-13009 [N = 2: 1 female, 1 male]).

Appendix 2

Table A2. Mann-Whitney test of independent series: "aura" – *Ph. m. "aurantiacocaudatus"*, "ir" – *Ph. m. khorasanus* ssp. n., "*myst*" – *Ph. m. mystaceus* sensu stricto, "galli" – *Ph. m. "galli"*; for abbreviations, see Materials and Methods. Significant values of $p \leq 0.05$ are marked with bold and an asterisk.

	ALL							**Sexes**
	aura-ir	**ir-myst**	**ir-galli**	**myst-aura**	**aura-galli**	**galli-myst**	**ir-all**	**f-m**
1. SVL	0.321	**0.000***	**0.013***	**0.000***	**0.008***	**0.000***	**0.005***	**0.007***
2. TL	**0.044***	**0.000***	**0.001***	**0.000***	**0.027***	**0.000***	**0.000***	**0.005***
3. SVL/TL	**0.008***	**0.000***	0.115	**0.001***	**0.006***	**0.000***	**0.001***	0.591
4. SLbA	**0.015***	**0.004***	**0.031***	0.178	0.855	0.180	**0.014***	0.813
5. SL	**0.007***	**0.001***	**0.050***	**0.003***	0.455	**0.005***	**0.003***	0.355
6. TL-black/TL	**0.023***	**0.000***	**0.001***	**0.002***	**0.016***	0.499	**0.001***	0.527
7. SSbNb	0.351	0.623	**0.036***	0.412	**0.037***	**0.003***	0.140	0.201
8. SbN-SpN	0.596	0.597	0.158	0.050	**0.000***	0.118	0.570	0.169
9. SpN	0.927	0.923	0.103	**0.000***	**0.002***	**0.009***	0.457	**0.022***
10. hSpN SbN	**0.015***	**0.000***	**0.033***	0.127	0.758	0.096	**0.001***	0.259
11. SbN-L	**0.010***	0.589	0.097	**0.000***	0.992	**0.016***	0.144	0.234
12. WS&BL	0.111	**0.000***	**0.027***	0.026	0.026	NA	**0.002***	0.524
13. aIMd-IL	**0.017***	0.392	0.053	0.113	0.771	0.252	**0.030***	0.091
14. SuSSCF	0.029	**0.004***	0.912	0.164	**0.000***	**0.000***	0.174	0.839
15. pSL-CF	0.272	**0.026***	**0.004***	**0.042***	**0.000***	**0.025***	**0.007***	0.857
16. ILbA	**0.006***	**0.046***	**0.002***	**0.022***	0.510	**0.002***	**0.003***	0.640
17. IL	**0.023***	**0.017***	0.059	0.645	**0.013***	0.059	**0.022***	0.588
18. SLIII	**0.009***	**0.011***	**0.008***	**0.011***	0.738	**0.002***	**0.001***	NA
19. FrIII	0.341	**0.000***	0.056	**0.000***	**0.000***	0.889	0.283	
20. SLIV	**0.000***	0.087	**0.000***	0.087	0.463	**0.026***	**0.000***	
21. FrIV	**0.001***	**0.000***	0.937	**0.000***	**0.000***	**0.026***	0.563	

Table A2. Continued.

	MALES							FEMALES						
	aura-ir	**ir-myst**	**ir-galli**	**myst-aura**	**aura-galli**	**galli-myst**	**ir-all**	**aura-ir**	**ir-myst**	**ir-galli**	**myst-aura**	**aura-galli**	**galli-myst**	**ir-all**
1. SVL	0.432	0.142	1.000	**0.004***	0.533	**0.002***	0.110	0.147	**0.001***	**0.003***	**0.000***	**0.007***	**0.001***	**0.003***
2. TL	0.694	**0.034***	0.160	**0.002***	0.328	**0.000***	0.726	**0.015***	**0.001***	**0.003***	**0.000***	0.052	**0.000***	**0.001***
3. SVL/TL	0.260	**0.033***	**0.040***	**0.047***	0.947	**0.009***	**0.022***	**0.005***	**0.002***	0.921	**0.006***	**0.000***	**0.000***	**0.045***
4. SLbA	0.279	**0.028***	0.550	0.057	0.215	**0.003***	0.281	**0.039***	0.086	**0.028***	0.861	0.547	0.837	**0.040***
5. SL	**0.043***	**0.032***	0.293	**0.037***	0.063	**0.005***	**0.043***	**0.048***	**0.009***	**0.045***	**0.038***	0.771	0.101	**0.023***
6. TL-black/TL	0.091	**0.033***	**0.040***	0.395	0.141	0.186	**0.032***	0.186	**0.004***	**0.048***	**0.002***	0.263	0.077	**0.018***
7. SSbNb	0.715	1.000	0.197	0.450	0.089	**0.010***	0.537	0.457	0.601	0.106	0.752	0.148	0.073	0.230
8. SbN-SpN	0.330	0.892	0.593	0.178	**0.017***	0.296	0.892	0.804	0.526	0.125	0.150	**0.007***	0.229	0.453
9. SpN	0.436	0.361	0.091	0.663	**0.040***	0.143	0.192	0.901	0.844	0.109	0.932	0.034	**0.037***	0.928
10. hSpN SbN	0.103	0.049	0.176	0.441	0.813	0.392	0.053	0.063	**0.003***	**0.041***	0.174	0.899	0.156	**0.006***
11. SbN-L	0.125	0.797	0.223	**0.040***	0.662	0.102	0.349	**0.043***	0.582	0.243	**0.002***	0.851	0.066	0.314
12. WS&BL	0.192	**0.034***	0.061	0.279	0.338	NA	0.118	0.231	**0.006***	**0.003***	0.045	**0.030***	NA	**0.011***
13. aIMd-IL	0.528	0.454	0.294	0.520	0.466	1.000	0.611	**0.002***	0.130	**0.007***	0.097	0.818	0.208	**0.004***
14. SuSSCF	0.608	0.310	0.648	0.482	0.092	**0.030***	1.000	**0.021***	**0.008***	0.913	0.221	**0.000***	**0.000***	0.090
15 pSL-CF	0.641	0.892	0.355	0.283	**0.032***	0.154	0.643	0.099	**0.021***	**0.003***	0.110	**0.002***	0.119	**0.004***
16. ILbA	**0.037***	0.054	**0.049***	0.261	0.207	0.070	**0.026***	0.091	0.351	**0.039***	**0.039***	0.966	**0.016***	**0.036***
17. IL	0.480	0.633	0.227	0.710	0.216	0.154	0.354	**0.031***	**0.012***	**0.010***	0.354	**0.038***	0.261	**0.034***

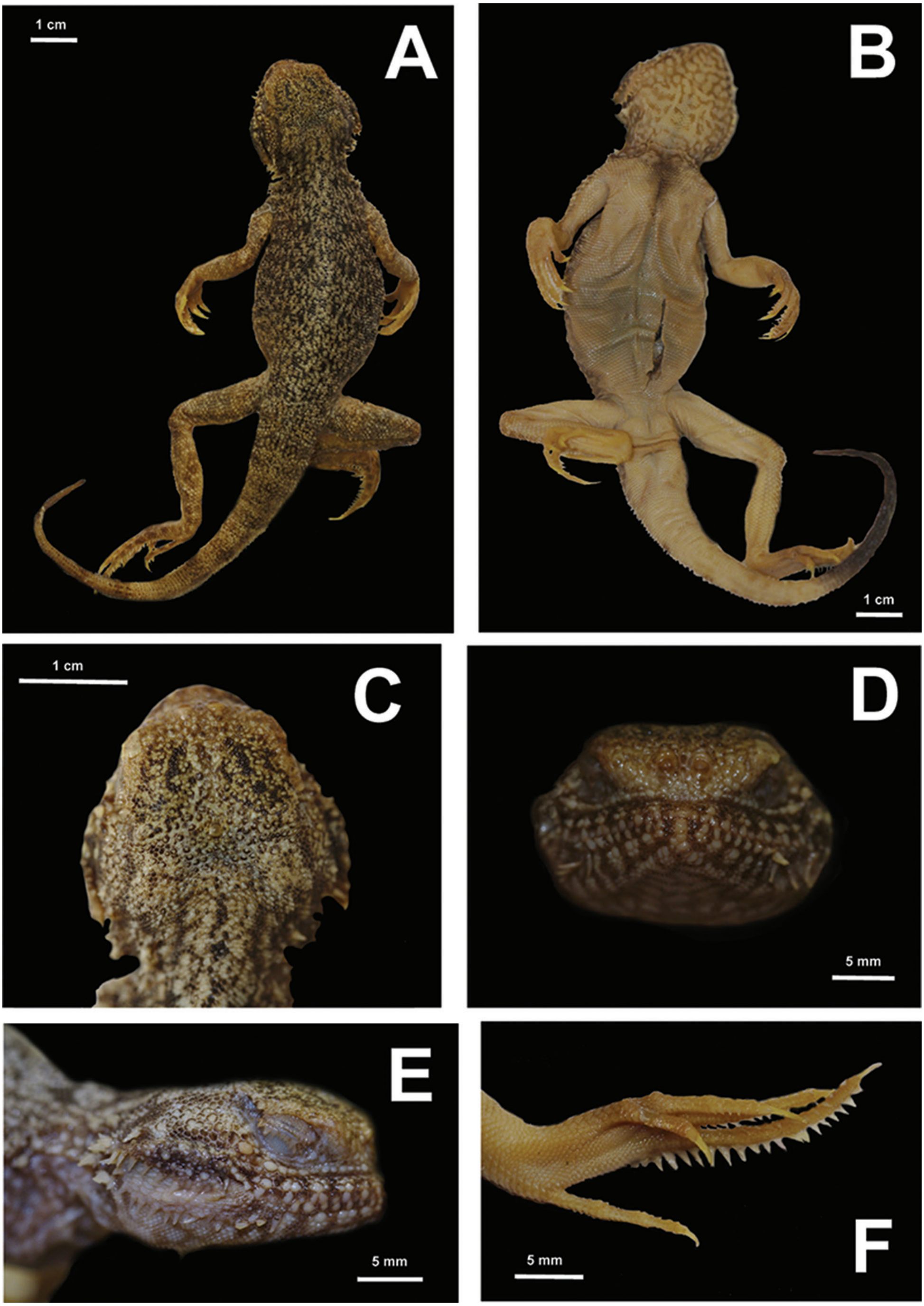

Figure 10. ZMMU R-6413, lectotype of *Phrynocepahlus mystaceus galli* Krassowsky, 1932 in preservative: **A** dorsal view **B** ventral view **C** head in dorsal view **D** head in frontal view **E** head in lateral view **F** left foot in thenar view (photographs by E. N. Solovyeva).

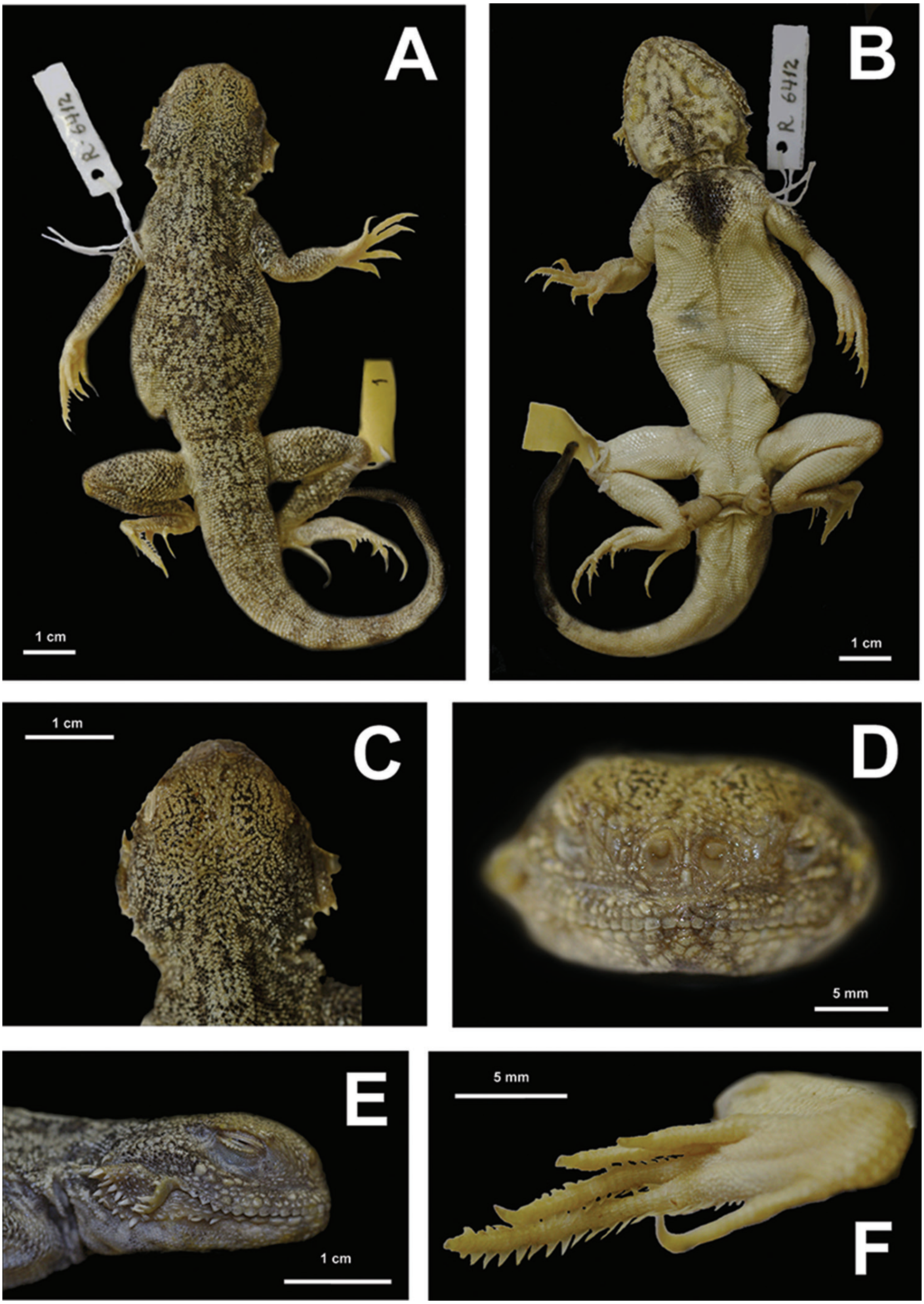

Figure 11. ZMMU R-6412, holotype of *Phrynocepahlus mystaceus aurantiacocaudatus* Semenov & Shenbrot, 1990 in preservative: **A** dorsal view **B** ventral view **C** head in dorsal view **D** head in frontal view **E** head in lateral view **F** right foot in thenar view (photographs by E. N. Solovyeva).

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A review of the genus *Takecallis* Mastumura in Korea with the description of a new species (Hemiptera, Aphididae)

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Abstract

The aphid genus, *Takecallis* Mastumura, 1917, was reviewed from Korea. Four species, *T. alba* Y. Lee, **sp. n.**, *T. arundicolens* (Clarke), *T. arundinariae* (Essig), and *T. taiwana* (Takahashi), are recognized in Korea and morphological and molecular evidence are presented. Species descriptions and illustrations are given for the four species. A key to Korean species and the results of COI sequence analyses are also provided.

Keywords

Aphid, Bamboo pest, Calaphidinae, COI, Panaphidini

Introduction

The genus *Takecallis* was established by Matsumura (1917) based on the type species *T. arundicolens*. This is one of the small aphid genera of the tribe Panaphidini (Aphididae: Calaphidinae). In this genus, six species are known around the world (Remaudière and Remaudière 1997, Favret 2017). All known species have been described from Southeast Asian countries such as China (Qiao and Zhang 2004), India (Chakrabarti 1988), Japan (Higuchi 1968), Korea (Quednau and Lee 2001), and Taiwan (Quednau 2003).

However, some species such as *T. arundicolens*, *T. arundinariae*, and *T. taiwana* were introduced into Australia (Valenzuela et al. 2010), England (Higuchi 1968), Hungary (Basky and Neményi 2014), Netherland (Pion 2009), New Zealand (Higuchi 1968), North and South America (Delfino 2001, Foottit et al. 2006, Gonzáles et al. 2000, Halbert et al. 2000, Lazzari et al. 1999, Simbaqueba et al. 2016), and South Africa (Quednau 1962). *Takecallis* species have a monoecious holocyclic life cycle on various bamboos (*Arundinaria* spp., *Bambusa* spp., *Dendrocalamus* spp., *Phyllostachys* spp., *Pleioblastus* spp., *Pseudosasa* spp., *Sasa* spp., and *Yushania* sp.) belonging to the family Poaceae (Qiao and Zhang 2004, Quednau 2003).

In Korea, three species, *Takecallis arundicolens*, *T. arundinariae*, and *T. taiwana*, have so far been recorded in this genus (Quednau and Lee 2001). However, recent DNA barcoding revealed that there is an undescribed species in Korea (Lee et al. 2017). In this study, a large number of *Takecallis* samples were collected in Korea and examined together with museum specimens. We also conducted molecular analyses based on the partial mitochondrial cytochrome oxidase subunit I (COI) of fresh-ethanol preserved samples. Generally, COI barcoding provides a good enough resolution for species identification in aphids (Foottit et al. 2008, Lee et al. 2011).

A total of four species were recognized in Korea. Both morphological and molecular evidence strongly indicated that *T. alba* Y. Lee, sp. n. is a valid species. Here, together with a description of the new species, photographs of live aphids and illustrations are provided along with a key to species of the genus *Takecallis* in Korea. Pairwise distance analyses and a neighbor-joining tree based on the partial COI sequence are also provided.

Materials and methods

Aphid samples were collected in South Korea from 1999 to 2015. All samples were preserved in 90–95% ethanol for over one month, and then mounted in Canada balsam, following the methods of Blackman and Eastop (2000) and Martin (1983). Illustrations for each species were taken with a digital camera attached to the microscope (Leica 400B, Leica Microsystems, Germany) at a resolution of 600 dpi. Measurements for each specimen are taken from the digital images by using image analysis software, Active measure ver. 3.0.3 (Mitani Co. Ltd, Japan). All specimens are deposited in the National Academy of Agricultural Science (NAAS), Jeonju-si, Republic of Korea and the College of Agriculture and Life sciences, Seoul National University, Republic of Korea (CALS SNU).

Aphid samples were identified using keys to *Takecallis* species by Higuchi (1972) and Quednau (2003). For further confirmation, DNA barcoding results were also applied.

Abbreviations used for diagnosis, description, figures, and Table 1 are:

BL	body length;
ANT	antennae;
ANT I-VI	antennal segments I–VI;

BASE	basal part of last antennal segment;
PT	processus terminalis of last antennal segment;
Ls ANT III	longest setae on ANT III;
BD III	basal diameter of ANT III;
URS	ultimate rostral segment;
Co	costa;
Cu	cubitus;
M	media;
Pts	pterostigma;
Rs	radial sector;
FEM	hind femur;
TIB	hind tibiae;
HT 2	second segment of hind tarsus;
SIPH	siphunculus;
ABD TERG I-VIII	abdominal tergites I-VIII.

Provincial names in South Korea for the collection data are abbreviated as follows: CN, Chungcheongnam-do; GB, Gyeongsangbuk-do, GN, Gyeongsangnam-do; GW, Gangwon-do; JJ, Jeju-do; JN, Jeollanam-do.

Main morphological characters such as measurements (in mm), number of setae on antennal segments, number of rhinaria, and body part ratios of Korean *Takecallis* are given in Table 1.

In total, 63 COI sequences of five *Takecallis* species were downloaded from GenBank (Suppl. material 1). All sequences were aligned using MEGA 7 (Kumar et al. 2016). Intra- and inter-specific distances were calculated by a pairwise distance method based on the Kimura-2-Parameter (K2P) model (Kimura 1980). A neighbor-joining analysis (NJ) based on the K2P model for the final data set of 658 bp was also constructed.

Taxonomy

Takecallis Mastumura, 1917

Takecallis Matsumura, 1917: 354, 373.

Type-species. *Takecallis bambusae* Matsumura, 1917 (= *T. arundicolens*) by original designation.

Generic diagnosis. Alatae: Morphological features of *Takecallis* are similar to *Subtakecallis* Raychaudhuri and Pal in having a nose-like processus on the clypeus, and spinal abdominal setae surrounded by cribriform wax glands. However, this genus can be distinguished from the above genus by the following characters: PT/BASE ≥ 1.00 and spinal abdominal setae often at low elevations. Apterae are unknown.

Host plant. *Takecallis* species occur on various bamboos such as *Arundinaria* spp., *Bambusa* spp., *Dendrocalamus* spp., *Pseudosasa* spp., *Phyllostachys* spp., *Pleioblastus* spp., and *Sasa* spp. (Poaceae).

Distribution. This genus is native to the Oriental region, but one or more species occur as introduced populations in Australian, Ethiopian, Palearctic, Nearctic, and Neotropical regions.

Takecallis alba Y. Lee, sp. n.

http://zoobank.org/974AEFD4-E563-4944-8C2B-3039BC4099E9

Figs 1A, 2, Table 1

Material examined. Holotype: 1 alate viviparous female, Mt. Hwangbyeong, Pyeongchang-gun, GW, South Korea, 37°42'27"N, 128°41'14"E, on *Sasa* sp., Y. Lee leg., 29.viii.2013, no. 130829YR-11; *Paratypes*: 10 alate viviparous females, same data as the holotype; 7 alate viviparous females, Mungyeong-eup, Mungyeong-si, GN, South Korea, 36°47'11"N, 128°09'29"E, on *Pseudosasa* sp., S. Lee leg., 18.v.2005, no. 050518SH-38; 6 alate viviparous females, Mt. Hwangbyeong, Pyeongchang-gun, GW, South Korea, 37°42'27"N, 128°41'14"E, on *Sasa* sp., Y. Lee leg., 15.viii.2013, no. 130815YR-12; 5 alate viviparous females, Mt. Deokyousan, Muju-gun, JB, South Korea, 35°54'23"N, 127°48'51"E, on *Pseudosasa* sp., H. Lee leg., 30.vi.2014, no. 140630YR-2; 2 alate viviparous females, Inje-gun, Hangye-ri, Hangyeryng, GW, South Korea, 38°6'31"N, 128°24'49"E, on *Sasa* sp., Y. Lee leg., 15.vi.2015, no. 150615YR-3.

Etymology. The species name *alba* is derived from Latin, referring to its pale body color.

Diagnosis. *T. alba* sp. n. is morphologically close to *T. assumenta* Qiao and Zhang and *T. affinis* Ghosh. However, this species can be distinguished from the latter two species by the following characters: URS with four accessory setae (accessory setae absent in *T. assumenta*, two accessory setae in *T. affinis*), URS 0.45–0.50 × HT 2 (0.43 in *T. assumenta*, 0.32–0.41 in *T. affinis*), ANT III with 4–7 transversely elliptical secondary rhinaria densely concentrated on very short dark section of proximal 3rd of ANT III (6–10 elliptical secondary rhinaria, on basal 1/3 of the segment in *T. assumenta*, 10–16 subcircular secondary rhinaria on basal 2/5 of the segment in *T. affinis*).

Description. *Alate viviparous female*: *Color in life*. Head pale to yellow, compound eye red. ANT pale, marginal border of ANT I-II dusky, the top end of 1/3 of the segment, and distal joint of ANT III dark, distal joint of ANT IV - BASE dusky. Thorax and ABD TERG pale yellow to bright yellow. Legs pale, distal 2/5 of FEM with dark spot, tarsi dark. Wing veins dark, margins of wing veins with dark spots. SIPH pale. Cauda slightly dark. Entire body covered with white wax.

Morphology. Body oval, BL 2.08–2.51 mm long. Head with three pairs of anterior and two pairs of posterior short and pointed discal setae about 0.02–0.03 mm long, median protrusion on frons developed, epicranial suture and antennal tubercle developed, head dorsum without tubercles. ANT 6-segmented, 1.48–1.70 × BL, ANT III longest with 4–7 transversely elliptical secondary rhinaria in a row

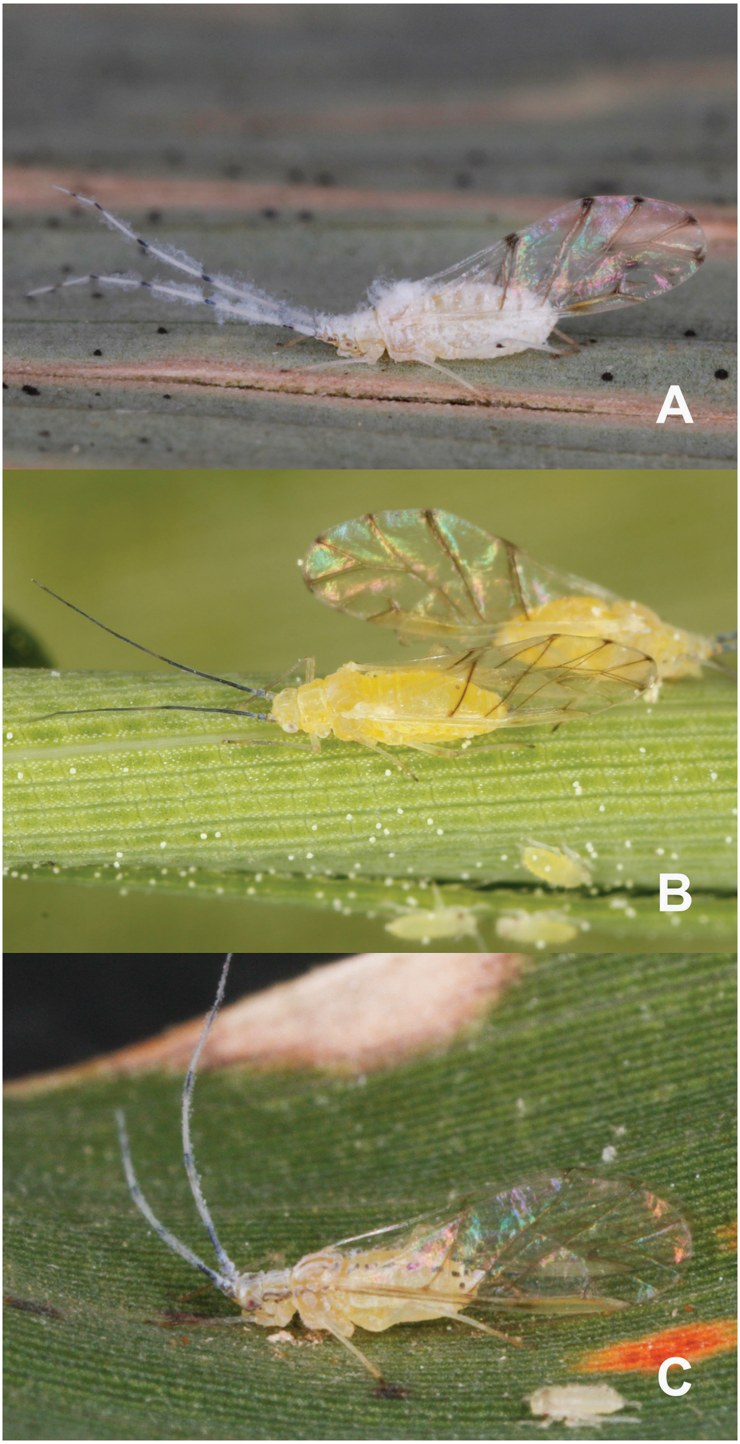

Figure 1. Photographs of live *Takecallis* spp. **A** alate viviparous female of *T. alba* sp. n. **B** alate viviparous female of *T. arundicolens* **C** alate viviparous female of *T. arundinariae*.

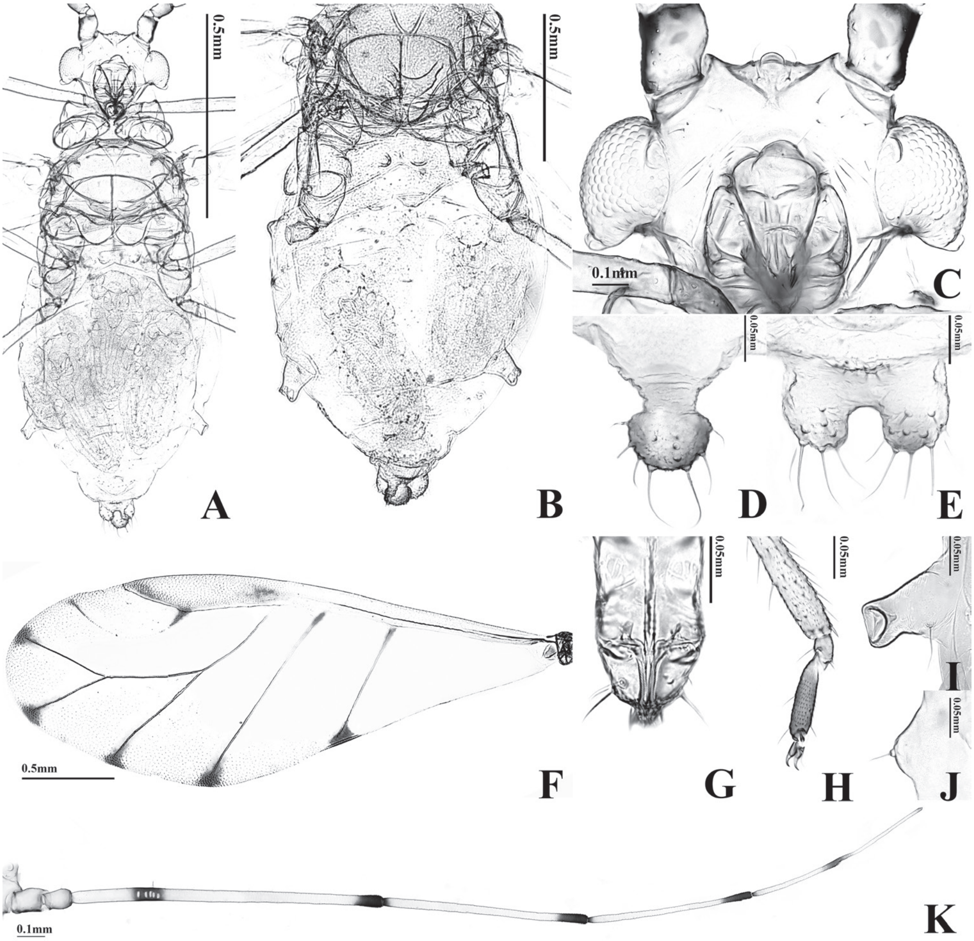

Figure 2. Alate viviparous female of *T. alba* sp. n.: **A** body **B** dorsal ABD TERG **C** head **D** cauda **E** anal plate **F** forewing **G** URS **H** HT 2 **I** SIPH **J** 4th marginal tubercle **K** ANT.

on the top end of 1/3 of the segment, Ls ANT III 0.25–0.50 × BD III, ANT IV-VI imbricated, ANT IV without secondary rhinaria, ANT IV longer than ANT V, PT 0.89–1.11 × BASE. Clypeus with nose-like processus bearing two hairs. Rostrum very short, barely reaching to fore coxae, URS short blunted, 0.05 mm long with four accessory setae, URS 0.13–0.15 × BASE, 0.45–0.50 × HT 2. Thorax smooth without tubercles. Fore coxae enlarged. Longest setae on TIB 0.06–1.00 × middle width of TIB, first tarsal segments with 6–7 setae, HT 2 0.10–0.11 mm long. Wing vein Pts of forewing slightly dark, margins of wing veins Cu1b, Cu1a, and M with dark spots. Dorsal ABD TERG I–VII with a pair of spinal setae on small elevations, ABD TERG VIII with a pair of spinal setae on a single elevation, ABD TERG margin I–IV with a single seta on cone-shaped marginal tubercle, 4th marginal tubercle 0.04–0.05mm. SIPH cylindrical 0.08–0.11 mm long, bearing 0.03–0.05 mm of

Table 1. Biometric data of *Takecallis* species in Korea.

	Body parts	***T. alba* sp. n. (n = 20)**	***T. arundicolens* (n = 20)**	***T. arundinariae* (n = 20)**	***T. taiwana* (n = 14**
Length (mm)	BL	2.08–2.51	1.57–1.89	1.90–2.65	2.21–2.48
	ANT	3.36–4.00	2.36–2.51	2.54–3.41	1.61–1.88
	ANT I	0.12	0.07–0.09	0.09–0.12	0.08
	ANT II	0.09–0.11	0.07–0.08	0.09–0.12	0.06–0.09
	ANT III	1.07–1.33	0.67–0.72	0.70–1.11	0.57–0.67
	ANT IV	0.73–0.91	0.46–0.51	0.54–0.85	0.31–0.36
	ANT V	0.60–0.69	0.46–0.48	0.48–0.77	0.26–0.33
	BASE	0.34–0.40	0.27–0.31	0.26–0.40	0.15–0.20
	PT	0.34–0.44	0.33–0.36	0.31–0.40	0.17–0.19
	URS	0.05	0.05–0.06	0.05–0.06	0.07
	FEM	0.55–0.69	0.41–0.46	0.53–0.66	0.46–0.50
	TIB	0.87–1.15	0.72–0.80	0.91–1.21	0.79–0.88
	HT 2	0.10–0.11	0.09–0.10	0.10–0.12	0.11–0.13
	SIPH	0.08–0.11	0.04–0.05	0.05–0.08	0.04–0.05
	Cauda	0.12–0.14	0.14–0.15	0.11–0.16	0.15–0.20
	Ls ANT III	0.01–0.02	0.01	0.01	0.01–0.02
No. of setae on	ANT I	5–6	4–5	3–5	3–4
	ANT II	2–3	2–3	2–3	3
	ANT III	24–39	13–23	18–28	17–21
	BASE	1	1	1	1
	URS (accessory setae)	4	4–5	4	4–5
	SIPH	1	1	1	0
	ABD TERG VIII	2	2	2	2
	Cauda knob	9–12	9–13	10–15	12–13
	Each lobe of anal plate	7–10	7–8	8–12	10
No. of rhinaria on	ANT III	4–7	4–7	5–10	5–7
	ANT IV	0	0	0	0
	ANT V	1	1	1	1
Ratio (times)	ANT / BL	1.48–1.70	1.30–1.60	1.22–1.39	0.73–0.79
	PT / BASE	0.89–1.11	1.10–1.26	0.94–1.27	0.90–1.06
	PT / ANT III	0.31–0.34	0.47–0.50	0.35–0.47	3.32–3.72
	URS / HT 2	0.45–0.50	0.56–0.67	0.42–0.60	0.54–0.64
	URS / BASE	0.13–0.15	0.16–0.20	0.13–0.23	0.35–0.44
	SIPH / BL	0.03–0.04	0.02–0.03	0.02–0.03	0.02
	SIPH / ANT III	0.06–0.09	0.06–0.07	0.06–0.09	0.07–0.10
	SIPH / FEM	0.13–0.17	0.09–0.12	0.09–0.13	0.09–0.12
	SIPH / Cauda	0.57–0.79	0.27–0.36	0.36–0.64	0.20–0.35
	Ls ANT III / BD III	0.25–0.50	0.33	0.20–0.33	0.33–0.67

single seta. Cauda knobbed 0.12–0.14 mm long with 9–12 setae. Anal plate bilobed, each lobe with 7–10 setae.

Distribution. This species has so far been collected from Gyeongsangnam-do, Gangwon-do, and Jeollanam-do of South Korea.

Host plants. This species feeds on the underside of leaves of *Pseudosasa* sp., and *Sasa* spp. (Poaceae). The host plants were identified by the first author using Lee (2003).

Remarks. This species was first referred to as *Takecallis* sp. in Lee et al. 2017.

Takecallis arundicolens (Clarke, 1903)

Figs 1B, 3, Table 1

Takecallis bambusae Matsumura, 1917.

Material examined. 2 alate viviparous females, Naksan-temple, Ganghyeon-myeon, Yangyang-gun, GW, South Korea, 38°7'25"N, 128°37'38"E, on *Sasa* sp., S. Lee leg., 25.vi.2003, no. 030625SH-62; 1 alate viviparous female, Namheae-gun, GN, South Korea, 34°50'15"N 127°53'32"E, on *Sasa* sp., S. Lee leg., 7.iv.2006, no. 060407SH-16; 1 alate viviparous female, Seobjikoji Beach, Seoguipo-si, JJ, South Korea, 33°25'24"N, 126°55'45"E, on *Sasa* sp., S. Lee leg., 27.iv.2006, no. 060427SH-55; 3 alate viviparous females, Ehwa womans univ., Deahyeon-dong, Seodaemun-gu, Seoul, South Korea, 37°33'42"N, 126°56'48"E, on *Arundinaria* sp., Y. Lee leg., 18.x.2011, no. 111018YR-1; 2 alate viviparous females, Taean-gun, CN, South Korea, 36°47'47"N, 126°09'04"E, on *Sasa* sp., Y. Lee and H. Lee leg., 10.v.2014, no. 140510YR-17; 1 alate viviparous female, Is. Odongdo, Yeosu-si, JN, South Korea, 34°44'51"N, 127°45'52"E, on *Sasa* sp., Y. Lee and H. Lee leg., 16.vii.2014, no. 140716YR-1.

Description. *Alate viviparous female*: *Color in life*. Head pale to bright yellow, compound eye pale. ANT I concolorous with head, ANT II slightly dusky, basal 1/3 and distal 1/3 of ANT III dark, basal half of ANT IV-V, and ANT VIb dusky. Thorax concolorous with head or slightly darker. ABD TERG pale yellow to bright yellow. Legs pale, tarsi dark. Wing veins dark. SIPH pale. Cauda dark. Entire body slightly covered with white wax.

Morphology. Body oval, BL 1.57–1.89 mm long. Head with 3 pairs of anterior and 2 pairs of posterior short and pointed discal setae about 0.02–0.04mm, median protrusion on frons developed, epicranial suture and antennal tubercle developed, head dorsum without tubercles. ANT 6-segmented, 1.30–1.60 × BL, ANT III longest with 4–7 transversely elliptical secondary rhinaria in a row on 1/3 of the segment, Ls ANT III 0.33 × BD III, ANT IV–VI imbricated, ANT IV without secondary rhinaria, BASE with a single seta, PT 1.10–1.26 × BASE. Clypeus with nose-like processus, rostrum very short, reaching to fore coxae, URS short blunted, 0.05–0.06 mm long with 4–5 accessory setae, URS 0.16–0.20 × BASE, 0.56–0.67 × HT 2. Thorax smooth and without tubercles. Fore coxae enlarged, longest setae on TIB almost same length as middle width of TIB, first tarsal segments with 5–7 setae, HT 2 0.09–0.10 mm long. Wing vein Pts of forewing slightly dark. Dorsal ABD TERG I–VII with a pair of spinal setae on small elevations, ABD TERG VIII with a pair of spinal setae, ABD TERG margin I-IV with a single seta on cone-shaped marginal tu-

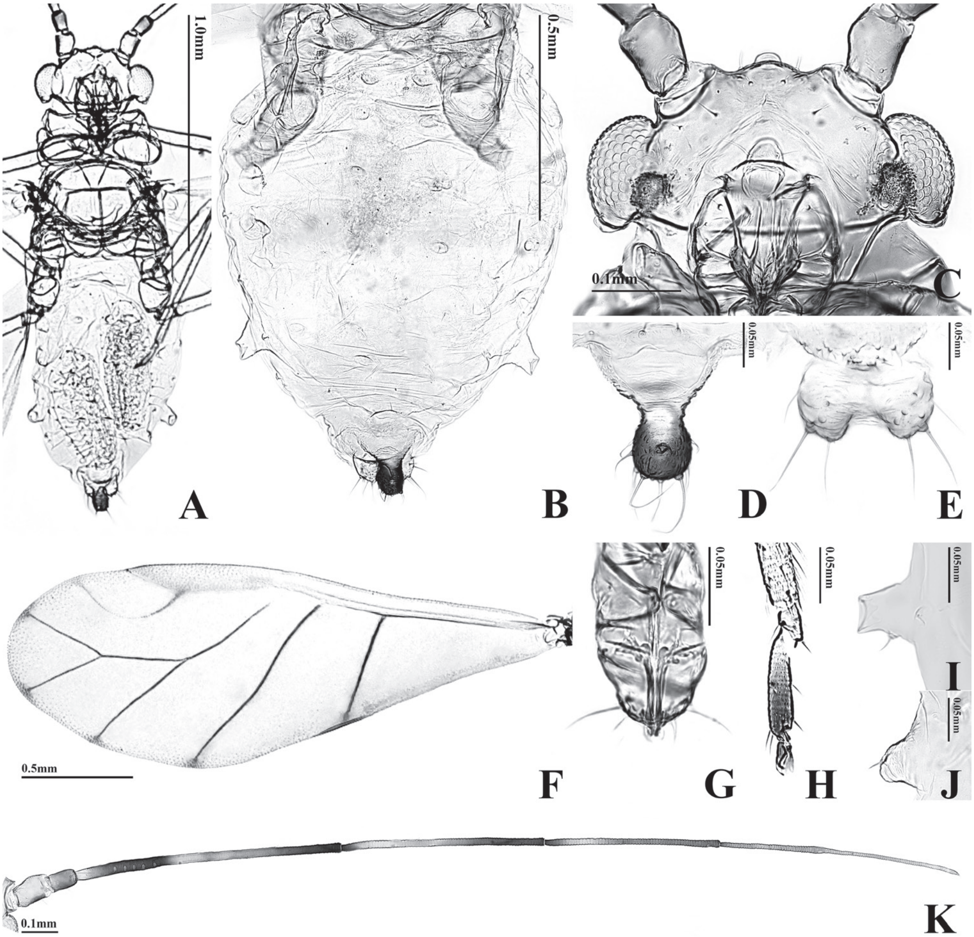

Figure 3. Alate viviparous female of *T. arundicolens*: **A** body **B** dorsal ABD TERG **C** head **D** cauda **E** anal plate **F** forewing **G** URS **H** HT 2 **I** SIPH **J** 4th marginal tubercle **K** ANT.

bercle, 4th marginal tubercle 0.04–0.05mm. SIPH cylindrical 0.04–0.05 mm long with 0.02–0.03 mm of single seta. Cauda knobbed 0.14–0.15 mm long with 9–13 setae. Anal plate bilobed, each lobe with 7–8 setae.

Distribution. This species is originally distributed in East Asian countries; Korea (Paik 1965), China (Qiao and Zhang 2004), Japan (Higuchi 1968), and eastern Russia (Blackman and Eastop 2017). It has been introduced into Europe (Pons and Lumbierres 2004, Stroyan 1964), and USA (California) (Clarke 1903). However, the recent DNA barcoding result revealed that European populations are genetically different from Asian populations (Lee et al. 2017).

Host plants. *Arundinaria* spp., *Bambusa* spp., *Phyllostachys* spp., and *Sasa* spp. (Poaceae).

Takecallis arundinariae **(Essig, 1917)**

Figs 1C, 4, Table 1

Takecallis arundinariae Blackman, 1980.
Myzocallis bambucifoliae Takahashi, 1921.
Myzocallis bambusifoliae Takahashi, 1921.

Material examined. 1 alate viviparous female, Seoul, South Korea, 37°34'31"N, 126°59'51"E, on *Sinoarundinaria reticulata*, W.H. Paik leg., 15.v.1960, no. 1258; 1 alate viviparous female, Seoul, South Korea, 37°34'31"N, 126°59'51"E, on *Sasa kurilensis*, W.H. Paik leg., 3.xi.1971, no. 6924; 5 alate viviparous females, Hwasun, JN, South Korea, 35°3'52"N, 126°59'11"E, on unknown host, S. Lee leg., 31.iii.1999, no. 990331SH-1; 6 alate viviparous females, Chupungryeong, Gimcheon, GB, South Korea, 36°13'9"N, 127°59'51"E, on *Sasa* sp., S. Lee leg., 12.v.1999, no. 990512SH-30; 5 alate viviparous females, Sanpo-myeon, Naju-si, JN, South Korea, 35°2'22"N, 126°48'21"E, on *Phyllostachys bambusoidea*, G.M. Kwon leg., 12.i.2000, no. 000112GM-04; 5 alate viviparous females, Namyang-myeon, Goheung-gun, JN, South Korea, 34°43'42"N, 127°20'10"E, on *Phyllostachys bambusoidea*, S. Lee leg., 14.iii.2000, no. 000314SH-2; 3 alate viviparous females, Sacheon-gun, GN, South Korea, 37°48'39"N, 128°51'17"E, on *Phyllostachys bambusoidea*, S. Lee leg., 16.iii.2000, no. 000316SH-6; 4 alate viviparous females, Namhae-gun, GN, South Korea, 34°49'58"N, 127°53'53"E, on *Gramineae* sp., S. Lee leg., 8.iv.2006, no. 060408SH16; 2 alate viviparous females, Taean-gun, CN, South Korea, 36°44'44"N 126°17'52"E, on *Phyllostachys* sp., Y. Lee and H. Lee leg., 10.v.2014, no. 140510YR-17.

Description. *Alate viviparous female*: *Color in life*. Head pale to bright yellow with black stripe on head dorsum, compound eye pale red. From ANT I to basal half of ANT III dark, from distal joint of ANT III to BASE dusky. Thorax pale yellow with dark stripe pattern. ABD TERG pale yellow with pair of dark dorsal tubercle. Legs pale, tarsi dark. Wing veins dark. SIPH and cauda pale. Entire body slightly covered with white wax.

Morphology. Body oval, BL 1.90–2.65 mm long. Head with three pairs of anterior and two pairs of posterior short and pointed discal setae about 0.010.02mm, median protrusion on frons developed, epicranial suture and antennal tubercle developed, head dorsum without tubercles. ANT 6-segmented, 1.22–1.39 × BL, ANT III longest with 5–10 transversely elliptical secondary rhinaria in a row on 1/4 of the segment, Ls ANT III 0.20–0.33 times as long as BD III, ANT IV-VI imbricated, ANT IV without secondary rhinaria, PT 0.94–1.27 times as long as BASE. Clypeus with nose-like processus, rostrum very short, passing over fore coxae, URS short blunted, 0.050.06 mm with four accessory setae, URS 0.13–0.23 × BASE, 0.42–0.60 × HT 2. Thorax smooth and without tubercles. Fore coxae enlarged, longest setae on TIB 0.75–1.25 × middle width of TIB, first tarsal segments with 5–7 setae, HT 2 0.10–0.12 mm long. Wing veins Co and Pts of forewing slightly dark. Dorsal ABD TERG I–VII with a pair of spinal setae on small elevations, ABD TERG VIII with a pair of spinal setae. SIPH cylindri-

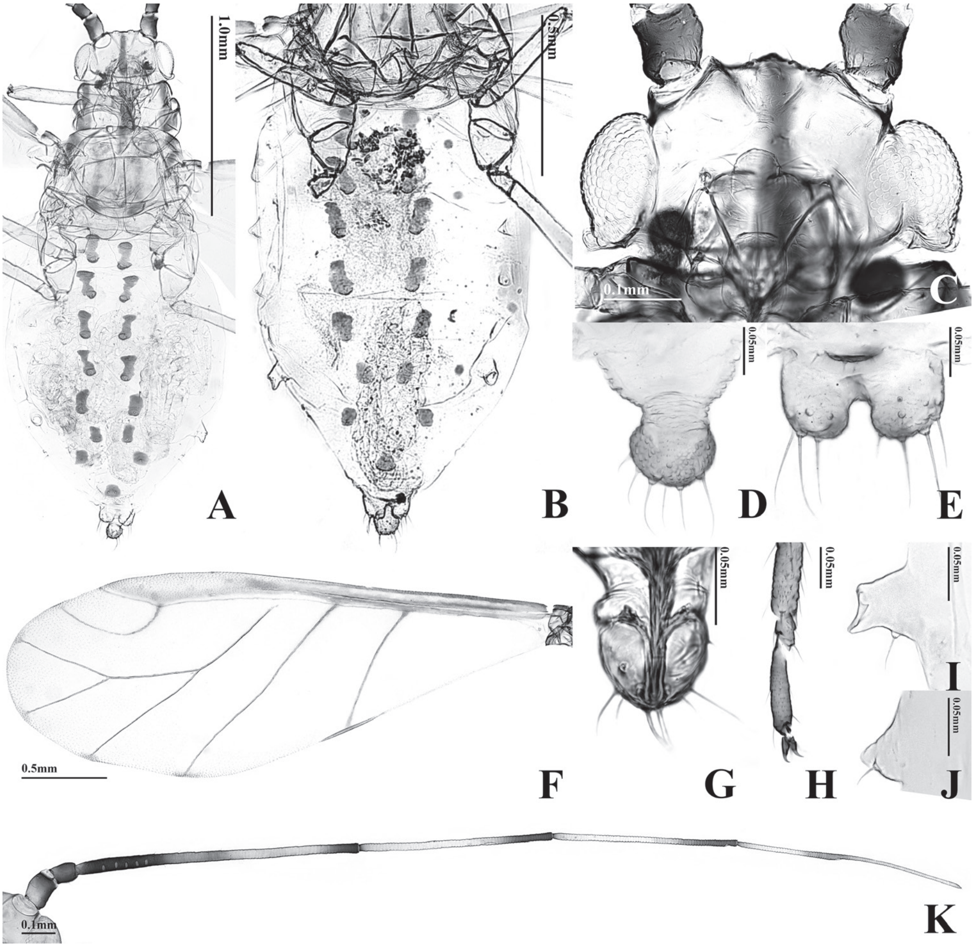

Figure 4. Alate viviparous female of *T. arundinariae*: **A** body **B** dorsal ABD TERG **C** head **D** cauda **E** anal plate **F** forewing **G** URS **H** HT 2 **I** SIPH **J** 4th marginal tubercle **K** ANT.

cal, 0.05–0.07 mm long bearing about 0.03–0.06 mm of single seta. Cauda knobbed 0.11–0.16 mm long with 10–15 setae. Anal plate bilobed, each lobe with 8–12 setae.

Distribution. This species is originally distributed in south-east Asian countries; Korea (Paik 1965), China (Qiao and Zhang 2004), India (Gosh et al. 1971), Japan (Higuchu 1968), Taiwan (Higuchu 1968), and eastern Russia (Blackman and Eastop 2017). It has invaded Europe (Barbagallo and Ortu 2009, Basky and Neményi 2014, Giacalone and Lampel 1996, Higuchi 1968, Limonta 1990, Piron 2009, Tistispis et al. 2007), Australia (Valenzuela et al. 2010), New Zealand (Blackman and Eastop 2017), North America (Coffelt and Schultz 1990), and South America (Foureaux and Kato 1999, Lazzari et al. 1999, Simbaqueba et al. 2016).

Host plants. *Arundinaria* spp., *Bambusa* spp., *Dendrocalamus* spp., *Phyllostachys* spp., *Sasa* spp., and *Sinoarundinaria reticulata* (Poaceae).

Remarks. Among the examined specimens, it is described that four alate viviparous females were collected on *Gramineae* sp. However, this host plant is probably not a true host plant due to *Takecallis* species being recorded only on bamboo species, and is probably a misidentification.

Takecallis taiwana (Takahashi, 1926)

Fig. 5, Table 1

Therioaphis tectae Tissot, 1932.

Material examined. 6 alate viviparous females, Seoguipo-si, JJ, South Korea, 33°15'3"N, 126°32'38"E, on *Sasa* sp., W.H. Paik leg., 25.iv.1971, no. 6196; 8 alate viviparous females, Seoguipo-si, JJ, South Korea, 33°15'3"N, 126°32'38"E, on *Sasa* sp., W.H. Paik leg., 15.x.1971, no. 6799.

Description. *Alate viviparous female*: *Color in life*. Not available in this study.

Morphology. Body oval, BL 2.21–2.48 mm long. Head with three pairs of anterior and two pairs of posterior pointed discal setae about 0.04–0.05mm, median protrusion on frons developed, epicranial suture and antennal tubercle developed, head dorsum with a central black stripe, spinal tubercle not developed. ANT 6-segmented 0.73–0.79 × BL, ANT III longest with 5–7 transversely elliptical secondary rhinaria in a row on basal 1/3 of the segment, longest setae on ANT III 0.33–0.67 × BD III, from distal half of ANT III to ANT VI imbricated, ANT IV without secondary rhinaria, PT 0.901.06 × BASE. Clypeus with nose-like processus, rostrum very short, reaching to fore coxae, URS short blunted 0.07 mm long with 4–5 accessory setae, URS × 0.35–0.44 BASE, 0.54–0.64 × HT 2. Thorax smooth, without tubercles. Fore coxae weakly enlarged, longest setae on TIB 0.08–1.00 × middle width of TIB, first tarsal segments with 5–7 setae, HT 2 0.11–0.13 mm long. Wing veins Co and Pts of forewing slightly dark. Dorsal ABD TERG I–VII with a pair of spinal setae on small elevations, ABD TERG VIII with 2 setae. SIPH cylindrical, 0.04–0.06 mm long. Cauda knobbed 0.15–0.20 mm long with 12–13 setae. Anal plate bilobed, each lobe with ten setae.

Distribution. This species is widely distributed in Southeast Asia; Korea (Paik 1965), China (Qiao and Zhang 2004), Japan (Higuchi 1968), and Taiwan (Higuchi 1968). It has been introduced into Europe (Higuchi 1968, Maslyakov and Izhevsky 2011, Ripka 2008, Simala et al. 2008), South Africa (Quednau 1962), New Zealand (Blackman and Eastop 2017), North America (Halbert et al. 2000), and South America (Foureaux and Kato 1999, Lazzari et al. 1999).

Host plants. *Arundinaria* spp., *Bambusa* spp., *Phyllostachys* spp., and *Sasa* spp. (Poaceae).

Remarks. This species was misidentified as *T. sasae* by Paik (1972) in Korea. Later it was revised to *T. taiwana* by Quednau and Lee (2001).

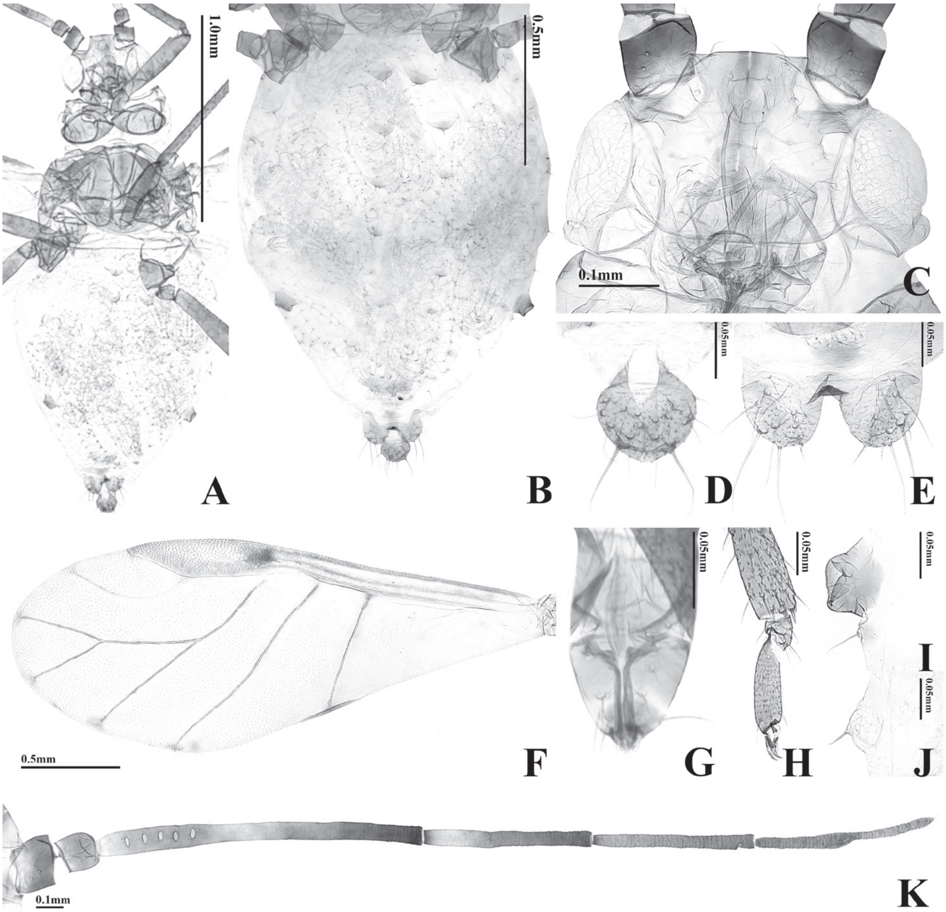

Figure 5. Alate viviparous female of *T. taiwana*: **A** body **B** dorsal ABD TERG **C** head **D** cauda **E** anal plate **F** forewing **G** URS **H** HT 2 **I** SIPH **J** 4th marginal tubercle **K** ANT.

Key to species of the genus *Takecallis* in Korea (Fig. 6)

1 ANT shorter than BL, marginal seta on ABD TERG VI not positioned on base of SIPH (Fig. 5I), URS 0.35–0.44 × BASE .... ***T. taiwana***

– ANT longer than BL, marginal seta on ABD TERG VI positioned on base of SIPH (Figs 2I, 3I, 4I), URS 0.13–0.23 × BASE... **2**

2 ABD TERG with dark spinal sclerites (Fig. 4B), cauda pale (Fig. 4D) .... ***T. arundinariae***

– ABD TERG without dark spinal sclerites (Figs 2B, 3B), cauda slightly dusky or blackish (Figs 2D, 3D) **3**

3 ANT 3.36–4.00mm, secondary rhinaria densely concentrated on very short dark section of proximal third of ANT III (Fig. 2K) .... ***T. alba* sp. n.**

– ANT 2.36–2.51mm, secondary rhinaria spread over longer dark section occupying most of proximal third of ANT III (Fig. 3K) .... ***T. arundicolens***

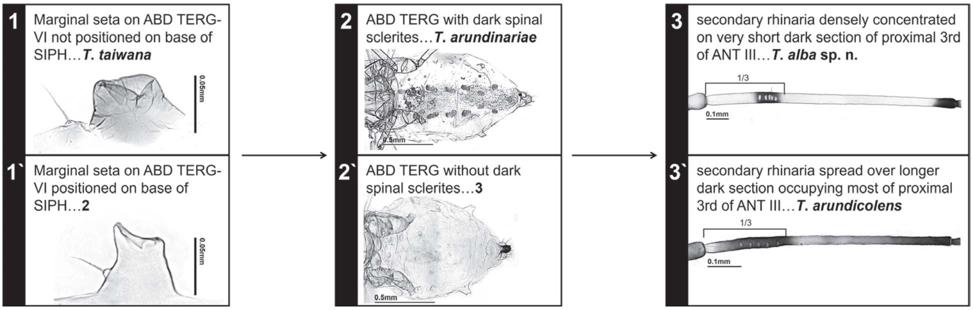

Figure 6. Pictorial key to species of the genus *Takecallis* in Korea.

Table 2. Intra- and inter-specific pairwise genetic divergence (%) based on K2P model for five *Takecallis* species

	***T. alba* sp. n. (n = 8)**	***T. arundicolens* (n = 35)**	***T. arundinariae* (n = 11)**	***T. sasae* (n = 8)**	***T. taiwana* (n = 1)**
T. alba sp. n.	0				
T. arundicolens	9.36–12.58	0–9.36			
T. arundinariae	9.94	7.78–12.87	0		
T. sasae	13.46	10.51–12.49	14.44	0	
T. taiwana	11.14	9.58–13.50	14.44	5.71	0

Molecular analyses and discussion

The NJ tree of partial COI sequences suggested that 63 sequences are distinctly divided into six groups (Fig. 7). This result clearly represented each morpho-specific group except the *T. arundicolens* complex. The *T. arundicolens* complex was separated into two genetically distinct groups (Fig. 7). Genetic distances between the two *T. arundicolens* groups ranged from 7.16 % to 9.36 %. These intraspecific divergence values are much higher than the general species delimitation value of 2.5 % in the subfamily Calaphidinae (Lee et al. 2017). In the previous study, Lee et al. (2017) suggested that this species complex seems to include at least 2 distinct species. However, it is very difficult to determine which one is the original species because morphological differences between genetically distinct groups were only observed in alatoid nymphs (Lee et al. 2017). Therefore, to solve this issue explicitly, additional studies are needed.

Except for the *T. arundicolens* complex, the rest of the four species showed 0 % of intraspecific genetic divergence (Table 2). Interspecific distances among the five species ranged from 5.71 % to 14.44 % (Table 2). *T. sasae* and *T. taiwana* showed the lowest interspecific distance level (Table 2). Overall mean genetic distance was 8.91 % for the 63 partial COI sequences of the five *Takecallis* species.

Molecular evidence strongly indicates the validity of *T. alba* sp. n. All of the individuals of *T. alba* sp. n. were grouped together and this group was clearly separated from other species groups with a high interspecific distance level that ranged from 9.36 % to 13.46 % (Table 2). Morphological characteristics of this species correspond

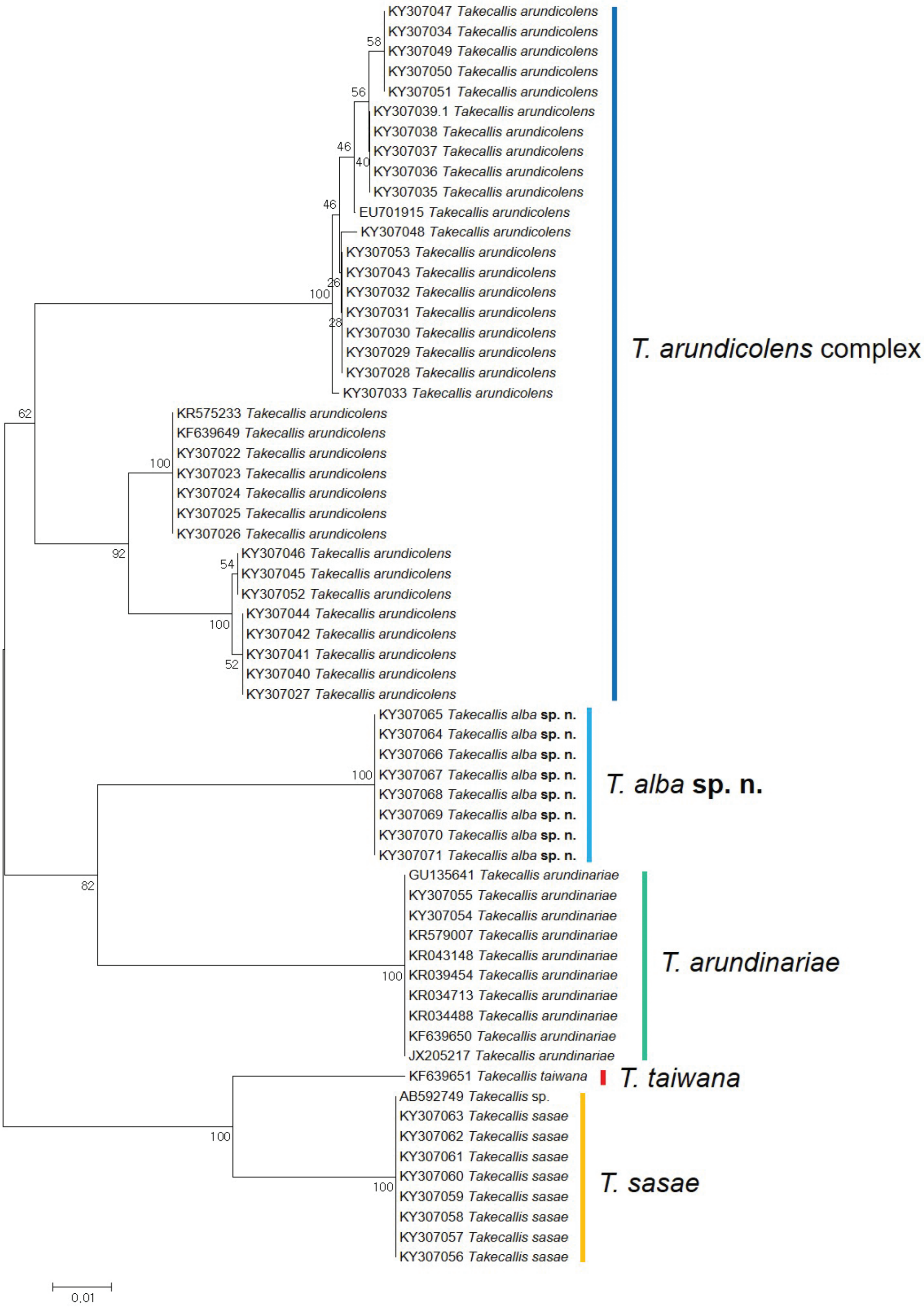

Figure 7. Neighbor-joining tree of COI partial gene sequences of *Takecallis* spp. (63 sequences of five species).

to molecular evidence. Although we could not test all *Takecallis* species from all over the world, this species also has morphological characteristics that distinguish it from all known species. Morphologically, *T. alba* sp. n. is most similar to *T. affinis* and *T. assumenta*. However, its number of accessory setae on URS and the arrangement of secondary rhinaria on ANT III are clearly distinct from the above two species.

In the present study, four *Takecallis* species were recognized from Korea. Our study demonstrated that the four species are clearly separated based on morphological and molecular evidence. However, the taxonomic status of genetically distinct groups within the *T. arundicolens* complex still needs to be resolved.

Acknowledgements

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COMMENTARY

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On 'lost' indigenous etymological origins with the specific case of the name *Ameiva*

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Abstract

Modern biology builds upon the historic exploration of the natural world. Recognizing the origin of a species' name is one path to honor the historic exploration and description of the natural world and the indigenous peoples that lived closely with organisms prior to their description. While digitization of historic papers catalogued in databases such as the Biodiversity Heritage Library (BHL) allows for searching of the first use and origin of names, the rapid pace of taxonomic publishing can occlude a thorough search for etymologies. The etymological origin of the genus name *Ameiva* is one such case; while unattributed in multiple recent works, it is of Tupí language origin. The first description was in the *Historiae Rerum Naturalium Brasiliae* by George Marcgrave (1648). *Ameiva* was the name used by Marcgrave's Amerindian hosts in 17th century Dutch Brazil, where local people spoke the now extinct language Tupí. The Tupí origin was not lost, however, until as recently as the 2000s. Herein, the pre- and post-Linnaean use of the name *Ameiva* is traced and when the name is attributed to the Tupí language and to Marcgrave through time it is noted. The opportunity to discover and/or recover etymological origins, especially names from extinct and indigenous languages, provides insight into the early Western sciences. Careful study of etymology by naturalists is consistent with the idea that science is an evolving process with many predecessors to appreciate.

Keywords

Etymology, indigenous languages, Neotropics, reptiles, seventeenth century, zoological nomenclature

Introduction

Our understanding of the relationships of species is evolving rapidly. As a result, scientists are continually revising circumscriptions, proposing new names, and resurrecting old names. Scientific names derived from indigenous, Latin, and Greek words, technical terms, and given in honor of people and places are attributed when known (e.g., Jaeger 1959). The use of indigenous names is frequent, honoring local peoples and places. Best practices associated with indigenous and traditional languages include consultation with native speakers for new attributions or changes to taxa names (e.g., Maori: Whaanga et al. 2013). Sometimes, indigenous words are 'Latinized' using the Roman alphabet or with changed endings to follow nomenclatural rules (ICZN Article 11.2-3). In this case, preventative loss of the meaning of the name would ideally involve careful notation of etymological origins. Tracing the early roots of indigenous names given in the past is one way to record historic scientific efforts, honor cultural exchange between Western scientists and the world (Agrawal 1995), and correct mistranslations.

Seventeenth century European naturalists described the fauna and flora of the world widely. Their work is echoed across a multitude of names in use today, common and scientific, derived from indigenous languages. The Sydney language word 'waratah' is the common name for the national flower of Australian state New South Wales *Telopea speciossissima* (Sm.) R.Br., while the manatee *Trichechus manatus* Linnaeus, 1758 is a cognate of the Caribbean Taino language 'manati'. The South American tegu lizard *Tupinambis teguixin* Linnaeus, 1758 is a direct cognate from the extinct language Tupí. The language was spoken widely among Tupinambá people and become the língua geral or the most common unifying Tupí language of the 50 or more languages spoken amongst Tupi-Guarani speaking peoples (Walker et al. 2012).

One of these suspected cognates is *Ameiva* (Meyer, 1795), the modern generic name of a group of more than 36 lizard species distributed throughout Central and South America and the Caribbean. The specific etymology of the name *Ameiva* is marked as 'unknown' in some modern taxonomic revisions (Harvey et al. 2012), scientific dictionaries (Beolens 2011), and online databases (Uetz 2015)). If *Ameiva* was Amerindian in origin, usage in natural history literature could help to discover its etymological origins. A digital online resource, the Biodiversity Heritage Library (BHL), allowed a precursory universal search for its use in historic Western science texts and facilitated a starting point. The taxonomic record of the name *Ameiva* was traced to and after Linnaeus, and other scholarship was traced to determine the origin of the name *Ameiva*.

The *Historiae Rerum Naturalium Brasiliae* (Marcgrave, 1648)

The earliest use of the name *Ameiva* within the BHL was found in the Latin-language *Historiae Rerum Naturalium Brasiliae* by George Marcgrave of the Dutch Republic (Marcgrave 1648). Texts from ancient languages can be difficult to assess, therefore a translation of the original 17th century Latin text is provided (Table 1, Fig. 2). Lizards named *Ameiva* were described in the *Historiae Rerum Naturalium Brasiliae* in 1648 by a brief paragraph, describing the morphology and some behaviors (Marcgrave 1648: 238; Fig. 2).

Table 1. Latin to English translation related to the genus *Ameiva* from Marcgrave (1648, p. 238). To view the text as set in the original publication see Figure 2.

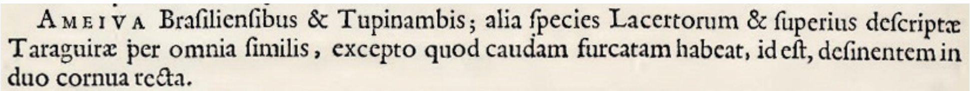
p. 238	p. 238
Ameiva Brafiiienfibus & Tupinambis; alia species Lacertorum & superius descriptae Taraguirae per omnia fimilis, excepto quod caudam furcatam habeat; id eft,definentem in duo cornua rexta.	*Ameiva* Brasiiiensibus & Tupinambis; everything is like the other species of lizards described in the group of Taraguirae, except that it has a bifurcated tail. The two tail lengths are straight but vary in measurement.

CAP. XII.

Tejuguacu & Temapara. Taraguira. Americima. Carapopeba. Ameiva. Taraguico Aycuraba. Tejunhana. Omnes Lacerti.

TEIVGVACV & TEMAPARA Tupinambis: Lacertus egregius: corporis figura, capitis, oculorum, oris, pedum, caudæ, convenit cum Senembi; in hoc autem differt. Primo totius corporis color eſt niger, elegantiſſimis maculis albis inſperſis variegatus, & verſus extremam caudam, ſex quaſi aculeos habet albos. Secundo cauda craſſior in exortu. Tertio caret illis dentatis aculeis per dorſi longitudinem. Quarto in pedibus poſterioribus exterior digitus paulo remotior eſt à reliquis, & brevior. Quinto linguam habet bifidam longam, rubram, glabram, quam velociſſime inſtar ſerpentis ad digiti longitudinem protrudit & retrahit. Nullam edit vocem: patiens eſt: lubenter veſcitur ovis, quæ cruda ſorbet. Inediæ patientiſſimum animal: detinebam alligatum, ſed cum ſe à vinculis abſolviſſet, liberum permiſi per cubiculum currere, & interdum dedi hauſtum aquæ è vitro bibendum, ita adhuc obambulat quando ſitit, alias toto die in angulo ſuo ordinario ſedet. Libenter incumbit cineribus calidis: vigeſimo Martii quiſpiam partem caudæ calcando detriverat; poſtea tamen de novo ad duos digitos crevit; primo Julii moriebatur inedia plane tabidus factus, ſeptem enim menſium ſpacio nihil comederat, tantum aliquando hauſtum aquæ ſumſerat lambendo. Caro ejus comeditur.

Figure 1. Marcgrave's watercolors are highly accurate. The watercolor related to the tegu lizard, *Tupinambis teguixin*, is reproduced here set within text (Marcgrave 1648, p. 237).



AMEIVA Braſilienſibus & Tupinambis; alia ſpecies Lacertorum & ſuperius deſcriptæ Taraguiræ per omnia ſimilis, excepto quod caudam furcatam habeat, id eſt, deſinentem in duo cornua recta.

Figure 2. The passage related to the species *Ameiva* that is treated in Marcgrave (1648, p. 238). The Latin text is translated side-by-side with the English in Table 1.

The first Western use of *Ameiva* in *Historiae Rerum Naturalium Brasiliae* is largely corroborated by taxonomic scholars, both before and after the 1758 publication of Linnaeus' momentous taxonomic work *Systema Naturae*. Marcgrave wrote and illustrated *Historiae Rerum Naturalium Brasiliae* as an eight-volume book describing the plants, fishes, amphibians, reptiles, birds, mammals, and people of Brazil over a journey beginning in 1638 with Prince John Maurice of Nassau-Siegen (Gudger 1914). Marcgrave accompanied his notes with watercolor paintings made from life in the field over six years and six months (Gudger 1912). Marcgrave returned the final manuscript on a ship bound for Amsterdam but contracted a fever in 1643, and succumbed to death in 1644 without seeing its publication. Jan de Laet, Prefect of the Dutch West India Company, published Marcgrave's manuscript posthumously (Gudger 1912). The 303-page Latin document contains 429 plates based on Marcgrave's original watercolor paintings (e.g., see Fig. 1). A later twelve-volume anthology included an additional four chapters related to medical cures of the Americas and become the most popular version of the text (Piso 1658).

The information related to *Ameiva* is accurate based on scientific knowledge of the genus today with the exception ofa description of a bifurcated tail as a diagnostic character. In fact, tail regeneration is quite common across many species and families of lizards and may present as a bi- or trifucation (Bateman and Fleming 2009). Marcgrave's descriptions of many taxa are highly accurate to this day, specifically depictions of the morphology of fishes and plants (Gudger 1916). The descriptions are referenced by Linnaeus and in pre- and post-Linnaean travel and scientific writings today due to their accurate details (Whitehead 1979).

Taxonomic nomenclature of *Ameiva* by Linnaeus (1758)

Linnaeus described *Lacerta (= Ameiva) ameiva* in *Systema Naturae* (1758: 203), and cited Seba (1734) and two student dissertations from *Ameoenitates Academicae I* (Linnaeus 1749: 127, 293) in the species description (Bauer 2012; Liner 2012). In Seba's *Thesaurus rerum naturalium* (Seba, 1734), Seba referenced *Ameiva* from a work of Johannes Jonston (1650:140, table 88, Fig. 2). Barthold Rudolph Hast's student thesis '*Amphibia Gyllenborgiana*' indicated that Seba described *Ameiva* (1734: 127). Later, Lars Balk (1746) defended a dissertation describing the collections in the '*Museum Adolpho-Fridericianum*' and referenced Hast's thesis to describe *Ameiva*. Jonston (1650), precursor of Seba, would be a contemporary of Marcgrave. Pre-Linnaean scholarship attributing the description of *Ameiva* to Marcgrave (1648) also exists.

Historic pre-Linnaean scholarship using *Ameiva*

Outside of Amsterdam, naturalists cited the *Historiae Rerum Naturalium Brasiliae* for many years after its publication (Jonston 1650; Piso 1658; Ray 1686). Johannes Jonston, of the Leszno Academy in Poland, wrote a four volume work, the *Historiae naturalis de quadrupedibus libri* or the 'Natural History of the Four-footed Beasts' in 1650, just two years after publication of Marcgrave (Bauer 2012). He described *Ameiva* more than a dozen times across the four volumes. While his contemporaries Piso (1658) and Ray (1686) referenced and quoted various parts of Marcgrave's work in their natural history writing, Jonston (1650) went so far as to say that Marcgrave was the first to describe American *lacerti* 'lizards'. Thus, Seba (1734) described *Ameiva* for Linnaeus *sensu* Jonston (1650) and ultimately Marcgrave (1648).

Post-Linnaean scholarship using the name *Ameiva*

Post-Linnaean publications (Spix 1824; Stejneger 1904) and travelogues (Wied 1825: 86) attributed *Ameiva* to Marcgrave, too. Notably, the German scholar Spix described the 'brilliantly colored' *Ameiva* lizards referenced by Marcgrave during the 1817 Bavarian Wied-Neuwied expedition to Brazil (Spix 1824: p. 245). More recently, Stejneger (1904) attributed the name *Ameiva* to Marcgrave in *The Herpetology of Porto Rico* (p. 612). Jaeger (1955) described the word as a root, writing '*Ameiv*— name of a kind of lizard' and Aboriginal in origin. Last, Gotch (1986) recognized both *teju* and *ameiva* as Tupí words.

The species name became generic when Meyer (1795) elevated *Ameiva* from within *Lacerta* Linnaeus, 1758 to a genus with fourteen species of lizards. Meyer (1795) diagnosed *Ameiva* as lizards with five regular parietal scales, prefrontal scales separated from nasal scales, homogeneous lamellae of the toes, without preanal spurs. Meyer (1795) spelled the genus name *Ameiua,* likely because letters like "u" and "j" do not occur in the classical Latin alphabet. Throughout historic scholarship, *Ameiva* reverted to its historic spelling. Other spellings were mistakes, therefore ignored and quickly reverted to *Amaiva* (Kuhl, 1820), *Amieva* (Gray, 1840), and *Amiva* (Cope, 1887).

At the same time, the word *Ameiva* was translated to local languages. Merrem (1820) translated species names into German words that made biological sense to local readers. *Ameiva* was assigned to the German *Warnender,* a 'traveler'. Blasius Merrem studied reptile and amphibian biology, and he had knowledge of the *Ameiva* as actively foraging lizards. The translation to 'traveler' provides context for later species naming including *Ameiva exsul*. Cope (1862) gave the Puerto Rican area *Ameiva* the specific name '*exsul'* without providing context in the original description. The Latin word *'exsul'* has multiple meanings including 'banished person.'exile', 'wanderer', and 'traveler'. Stejneger (1904: 612) knew Cope personally and added a footnote to *The Herpetology of Porto Rico* (Stejneger 1904) that the Latin '*exsul'* translates to 'wanderer or traveler'. At least one later scholar without knowledge of the Stejneger (1904) footnote, translated '*exsul'* as a 'banished person or exile', losing the spirit of the original attribution (Brown 1954). Although relatively minor consequences exist in the misunderstanding of the original meaning of *Ameiva*, this represents just one example of the importance ofmaintaining a clear and consistent record of etymologies.

Other authors developed Marcgrave's work with their own flourishes. Owens (1742), a popular 18th century writer, described *Ameiva* as fearful creatures with two tails, concluding that "….this Article [*forked tail*] seems to differ from all other sanguineous Animals….I have never heard of any else furnished with two Tails (p. 122)". As stated earlier, birfucation is common amongst Neotropical lizards. Owens' (1742) other colorful writing declared the *Tejuguacu* (=*Tupinambis teguixin*) could for "…six or seven moons, live without any sustenance, but air, the fluid in which we all breathe". Marcgrave did tether a tegu to a leash in his Brazilian residence (Fig. 1). The tegu refused to eat and wasted away to its death after living without sustenance for seven months: "septem enim mensium spacio nihil comederat".

Records of extinct languages

Now establishing that Marcgrave ran into the word *Ameiva* in South America, of local Amerindian origin, a problem exists in independently verifying the word by Tupí language authorities. The word *Ameiva* was not found in available Tupí dictionaries and references searched (da Silveira Bueno 1998; Tibirçá 2001; Chiaradia 2015). Henrique Caldeira Costa (pers. comm.) speculates that the name *Ameiva* is actually a contraction of two Tupí words, 'Ambere' and 'Aíba' meaning 'lizards that are not fit to eat'. Chiaradia (2015) defines 'Aimbere' or 'Ambere' as 'one who writhes', a reasonable translation for lizards. Tibirçá (2001) translates 'amberé' to the Portuguese 'lagartixa', a small lizard. Chiaradia (2015) and Tibirçá (2001) translates 'Aiba' to 'something that is not good to eat' or 'bad/evil'.What Marcgrave may have heard spoken in 1640 was 'amberé – aíba' or writhing, inedible lizards (H. Cladeira Costa, pers. comm.). Only the history of the name *Ameiva* was discussed herein; however, Marcgrave described many more Neotropical species. Other examples in the botanical and zoological literature from the Tupí language groups include *piranha*, *jacaranda*, *petunia,* and *jaguar*.

Conclusions

An Amerindian origin exists for the word *Ameiva*, possibly old Tupí, first introduced into the Western science vernacular by Marcgrave (1648). Etymology is germane in a world of rapid scientific discourse. Negligible time is required to determine the origin of many scientific names unattributed in modern scholarly works. With the digitization of historic texts in online databases (i.e., Biodiversity Heritage Library), we are linked more easily today to our academic predecessors than any other time in the recent past (Pilsk et al. 2010). Correcting the attribution of the word *Ameiva* honors scientific inquiry of the past and historic contributions of indigenous people to Western scientific nomenclature. The *Historiae Rerum Naturalium Brasiliae* is digitized and searchable and so may provide important insights into the behavior, distribution, and historic assemblage of species in eastern South America (Lees and Pimm 2015). For groups in need of taxonomic and phylogenetic revision, like the family *Teiidae* containing the genus *Ameiva* (Harvey et al. 2012; Pyron et al. 2013), great opportunity exists for recovering lost etymologies and new honorarium.

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